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Body condition of phocid seals during a period of rapid environmental change in the Bering Sea and Aleutian Islands, Alaska

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ABSTRACT

A warming climate has driven rapid physical changes in the Arctic environment, particularly in the Bering Sea. Biological changes are also increasingly evident in the Bering Sea and adjacent waters. The ecological results have been profound and relatively well documented for fishes and lower trophic levels. Upper trophic predators such as marine mammals, however, have been underrepresented in ecosystem surveys, models, and efforts to practice ecosystem-based fisheries management. We used multiple linear regression to model body condition (mass/length) as a function of species, age class, sex and year for ribbon and spotted seals in the Bering Sea, and harbor seals in the Aleutian Islands, from 2007 to 2018, for evidence of recent trends that might reflect trophic or bottom-up changes in the ecosystem. Model-averaged coefficients (in kg cm^{-1}, relative to the overall mean) indicated that body condition was lower for subadults (−0.063; 95% CI: −0.074 – −0.051) and pups (−0.120; 95% CI: −0.129 – −0.112) than for adults (0.183, the negative sum of the subadults and pups coefficients). Body condition for males (0.010; 95% CI: 0.002–0.019) was higher than for females (−0.010). Overall, body condition declined annually (−0.014 per year; 95% CI: −0.025 – −0.004), and there was an additive annual decline in body condition of seal pups across all species and sexes (−0.020; 95% CI: −0.030 – −0.011). An additive annual increase in body condition of spotted seals across all sexes and age classes (0.013; 95% CI: 0.004–0.022) mitigated the annual declines for this species. Model-averaged fitted values therefore indicated annual declines in body condition for ribbon and harbor seals of all sex and age classes, and for spotted seal pups. We relate these declines to the trend in Bering Sea ice extent and to recent, rapid changes brought on by the significant Northeast Pacific marine heat wave of 2014–2016 and its lingering effects through 2018 and 2019. The results indicate that these typically resilient, long-lived, generalist predators can be impacted by bottom-up forcing.

1. Introduction

The Arctic is changing, driven largely by a warming climate in which temperatures have risen 2 ° to 3 °C since 1880 and 0.75 °C in just the past decade (Post et al., 2019). In the past five years, environmental change has been extremely rapid in the Arctic, particularly in the Bering Sea, where short-term variability superimposed on the long-term warming trend reflects 'teleconnections' from tropical and temperate Pacific waters in the form of marine heat waves (Di Lorenzo and Mantua, 2016). Globally, marine heat waves are becoming more frequent and intense (Frölicher et al., 2018). The annual extent of seasonal sea ice in the Bering Sea has fluctuated dramatically, with record highs and lows for the satellite era occurring in the past decade (Fetterer et al., 2017, updated daily). Biological changes are also increasingly evident in the Bering Sea and adjacent waters, including changes in species distributions (Dunmall et al., 2013; Grebmeier, 2012; Mueter and Litzow, 2008; Stafford, 2019; Stevenson and Lauth, 2019), individual condition and health (VanWormer et al., 2019), or vital rates of mortality and reproduction (Piatt et al., 2020). Cumulatively, recent changes in the region have risen to a level that has been characterized as 'ecosystem transformation' (Huntington et al., 2020).

The ramifications of environmental and biological change for ecosystem structure, dynamics and function are complex; identifying and understanding them likely will require broad examination across trophic levels. Lower- and mid-trophic species are relatively well-monitored, especially in association with fisheries oceanography studies in the southeastern Bering Sea. Many upper trophic species, however, are comparatively rare and difficult to observe or monitor, and

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challenging to include in integrated ecosystem surveys (e.g. Duffy-Anderson et al., 2019; Van Pelt et al., 2016), models (e.g. Punt et al., 2016), and fisheries management schemes (e.g. Siddon and Zador, 2019). Upper trophic species, especially long-lived generalist predators, may be relatively resilient to 'bottom-up' variability, in some cases making them insensitive as sentinel indicators of ecosystem shifts. When changes in body condition or growth rates do appear in generalist top predators, however, the changes are likely to reflect broad underlying ecological shifts in multiple prey species. Since 2007, we have studied three species of phocid seals that are abundant upper trophic predators in the Bering Sea and Aleutian Islands, for evidence of recent trends that might reflect trophic or bottom-up changes in the ecosystem.

Ribbon seals (*Histriophoca fasciata*) and spotted seals (*Phoca largha*) are ice-associated species with their main breeding distributions in the seasonally ice-covered waters near the continental shelf breaks of the Bering Sea and Sea of Okhotsk (Burns, 1981; Lowry, 1985b). These species depend on the sea ice during key life history events, including pupping, breeding, and molting. In summer, ribbon seals disperse to a variety of offshore foraging habitats in the Gulf of Alaska, North Pacific Ocean, Bering Sea, and Chukchi Sea, while spotted seals divide their time between coastal haul-out sites and foraging zones off the shores of Alaska and Russia. Both species' main prey, at least during the spring and summer when most samples have been collected, consists of fish, squid, and crustaceans (Boveng et al., 2009, 2013; Dehn et al., 2007; Quakenbush et al., 2009), and there is little evidence for substantial niche separation between the two species (Wang et al., 2016).

Harbor seals (*Phoca vitulina richardii*) have a broad range in temperate and sub-Arctic waters of the northeast Pacific Ocean and are found in all coastal Alaska waters north to at least Bristol Bay and the Pribilof Islands (Muto et al., 2018). Harbor seals forage mostly near shore, and their diet is also mainly fish, squid, and crustaceans, but they do not depend on sea ice for life history events, like ribbon and spotted seals, and therefore may respond differently than those species to ecological change. The primary objective of this study was to assess trends in body condition of ribbon and spotted seals in the Bering Sea and harbor seals in the Aleutian Islands during a period of rapid ecological change.

2. Methods

We captured, sampled, and released ribbon and spotted seals during April to early June of 2007, 2009, 2010, 2014, 2016, and 2018 at the edge of the pack ice in the Bering Sea. Harbor seals were captured, sampled, and released in the Aleutian Islands during September in 2014–2016 (Fig. 1). We used long-handled fish-landing nets to catch ribbon and spotted seals in the Bering Sea, the same nets to catch harbor seals on beaches or exposed reefs in the Aleutian Islands, and tangle nets deployed from small boats to catch harbor seals in nearshore waters of the Aleutians (Jeffries et al., 1993).

Seals were transferred from capture nets to hoop nets and physically restrained or sedated with an intravenous injection of diazepam (2010; 0.1 mg per kg body mass) or midazolam (2014–2018; 0.1 – 0.2 mg per kg body mass). We occasionally used an intramuscular injection of midazolam depending on the seal's behavior and temperament during capture and initial handling. Prior to releasing sedated seals, the effects of sedation were reversed with an intramuscular injection of flumazenil (0.01 mg per kg of body mass; West-Ward Pharmaceuticals, NJ, USA) if the attending veterinarian determined it was needed.

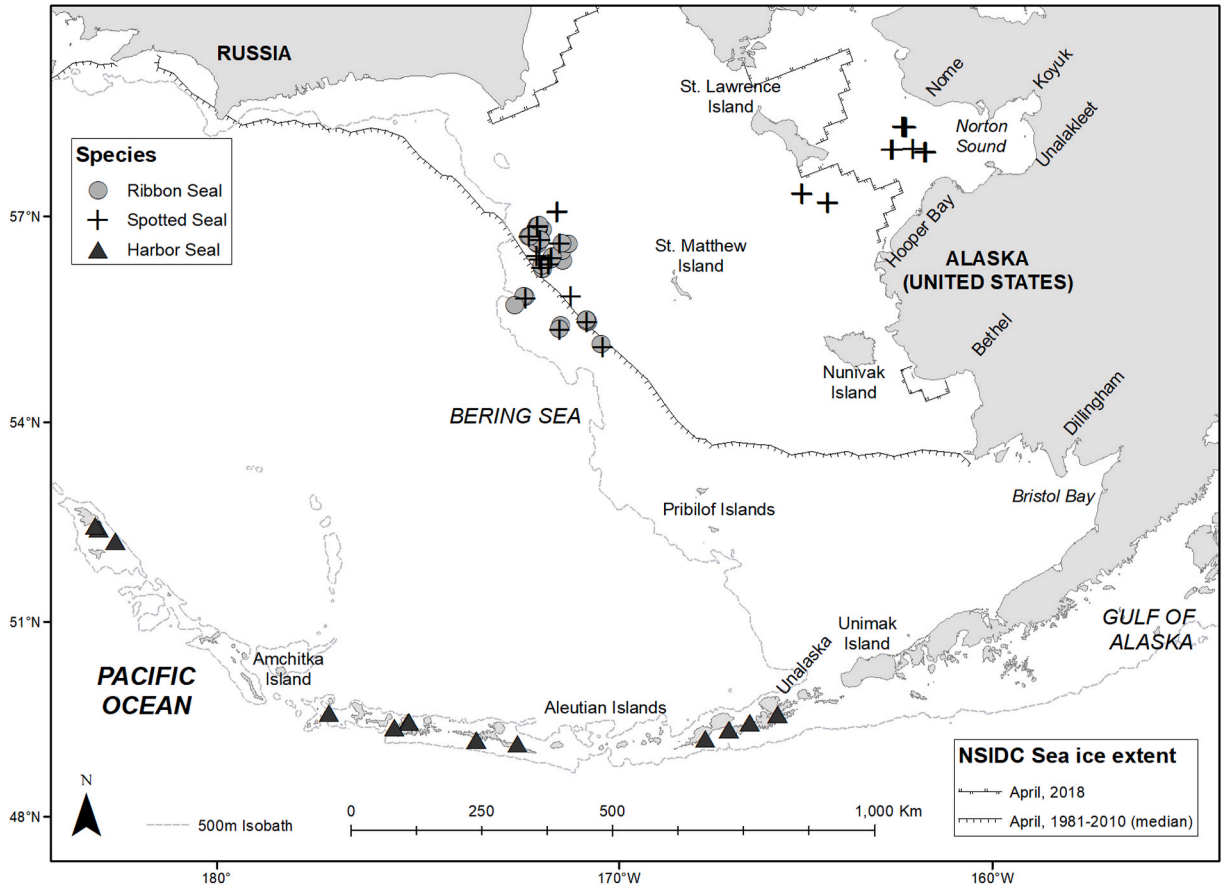

Fig. 1. Locations where spotted, ribbon, and harbor seals were weighed and measured in 2007–2018. The 500 m isobath is shown as a reference for the continental shelf break. Sea-ice extent from the National Snow and Ice Data Center (NSIDC) is shown as the average for the month of April, 2018, and as the median from April, 1981–2010, for comparison (Fetterer et al., 2017, updated daily).

We weighed seals in a sling suspended from a bipod and hanging scale (Dynalink, 250 kg) on ice floes in the Bering Sea or with a crane and hanging scale on a ship in the Aleutians. All seals were weighed (to the nearest 0.1 kg) and measured (length of prone seal in a straight line from nose to tip of tail, axillary girth, maximum girth, hip girth; to the nearest 0.5 cm), and age class and sex were determined. We classified individuals into four age classes: pup (<1 y, not weaned), young-of-the-year (<1 y, weaned), subadult (1–4 y), and adult (>4 y) (Burns, 1981; Pitcher and Calkins, 1979). We used morphological characteristics (e.g. size, color and, for ribbon seals, distinctness of ribbons) to distinguish between subadults and adults (Burns, 1969, 1981; Naito and Oshima, 1976; Quakenbush et al., 2009; Tikhomirov, 1968).

We used a portable ultrasound scanner to measure blubber depth at four different sites on the seals sampled in 2014–2018. Owing to the relatively short period of ultrasound sampling, we did not use blubber depth for our evaluation of trends in body condition. However, we report linear regression results on the relationships between blubber depth, body condition, and girths in the Supplemental Materials, because they may be useful in future analyses for evaluating relative changes in fat mass versus lean body mass in response to ecological change.

We used multiple linear regression to model our index of body condition, mass per standard length (in kg cm^{-1}), as a function of species, age class ('age_class'), sex, year, and a linear or quadratic trend for day of year ('Day' and 'Day2' respectively). Year was modeled as fully time-dependent ('year'), a linear trend ('Year'), or a quadratic trend ('Year2'). Having found little support for 'year', 'Day2', 'Day:Year', or higher-order annual trend effects in preliminary analyses, we included all possible combinations of main effects and two-way interactions of species, age class, sex, Day, Year, and Year2 (except 'Day:Year') in our candidate model set. To help with numerical stability and to facilitate interpretation of estimated coefficients across models, all categorical covariates were centered at zero, 'Day' was standardized, and the annual trend covariates ('Year' and 'Year2″) were orthogonalized. The bias-corrected Akaike's Information Criterion (AICc; Burnham and Anderson, 2002) was used to calculate model weights. Using AICc model weights, standardized estimated coefficients were model-averaged based on Lukacs et al. (2009) and Cade (2015). Model-averaged estimates for body condition were calculated from the predictions for each individual model, based on AICc weights. All analyses were performed with MuMIn package version 1.43.15 (Bartoń, 2019) in R 3.6.3 (R Core Team, 2019). The R code and data are available in Boveng et al., (2020).

3. Results

We weighed and measured 98 ribbon seals and 94 spotted seals at the pack ice edge in the Bering Sea between 2007 and 2018, and 80 harbor seals in the Aleutian Islands between 2014 and 2016 (Table 1). We combined weaned pups and maternally-dependent pups into the single age class 'pup' for analysis.

The most general model, including all two-way interactions, explained much of the variation in body condition (multiple R^2 = 0.891), but it was not well supported by AICc (Delta AICc = 65.5). There was considerable model selection uncertainty among the more parsimonious models, with the minimum AICc model only receiving 7% of the model weight (Supplemental Material Table S1). Although AICc weights can in some cases be a poor indictor of the relative importance of predictors (Cade 2015), the sums of AICc weights across all models that included a particular variable were largest for 'age_class' (100% of AICc weight), 'Day' (100%), 'species' (100%), 'Year' (100%), 'sex' (99.8%), 'age_class:Year' (98.6%), 'species:Year' (96.9%), 'Day:species' (96.2%), 'Year2' (92.0%), and 'age_class:Day' (83.9%).

Model-averaged coefficient estimates indicated several significant effects on body condition based on species, age class, sex, day, year, or two-way interactions (Table 2; note that all the predictors were centered at zero, making the coefficients relative to the mean body condition across species, age class, sex, day, and year, and—because only $n-1$ coefficients can be independently estimated for a categorical predictor with n levels—the remaining coefficient is simply the negative sum of the $n-1$ estimated values). Body condition evaluated across all species was lower for subadults (−0.063 kg cm^{-1}; 95% CI: −0.074 – −0.051) and pups (−0.120 kg cm^{-1}; 95% CI: −0.129 – −0.112), than for adults (0.183 kg cm^{-1}). Males were found to have higher body condition (0.010 kg cm^{-1}; 95% CI: 0.002–0.019) than females (−0.010 kg cm^{-1}). There was a negative 'Year' effect on body condition across all species,

Table 1
Numbers of ribbon, spotted, and harbor seals, by age class and sex, sampled for body condition in 2007–2018.

Species	Sex	Adult	Subadult	Pup	Total
Ribbon	Female	29	11	8	48
	Male	24	13	13	50
	Total	**53**	**24**	**21**	**98**
Spotted	Female	14	5	29	48
	Male	5	10	31	46
	Total	**19**	**15**	60	**94**
Harbor	Female	23	14	7	44
	Male	17	13	6	36
	Total	**40**	**27**	**13**	**80**
Grand total		**112**	**66**	**94**	**272**

Table 2
Model-averaged predictors for seal body condition (mass per standard length) of ribbon, spotted, and harbor seals sampled between 2007 and 2018 in the Bering Sea and Aleutian Islands, Alaska. For each predictor with >0.5 proportion of AICc model weights ('Weight'), model-averaged estimates ('Estimate'), 95% confidence intervals (Lower, Upper), and p-value codes are provided, where '***' indicates $p < .001$, '**' indicates $p < .01$, '*' indicates $p < .05$, and '.' indicates $p < .1$. For predictors that are factors, results are provided for each factor level ('Factor'). For a categorical (factor) regression predictor with n levels, only n-1 coefficients and their confidence limits can be estimated. The factor levels we estimated for 'age_class' are subadult and pup, levels for 'species' are spotted and harbor seals, and the level for 'sex' is male. Because all covariates were centered at zero, the estimated coefficients are relative to the mean body condition across species, age class, sex, day, and year. The coefficient for the remaining factor level is therefore the negative sum of the other coefficients. For example, the 'age_class' coefficient for adults is the negative sum of the other age classes (0.063 + 0.120 = 0.183).

Predictor	Weight	Factor	Estimate	Lower	Upper	p-value
age_class	1.000	subadult	-0.063	-0.074	-0.051	***
		pup	-0.120	-0.129	-0.112	***
Day	1.000		-0.012	-0.022	-0.003	**
species	1.000	spotted	0.008	-0.003	0.019	
		harbor	0.008	-0.003	0.018	
Year	1.000		-0.014	-0.025	-0.004	**
sex	0.998	male	0.010	0.002	0.019	*
age_class:Year	0.986	subadult	0.002	-0.006	0.010	
		pup	-0.020	-0.030	-0.011	***
species:Year	0.969	spotted	0.013	0.004	0.022	**
		harbor	-0.004	-0.013	0.005	
day:species	0.962	spotted	0.010	0.002	0.019	*
		harbor	0.012	0.004	0.020	*
Year2	0.920		0.006	-0.003	0.015	
age_class:Day	0.839	subadult	-0.002	-0.010	0.006	
		pup	0.012	0.004	0.023	.
sex:Year	0.796	male	0.006	-0.004	0.018	
age_class:sex	0.704	subadult:male	-0.004	-0.014	0.002	
		pup:male	-0.007	-0.019	-0.002	
sex:species	0.677	male:spotted	0.006	0.000	0.017	
		male:harbor	0.003	-0.009	0.016	
age_class:Year2	0.612	subadult	0.000	-0.008	0.008	
		pup	-0.006	-0.018	-0.001	
sex:Year2	0.541	male	-0.005	-0.017	0.000	

sexes, and age classes (−0.014 kg cm^{-1} per year); 95% CI: −0.025 – −0.004). There was an additional negative 'age_class:Year' effect on body condition for the pup age class (−0.020; 95% CI: −0.030 – −0.011) and a positive 'species:Year' effect on body condition for spotted seals (0.013; 95% CI: 0.004–0.022). There were several effects related to 'Day' that differed between species and age class (Table 2).

Model-averaged predictions (i.e. fitted values) for body condition demonstrate the additive and interaction effects of the covariates, depicted by year (Fig. 2) and day (Fig. 3) for each species, age class, and sex. For example, the predicted decline in spotted seal pup body condition (Fig. 2) reflects the net effect of the 'age_class:Year' term for pups (Table 2; −0.020 kg cm^{-1} per year), mitigated by annual increases in body condition for this species as a whole (0.013 kg cm^{-1} per year), plus the contributions of the main 'Year' effect and other covariates. With no such mitigation for the other species, the fitted values indicate an annual decline in body condition of ribbon and harbor seals for all sex and age classes. The model also predicts significant daily decreases in body condition for ribbon seals of all age classes during April and May, weak

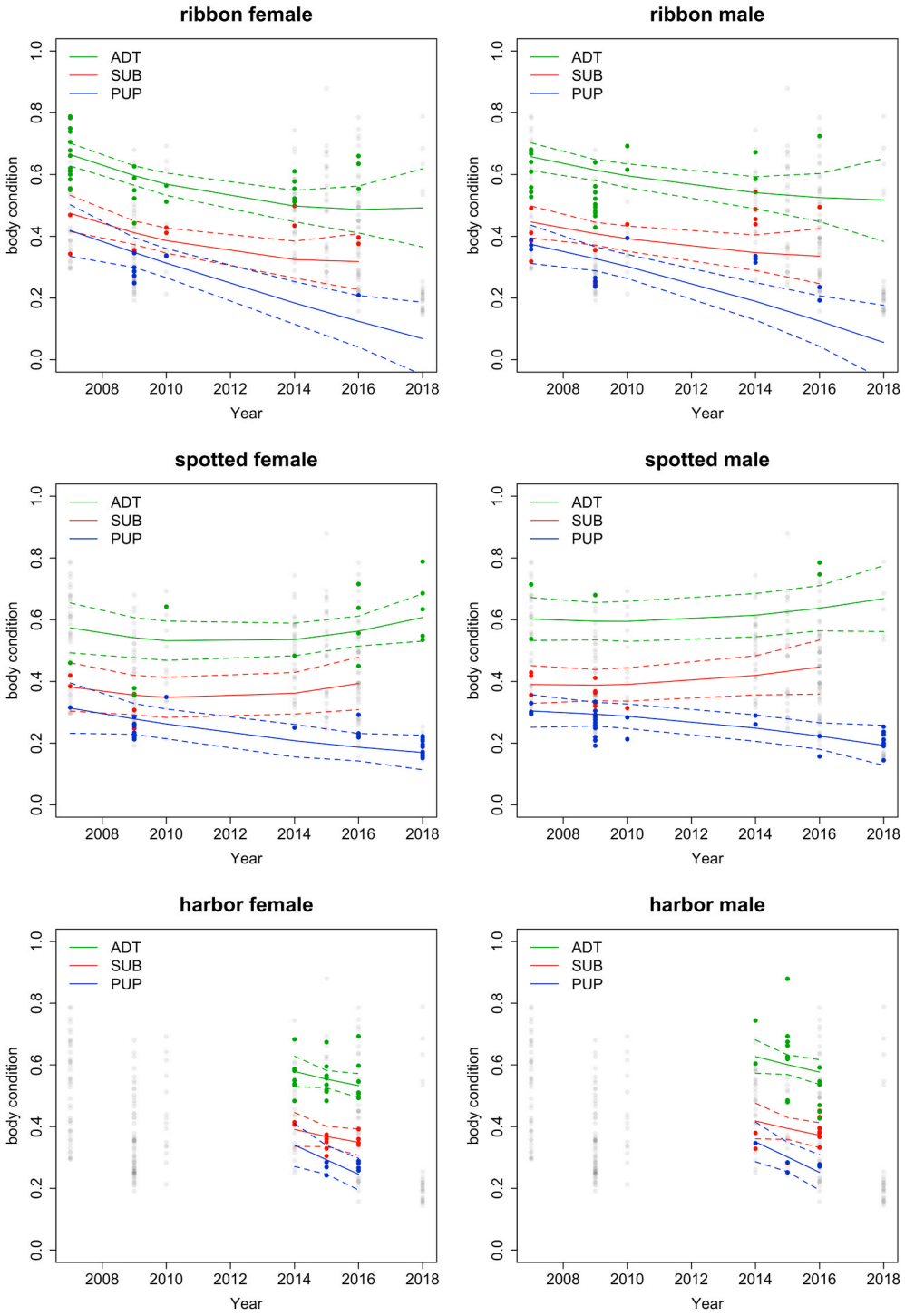

Fig. 2. Model-averaged estimates for body condition by year for each species, age class, and sex of ribbon seals, spotted seals, and harbor seals. For each species and sex, colored symbols depict the data for each age class, superimposed on the light gray symbols for the entire data set. Predictions are based on the mean value for 'Day' for each species.

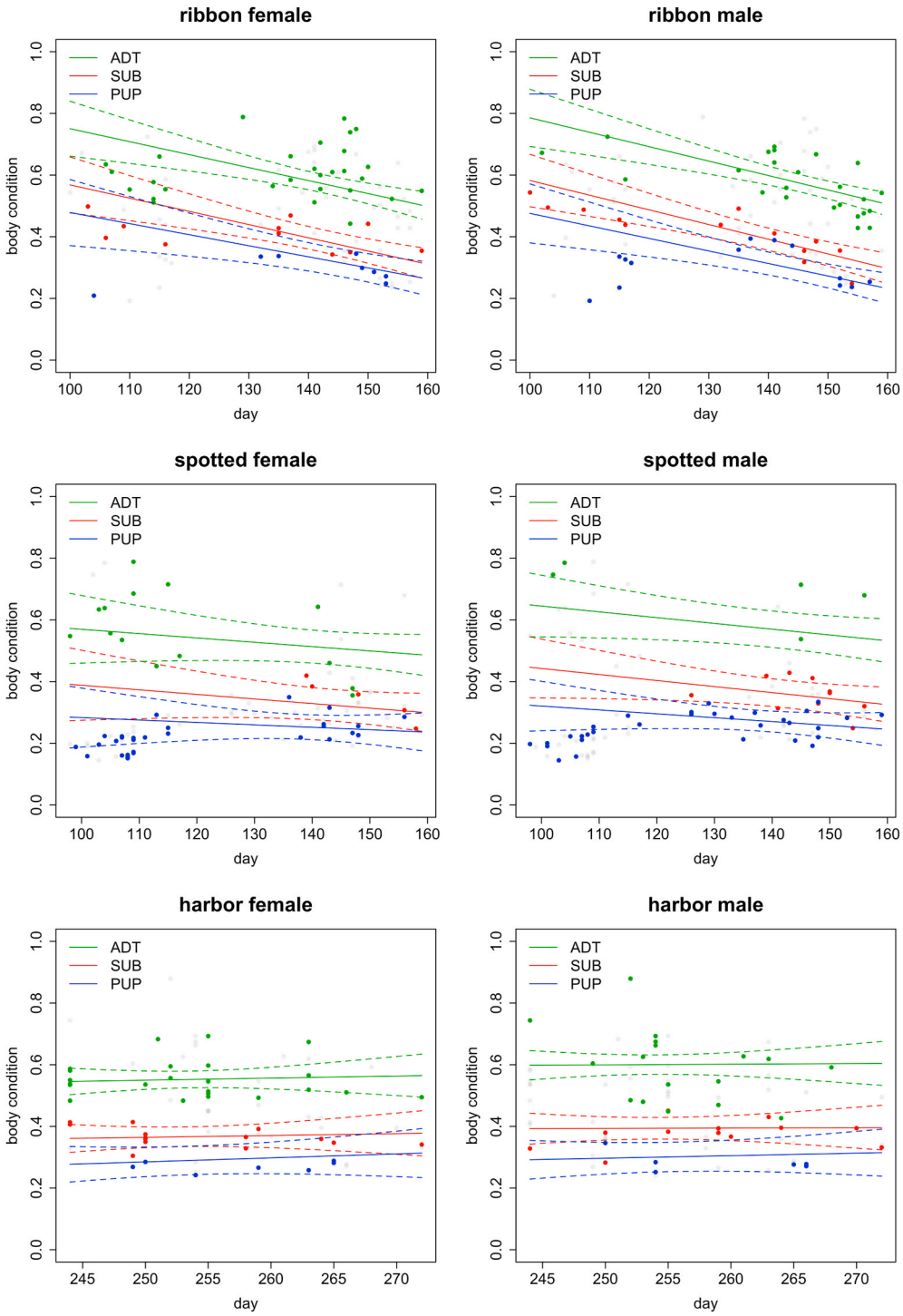

Fig. 3. Model-averaged estimates for body condition by day of year for each species, age class, and sex of ribbon seals, spotted seals, and harbor seals. For each species and sex, colored symbols depict the data for each age class, superimposed on the light gray symbols for the entire data set. Predictions are based on the median value for 'Year' for each species.

evidence of daily decreases for spotted seals during April and May, and weak evidence of daily increases for harbor seals during September (Fig. 3).

4. Discussion

4.1. Daily changes in body condition

Despite our modest sample of weights and measurements across years, we detected notable temporal trends in body condition. Although these differed by species and age class, our models produced sensible predictions of daily changes in body condition. The ribbon and spotted seals were sampled from early April until early June, the period in which births, nursing, mating, and molting take place. These critical life-history events are energy intensive and, at times, they restrict or preclude foraging, requiring most non-pups to rely upon stored reserves compared to other times of the year (Ochoa-Acuna et al., 2009). Thus, most phocid seal non-pups lose mass and body condition during this

period. Pups, on the other hand, are expected to gain mass while nursing, followed by a period of relatively stable or declining mass after weaning (Naito and Nishiwaki, 1972; Tikhomirov, 1968), while gaining foraging proficiency. The negative slopes for non-pup ribbon and spotted seals (Fig. 3) indicate that on average those groups declined daily in body condition over the within-year sampling periods. For pups of both species, particularly ribbon seal pups, the trend lines also indicate a daily net loss of condition. Our relatively small sample did not support higher-order daily effects, and a linear effect is incapable of expressing the initial daily increase and later decrease expected for pups, but there is a hint of that pattern in the distribution of the pups' data points.

The harbor seals in our study were sampled over the month of September, a short period well after harbor seals' birth-nursing-mating period and after most individuals have completed their molt. Harbor seals of all age classes would be expected to be slowly gaining mass during this period, but because the samples were all collected within a 30-day range of dates, our data would have little power to detect this trend. Indeed, the predicted daily body conditions for harbor seals (Fig. 3) are flat or only slightly increasing, consistent with this expectation. Therefore, we note that despite having relatively small samples within species, sex, and age class categories, our body condition data reproduced the expected within-year patterns for all three species.

4.2. Interannual changes in body condition

The interannual declines in body condition of ribbon and spotted seal pups may indicate that nutrition was limiting during the perinatal and maternal provisioning period. Poor foraging conditions for mothers during the winter could also have impacted pup condition, particularly for ribbon seals, in which adult body condition appeared to decline over the course of our study. These are periods when both species are typically concentrated near the southern sea-ice edge in the Bering Sea. Historically, that sea-ice edge has occurred near the continental shelf break, a productive region where upwelling and spring sea-ice melt combine to generate favorable conditions (Mizobata et al., 2006; Springer et al., 1996) for these upper-trophic predators when energetic demands, particularly on pregnant or lactating females, are very high. Both ribbon and spotted seal mothers are believed to continue at least some foraging during lactation (Burns, 1981; Quakenbush, 1988). Although few details of their foraging ecology at that time of year have been documented, ribbon seals are more adapted for deep diving and foraging off the shelf than spotted seals. This difference led Boveng et al. (2013) to predict that ribbon seal reproductive success in the Bering Sea would be sensitive to future declines in ice extent because a northward retraction of the ice edge, away from deeper water over the shelf slope, would displace mother-pup pairs from preferred foraging habitat. Spotted seals, however, were anticipated to be more resilient due to foraging habits more suited to the waters and epibenthic communities on the shelf, where the receding ice edge would occur for the foreseeable future (Boveng et al., 2009). During our study in 2007–2018, sea-ice extent in the Bering Sea fluctuated considerably, with a record high of 1,036,921 km^2 in 2012, a record low of 137,096 km^2 in 2018, and an average decline of about 47,000 $km^2\ y^{-1}$ (Fetterer et al., 2017, updated daily). Over the period 2007–2018, ribbon seal adult body condition in our samples declined, while spotted seal adult condition remained relatively stable or increased slightly (Fig. 2). These patterns seem consistent with the predictions that the reproductive success of ribbon seals would be more sensitive than that of spotted seals to declining sea ice extent and northward retraction of the spring ice edge, away from the Bering Sea shelf break (Boveng et al., 2009, 2013).

Since 2000, conditions have alternated between relatively cold and warm periods that have been termed 'stanzas' (Stabeno et al., 2012). Warm and cold stanzas have been associated with different Bering Sea trophic conditions, posed as the oscillating control hypothesis (OCH), in which alternating cold and warm stanzas are associated with oscillating bottom-up and top-down control of southeastern Bering Sea pelagic ecosystem function (Hunt et al., 2002) and pollock recruitment (Hunt et al., 2011). After the extreme warm year in 2018, similar trophic implications of cold and warm conditions have been examined in the northern Bering Sea, as well (Duffy-Anderson et al., 2019). The typical pattern in warm periods is an early sea-ice retreat that delays stratification needed to initiate the spring phytoplankton bloom; the phytoplankton, and the zooplankton species that graze on them, tend to be smaller and less lipid rich than the species prevalent in cold years with later ice retreat. More production is expected to accrue to the pelagic system than the benthos during the warm years. Cascading impacts on the quality and abundance of young pollock, other forage fish, and their predators in warm years tend to be negative but complex (Duffy-Anderson et al., 2019; Hunt et al., 2011; Piatt et al., 2020). Hunt et al. (2002) predicted from the OCH that piscivorous pinnipeds (i.e. seals) would prosper in cold years, from reduced competition with large fish for cold-water forage fish, and during the transitions from cold to warm stanzas, nourished by abundant young of large predatory fish species. Our samples in 2007–2010 were collected during a cold stanza, and in 2014–2018, during a warm stanza, with no sampling in the transition years. A simple explanation of the net declines in seal pup body condition we observed could be built on an appeal to the OCH, but without a better understanding of the actual diets of ribbon and spotted seals during the contrasting stanzas—particularly the degree of dependence on forage fish versus large predatory fish—it will remain difficult to do more than speculate about the trophic mechanisms that might underlie declines in seal body condition.

Among the potential relationships between declining sea-ice extent and seal body condition that merit further study is the degree to which ribbon and spotted seals derive energy from sympagic (ice-associated) versus pelagic (water column) primary productivity during the critical gestation, birthing, and nursing periods. Wang et al. (2016) found that a majority of carbon in fatty acids of bearded (*Erignathus barbatus*) and spotted seals' blubber was derived from sympagic production during the relatively cold, high-ice years 2007–2012, and the contribution from sympagic production in bearded seal blubber was significantly greater during those cold years than during warmer years of 2002–2005. Ribbon seals in that study were only sampled in the warmer years, but the data suggested that their blubber fatty acids were derived from sympagic production in high proportions similar to those in spotted seals. Without the contrast from ribbon seal samples in the colder years, it is not possible to assess whether ribbon seals—which seem to have a preference for foraging at the shelf break or in deeper waters—are more dependent than spotted seals on pelagic than on sympagic production. Wang et al. (2016) noted that unless there are differences in prey quality or quantity stemming from sympagic and pelagic carbon sources, shifts between them may not have substantial effects on seals' growth or vital rates. Studies to investigate whether such differences occur in quantity or quality of key forage fish as a result of variability in sea-ice extent could be highly valuable to understanding upper predators' responses in the northern Bering Sea ecosystem.

The edge of the pack ice in mid-April 2018 was hundreds of kilometers farther north than in any other year that we sampled (Fig. 1). In 1967, prior to satellite observations that began in 1979, spring conditions were in many ways similar to those in 2018 and 2019, resulting in distributions of ice-associated pinnipeds far north of where they usually had occurred (Burns, 1968, 1970). Ribbon seals were abundant throughout the northeastern Bering Sea by April 20, 1967. Even the oldest hunters in the region did not recall seeing as many ribbon seals in previous years (Burns, 1968), and the harvest of ribbon seals was the highest ever recorded in Alaska (Lowry, 1985a). In contrast, during the third week of April in 2018, while working at the ice edge in the northeastern Bering Sea, we encountered very few ribbon seals and captured none for sampling, unlike previous years when the pack ice was farther south and we typically captured more ribbon than spotted seals. It is unclear whether or where ribbon seals were successful at

producing and rearing pups in 2018, and the same questions are relevant to 2019, when ice extent was similarly low but we had no seal observations or samples.

A long-term program has been conducted by the Alaska Department of Fish and Game (ADF&G) to monitor health and condition—among many other aspects—of bearded, ringed (*Pusa hispida*), spotted, and ribbon seals (Crawford et al., 2015; Quakenbush and Citta, 2008; Quakenbush et al., 2009). The program samples relatively large numbers of seals in collaboration with Alaska Native subsistence hunters, and has potential for greater statistical power to detect trends than our samples from live-caught seals. Spotted and ribbon seal results from the harvest samples have not yet been published for the years overlapping with our study period. For ringed seals, they found that several indices of condition were lower during a period of greater ice concentration (1975–1984) than during a more recent period (2003–2012) with less ice (Crawford et al., 2015). Correlations of the ringed seal indices with May sea-ice area of ≥50% concentration across the years of the two periods were mostly negative, though only two of five indices were significant. For bearded seals, comparisons between these two periods were mixed, and correlations with sea ice were non-significant. Thus, detecting climate-driven change in ice-associated seals appears to be complex, perhaps a reflection of the resilience of these generalist, high-trophic level predators to the typical interannual variability of the Arctic. However, ambiguities in the ADF&G results obtained through 2012 may reflect a lack of changes as profound as those observed in the Pacific Arctic during 2018 and 2019 (this issue).

During the period of our study, NOAA declared two unusual mortality events (UMEs) for seals in Alaska. The first UME (Burek-Huntington et al., 2012; NOAA, 2020), which occurred from May 2011 to December 2016, involved all four species of ice seals in Alaska (ribbon, spotted, bearded, and ringed). The primary symptoms in that UME were hair loss, delayed molting, skin ulcers, lethargy, and labored breathing, but after extensive testing, a definitive cause for the UME was not determined. The second UME, for bearded, ringed, and spotted seals, was declared in September 2019, after large numbers of dead and stranded seals were found in the Bering and Chukchi seas beginning in June 2018 (NOAA, 2020). Ribbon seals seemed to be rare or absent from the reported strandings, though many of the carcasses were decomposed and not conclusively identified. Ribbon seals are also less coastal in their distribution at sea and may simply be underrepresented in the strandings. The investigation of this UME has just begun, but it appears to be different from the first, and more likely food related; most of the stranded seals have been young and/or emaciated. Although no clear link to climate-related changes has been established for the first UME, the co-occurrence of the second UME with record low sea-ice extent and absence of ice from vast portions of the birthing and nursing areas for all four species of ice-associated seals in the Bering Sea is strongly suggestive of a major climate-related impact to the seal populations.

Our harbor seal data comprise just three annual sampling events in September, 2014–2016, near the end of the longer data set for spotted and ribbon seals, a limited perspective for evaluating trends. Still, the apparent rate of decline in harbor seal body condition was striking. A simple linear model including only 'species*Year' effects indicated an annual decline in body condition of 45 g body mass per centimeter of body length. Harbor seals are among several ecosystem components that have undergone long-term declines in the Aleutian Islands. They declined precipitously between about 1980 and 1999, particularly in the western Aleutians, where counts dropped by 86% (Small et al., 2008) and have not shown an indication of substantial recovery (Muto et al., 2019). The timing and regional pattern of greatest declines and failure to recover in the western Aleutian Islands mirror those observed in Steller sea lions (Small et al., 2008), plausibly suggestive of a low-frequency 'ecosystemic' common cause, but we are aware of no consensus view on what that cause may be. We suspect, though, that the recent declines we estimated in harbor seals' body condition could be more of an acute response to short-term environmental variation than a continued chronic response to whatever may have caused the western Aleutian harbor seal numbers to decline.

The sampling period for harbor seals coincided with a very strong marine heat wave in the Northeast Pacific Ocean that had strong effects on most marine ecosystem components being monitored in the region (e.g., Siddon and Zador, 2019). Piatt et al. (2020) offered a plausible synthesis of physical and biological oceanographic indicators, forming an explanation for the mass die-off of common murres (*Uria aalge*) that occurred in the northeast Pacific during 2015–2016: In simple terms, the warm waters of the marine heatwave reduced phytoplankton and zooplankton productivity, thereby reducing prey availability to forage fish. At the same time, prey requirements of forage fish increased due to the metabolic effect of warmer temperatures, leading to poorer condition and nutritional content. The metabolic effect also increased the needs of larger predatory fish that compete with murres for their forage-fish prey. The murres, with high endothermic metabolic demands of their own, were effectively squeezed between the jaws of an 'ectothermic vise' because of their heavy reliance on forage fish (Piatt et al., 2020). Harbor seals are generalist predators with diets that vary seasonally and throughout their range. Their diet is poorly characterized for the Aleutian Islands, based on stomach contents from 18 harbor seals taken on Amchitka Island in the months of January, March, and April (Kenyon, 1965; Wilke, 1957). Prey items found were primarily Atka mackerel (*Pleurogrammus monopterygius*) and octopus (*Octopus rubescens*; and *Octopus* sp.). Fringed greenling (*Hexagrammos lagocephalus*), Alaska pollock (*Gadus chalcogrammus*), and unidentified fish and crab were also found in single stomachs or trace amounts. Forage fish, such as sand lance, herring, smelt, and capelin weren't noted in the Aleutian harbor seal samples but are staples commonly occurring in the few studies that have been conducted on harbor seal diet in the Gulf of Alaska and Bering Sea (Jemison, 2001; Pitcher, 1980a, 1980b). Because of the opportunistic nature of harbor seal prey selection, a broader mechanism than forage fish being caught in an ectothermic vise will be required to understand whether and how the marine heatwave of 2014–2016 impacted body condition of harbor seals in the Aleutians.

As an integrated understanding of the Bering Sea and Aleutian Islands ecosystem dynamics continues to develop, we will attempt to link our observations of species- and age-specific trends in ribbon, spotted, and harbor seal body condition with documented changes in their prey species and their prey's neighbors in the food web. With high metabolic rates and populations numbering in the hundreds of thousands to low millions in the Pacific Arctic, these seals are significant consumers that have the potential to shape the trophic web. As long-lived generalist predators, they are often thought to be relatively resilient or insensitive to bottom-up variability. In normal or average conditions, their ability to switch among a diverse suite of prey allows them to smooth over variability in one or a few food species, but when they do exhibit a decline in body condition, the trend is likely to reflect strong or broad underlying ecological changes such as those that now seem to be occurring with unprecedented frequency. That studies such as Crawford et al. (2015) and Wang et al. (2016) have found some, but sometimes conflicting, evidence of climate-induced signals in seals may reflect that the ecological variability experienced in the past two decades is just beginning to exceed the long-term bounds to which these species are adapted. If conditions like those recorded in 2018 and 2019 become more frequent as predicted (Oliver et al., 2018), impacts from loss of sea ice on the condition and vital rates of phocid seals are likely to become clearer.

Credit author statement

Peter Boveng: Conceptualization, Investigation, Writing – Original Draft, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **Heather Ziel:** Methodology, Validation, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing. **Brett McClintock:** Software, Formal analysis, Investigation,

Writing – Original Draft, Writing – Review & Editing. **Michael Cameron:** Investigation, Writing – Review & Editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.dsr2.2020.104904.

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Re-examination of population structure in Arctic ringed seals using DArTseq genotyping

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ABSTRACT: Although Arctic ringed seals *Phoca hispida hispida* are currently abundant and broadly distributed, their numbers are projected to decline substantially by the year 2100 due to climate warming. While understanding population structure could provide insight into the impact of environmental changes on this subspecies, detecting demographically important levels of exchange can be difficult in taxa with high abundance. We used a next-generation sequencing approach (DArTseq) to genotype ~5700 single nucleotide polymorphisms in 79 seals from 4 Pacific Arctic regions. Comparison of the 2 most geographically separated strata (eastern Bering vs. Beaufort Seas) revealed a statistically significant level of genetic differentiation (F_{ST} = 0.001, p = 0.005) that, while small, was 1 to 2 orders of magnitude greater than expected based on divergence estimated for similarly sized populations connected by low (1 % yr^{-1}) dispersal. A relatively high proportion (72 to 88 %) of individuals within these strata could be genetically assigned to their stratum of origin. These results indicate that demographically important structure may be present among Arctic ringed seals breeding in different areas, increasing the risk that declines in the number of seals breeding in areas most negatively affected by environmental warming could occur.

KEY WORDS: *Phoca hispida hispida* · Population structure · Pacific Arctic · Climate warming · DArTseq

1. INTRODUCTION

Over the past several decades, Arctic warming has occurred at twice the rate of the global average (IPCC 2013), resulting in reductions in the extent, thickness, and seasonal duration of sea ice (Stroeve et al. 2012, Lindsay & Schweiger 2015, Wang & Overland 2015). These reductions are expected to have significant, but not uniform, consequences for species that depend on sea ice for important aspects of their life history (Moore & Huntington 2008, Kovacs et al. 2011, Laidre et al. 2015, Descamps et al. 2017). One such species is the ringed seal *Phoca hispida*, which builds subnivean lairs on the ice that are essential for protecting pups from thermal stress and predation (Smith & Stirling 1975, Smith 1976, Gjertz & Lydersen 1986, Lydersen & Smith 1989, Hammill & Smith 1991, Smith et al. 1991, Furgal et al. 1996). Although ringed seals in some areas appear to have adjusted to contemporary ice conditions (Bering and Chukchi Seas; Crawford et al. 2015), ringed seals in other regions have shown declines in body condition,

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reproduction, and pup survival that are thought to be correlated with earlier spring sea ice retreat and declines in snow depth (Ferguson et al. 2005, 2017, Stirling 2005, Harwood et al. 2012a, 2015a, Iacozza & Ferguson 2014).

Ringed seals (subspecies *P. h. hispida*) are currently abundant and broadly distributed throughout the Arctic. While most of what is known about their population size is limited to surveys of only parts of their range (e.g. Bengtson et al. 2005, Conn et al. 2014), combined regional estimates suggest an abundance of around 3 million individuals (Laidre et al. 2015). Despite these large numbers, a status review conducted by NOAA Fisheries concluded that it is likely that the number of Arctic ringed seals will decline substantially by the year 2100 and that seals will no longer persist in substantial portions of their range in the foreseeable future (Kelly et al. 2010b). Following this review, Arctic ringed seals were listed as threatened under the US Endangered Species Act (77 FR 76706), a decision that was vacated in 2016[1] but subsequently reversed[2]. Although the status review considered the extinction risk to the Arctic subspecies as a whole, it was noted that such risk would be elevated if population structure exists (Kelly et al. 2010b). The effects of climate warming have not been uniform across the Arctic, with more marked sea ice declines in some areas than in others (Frey et al. 2014, 2015, Peng & Meier 2017, Wang et al. 2017). As such, ringed seals in some parts of the subspecies' range could be more severely impacted by Arctic warming than those in other areas.

The extent of such impacts will depend in part on the factors driving breeding habitat selection in Arctic ringed seals. If selection of breeding and pupping habitats is most strongly driven by habitat quality, then seals that once used a region that now has little to no ice or snow cover will move to less impacted regions in subsequent years. However, if seals exhibit high natal fidelity (i.e. return to reproduce in the same area where they were born), they may continue to return to an area of suboptimal quality even if high pup mortality and/or low breeding success occurs, as has been observed among Northwest Atlantic harp seals (Stenson & Hammill 2014). If this pattern of behavior persists, the number of seals breeding in those areas most negatively affected by environmental warming could decline, potentially leading to a loss of genetic diversity important to the evolutionary potential of the species.

The drivers behind breeding site selection in ringed seals are not well understood. Tagging studies have shown that ringed seals can range widely during the open water season (Heide-Jørgensen et al. 1992, Teilmann et al. 2000, Born et al. 2004, Freitas et al. 2008, Kelly et al. 2010a, Crawford et al. 2012, Harwood et al. 2012b). During the winter and spring subnivean period, however, Arctic ringed seals maintain much smaller ranges, and most tagged seals, including adult males and females as well as subadults, demonstrated breeding site fidelity across years (Kelly et al. 2010a, Martinez-Bakker et al. 2013, Luque et al. 2014, Harwood et al. 2015b). While this pattern of behavior indicates that the choice of where to reproduce is not random, the limited duration of tagging studies makes it difficult to identify the temporal scale over which breeding site fidelity is maintained and thus what factors influence its development.

If fidelity to breeding sites is driven by the return to natal sites, then genetic differences between regions should build over time. Estrous in ringed seals occurs during lactation (McLaren 1958, Atkinson 1997); thus, the timing and location of breeding and whelping are closely linked. Given this link, natal site fidelity could not only lead to maternally driven structure but may also influence gene flow between regions, as has been seen among ringed seals inhabiting Lake Saimaa, Finland (*P. h. saimensis*), where significant and substantial genetic differentiation between areas within the lake has been revealed at both mitochondrial and nuclear markers (mitochondrial F_{ST} = 0.444, nuclear F_{ST} = 0.107; Valtonen et al. 2012, 2014).

Detecting genetic differences between areas among Lake Saimaa seals is facilitated by the small effective size of this subspecies (~139–150 mature individuals; Sipilä 2016), which allows genetic differences between regions to develop more quickly. However, detecting restricted dispersal in highly abundant taxa, such as the Arctic ringed seal, is much more challenging. For example, F_{ST}, which is commonly calculated as a metric of connectivity between groups, can be estimated under Wright's island model as

$$F_{ST\text{-}mtDNA} \approx 1/(2N_e m - 1)$$

$$F_{ST\text{-}SNP} \approx 1/(4N_e m - 1)$$

where N_e represents the effective population size of the groups being compared (Wright 1965), m represents the fraction of immigrants within a group, and SNP is single nucleotide polymorphism. As described in Lowe & Allendorf (2010), the extent to which gene flow affects evolutionary processes (i.e. genetic con-

[1]Alaska Oil and Gas Association vs. National Marine Fisheries Service, case no. 4:14-cv-00029-RRB

[2]Alaska Oil and Gas Association vs. National Marine Fisheries Service, case no. 16-35380

nectivity) depends primarily on the absolute number of dispersers (i.e. $N_e m$), while demographic connectivity, or the degree to which population growth and vital rates are affected by dispersal, is dependent on the relative contribution of net immigration to total recruitment (i.e. m). Both types of connectivity are important, as genetic cohesion acts to maintain the evolutionary potential of the species, while demographic cohesion is needed to avoid depletion in the face of localized threats. Given the relationships described above in Wright's (1965) equation, the effect size (here, the magnitude of F_{ST}) associated with a particular threshold of genetic connectivity ($N_e m$) remains the same irrespective of population size. However, for the same m, the F_{ST} for a small population will be much larger, and much more easily detected, than the F_{ST} for a large population. For example, if we assume that the mature ringed seals that inhabit Lake Saimaa are evenly distributed across the 4 regions compared in the Valtonen et al. (2012) study and that those 4 regions are connected by approximately 1% dispersal per year (see Section 2.3.5 below for details), then the mtDNA F_{ST} would be 0.17. However, in a hypothetical situation in which ~15 000 ringed seals were evenly distributed across 4 regions in the Arctic and connected by the same level of dispersal, the mtDNA F_{ST} would be several orders of magnitude lower (F_{ST} = 0.001) and thus much more difficult to detect.

Thus far, genetic analyses have largely failed to detect population structure within Arctic ringed seals. Davis et al. (2008) compared the microsatellite genotypes (n = 11 loci) of seals sampled at 8 sites ranging from Saint Lawrence Island, Alaska, through the Canadian Arctic, Greenland, and Norway and into the White Sea in Russia. Though seals sampled in the White Sea were significantly differentiated from seals sampled at all other sites (Φ_{ST} = 0.0180–0.0306, $p < 0.001$), the remaining comparisons yielded very small estimates of differentiation, most of which were not significant. However, sample sizes for some of the areas were relatively low and may have included seals sampled outside of the breeding season. Subsequent analysis by Martinez-Bakker et al. (2013) used a panel of 9 microsatellite loci and included only samples collected during the breeding season at 9 sites ranging from the Chukchi Sea to the eastern Beaufort Sea. Though small but significant differences in mtDNA control region sequences were found between some strata, a geographic pattern among the sites with significant differences was not evident, with no differences detected among some of the most distant sites, while in other cases neighboring sites showed statistically significant differences. In general, the degree of differentiation between sites was markedly low, and the authors concluded that gene flow among these Arctic sites was relatively high (Martinez-Bakker et al. 2013).

While the failure to identify clear patterns of genetic differentiation among Arctic ringed seals suggests genetic connectivity between ringed seal breeding sites, it is possible that weak, but demographically important, population structure exists but was not detected in these previous studies. Traditional genetic markers, such as those used in the above studies, may have little statistical power to detect small, but biologically significant, levels of genetic differentiation between areas (Waples & Gaggiotti 2006, Lowe & Allendorf 2010). However, recent advances in high-throughput next-generation sequencing (NGS) technologies have provided a cost-effective means to simultaneously discover and genotype large numbers (hundreds to thousands) of SNPs, even in species for which little to no genomic information is available (Baird et al. 2008, Davey et al. 2011, Peterson et al. 2012). The production of these extensive datasets can substantially increase the power and precision of genetic analyses even with limited sample sizes (e.g. Willing et al. 2012, Nazareno et al. 2017), allowing previously undetected patterns of demographic and evolutionary structure to be resolved (Corander et al. 2013, Reitzel et al. 2013, Benestan et al. 2015). In addition, genome-wide scans of diversity using NGS technologies allow outlier loci, putatively under divergent selection, to be identified (Benestan et al. 2016, Gleason & Burton 2016); analysis of these loci can reveal patterns of adaptive variation with important implications for developing conservation and management strategies (Funk et al. 2012, 2018).

In this study, we take advantage of recent advances in NGS to discover and genotype a large panel of SNP markers (n = 5699 loci) in ringed seals (n = 79) sampled during the breeding season in 4 regions of the Pacific Arctic and use this dataset to evaluate population structure in Arctic ringed seals. Compared to previous studies, which relied on ≤11 microsatellite loci, the number and genome-wide placement of the SNPs analyzed should greatly increase the power to detect subtle differences between regions. The results of this study will increase our understanding of genetic and demographic connectivity between regions so that the effect of regional depletions of Arctic ringed seals by hunting or climate warming can be evaluated.

2. MATERIALS AND METHODS

2.1. Samples

Tissue samples (n = 113) were collected from ringed seals in the Pacific Arctic region during the spring breeding season (March through May between 2000 and 2017; Fig. 1) and archived in the Southwest Fisheries Science Center's Marine Mammal and Sea Turtle Research Tissue Collection. The majority of these samples (n = 59) were collected during a biomonitoring program run by the Alaska Department of Fish and Game from seals legally harvested for subsistence by Alaska Natives. The remaining samples were collected from the remains of seals that were killed by polar bears (n = 54). Following collection, the samples were either stored at −80°C or were preserved in a salt-saturated 20% DMSO solution or 100% ethanol and subsequently archived in a −20°C freezer.

Ringed seals are distributed throughout the study area, making it difficult to identify biologically relevant boundaries by which to divide groups for comparison. Thus, for analyses requiring that *a priori* groups be identified for comparison, we stratified the samples into 4 geographic areas. These strata correspond to 4 of the 5 Alaska Native regions that harvest ice seals and are represented by the Ice Seal Committee, which is the tribally authorized Alaska Native organization that co-manages ice seals in partnership with NOAA Fisheries. The fifth and southernmost Alaska Native region is Bristol Bay. Few samples were available from this area; thus, it was not included as a stratum in our analysis. The 4 strata analyzed include (1) the eastern Bering Sea, (2) the northern Bering Sea, (3) the southeastern Chukchi Sea, and (4) the Beaufort Sea (Fig. 1). The eastern Bering Sea and the Beaufort Sea strata also align with 2 of the 3 biogeographic provinces (Eastern Bering Shelf and Beaufort Sea provinces, respectively) that have been delineated within the study area (Sigler et al. 2011), although the third province (Chirikov–Chukchi Province) includes both the northern Bering and southeastern Chukchi strata used in our analysis. A list of sample information is included in Table S1 in the Supplement at www.int-res.com/articles/suppl/n044p011_supp.pdf.

2.2. DArTseq library preparation and high-throughput sequencing

Many NGS methods rely on the use of restriction enzymes (REs) to reduce genome complexity prior to high-throughput sequencing. DArTseq (RADseq; Davey et al. 2011), which was developed by Diversity Arrays Technology (DArT P/L), is one such method (Jaccoud et al. 2001, Sansaloni et al. 2011, Kilian et

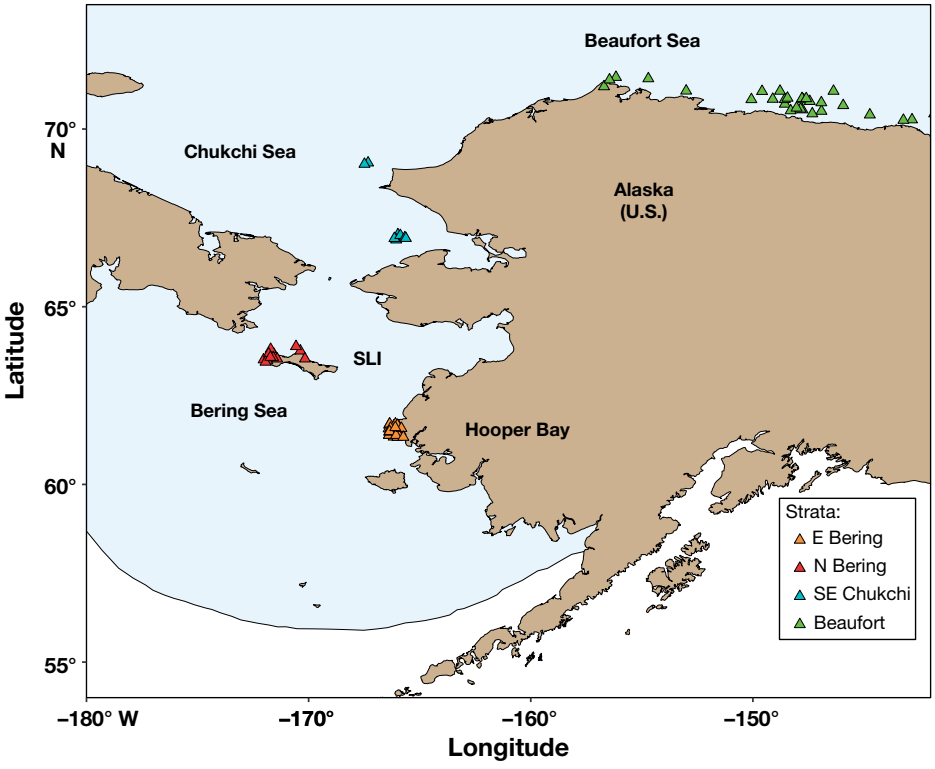

Fig. 1. Collection locations for analyzed samples. The approximate range of ringed seals within this area is shown in light blue. Triangles indicate the collection locations (some of which are approximated) for the analyzed samples. The color of symbols denotes the stratum to which the collected sample belongs (see legend). Places mentioned in the text are shown, including Hooper Bay and Saint Lawrence Island (SLI)

al. 2012). In brief, this method entails a complexity reduction step in which DNA is digested with a combination of REs which act to exclude repetitive regions of the genome while targeting low copy sequences. The resulting libraries are then sequenced on a high-throughput sequencing platform, allowing for high read coverage of regions most likely to be informative in population studies.

DNA was extracted from these samples using the Machinery-Nagel NucleoMag® tissue extraction kit and following the manufacturer's protocol (see www.mn-net.com), with the exception that the proteinase K tissue lysis step was extended to include an overnight digestion at 37°C followed by a 3 h digestion at 55°C. DNA was quantified on a fluorometer using Quant-iT PicoGreen, and DNA integrity was assessed by electrophoresing 100 ng of DNA on a 1% ethidium bromide (EtBr)-stained agarose gel at 70 to 80 V for approximately 1 h alongside a 1 kb DNA size standard. Those extracts that demonstrated the presence of high molecular weight DNA on the gel and that contained at least 500 ng of DNA were retained in the study, and additional samples were selected as needed to replace those that produced insufficient DNA.

A trial RE digest was conducted for a subset (n = 6) of the selected samples to ensure that DNA quality was sufficient for restriction digest to be successful. For this trial, 100 ng of DNA from each sample was digested in a 50 µl reaction that contained 1 µl (10 units) of the HindIII RE, 5 µl of NEB buffer, and (for the remaining volume) DNase/RNase-free distilled water. The digestion was conducted in a thermocycler for 3 h at 37°C. The resulting digests were electrophoresed alongside the original undigested DNA extractions (from the same samples) and 5 µl of lambda DNA-HindIII digest on a 1% agarose gel (pre-stained with EtBr) at 50 V for a total of 2 h.

Although sample selection was initially based on maintaining relatively even coverage of the 4 regions, not all of the samples chosen produced DNA of sufficient quantity and quality for the DArTseq library preparation protocol. In total, 89 samples from the eastern Bering Sea (n = 34), northern Bering Sea (n = 14), southeastern Chukchi Sea (n = 9), and Beaufort Sea (n = 32) were included (Table S1). Approximately 50 to 100 ng of DNA from each of these samples was diluted in 10 to 20 µl TE buffer and then shipped to DArT P/L at the University of Canberra, Australia, for library preparation and sequencing.

Once at the DArT P/L laboratory, DNA quality was re-evaluated. For a subset of samples, the DNA provided was divided in 2 parts prior to digestion/ligation to act as technical replicates to be assessed for scoring consistency (referred to as the reproducibility score, used in filtering below). Double digestion was performed using methylation-sensitive REs as described by Kilian et al. (2012). The only modification to this protocol was that the single PstI-compatible adaptor was replaced with 2 different adaptors corresponding to the PstI and SphI RE overhangs. The PstI-compatible adapter was designed to include the Illumina flowcell attachment sequence, a sequencing primer, and a staggered, varying length barcode region, similar to the sequence reported by Elshire et al. (2011). The SphI-compatible adapter simply comprised the Illumina flowcell attachment region and SphI overhang sequence. Ligated fragments with both a PstI and SphI adaptor were amplified by PCR using an initial denaturation step of 94°C for 1 min, followed by 30 cycles with the following temperature profile: denaturation at 94°C for 20 s, annealing at 58°C for 30 s, and extension at 72°C for 45 s, with an additional final extension at 72°C for 7 min. Equimolar amounts of amplification products from each sample were combined before single-end sequencing for 77 cycles on an Illumina Hiseq2500 to yield approximately 2.5 million reads per sample.

Samples were genetically sexed by amplification and real-time PCR (Robertson et al. 2018). Samples from 1 male and 1 female for which sex had been determined via examination of a stranded animal were included as positive controls in all amplifications. Sex was determined by the amplification pattern: males had 2 products and females had 1.

2.3. Data analysis

2.3.1. Pipeline processing

The FASTQ-formatted sequences generated from the sequencing lane were processed using proprietary diversity array technology (DArT P/L) analytical pipelines. In summary, the primary pipeline filters out poor-quality sequences and corrects low-quality bases from singleton tags using collapsed tags as a template. More stringent selection criteria are applied to the barcode region to ensure that sequences are reliably assigned to the appropriate sample. Identical sequences are then collapsed into fastqcoll files that are processed through the secondary DArT P/L pipeline, which uses proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14) to produce

genotypes for each sample. Multiple samples were processed from DNA to allelic calls as technical replicates; those loci with reproducibility scores less than 90% across replicates were removed. Calling quality was assured by high average read depth per locus (mean across all markers of 31.2 reads per locus, minimum of 5 reads per locus). The mean call rate across loci was 84.8%. Given that a ringed seal reference genome is not yet available, mapping efficiency of reads against such a reference could not be evaluated. The data were converted to a matrix of SNP loci by individuals, with the contents stored as integers: 0, homozygote, reference state; 1, heterozygote; and 2, homozygote, alternate state.

2.3.2. Additional filtering

For this analysis, only the codominant SNP genotype data (allSnps_singlerow_dartseq.csv) received from DArT P/L were analyzed. The genotype data were imported into R (v3.5.0) and then filtered to remove SNPs that had average read counts less than 10. SNPs with high read depth, which can represent false heterozygotes caused by copy number variations or paralogous sequences, were also removed. For this criterion, we first calculated the average read depth (d) across all loci and then removed those loci with average read depths greater than $d + 4 \times \sqrt{d}$, which has been shown to be effective in reducing the number of false heterozygotes (Li 2014). We then used the package dartR 1.1.11 (Gruber et al. 2018) to remove loci that (1) had reproducibility scores, which were calculated based on the technical replicates described above, less than 100%; (2) had been called in less than 90% of individuals; (3) were monomorphic; and (4) were secondary SNPs (e.g. SNPs that were found on the same sequence fragment as 1 or more other SNPs), in which case only the locus with the highest polymorphic information content was retained. After this filtering was completed, we removed individuals that were missing >20% of the data.

The remaining filtering steps were conducted in R using the strataG package (Archer et al. 2017). These steps included removing (1) loci with minor allele frequencies ≤0.05, (2) any loci that were not bi-allelic, (3) loci that had expected heterozygosity greater than 0.6, (4) one of each pair of samples that shared 80% or more of their alleles (i.e. samples that were likely collected from the same individual), and (5) loci that were out of Hardy-Weinberg equilibrium in 3 or more of the *a priori* defined geographic strata.

2.3.3. Identification of outlier loci and analysis of relatedness

We used the R package OutFLANK v0.2 (Whitlock & Lotterhos 2015) to identify SNPs that might be under diversifying selection. This approach is based on identifying loci with atypical values of F_{ST} as outliers. OutFLANK uses an improved method for deriving the null distribution of population differentiation for presumed neutral loci and results in fewer false positives than some other methods. We ran OutFLANK with a 5% left and right trim of the null distribution of F_{ST} and a false discovery rate (q-value) of 5%.

The R package related 1.0 (Pew et al. 2015) was used to estimate pairwise relatedness (r) between all pairs of genotyped individuals based on the allele frequencies of the full sample set. The 'compareestimators' function was then used to generate simulated genotypes for 200 dyads representing 4 categories of known relatedness (unrelated, half sibling, full sibling, and parent–offspring) based on the allele frequencies of a subset of the SNP data (n = 500 randomly chosen loci). The distribution of r values for simulated individuals within each category was plotted against those generated from the sampled individuals to visualize how well our dataset was able to discriminate between relationship categories. Although related allows for coefficients to be generated under 7 different estimators, here we present only the results based on the Lynch & Ritland (1999) estimator, which had the highest correlation coefficient between the observed and expected relatedness values.

2.3.4. Comparisons between geographic strata

The R package strataG (Archer et al. 2017) was used to generate summary statistics describing the genetic diversity retained in each stratum, including the observed and expected heterozygosity and the number of alleles. This package was also used to estimate genetic differentiation (F_{ST}) between pairs of the *a priori* defined geographic strata. Significance of comparisons was evaluated via bootstrapping across 1000 permutations. A correction for multiple tests was not applied when interpreting the results of the pairwise comparisons, as each comparison was testing an independent hypothesis. Corrections for multiple tests effectively reduce the critical value (α), or Type I error rate, at the expense of the Type II error rate (Perneger 1998); as such, inappropriate application of correction factors can have serious conservation management implications.

To evaluate the impact of sample size on our comparisons, we randomly subsampled the 2 largest strata (the eastern Bering Sea stratum and the Beaufort Sea stratum) to create 20 datasets in which the sample sizes were equivalent to (1) the sample size of the southeastern Chukchi Sea stratum (n = 8), (2) the sample size of the northern Bering Sea stratum (n = 14), (3) a sample size of 20, and (4) a sample size of 25. Pairwise comparisons were then conducted for these datasets (60 in total), and the proportion of significant tests was counted.

Pairwise comparisons between regions were repeated after subdivision of strata by sex to explore whether sex-based differences in structure could be detected.

2.3.5. Calculating expected effect size

When interpreting the results of genetic analyses to delineate management units, Palsbøll et al. (2007) advocate placing the focus on the amount of genetic divergence rather than on rejecting panmixia, to address the case where statistical power is too low to reject panmixia among demographically independent units and where the statistical power is sufficient to reject panmixia even among demographically correlated groups. This approach involves, first, determining the amount of population genetic divergence that corresponds to the relevant dispersal rate and, second, comparing the empirical results with this criterion, as done by Martien et al. (2012).

Here, we used this approach and Wright's (1965) equation (see Section 1) to calculate the expected magnitude of F_{ST} between demographically independent stocks of Arctic ringed seals. Limited information is available on the dispersal rate at which groups become demographically independent, although simulations based on cetaceans have shown that if the dispersal rate between 2 groups is less than a few percent per year, 1 group could eventually be extirpated if the groups are managed as a single unit and human-caused mortality is disproportionately focused on only one of the groups (Taylor 1997). Generation length for ringed seals was estimated to be approximately 18 yr by Pacifici et al. (2013), based on a maximum life span of 46 yr and an age of first reproduction of 6.9 yr. No information on the generation length of ringed seals within our study area was available, but to identify a lower bound on generation time, we also calculated the effect size based on a maximum life span of 15 yr (the average age of females in Lydersen & Gjertz 1987) and an age of first reproduction of 4 yr. Based on these parameters, a 1% dispersal rate per year translates into a per generation rate of 0.07 to 0.18 (m in the equation noted in Section 1). Although the abundance of ringed seals in the strata we compared is not known, aerial surveys of the eastern Chukchi Sea indicated that ~231 000 seals were present in May and June of 1999 and 2000 (Bengtson et al. 2005), while aerial surveys of the eastern Bering Sea during April and May of 2012 indicated that ~170 000 seals were present (Conn et al. 2014). Using these abundance estimates to represent the census size (N_c) of our strata and further assuming that ringed seals have an N_e:N_c ratio at the low to middle range of that typical of mammals (0.2; Frankham 1995), we calculated an input N_e value that ranged from 34 000 to 46 200 seals.

2.3.6. Clustering analyses

Two methods were used to evaluate the number of genetic clusters (K) that could be detected in our dataset. Model-based Bayesian clustering was conducted using the program STRUCTURE 2.2.3 (Pritchard et al. 2000). This analysis was based on a model of admixture with correlated allele frequencies. Given that the inclusion of related individuals may lead to false inferences in STRUCTURE analyses (Anderson & Dunham 2008, Rodríguez-Ramilo & Wang 2012), 1 of the individuals in the single putative paternal half-sibling pair that was identified (described in Section 3.1) was removed prior to conducting this analysis. In addition, the number of sampled individuals per stratum has been shown to have a strong impact on the ability of STRUCTURE to correctly estimate the number of clusters (Fogelqvist et al. 2010, Puechmaille 2016). Given that the sample sizes representing the eastern Bering Sea stratum (n = 28) and the Beaufort Sea stratum (n = 29) were similar and markedly larger than those representing the other 2 strata (n = 14, northern Bering Sea; n = 8, southeastern Chukchi Sea), the STRUCTURE analysis was run using both the full SNP dataset (all 4 strata) and only the 2 strata with similar and larger sample sizes. Both the 2- and 4-strata analyses were initially run with no *a priori* information on geographic origin of the samples included. To evaluate whether the performance of STRUCTURE improved when information on the geographic location of sampling was included *a priori*, the 2-strata analysis was also run with the inclusion of a location prior (i.e. LOCPRIOR = 1).

For all 3 analyses, the model was run using values of K ranging from 1 to 10, with 3 replicate runs for each value of K and a burn-in of 200 000 steps followed by 1 000 000 Markov chain Monte Carlo iterations. The output from replicate runs was summarized using STRUCTURE HARVESTER (Earl 2012). The most parsimonious number of clusters present within the dataset was inferred based on the K value that had the highest $\ln\Pr(X|K)$ or, for $K > 2$, the ad hoc statistic delta K (Evanno et al. 2005). Membership scores for each value of K were aligned and averaged over replicates using CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007), and barplots were generated to visualize the results using the R package pophelper (Francis 2017).

While STRUCTURE forms genetic clusters of individuals by minimizing departure from Hardy-Weinberg and linkage disequilibria, discriminant analysis of principal components (DAPC) is a non-model based method that optimizes genetic separation between groups and minimizes variation within groups (Jombart et al. 2010). We ran DAPC using the R package adegenet v2.1.3 (Jombart 2008) and following the recommendations of the online tutorial provided by the developers (Jombart & Collins 2015). First, the function 'find.clusters' was run to identify the most likely number of clusters in the data in the absence of *a priori* sampling information. This function converts the genotypic data into uncorrelated principal components (PCs) and ranks the clustering solutions for different values of K using the Bayesian information criterion (BIC). For the ringed seal dataset, BIC increased in a roughly linear fashion with increasing values of K, and the contribution of additional PCs to the total variance did not asymptote (Fig. S1a). The lowest BIC was found at $K = 1$, indicating that no distinct genetic clusters were detected within the SNP dataset (Fig. S1b).

Given this result, we proceeded by running the DAPC (function 'dapc' in adegenet) with sampling locality provided as a prior. We initially ran the DAPC with the number of PCs retained limited to the number of samples divided by 3, which is the maximum number recommended by the developers. We then used the function 'optim.a.score' with 100 simulations to identify the optimal number of PCs to retain without leading to overfitting of the discriminant functions, which can result in spurious discrimination of any set of clusters. The *a*-score is a measure of the proportion of successful re-assignments to the *a priori* defined clusters corrected for the number of retained PCs. The DAPC was then re-run using the optimal number of PCs and retaining 3 discriminant functions (the number of putative populations minus 1, as recommended). The function 'scatter.dapc' in adegenet was then used to plot the results of the DAPC analysis when sampling locality was used as a prior. Inertial ellipses were drawn to encompass 67% of the variance (the default value) within each stratum.

2.3.7. Assignment test

We used the R package assigner v0.5.7 (Gosselin et al. 2016) to evaluate whether our dataset could be useful in assigning individuals to the group in which they were sampled. Given the small sample sizes for the northern Bering Sea (n = 14) and particularly the southeastern Chukchi Sea (n = 8) stratum, we restricted these analyses to using only the eastern Bering Sea (n = 28) and the Beaufort Sea (n = 29) strata, which had relatively high sample sizes and also represent the 2 most geographically disparate strata. Although the sample sizes were similar for these 2 strata, we created 10 subsets of the data by randomly subsampling 28 individuals from each stratum to avoid any bias created by uneven sample sizes. Assigner employs the gsi_sim algorithm (Anderson et al. 2008) to conduct a self-assignment analysis using a leave-one-out (LOO) approach. Each individual is sequentially removed from the set of baseline samples, and the remaining individuals are used to calculate F_{ST} at each locus. The loci are ranked according to the F_{ST} values, and the chosen number of loci are retained based on that ranking. These loci are then used to assign the LOO individual to the stratum that has the highest probability of producing its genotype. This approach (training, holdout, LOO [THL] method) was used to avoid high-grading bias (Anderson 2010) while maximizing the sample size of individuals retained in the training dataset from which the baseline allele frequencies for each stratum are calculated. Assignment success is then estimated as the proportion of individuals that are correctly assigned to their sampling location of origin. The analysis was run both with and without imputing missing genotype data; imputation was conducted using the random forest algorithm based on 100 trees. For each subsample, we ran the THL analysis for 10 iterations and with the number of ranked SNPs ranging from 10% of the total number to the full dataset.

To further evaluate the results of the assignment test, we created 20 subsets of the data in which the individuals included in the original analysis were randomly assigned to 1 of 2 strata without replace-

ment. The analysis described above was then repeated with the proportion of ranked SNPs ranging from 50 to 80% of the total. The assignment success based on these randomized datasets was then compared to the empirical results.

3. RESULTS

The analyses presented here utilized only the codominant SNP data provided by DArT P/L, which included 100 281 SNPs genotyped. The species of 2 of the 89 samples genotyped were determined via mitochondrial DNA control region sequencing to have been misidentified, and these 2 samples (1 from the southeastern Chukchi Sea stratum and 1 from the Beaufort Sea stratum) were removed from the analysis prior to filtering. A summary of the number of loci and/or samples removed at each filtering step is shown in Table 1. Our final SNP dataset included 79 individuals with 80% or greater locus coverage for 5699 bi-allelic SNP loci. Measures of SNP diversity were similar among strata (Table 2), with average expected heterozygosity ranging from 0.250 to 0.263 and average observed heterozygosity ranging from 0.219 to 0.228.

3.1. Identification of outlier loci and relatedness analysis

The OutFLANK analysis did not identify any loci as being putatively under selection given a q-value threshold of 0.05. The OutFLANK approach generally has a low rate of false positives and has been noted as being only suited for identification of loci under strong spatially diversifying selection (Whitlock & Lotterhos 2015).

The mean relatedness coefficient calculated for all pairs of genotyped individuals was −0.013 (SD = 0.0144). All except 1 pair of individuals had coefficients that fell at or below 0.066. The coefficients estimated from the sampled individuals fell within the range of those calculated from the simulated genotypes of unrelated individuals (−0.108 to 0.122, median = −0.002; Fig. S2), where unrelated refers to individuals that do not fall in the other 3 categories (i.e. parent–offspring, full sibling, half sibling). The one exception to this pattern was a pair of individuals that were sampled during the same year in Hooper Bay, Alaska, and had a relatedness coefficient of 0.237. This value is similar to what would be expected between half-sibling pairs, and it fell in the middle of the distribution calculated for the simulated half-sibling pairs. Review of the harvest data associated with these 2 samples revealed that they were part of the same cohort (one was estimated to be 10 mo old, the other 12 mo old, both males). Given that they did not share the same mtDNA haplotype, these 2 individuals appear to be paternal half siblings that are the offspring of a single male mating with 2 different females in the same year (2007).

3.2. Comparisons between geographic strata

Overall differentiation was low but significant (F_{ST} = 0.001, p = 0.028; χ^2 p-value = 0.012). Pairwise comparisons between the *a priori* defined geographic strata revealed low but statistically significant levels of genetic differentiation between the eastern Bering Sea stratum and the Beaufort Sea stratum (F_{ST} = 0.001, p = 0.005; Table 3). With the exception of the comparisons

Table 1. Number of putative single nucleotide polymorphisms (SNPs) retained after each filtering step. Samples flagged for removal included n = 2 samples identified in the mtDNA analysis as bearded seals, n = 2 from stranded individuals that may not have died during the breeding season, n = 3 samples collected from regions outside the study area (Okhotsk Sea), and n = 2 samples identified as duplicates (i.e. samples collected from the same animal). Secondary loci are defined as SNPs found on the same sequence fragment as 1 or more other SNPs. When secondary loci were identified, only the locus with the highest polymorphic information content was retained. *d*: average read depth across all loci; HWE: Hardy-Weinberg equilibrium

Criterion	No. of individuals	No. of loci removed	No. of loci remaining
Unfiltered dataset[a]	90	0	100 281
Avg. read count <10	90	18 613	81 668
Avg. read count $>d + 4 \times \sqrt{d}$[b]	90	16 483	65 185
Remove flagged individuals	81	13 346	51 839
Reproducibility <1.0	81	5700	46 139
Call rate ≤0.9	81	17 031	29 108
Secondary loci	81	8584	20 524
Individual coverage <0.8	79	148	20 376
Minor allele frequency <0.05	79	14 596	5780
Heterozygosity >0.60	79	0	5780
Out of HWE in 3 or more strata	79	81	5699

[a]Four samples failed to pass the DArT quality assurance/quality control screening process and were removed prior to any filtering;
[b]This step is intended to remove loci with excessive coverage, which may represent paralogs or repetitive elements.

Table 2. Single nucleotide polymorphism diversity indices for samples after subdivision into strata. n: no. of samples; *P*: proportion of loci genotyped within the stratum, H_e: mean expected heterozygosity across loci; H_o: mean observed heterozygosity across loci

Stratum	n	*P*	H_e	H_o
E Bering	28	0.982	0.260	0.219
N Bering	14	0.987	0.257	0.228
SE Chukchi	8	0.984	0.250	0.223
Beaufort	29	0.979	0.263	0.224
All	79	0.991	0.267	0.223

involving the northern Bering Sea, the magnitude of the remaining pairwise comparisons was similar (F_{ST} = 0.001) but not significant.

When individuals were randomly subsampled from the eastern Bering Sea and the Beaufort Sea strata and then compared to each other, only 2 of 20 comparisons were statistically significant with a sample size of 8, while 4 of 20 were statistically significant with a sample size of 14 (Table S2). When sample sizes were increased to 20 per stratum, 14 of 20 were statistically significant, and at 25 samples per stratum, all but one test (of 20) were significant.

When the samples were subdivided by sex, the comparison between the eastern Bering Sea and the Beaufort Sea remained statistically significant for both males and females (females, F_{ST} = 0.003, p = 0.016; males, F_{ST} = 0.002, p = 0.016). No other sex-specific comparisons were significant (Table 3).

3.3. Calculating expected effect size

If we assume a generation length between 7 and 18 yr, a dispersal rate of 1% per year, and effective population sizes ranging from 34 000 to 46 200 seals, then we would expect a maximum $F_{ST\text{-}SNP}$ value between 1×10^{-4} (minimum 3×10^{-5}). The empirical estimates of F_{ST} based on the SNP data (F_{ST} = 0.001) are approximately 1 to 2 orders of magnitude higher than these expected effect sizes for all pairwise comparisons, except for 2 (both of which include the northern Bering Sea).

3.4. Clustering analyses

The results of the STRUCTURE analysis using the full SNP dataset (with 1 of the samples comprising the putative half-sibling pair removed) are shown in Fig. 2a. The Δ*K* method (Evanno et al. 2005) identified *K* = 2 as the most likely number of clusters (Fig. S3). However, at *K* = 2, there was no clear geographic pattern to the assignments, although some individuals did assign strongly to one of the putative clusters (Fig. 2a). However, in general the number of individuals that were assigned to each cluster was roughly even, and many individuals were relatively admixed, both of which are suggested to be indicative of a lack of population structure (Pritchard et al. 2010). Given these results, and that the mean estimated lnP(*K*) was highest for *K* = 1 (albeit only slightly), which cannot be evaluated using the Δ*K* method, we consider it most likely that there is a single cluster. When *a priori* information on the geographic location of sampling was incorporated, the results were similar for *K* = 2, and runs for *K* = 3 and higher tended to lump all individuals in a single cluster, with the exception of those samples with the highest amounts of missing data (Fig. 2b).

No further resolution was obtained when we repeated the STRUCTURE analyses using only the eastern Bering Sea and the Beaufort Sea strata (Fig. 2c), which contained similar sample sizes and thus should not be subject to biases due to uneven sampling. Although the Δ*K* method identified *K* = 5 as the optimal clustering solution, the mean estimated lnP(*K*) remained highest for *K* = 1 (Fig. S3), and no geographic pattern was apparent when the barplots showing individual assignments were examined (Fig. 2c).

As noted in Section 2.3.6, running the *K*-means clustering method in the absence of information on the geographic origin of samples found that the lowest BIC was for a single cluster (Fig. S1b). We then ran the DAPC model with the samples stratified *a priori* by geographic location and with the number of PCs set to the number of samples divided by 3, as recommended by the developers. Optimizing the *a*-score for this initial DAPC model indicated that 22 PCs should be retained. However, the *a*-score was low (*a*-score maximum = 0.148), indicating weak or unstable discrimination of individuals into the *a priori* defined groups. A clear peak in the *a*-score across the range of PCs tested was not observed (Fig. S1c). The 3 discriminant functions that were retained (the number of groups minus 1) explained 33.2% of the variance. The scatter plots showed only slight separation between the mean values representing each group, and the ellipses encompassing 67% of the variance within groups showed substantial overlap (Fig. 3).

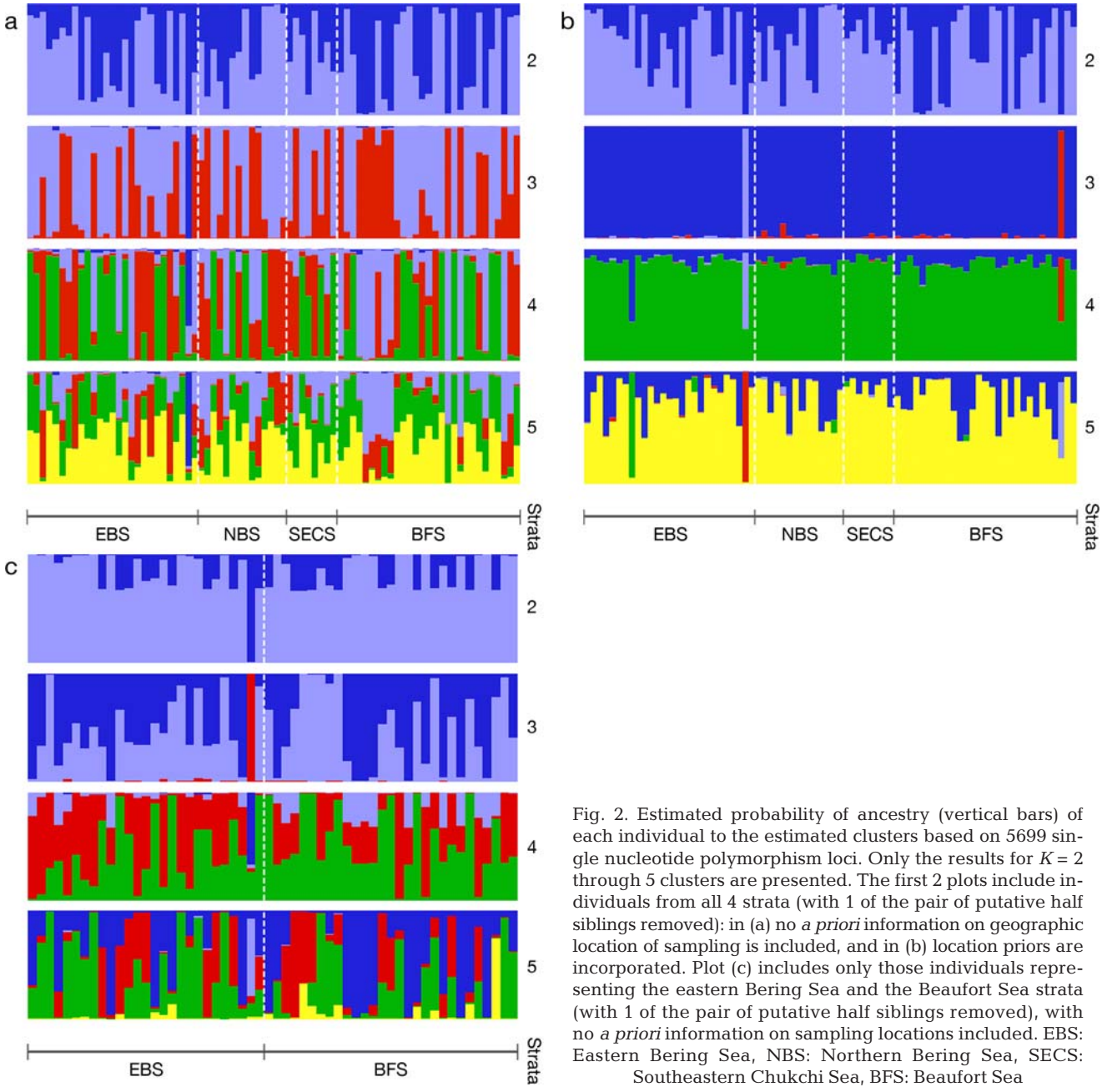

Fig. 2. Estimated probability of ancestry (vertical bars) of each individual to the estimated clusters based on 5699 single nucleotide polymorphism loci. Only the results for $K = 2$ through 5 clusters are presented. The first 2 plots include individuals from all 4 strata (with 1 of the pair of putative half siblings removed): in (a) no *a priori* information on geographic location of sampling is included, and in (b) location priors are incorporated. Plot (c) includes only those individuals representing the eastern Bering Sea and the Beaufort Sea strata (with 1 of the pair of putative half siblings removed), with no *a priori* information on sampling locations included. EBS: Eastern Bering Sea, NBS: Northern Bering Sea, SECS: Southeastern Chukchi Sea, BFS: Beaufort Sea

3.5. Assignment test

Assignment success was maximized when missing genotype data were imputed using the random forest algorithm; thus, only those results are reported here. When overall assignment success was defined as the proportion of samples that were correctly assigned back to their sampling origin with a probability of 50% or greater, the overall assignment success was greater than 70% once 1140 or more loci were analyzed and was maximized at 80% correctly assigned when 2850 loci were analyzed (Fig. 4). When the threshold for assignment of an individual to a given stratum was raised to a probability of 70% or greater, the values remained similar, with up to 79% being assigned correctly (Table 4). Assignment success was somewhat higher for the Beaufort Sea stratum (reaching a high of 88% correct assignments) than for the eastern Bering Sea stratum (72% correct assignments) (Fig. 4). When all loci were included in the analysis, assignment success was 72 to 76% with the 50% probability

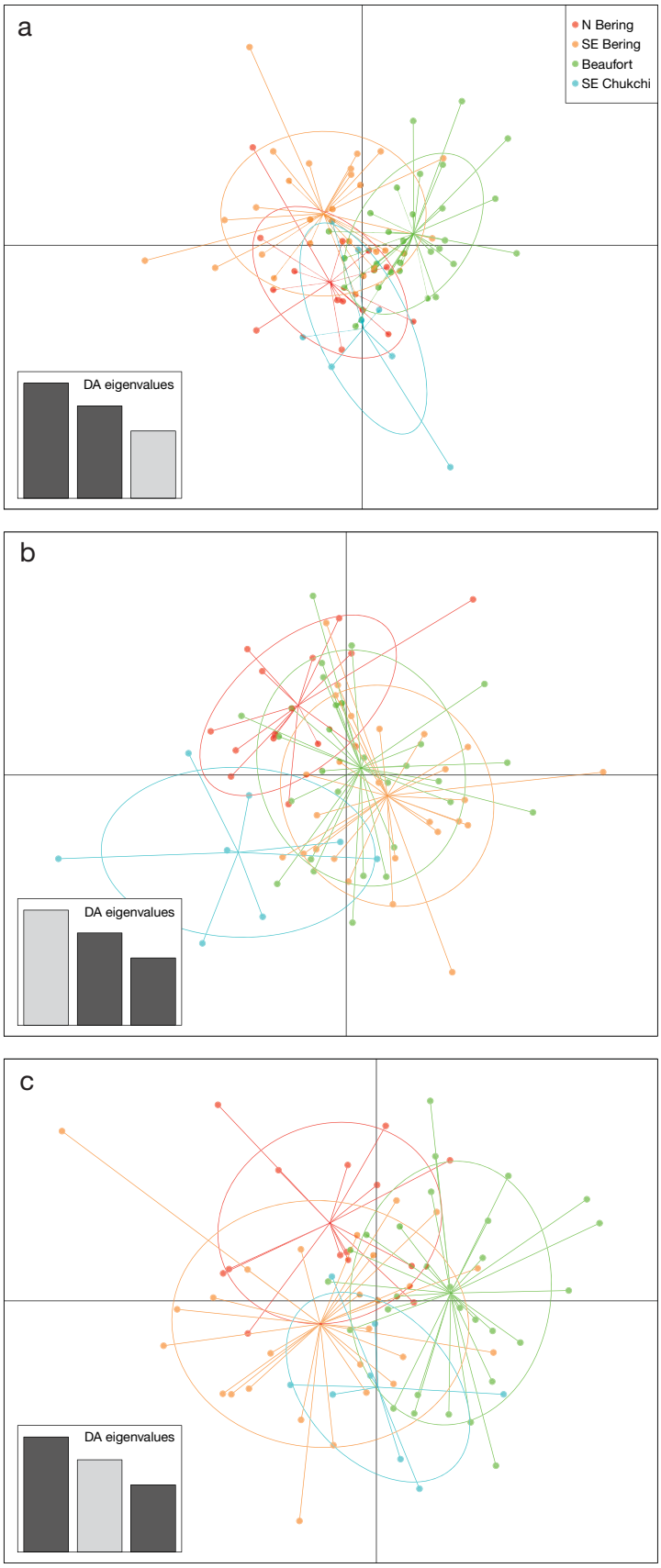

Fig. 3. Results of running a discriminant analysis (DA) of principal components on the single nucleotide polymorphism genotype data using sampling locality as a prior and retaining 22 principal components. Points represent individual genotypes, color coded by their original sampling locality. The inertial ellipses represent 67% of the variance within each stratum: (a) first 2 discriminant factors, (b) second and third discriminant factors, and (c) first and third discriminant factors

threshold and 71 to 75% with the 70% probability threshold.

When the assignment test was repeated using the simulated datasets in which individuals were randomly grouped in 1 of 2 strata, the maximum assignment success was 55.5%, which is similar to what would be expected at random when assigning between 2 groups (Fig. 5).

4. DISCUSSION

Early studies of many commercially exploited marine fish and mollusc species found little to no genetic differentiation, which was typically attributed to a high degree of gene flow in marine environments and to the large effective sizes of many populations (Cano et al. 2008). In several cases, however, the advent of high-throughput sequencing and the associated ability to evaluate population structure at thousands (vs. tens) of loci has uncovered evidence of population structure (e.g. Benestan et al. 2015, Momigliano et al. 2017). Similarly, early studies of subspecies and stocks of spinner dolphins *Stenella longirostris* in the eastern tropical Pacific, which historically numbered in the low millions (Wade et al. 2007), found little evidence of genetic differences using traditional genetic markers (mtDNA and microsatellites), despite the presence of morphological differences (Dizon et al. 1994, Galver 2002). Using >3700 nuclear SNP loci identified through a genotyping by sequencing approach, however, Leslie & Morin (2016) were able to show genetic structure that supported the morphologically distinguished groups.

The issue with detecting population structure in taxa with high abundance is that the rate at which genetic differences evolve between groups is inversely proportional to

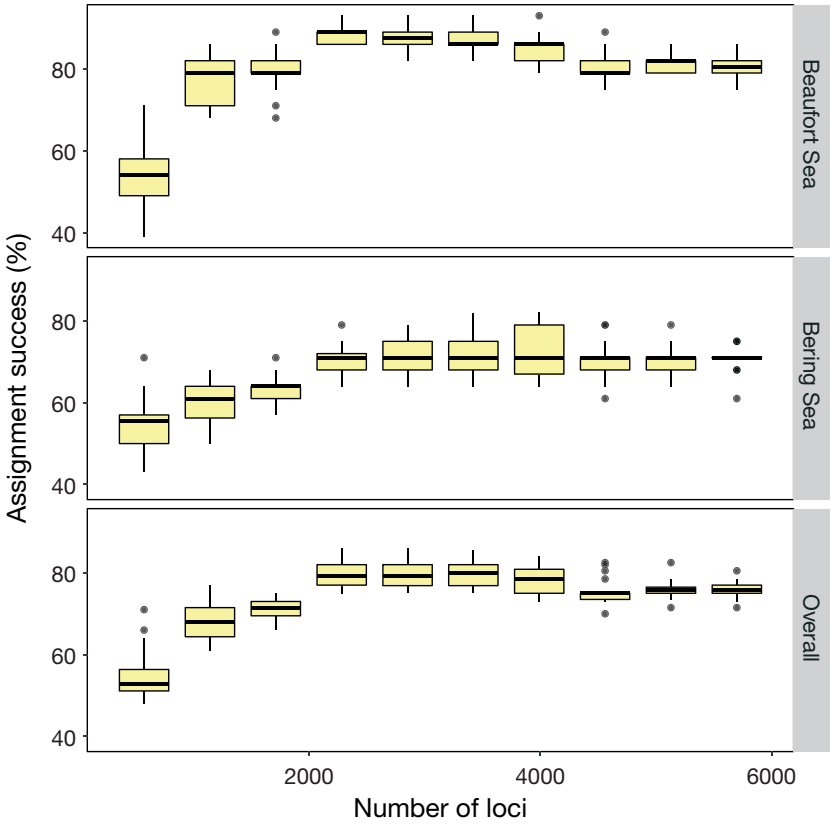

Fig. 4. Proportions of samples assigned correctly to their stratum of sampling origin (based on a 50% threshold) as the number of loci increased from 10% of all loci to the full dataset. For analysis based on only a proportion of the loci, loci were selected based on their ranking as derived from computing F_{ST} values between strata. Missing data were imputed based on the random forest algorithm

effective population size. In such scenarios, we would expect to find small effect sizes (i.e. F_{ST}) even when gene flow is limited (Taylor & Dizon 1996). While previous genetic studies have failed to identify clear patterns of population structure among Arctic ringed seals (Davis et al. 2008, Martinez-Bakker et al. 2013), those studies used a relatively low number of genetic markers (≤11 microsatellite loci) to compare geographic strata. As such, some question remained as to whether those datasets were powerful enough to detect small, but potentially biologically relevant, genetic differences between regions in this abundant subspecies.

To address this uncertainty, we increased the number of nuclear loci genotyped by several orders of magnitude (to ~5700 SNPs in 79 individuals). Low levels of divergence (F_{ST} < 0.001) between regions were detected in the SNP analysis, and only the comparison of the 2 most distant strata (the eastern Bering Sea and the Beaufort Sea strata) was statistically significant. No loci were identified as being putatively under selection in our

Table 3. Pairwise comparisons between strata with all samples included and after subdividing by sex. n: sample size. **Bold**: significant values (p < 0.05)

Comparison	F_{ST}	F_{ST} p
All samples		
E Bering Sea (n = 28) vs. N Bering Sea (n = 14)	0.000	0.490
E Bering Sea (n = 28) vs. SE Chukchi Sea (n = 8)	0.001	0.154
E Bering Sea (n = 28) vs. Beaufort Sea (n = 29)	**0.001**	**0.005**
N Bering Sea (n = 14) vs. SE Chukchi Sea (n = 8)	0.001	0.263
N Bering Sea (n = 14) vs. Beaufort Sea (n = 29)	0.000	0.316
SE Chukchi Sea (n = 8) vs. Beaufort Sea (n = 29)	0.001	0.207
Females only		
E Bering Sea (n = 12) vs. N. Bering Sea (n = 8)	0.002	0.204
E Bering Sea (n = 12) vs. SE Chukchi Sea (n = 5)	0.004	0.096
E Bering Sea (n = 12) vs. Beaufort Sea (n = 11)	**0.003**	**0.016**
N Bering Sea (n = 8) vs. SE Chukchi Sea (n = 5)	−0.001	0.693
N Bering Sea (n = 8) vs. Beaufort Sea (n = 11)	0.000	0.452
SE Chukchi Sea (n = 5) vs. Beaufort Sea (n = 11)	0.001	0.263
Males only		
E Bering Sea (n = 16) vs. N. Bering Sea (n = 6)	−0.002	0.877
E Bering Sea (n = 16) vs. SE Chukchi Sea (n = 3)	0.001	0.356
E Bering Sea (n = 16) vs. Beaufort Sea (n = 18)	**0.002**	**0.016**
N Bering Sea (n = 6) vs. SE Chukchi Sea (n = 3)	0.006	0.108
N Bering Sea (n = 6) vs. Beaufort Sea (n = 18)	0.000	0.522
SE Chukchi Sea (n = 3) vs. Beaufort Sea (n = 18)	0.002	0.271

Table 4. Results of running gsi_sim in the R package assigner. Values are averaged after 20 iterations. Due to limited sample sizes representing the northern Bering Sea and southeastern Chukchi Sea strata, only the eastern Bering Sea and the Beaufort Sea strata were analyzed here. For the upper half of the table, a sample is considered to be assigned correctly if it had a probability of assignment to its stratum of origin >50%. For the bottom half of the table, a sample was considered to be assigned correctly if it had a probability of assignment to its stratum of origin >70%

No. of loci	Beaufort Sea		Eastern Bering Sea		Overall
	No. of samples assigned correctly	Proportion of samples assigned correctly (%)	No. of samples assigned correctly	Proportion of samples assigned correctly (%)	Proportion of samples assigned correctly (%)
Stratum of origin >50%					
570	304	54	310	55	55
1140	430	77	336	60	68
1710	443	79	352	63	71
2280	494	88	396	71	79
2850	491	88	402	72	80
3419	486	87	404	72	79
3989	476	85	401	72	78
4559	448	80	394	70	75
5129	456	81	394	70	76
5699	453	81	397	71	76
Stratum of origin >70%					
570	288	51	299	53	52
1140	423	76	321	57	66
1710	439	78	345	62	70
2280	490	88	390	70	79
2850	485	87	394	70	78
3419	486	87	400	71	79
3989	471	84	396	71	77
4559	443	79	386	69	74
5129	451	81	388	69	75
5699	447	80	395	71	75

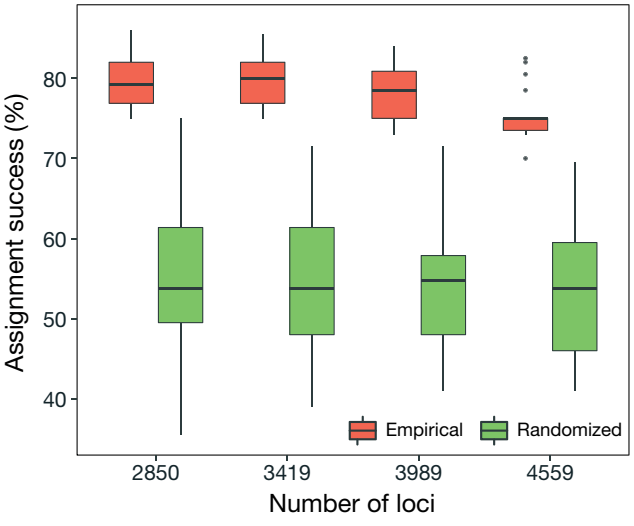

Fig. 5. Assignment success (proportions of samples that assigned to their stratum of origin at 50% or greater) in the empirical data (red) versus those generated from analysis of datasets in which individuals are randomly assigned to 1 of 2 strata of origin (green)

analysis, although additional exploration of the dataset could be valuable, as the approach we used is known to be conservative (Whitlock & Lotterhos 2015). The lack of evidence for local adaptation, together with the low magnitude of genetic differentiation identified between strata, suggests that gene flow between regions has been sufficient to avoid the loss of essential genetic variability needed to maintain the evolutionary potential of the subspecies. It is important to note, however, that large portions of the Arctic ringed seal's range were not analyzed (e.g. the Canadian Arctic and the Okhotsk Sea); thus, additional structure of evolutionary significance may have been identified with broader geographic sampling.

It is less clear how the low, and in 1 comparison statistically significant, levels of observed divergence relate to demographic connectivity, which depends on the relative contribution of dispersal to population dynamics and acts on ecological, rather than evolutionary, time scales (Waples & Gaggiotti 2006, Lowe & Allendorf 2010). The tipping point where populations become demographically independent usually occurs when the underlying genetic signal is weak, and in highly abundant species, genetic differentiation can persist despite a relatively low migration rate (Waples & Gaggiotti 2006). To provide some context in which our estimates of genetic divergence can be interpreted, we calculated the expected level of nuclear differentiation between areas when dispersal is low (1% per year). The empirical estimates of F_{ST} based on the SNP data (F_{ST} = 0.001 for all but 2 of the pairwise comparisons) are approximately 1 to 2 orders of magnitude higher than this expected effect size. Clearly, these estimates of differentiation rely on a number of assumptions, in terms of both the life history values used as well as the assumptions inherent in Wright's model (see Whitlock & McCauley 1999 for critique). However, while the actual rate could differ substantially, this exercise suggests that our results are consistent with what would be expected if some breeding sites used by Arctic ringed seals are connected by dispersal low enough to result in demographic independence.

Attempting to put the small but significant difference detected between the eastern Bering Sea and the Beaufort Sea in the pairwise comparisons of SNP data into the context of some of our other findings is challenging given the limitations of many genetic analytical approaches to discriminate between populations when divergence is low. For example, simulation testing of clustering methods, such as STRUCTURE (Pritchard et al. 2000, Evanno et al. 2005), has shown that such methods may have trouble accurately identifying the number of genetic clusters, and assigning individuals to those clusters, at low levels of genetic differentiation ($F_{ST} < 0.01$) (Latch et al. 2006, Waples & Gaggiotti 2006, Kalinowski 2011), an issue which may persist even when thousands of loci are analyzed (Benestan et al. 2015). The DAPC, which maximizes between-group variability while minimizing that within groups, has been able to at least partially separate out weakly differentiated stocks in some scenarios (e.g. Benestan et al. 2015, Leslie & Morin 2016, Dussex et al. 2018). When used with the ringed seal data, however, the DAPC was not able to identify genetic clusters in the absence of *a priori* information on geographic location of sampling and revealed substantial overlap of clusters even when geographic stratifications were incorporated.

Some support for the general pattern of population structure detected in our pairwise comparisons can be derived from the assignment test results. Assignment tests have been shown to lack power when differentiation is low, although their performance also depends on sample sizes and the number and variability of loci genotyped (Paetkau & Strobeck 1994, Berry et al. 2004, Paetkau et al. 2004, Hall et al. 2009, Lowe & Allendorf 2010). Here, we found that for the 2 most well-sampled groups, individuals could be assigned back to their stratum of origin with moderately high success (>70%) using the ~1100 loci that demonstrated the greatest differences between groups, with maximum assignment success (72% of the northern Bering Sea and 88% of the Beaufort Sea) being achieved when between 2280 and 3419 loci were used. While these patterns in assignment success suggest that a relatively large number of loci are needed to have a moderately high probability of correctly assigning samples to groups, the results indicate that some features (here, allele frequencies) differ between at least the 2 most well-sampled strata, which would be expected if population structure exists between those groups. Benestan et al. (2015) used similar methodology to conduct a LOO assignment test on American lobsters, which, like Arctic ringed seals, have high abundance and weak but significant levels of genetic differentiation ($F_{ST} \sim 0.001$). Similar to our results, they found that assignment success peaked at around 3000 loci. They also found that average assignment success increased with the number of individuals sampled, with mean assignment success remaining <75% when fewer than 30 individuals were sampled from each group.

4.1. Integrating other lines of evidence

In some cases where the genetic signal is small and/or its interpretation ambiguous, evaluating the genetic results in the context of other lines of evidence has proven informative. Within our study area, however, there are limited data from additional lines of evidence with which to corroborate the relevance of our genetic findings. The observations of ringed seals demonstrating fidelity to breeding sites over multiple years are congruent with what would be expected if natal fidelity is occurring, although the lack of known returns of seals to breed in regions where they were first identified as young of the year limits the inferences about population structure that can be drawn from the tagging data. In addition, the finding of a pair of putative paternal half siblings that were sampled in the same year at the same site is intriguing. Given the size of the population and that males are thought to maintain breeding territories throughout a given breeding season, it seems improbable that 2 seals sired by the same male in the same season would be sampled so close in proximity the following season if they were choosing wintering sites at random. While this finding suggests that at least some male yearling seals show an affinity for remaining in or returning to their natal site during their first winter post-weaning, ringed seals do not begin breeding until they are at least 6 or 7 yr old, and tagging data have shown that subadult seals overwinter near the ice edge, while breeding adults winter farther north in heavy and shorefast ice (Crawford et al. 2012). Thus, evidence that seals may show fidelity to their natal sites during their first year is not necessarily indicative of seals returning to their natal sites as breeding adults.

Some evidence that population structure may exist within the range of the Arctic ringed seal comes from recent studies identifying morphometric and life history differences among seals inhabiting the Canadian Arctic (Ferguson et al. 2018, 2019). Seals from sites located in the northern portion of the area studied (the Canadian High Arctic) were larger (both in

total length and girth) than their counterparts in the more southern waters of Hudson Bay. The northern seals grew more slowly, reaching asymptotic size 5 to 7 yr later than the southern seals; had lower fecundity and longer life spans; and exhibited sexual dimorphism that was not present among the southern seals. Within this area, latitudinal differences in diet and foraging behavior have also been observed (Yurkowski et al. 2016), and Ferguson et al. (2018, 2019) suggest that the divergent life history patterns that have emerged between the northern and southern seals are a response to differences in local environmental conditions. Of note, however, is that changes in growth rates and the average age of maturity of females have been observed to occur within areas over short time scales (between 1975–1984 and 2003–2012) in ringed seals in Alaska due to environmental conditions (Crawford et al. 2015), indicating there is flexibility within the species for both morphology and maturity.

4.2. Study design considerations

All samples analyzed in this study were collected opportunistically, either from harvested seals or research programs focused on other objectives (i.e. not on evaluating population structure). We only used samples that were collected during the breeding season to focus on the period when genetic differences by strata, if present, would be most pronounced in the dataset. This restriction, however, reduced the number of samples available for the study. Given these limitations, we increased power to detect demographically important genetic differentiation by increasing the number of loci genotyped, which, at least for microsatellite loci, has been shown to improve statistical power more than a larger sample size (Hale et al. 2012, Landguth et al. 2012). Note, however, that while the magnitude of differentiation (F_{ST} = 0.001) was similar among all comparisons except for 2 of those involving the northern Bering Sea, significant differentiation in the pairwise comparisons only occurred among the 2 strata with the highest (and roughly similar) sample sizes. In addition, comparisons based on random subsamples of individuals from those 2 strata did not consistently detect significant differentiation until the sample sizes reached 20 or higher. While the failure to detect significant differences in most of our comparisons could be a reflection of insufficient sampling, simulation-based studies have shown that with large numbers of SNPs (>1500 loci), accurate estimates of F_{ST} can be derived with as few as 2 samples (Nazareno et al. 2017).

Simulations have shown that in addition to sample size, sampling scheme can also affect inference of population structure (Schwartz & McKelvey 2009, Koen et al. 2013, Oyler-McCance et al. 2013, Landguth & Schwartz 2014). While the strata analyzed were represented by at least 8 samples genotyped at ~5700 loci, the sampling was uneven and patchy, and large portions of the range of the Arctic subspecies were not represented. Sample collection efforts occurred over a 12 yr period, during which time the sea ice conditions fluctuated. These fluctuations may have affected the distribution and behavior of the seals, but the limited number of samples collected from any area in a given year was too small to evaluate potential temporal changes in genetic diversity or structure. Most of the samples representing the 2 northernmost strata were collected from seals killed by polar bears and were broadly distributed throughout a larger area, while all of the Bering Sea samples were from harvested seals and were collected in a few communities (Hooper Bay in the eastern Bering Sea stratum and Savoonga and Gambell, which are about 60 km apart, on Saint Lawrence Island in the northern Bering Sea stratum). If adult seals show site fidelity to breeding areas, irrespective of whether such areas represent their natal sites, then more spatially concentrated sampling of the 2 southern strata relative to the northern strata could have increased the probability of sampling related individuals (i.e. half siblings). However, as long as each stratum is sampled randomly, the inclusion of close relatives by chance should not introduce a bias into our sample sets. While the 1 putative paternal half-sibling pair that was detected in our dataset was found in the eastern Bering Sea stratum, the individuals were sampled in different months of the same year (i.e. they were not sampled together). Repeating the pairwise comparisons after removing 1 of the half siblings did not change the results substantively (Table S3).

Our results, although robust, highlight the need for dedicated sampling efforts to (1) increase the sample sizes representing different areas, (2) fill in geographic gaps between sampling sites, and (3) broaden the temporal scale of sampling to evaluate the stability of the genetic signal.

5. SUMMARY AND FUTURE WORK

As the Arctic continues to warm, ringed seals face a variety of potential threats associated with declin-

ing habitat quality. Given that the magnitude of these threats to ringed seals is unlikely to be uniform across their range, understanding connectivity between areas, and the mechanisms underlying it, is important in predicting the overall risk to the subspecies. For example, if connectivity is low and fidelity to breeding areas is largely driven by the return to natal areas, the continued return of seals to reproduce and breed in areas with low snow cover and/or unstable ice would likely lead to low pup survival due to the collapse of subnivean lairs and the subsequent exposure of pups to cold, increased predation risk, and potential early separation from their mothers. Under this scenario, eventually the sites most negatively impacted by climate warming would no longer be used by seals for pupping and breeding in the spring, although seals might continue to use the area for foraging during the non-breeding season. However, if connectivity between breeding sites is high and selection of sites is driven by prey distribution, it is plausible that seals could choose to reproduce and breed in areas with high prey availability but poor ice stability and/or low snow cover. In such a case, overall declines in abundance associated with reduced pup survival might occur, but the breeding sites would continue to be used, acting as a population sink.

Our results, as well as those of previous studies (Davis et al. 2008, Martinez-Bakker et al. 2013), indicate that interchange between ringed seal breeding areas likely occurs. This connectivity, if it continues, may protect the seals from the loss of evolutionary potential and adaptive capacity as they navigate habitat alterations and loss in the face of environmental warming. However, the magnitude of nuclear differentiation we observed between the southernmost (eastern Bering Sea) and northernmost (Beaufort Sea) strata, while small, is markedly higher than that estimated assuming 1% dispersal per year, and the statistically significant differences between these areas suggest a lack of panmixia across our study area. In combination with the relatively high rate at which samples from these 2 strata could be genetically assigned to their region of origin, these results suggest that subtle, but demographically important, structure is present among breeding areas used by Arctic ringed seals in the Bering, Chukchi, and Beaufort seas, consistent with most seals returning to their natal sites to breed as adults. If such return continues despite reduced habitat quality at breeding sites most negatively affected by environmental warming, declines in the number of seals breeding in those areas could occur.

Our results suggest that a comprehensive analysis of samples collected during the breeding season and throughout the range of the Arctic subspecies, in combination with the large-scale genotyping approach used here, is needed to more fully assess demographic structure across the range and to thereby better understand the impacts of future environmental changes on ringed seals. One advantage of the approach used here is that it provides a resource from which SNP genotyping assays could be designed that would facilitate expanding the spatial as well as temporal scope of the current study. In particular, these data could be mined to identify a panel of the most informative SNPs that could be used with low-quality and/or historic samples, which might greatly expand the number of samples. Another genetic approach that has proven useful for characterizing population structure in weakly differentiated populations is kinship analysis (Kane & King 2009, Saenz-Agudelo et al. 2009, Palsbøll et al. 2010). Such an approach is challenging in large populations (Hellberg 2009) given the number of samples that need to be collected and genotyped. However, concentrated sampling of seals in a portion of the range that is continued over multiple breeding seasons could provide insight into whether natal fidelity occurs, in which case, with sufficient sampling one would expect to find individuals of breeding age that represent parent–offspring pairs.

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Title Page

Serologic survey for exposure to potential pathogens in ribbon seals *(Histriophoca fasciata*), spotted seals (*Phoca largha*), and bearded seals (*Erignathus barbatus*) in the Bering Sea

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Abstract

Little is known about exposure to diseases or the health of ice-associated seals of the Bering Sea, which include ribbon (*Histriophoca fasciata*), spotted (*Phoca largha*), and bearded seals (*Erignathus barbatus*). To assess exposure to several pathogens, ribbon and spotted seals were sampled from the pack ice of the Bering Sea, and bearded seals were sampled in Kotzebue Sound, Alaska. Serum was tested for antibodies against phocine herpesvirus-1 (PhHV-1), phocine distemper virus (PDV), influenza A, *Brucella* spp., *Coxiella burnetii, Leptospira interrogans*, *Toxoplasma gondii,* and *Sarcocystis neurona*. Spotted seals were positive for antibodies to PhHV-1 (76.6%), PDV (41.5%), influenza A (10.6%), *Brucella* spp. (9.5%), and *Coxiella burnetii* (8.3%); ribbon seals tested positive for PhHV-1 (9.5%), PDV (41.2%), influenza A (4.8%), and *Coxiella burnetii* (8.0%); and bearded seals tested positive for PhHV-1 (33.3%) and PDV (100%). All species were negative for antibodies to *Leptospira interrogans*, *Toxoplasma gondii*, and *Sarcocystis neurona*. Our study confirms that ice-associated seals in Alaska have been exposed to at least five pathogens, some of which could pose health concerns for indigenous Arctic communities. We recommend continued monitoring to identify human health concerns and to monitor changes in seal health that might be exacerbated by effects of climate warming.

KEYWORDS baseline health, Bering Sea, changing environment, ice-associated seals, pathogen, serology

Introduction

Ribbon seals (*Histriophoca fasciata*), spotted seals (*Phoca largha*), and bearded seals (*Erignathus barbatus*) are all ice-associated, with subarctic and Arctic distributions that include Alaska waters in the Bering, Chukchi, and Beaufort seas (Burns, 1981a, 1981b; Lowry, 1985). These three species are closely associated with, and dependent on sea ice during spring for critical life-history functions of whelping and nursing pups, mating, and molting their coats (Burns et al., 1981; Simpkins et al., 2003). All three species are sufficiently numerous to be ecologically important in the Bering Sea, and bearded and spotted seals are vitally important as traditional resources for nutrition and culture in coastal communities of Alaska (Bering Sea Elders Advisory Group, 2011; Kruse, 1991; Loring & Gerlach, 2009; OCEANA, 2016; Oceana and Kawerak Inc., 2014; Stephen R. Braund & Associates, 2010; Wolfe & Walker, 1987).

Because of these species' dependence on sea ice, there are increasing concerns about their fate in the warming Arctic. In the United States, concern about the effects of a changing climate on ice-associated seal populations was a primary reason behind petitions to list ribbon, spotted, and bearded seals under the Endangered Species Act (ESA) in 2007 and 2008 (Boveng et al., 2009; Boveng et al., 2013; Cameron et al., 2010). The southern distinct population segment (DPS) of spotted seals, which comprises seals located in the Yellow Sea and Sea of Japan, was listed as *Threatened* under the ESA in 2010 (National Marine Fisheries Service, 2010). The Beringia and Okhotsk bearded seal DPSs were listed as *Threatened* in 2012 (National Marine Fisheries Service, 2012).

Although many of the threats facing ice-associated seals stem simply from the potential for loss of breeding habitat (Laidre et al., 2008; Lomac-MacNair et al., 2018; Moore & Huntington, 2008), there may be other indirect but significant threats from reduced or altered distribution of ice. It is likely that changing sea ice extent and seasonal persistence will alter the distribution, quality, and quantity of prey

species (Burek et al., 2008; Huntington et al., 2020; Tynan & DeMaster, 1997). Altered prey dynamics combined with changes in seal distributions (Kovacs et al., 2011) could negatively affect seal foraging success, resulting in poor body condition (Boveng et al., 2020; Burek et al., 2008; Moore & Huntington, 2008). In the subarctic and Arctic, a warming climate could significantly change distributions, densities, or movement patterns of both seals and pathogens, as well as increase survival of pathogens in old and new habitats, potentially exposing seal species to new pathogens (Burek et al., 2008; Harvell et al., 1999; Moore & Huntington, 2008; VanWormer et al., 2019). Transmission rates of diseases, timing of pathogen shedding, population size and geographic distribution of pathogens, and the timing and persistence of infection are also likely to change with a warming climate (Burek et al., 2008; Jenkins et al., 2006; Kutz et al., 2005; Parkinson & Butler, 2005). Such shifts in exposure and transmission of diseases could have grave consequences for seal species, other wildlife, and even humans, as some pathogens are zoonotic (Bradley et al., 2005; Burek et al., 2008; Harvell et al., 1999; Moore & Huntington, 2008).

Serological surveys have been conducted for a variety of pathogens in marine mammals (Dubey et al., 2003; Gulland et al., 1996; Hinshaw et al., 1984; Kersh et al., 2012; Nielsen, Clavijo, et al., 2001; Nielsen, Stewart, et al., 2001; Osterhaus et al., 1990; Osterhaus et al., 1985). Previous studies have screened ribbon, spotted, and bearded seals for antibodies to *Brucella* spp., phocine distemper virus (PDV), phocine herpesvirus 1 and 2 (PhHV-1, PhHV-2), *Toxoplasma gondii*, and *Neospora caninum*. Spotted and bearded seals have also been tested for antibodies to canine distemper virus (CDV), and bearded seals have been tested for antibodies to San Miguel sea lion virus, influenza A, and *Leptospira interrogans* serovars *bratislava*, *canicola*, *grippotyphosa*, *hardjo*, *icterrohemorrhagiae*, and *pomona*. These studies found that variable proportions of ribbon, spotted, and bearded seals were positive for antibodies to PhHV-1 (Quakenbush et al., 2009, 2011a; Zarnke et al., 1997) and *Brucella* spp. (Foster et al., 2018; Nymo et al., 2018; Quakenbush & Citta, 2008; Quakenbush et al., 2009, 2011a). Antibodies to *T. gondii*

were found in bearded and spotted seals, and antibodies to *N. caninum* in one bearded seal (Dubey et al., 2003). One bearded seal also had a low titer to *Leptospira interrogans* serovar *grippotyphosa* (Calle et al., 2008). However, these previous studies included small numbers of ribbon (n<40) and spotted (n<50) seals, and all were from the Bering Strait and Chukchi Sea, prior to 2008. The serological surveys indicated that these seals had been exposed to a variety of pathogens, but the extent of actual infection and any effects on health of individuals were unknown. Because sample sizes were small, data on exposure to potential pathogens in ice-associated seals of the Bering Sea are still relatively limited.

The objective of this study was to test ribbon, spotted, and bearded seals for exposure to eight marine and terrestrial pathogens, including PhHV-1, influenza A, *Brucella* spp., PDV, *Coxiella burnetii*, *Leptospira* spp., *Toxoplasma gondii*, and *Sarcocystis neurona*. Obtaining baseline data on exposure to pathogens in these seals is important to provide a reference for detecting future changes and trends in health, including those that may be climate-related (Burek et al., 2008; Harvell et al., 1999). Additionally, since these species are important subsistence and cultural resources for Alaska Native communities, and bearded seals in particular constitute a large proportion of the subsistence harvest, knowledge about exposure to zoonotic pathogens that may affect human health is important.

Methods

Sampling

During May 2007, April 2008, May to June 2009, and May 2010, we captured, sampled, and released ribbon and spotted seals at the edge of the pack ice of the Bering Sea (Figure 1). We used small inflatable boats with 25 horsepower outboard engines to rapidly approach seals on ice floes, where they could be caught using long-handled salmon-landing nets. Seals were sampled on the ice floes on which they were caught. Seals less than one year of age were physically restrained, and individuals older than

one year were sedated with diazepam (Roche, Basel, Switzerland) injected intravenously into the extradural intravertebral vein (0.1 mg per kg body mass) to facilitate measurements, sample collection, and attachment of satellite tags (Boveng et al., 2007; Cameron et al., 2009). All seals that were given drugs were under veterinary care. Doxapram (Fort Dodge Laboratories, Inc. IA, USA) was administered orally (20 mg/ml) as needed following capture, primarily to ribbon seals, to stimulate respiration as some individuals tend to initiate a 'dive response' and breath-hold for long periods. Prior to releasing seals, the effects of sedation were reversed with an intramuscular injection of flumazenil (0.01 mg per kg body mass) (West-Ward Pharmaceuticals, NJ, USA) if the veterinarian determined it was needed.

In June 2009 and 2011, and July 2012, bearded seals were sampled on the pack ice in Kotzebue Sound, part of the Chukchi Sea, Alaska (Figure 1). Although we caught and sampled these seals in the Chukchi Sea, subsequent tracking by satellite tags confirmed that they were seasonal northward migrants associated with bearded seals that overwinter and breed in the Bering Sea, so we have included them in this study focused on seals of the Bering Sea. We used small inflatable or rigid-hull boats to search for bearded seals hauled out on ice floes. We approached seals on the ice, and when they went into the water, we deployed tangle nets from the boats and encouraged the seals to swim into the nets. Seals were removed from the nets and transferred to ice floes nearby for weighing, measuring, and sampling (Boveng & Cameron, 2013; Boveng et al., 2020). We used the same methods for handling and sampling bearded seals as described previously for ribbon and spotted seals.

We classified individuals into three age classes: young-of-the-year ('YOY', < 1 year, weaned), subadult (1-4 years), and adult (>4 years) (Boveng et al., 2009; Burns, 1981a; Burns & Frost, 1979). We used morphological characteristics (e.g., size, color, and distinctness of ribbons, for ribbon seals) to distinguish between subadults and adults. Seals were captured after pups were weaned, so we did not

sample any maternally dependent pups. The sex of each seal was determined by confirmation of a penile opening (males) or vaginal opening (females).

We collected blood samples from the extradural intravertebral vein (Geraci & Smith, 1975) using 18-gauge, 2.5- to 3.5-in. needles, depending on the size of the seal. Blood was drawn into SST Vacutainer® tubes (Becton Dickinson, Franklin Lakes, New Jersey) that contained serum separator gel. Blood tubes were kept in an insulated container to prevent freezing before processing, and then processed aboard a support vessel (ribbon and spotted seals) or at a shore-based laboratory (bearded seals) two to eight hours after collection. Serum separator tubes were centrifuged for 10 min at 10,000 rpm, and then 1-2 ml aliquots of serum were collected into vials and stored at -80 °C until analysis.

Serology

Sera were tested for antibodies to PhHV-1 using an indirect enzyme-linked immunosorbent assay (ELISA) at the Wildlife Health Center, School of Veterinary Medicine, University of California Davis (UC Davis) as previously described and validated by King et al. (2001) and Goldstein et al. (2003). Briefly, all samples were tested in duplicate at a 1:100 dilution, and compared to a positive reference sample with a PhHV-1-specific antibody level designated at 100 units/ml (U/ml). Results were estimated from a standard curve that was run on each plate, and samples with antibody concentrations <1.00 U/ml were considered to be negative; >1.00 and <10 U/ml were considered to be inconclusive; and ≥10.00 U/ml were considered to be positive for antibodies to a phocine herpesvirus (Table 1).

Samples were tested for antibodies to *Leptospira* spp. at the California Animal Health and Food Safety (CAHFS) laboratory at UC Davis. Standard microscopic agglutination microtiter procedure was used to test for exposure to *Leptospira interrogans* serovars (*pomona, hardjo, grippotyphosa,*

152 *icterohaemorrhagiae, canicola, and bratislava*). Titers >1:100 were considered positive for antibodies
153 (Table 1) (Colagross-Schouten et al., 2002).
154
155 Protozoal serology was performed at UC Davis (Conrad Laboratory) using an indirect fluorescent
156 antibody test (IFAT) for serum antibodies against *Toxoplasma gondii* and *Sarcocystis neurona*. The IFAT
157 was performed as described by Miller et al. (2002) with the cut-off for positive considered to be ≥1:320
158 (Table 1).
159
160 Sera were tested for antibodies to influenza A virus using a blocking-type enzyme-linked immunosorbent
161 assay (bELISA) (IDEXX Laboratories, Maine, USA) at the Southeastern Cooperative Wildlife Disease Study,
162 College of Veterinary Medicine, University of Georgia (Brown et al., 2009). Samples with S/N (serum
163 sample/negative control) values ≥ 0.50 were treated as negative responses (considered negative for the
164 presence of antibodies), and samples with S/N values < 0.50 were considered to be positive (Table 1).
165 Any sample that tested positive was retested to confirm results.
166
167 Antibodies to *Brucella* spp. were measured using the *Brucella* microagglutination test with *B. abortus*
168 antigen at the Bacterial Special Pathogens Branch of the Centers for Disease Control and Prevention,
169 Atlanta, Georgia. Titers <1:20 were considered negative for *Brucella* antibodies, titers >1:20 and <1:160
170 were considered inconclusive, and titers ≥1:160 were considered to be positive for antibodies (Table 1).
171 For reporting prevalence, we treated the inconclusive titers as negative.
172
173 A pan-*Brucella* real-time polymerase chain reaction (PCR) was performed on samples with positive and
174 inconclusive titers to detect the presence of *Brucella* spp. DNA (Probert et al., 2004). A *Brucella* species-
175 specific real-time PCR was then performed on samples that were PCR positive for *Brucella* DNA to

identify the *Brucella* species and assess relatedness to other known *Brucella* species. Additionally, a multiple-locus variable-number tandem repeat (VNTR) analysis on 15 loci (MLVA-15) (Le Flèche et al., 2006) was performed on positive samples to further examine where the *Brucella* spp. detected in the ribbon and spotted seal samples clustered in relation to other known *Brucella* species.

Serum samples were tested for antibodies against phase 1 and phase 2 of *Coxiella burnetii* using an indirect fluorescent antibody test (IFAT; Kersh et al. (2012)), at the Centers for Disease Control and Prevention, Atlanta, Georgia. Titers ≥1:128 were considered to be positive (Table 1).

Serum samples were tested for antibodies to PDV using microneutralization tests (Cosby et al., 1983) at the Centre for Infection and Immunity, Medical Biology Centre, Queen's University, Belfast, starting at 1 in 10 dilutions, followed by 2-fold dilutions. Sera were tested against the PDV/USA2006 and PDV/DK/2002 strains. Samples with titers greater than 1:32 were considered positive (Table 1).

Statistical analyses

We calculated overall proportion of samples positive for antibodies to pathogens in each species. The proportion positive was also calculated separately by year, age class, and sex of each species. Differences in antibody prevalence associated with species, year, age class, and sex were examined using Chi-square analysis (Zar 1996). Chi-square analyses were conducted in the R statistical environment (R Core Team, 2019).

Results

We collected blood samples from 63 ribbon and 50 spotted seals during April, May, and June of 2007-2010, and from seven bearded seals in June of 2009 and 2011, and July 2012. Not all seals were tested

for antibodies to each potential pathogen due to insufficient serum volume. We analyzed serum from 63 ribbon seals for antibodies to PhHV-1, *Toxoplasma gondii*, *Sarcocystis neurona*, and influenza A; 57 for *Leptospira* spp. and *Brucella* spp.; 25 for *Coxiella burnetii*; and 52 for PDV. We tested serum from 47 spotted seals for antibodies to PhHV-1, *Leptospira* spp., *Toxoplasma gondii*, *Sarcocystis neurona*, influenza A, and *Brucella* spp.; 24 for *Coxiella burnetii*; and 41 for PDV. We tested six bearded seal samples for antibodies to PhHV-1, *Leptospira* spp., *Toxoplasma gondii*, *Sarcocystis neurona*, and influenza A; five for *Brucella* spp.; four for *Coxiella burnetii*; and three for PDV (Table 2).

PhHV-1

The proportion of samples positive for antibodies to PhHV-1 was highest in spotted seals (76.6%), followed by bearded seals (33.3%), and then ribbon seals (9.5%; Table 2). Chi-square tests indicated that prevalence depended significantly on species (Pearson $\chi^2 = 49.68$, df=2, $p<0.001$), but possibly not on sex (Pearson $\chi^2 = 0.00$, df=1, $p=0.96$), year (Pearson $\chi^2 = 3.85$, df=4, $p=0.43$), or age (Pearson $\chi^2 = 0.25$, df=2, $p=0.88$).

Influenza A

The overall proportions of samples positive for antibodies to influenza A in both ribbon and spotted seals were low, but spotted seals had twice as many positive samples (10.6%) as ribbon seals (4.8%; Table 2). Spotted seals also had a greater number of seropositive individuals than ribbon seals in both YOY and adult age classes. No subadults of either species were positive for influenza A antibodies (Table 2). We failed to find a relationship between the prevalence of positives and species (Pearson $\chi^2 = 1.92$, df=2, $p=0.38$), sex (Pearson $\chi^2 = 0.07$, df=1, $p=0.79$), year (Pearson $\chi^2 = 1.47$, df=4, $p=0.83$), or age (Pearson $\chi^2 = 3.77$, df=2, $p=0.15$).

Brucella spp.

When comparing antibodies measured against *Brucella* spp., spotted seals were the only species with positive titers to *Brucella* spp (9.5%; Table 2). However, three ribbon seals (5.3%), five spotted seals (10.6%), and one bearded seal (20%) had titers that were inconclusive. We failed to find a significant relationship between *Brucella* prevalence of positives and sex (Pearson $\chi^2 = 0.00$, df=1, p=1.00), year (Pearson $\chi^2 = 3.65$, df=4, p=0.45), age (Pearson $\chi^2 = 4.84$, df=2, p=0.09), or species (Pearson $\chi^2 = 5.48$, df=2, p=0.06).

Real-time PCR was performed on 15 samples that had positive or inconclusive titers to *Brucella* spp., from three ribbon seals, 11 spotted seals, and one bearded seal. Eight of the 15 samples (1 ribbon, 7 spotted seals) were positive with a cycle threshold (Ct) of 37 or less, ranging from 25 to 37. One ribbon seal and two spotted seal samples with the lowest Ct values (25.7, 28, 28, respectively) were run in an MLVA-15 assay for genotyping. The assay produced a full MLVA profile (15/15 loci) for one spotted seal and partial profiles for the ribbon seal (11/15 loci) and one spotted seal (12/15 loci). Cluster analysis using the MLVA-15 data showed that the *Brucella* spp. in our samples clustered within the *B. abortus* clade and that they looked like outliers when clustered with the marine strains. Additionally, a *Brucella* species-specific PCR run on the eight positive samples indicated that the *Brucella* spp. did not cluster with *B. ceti* or *B. pinnipedialis*, the two marine *Brucella* species, but were more similar to *B. abortus* and *B. ovis*.

Phocine distemper virus

The proportions of samples positive for antibodies to PDV were high in all three seal species (Table 2). Bearded seals had the highest percent positive (100%), but sample size was small (n=3). All age classes of all species were positive for antibodies to PDV. Raw prevalence was markedly higher in 2009 than

2010 in ribbon and spotted seals, which were the only species sampled in both years. In 2009, 59% and 60% of spotted (n=29) and ribbon (n=30) seals, respectively, were seropositive; and in 2010, no spotted seals (n=12) and only 10% of ribbon seals (n=20) were positive for antibodies to PDV (Table 2). Chi-square tests indicated that prevalence varied significantly by year (Pearson $\chi^2 = 24.0$, df=1, $p<0.001$) and age (Pearson $\chi^2 = 7.5$, df=2, $p=0.02$), but possibly not by sex (Pearson $\chi^2 = 0.0$, df=1, $p=1.00$) or species (Pearson $\chi^2 = 4.2$, df=2, $p=0.13$). The significant chi-square test for an age effect is likely a result of the uneven distribution of ages sampled within years. In 2009, roughly equal numbers (n=20 or n=21) of each age class were sampled, but samples in 2010 were heavily biased towards YOY (n=20), with far fewer subadults (n=5) and adults (n=7).

Coxiella burnetii

The proportions of samples positive for antibodies to *Coxiella burnetii* were very similar in ribbon and spotted seals, 8% and 8.3%, respectively. Only YOY and adults of these species were seropositive, and all bearded seals were seronegative (Table 2). We failed to demonstrate a significant relationship between prevalence of positives and species (Pearson $\chi^2 = 0.36$, df=2, $p=0.84$), sex (Pearson $\chi^2 = 0.00$, df=1, $p=1.00$), year (Pearson $\chi^2 = 0.90$, df=4, $p=0.92$), or age (Pearson $\chi^2 = 2.10$, df=2, $p=0.35$).

Leptospira spp., *Toxoplasma gondii*, *Sarcocystis neurona*

Antibodies to these pathogens were not detected in samples from any of the three seal species (Table 2).

Discussion

Ribbon, spotted, and bearded seals sampled in the Bering Sea were positive for antibodies to four, five, and two pathogens, respectively. We only found significant differences in antibody prevalence among

272 species for PhHV-1, and among year and age class for PDV. PhHV-1 prevalence was highest in spotted
273 seals, followed by bearded seals, and lowest in ribbon seals. The prevalence of exposure to PDV in
274 ribbon and spotted seals, which were the only species sampled in both years, was higher in 2009 than in
275 2010. However, the differences in PDV prevalence detected among age classes was likely a result of
276 uneven distribution of ages sampled between the years, so a pattern of differences is not clear. Chi-
277 square tests found no significant differences for the prevalence of exposure among species, year, age
278 class, or sex for each of influenza A, *Brucella* spp., or *Coxiella burnetii*, though small sample sizes
279 provided low power to detect such effects (particularly for bearded seals).
280
281 The high percentage of samples positive for antibodies to PDV that we found for ribbon, spotted, and
282 bearded seals supports recent findings regarding the introduction and exposure to PDV in marine
283 mammals in the North Pacific Ocean. Serosurveys conducted in the North Pacific from the 1970s
284 through 2000 found that Steller sea lions (*Eumetopias jubatus*), northern sea otters (*Enhydra lutris*), and
285 harbor seals from Alaska, British Columbia, Washington, and Oregon, and ribbon, spotted, bearded, and
286 ringed seals from the Bering and Chukchi seas were all negative for antibodies to PDV (Burek et al.,
287 2005; Calle et al., 2008; Duignan et al., 1995; Hanni et al., 2003; Quakenbush & Citta, 2008; Quakenbush
288 et al., 2009, 2011a, 2011b; Zarnke et al., 2006). The first documentation of exposure to PDV in marine
289 mammals in the North Pacific was in northern sea otters in Alaska in 2004 (Goldstein et al., 2009).
290 However, antibodies to and infection with PDV has since been detected in spotted and ribbon seals,
291 northern fur seals (*Callorhinus ursinus*), Steller sea lions, and northern sea otters in the North Pacific
292 (Esquible et al., 2019; VanWormer et al., 2019). PDV antibodies and RNA were first detected in these
293 species after 2002, with peaks in infection and exposure occurring in 2003 and 2009 (VanWormer et al.,
294 2019). We also detected significantly higher prevalence of PDV in 2009 compared to 2010, which was
295 consistent with introductions following years when there was an open channel in the ice (VanWormer et

296 al., 2019). However, we are unable to determine whether this was due to a higher PDV prevalence in the
297 population, or whether it was an artifact of sampling biases.
298
299 The absence of PDV antibodies in marine mammals sampled from the Pacific Ocean, Bering, and Chukchi
300 seas prior to 2001, and the fact that PDV nucleic acid sequences from all species tested in the Pacific
301 were most similar to the PDV strain isolated from the 2002 PDV outbreak in Europe, suggests that the
302 virus may have been transmitted to the North Pacific after the European outbreak in 2002 (Goldstein et
303 al., 2009; VanWormer et al., 2019). VanWormer et al. (2019) showed that the years of higher prevalence
304 of PDV in the North Pacific followed years of poor Arctic sea ice that resulted in open water along
305 northern Russia to the Pacific. Movement data from satellite-tagged marine mammals has shown that a
306 likely mode of transmission is by intraspecies contact between pinniped species that have the potential
307 to travel long distances and transmit the virus (VanWormer et al., 2019). PDV has caused epizootics
308 resulting in mass mortality in harbor seal populations in northwestern Europe in 1988 and 2002, and off
309 the east coast of the U.S. in 2006 and 2018-2020 (Earle et al., 2011; Härkönen et al., 2006; Müller et al.,
310 2004; NOAA Fisheries, 2019; Osterhaus et al., 1990). No mortality events have been documented in
311 Pacific pinniped species associated with PDV to date, although the virus has contributed to some sea
312 otter and Steller sea lion deaths (Esquible et al., 2019; Goldstein et al., 2009). It remains unknown how
313 these ice-associated species will be affected by this virus. Zarnke et al. (2006) suggested that
314 morbilliviruses typically spread widely through immunologically-naïve populations, and with the recent
315 trend of reduced sea ice extent and open water along the coast of northern Russia, it is important to
316 continue to monitor for PDV in seal populations in Alaska.
317
318 Only spotted seals were seropositive for *Brucella* spp. antibodies, although all three seal species had
319 samples that were inconclusive for these antibodies. Using PCR, *Brucella* DNA was detected in six of nine

inconclusive samples, with one sample containing enough DNA to get a partial profile with MLVA genotyping. This difference between serologic testing and PCR results could be due to the timing of infection and development of an antibody response, or due to the antigen used in the test. Other studies also have noted differences in serologic and PCR test results (Maratea et al., 2003). In harbor seals, serological assays using different *Brucella* spp. antigens resulted in widely variable proportions of positives (16%-74%) (Hueffer et al. 2013), demonstrating the difficulty in determining which test to use. We chose to test for antibodies to *B. abortus* to examine the risk of exposure to infection in humans that may consume these seals.

Brucella spp. is a zoonotic pathogen that can cause abortion, fetal death, and reproductive disorders in animals (Nielsen et al., 1996; Probert et al., 2004). Brucellosis in humans causes a variety of symptoms including fever, malaise, myalgia and can become a chronic illness that affects several organs and tissues (Probert et al., 2004). Human brucellosis has been caused by five *Brucella* strains, including strains found in marine mammals (Probert et al., 2004). Cluster analysis indicated that the *Brucella* DNA present in seals in this study was most closely related to *B. abortus*, rather than to the marine *Brucella* species (*B. ceti*, *B. pinnipedialis*). The majority of brucellosis cases in humans is caused by *B. abortus* and *B. melitensis* (Franco et al., 2007), and only a few cases of brucellosis from a marine mammal strain have been documented (Sohn et al., 2003). The fact that the strain found in these seals is most similar to *B. abortus* could be a concern for human communities in the Arctic that depend on several marine mammal species for food. Bearded seals, in particular, are a significant part of the diet in many coastal northern Alaskan communities. Butchering seals, consuming raw or undercooked meat, and conducting post mortem examinations, are all activities with a risk of disease transmission to humans (Forbes et al., 2000); thus, education about *Brucella* may help to reduce the risk of zoonotic transmission in those communities.

Although there is evidence that phocid species in Arctic and subarctic North Pacific waters have been exposed to *Brucella* spp., the effects of *Brucella* spp. infection on the health of these animals remains unknown (Forbes et al., 2000; Zarnke et al., 2006). As in this study, other studies found that individuals that were positive for antibodies or had detectable *Brucella* spp. in tissues, appeared healthy with no clinical signs of brucella infection (Foster et al., 2018; Hoover-Miller et al., 2017; Nymo et al., 2018; Nymo et al., 2011; Zarnke et al., 2006).

We found that low numbers of ribbon (4.8%) and spotted seals (10.6%), but no bearded seals, were positive for antibodies to influenza A. These results are consistent with previous studies in Alaska and Arctic Canada, that found very low numbers of ringed seals (2.5%, 3%) and beluga whales (1.2%) positive for influenza A antibodies (Danner et al., 1998; Nielsen, Clavijo, et al., 2001). Our results support previous findings that exposure to influenza A in the subarctic and Arctic may be sporadic. Interestingly, antibodies were detected in young-of-the-year and in adults, but not in subadults. This could suggest that maternal antibodies transferred from mother to pup are what were measured in the young-of-the-year. Maternal antibodies are only present until three months of age in harbor seals (Ross et al., 1993). If this is also the case for spotted and ribbon seals, this may explain the absence of detectable antibodies in the subadult age class. Influenza A has caused epizootics of pneumonia that resulted in mass mortality of hundreds of harbor seals on the New England coast in the early 1980s (Geraci et al., 1982; Hinshaw et al., 1984) and 2011 (Anthony et al., 2012), as well as in northern Europe in 2014 (Bodewes et al., 2015). Although antibodies to influenza A do not appear to be widespread in marine mammals in the Arctic and subarctic, the potential for epizootics indicates that ice-associated seal populations should continue to be monitored for this infection.

As in previous studies that found exposure to PhHV-1 or related herpesviruses was common and geographically widespread in the subarctic and Arctic (Quakenbush et al., 2009, 2011a, 2011b; Zarnke et al., 1997), we found that all three ice seal species were seropositive for PhHV-1 antibodies, with increasing proportion-positive by age class. Previous studies found similar proportions of seropositive individuals in spotted and bearded seals (Quakenbush et al., 2011a; Zarnke et al., 1997), while the proportion of ribbon seals positive for PhHV-1 antibodies was slightly lower in those studies (Zarnke et al., 1997). Both studies that tested ribbon seals from the central Bering Sea (Zarnke et al. (1997) and this study) detected seropositive seals, while ribbon seals sampled from the northern Bering Sea were all negative (Quakenbush & Citta, 2008). The sample size of seals from the northern Bering Sea was small, so it is difficult to draw conclusions about differences in proportions of positives among ribbon seals sampled in different areas of the Bering Sea.

In this study, we found low proportions of ribbon seals (8%) and spotted seals (8.3%) positive for antibodies to *Coxiella burnetii*, and no bearded seals were positive. The first report of *C. burnetii* causing infection (placentitis) in a marine mammal was documented in a pregnant harbor seal that stranded in California (Lapointe et al., 1999). Since that first report, antibodies to *C. burnetii* have been documented in Steller sea lions, harbor seals, harbor porpoises, and northern fur seals, ranging from Oregon and Washington to Alaska (Duncan et al., 2012; Kersh et al., 2012; Kersh et al., 2010). Antibody prevalence was particularly high in fur seals (69%) and sea lions (59%) (Kersh et al., 2012; Minor et al., 2013).

Coxiella burnetii causes Q fever, a zoonosis found worldwide, with a wide range of wild and domestic animals that act as reservoirs of the pathogen. In humans, the primary manifestations of Q fever have been febrile and influenza-like illness, pneumonia, and hepatitis, and the most common mode of transmission to humans is by inhaling airborne particles from infected animals (Maurin & Raoult, 1999).

In 2010, Q fever was diagnosed in a human patient from a small community in Greenland, and a likely mode of transmission was from harbor or hooded seals, both of which are food sources for the community (Koch et al., 2010). Although we found low proportions of ribbon and spotted seals positive for antibodies to *C. burnetii*, any exposure is of concern because several Alaska Native communities depend on these species for subsistence (Bering Sea Elders Advisory Group, 2011; Kruse, 1991; Oceana and Kawerak Inc., 2014). Consequently, continued monitoring for this pathogen in ice seals is important for food safety for indigenous communities in Alaska.

In conclusion, antibodies to different pathogens varied among species, years, and age classes in ribbon, spotted, and bearded seals tested from the Bering Sea. These baseline data are important, considering that current climate models project a substantial decline in the extent of spring and summer sea ice throughout the Arctic region over the next several decades (Stroeve et al., 2012). To determine what effect, if any, loss of sea ice has on infection and pathogen transmission levels in ice-associated seals, it is necessary to first document current prevalence of exposure to potential pathogens and determine the natural range of variability in exposure by species and age class. For endemic pathogens, this requires knowledge of prevalence and typical (e.g., annual) variation in order to conduct comparative analyses. Documenting changes in epizootic prevalence may be more difficult, as other metrics (e.g., frequency of disease outbreaks) will likely need to be compared over different time scales. Such analyses can only be accomplished with regular health (e.g., serological) monitoring of ice-associated seal populations in the Bering Sea.

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Figure 1. Locations of samples collected from bearded, ribbon, and spotted seals in the Bering Sea and Kotzebue Sound from 2007-2010.

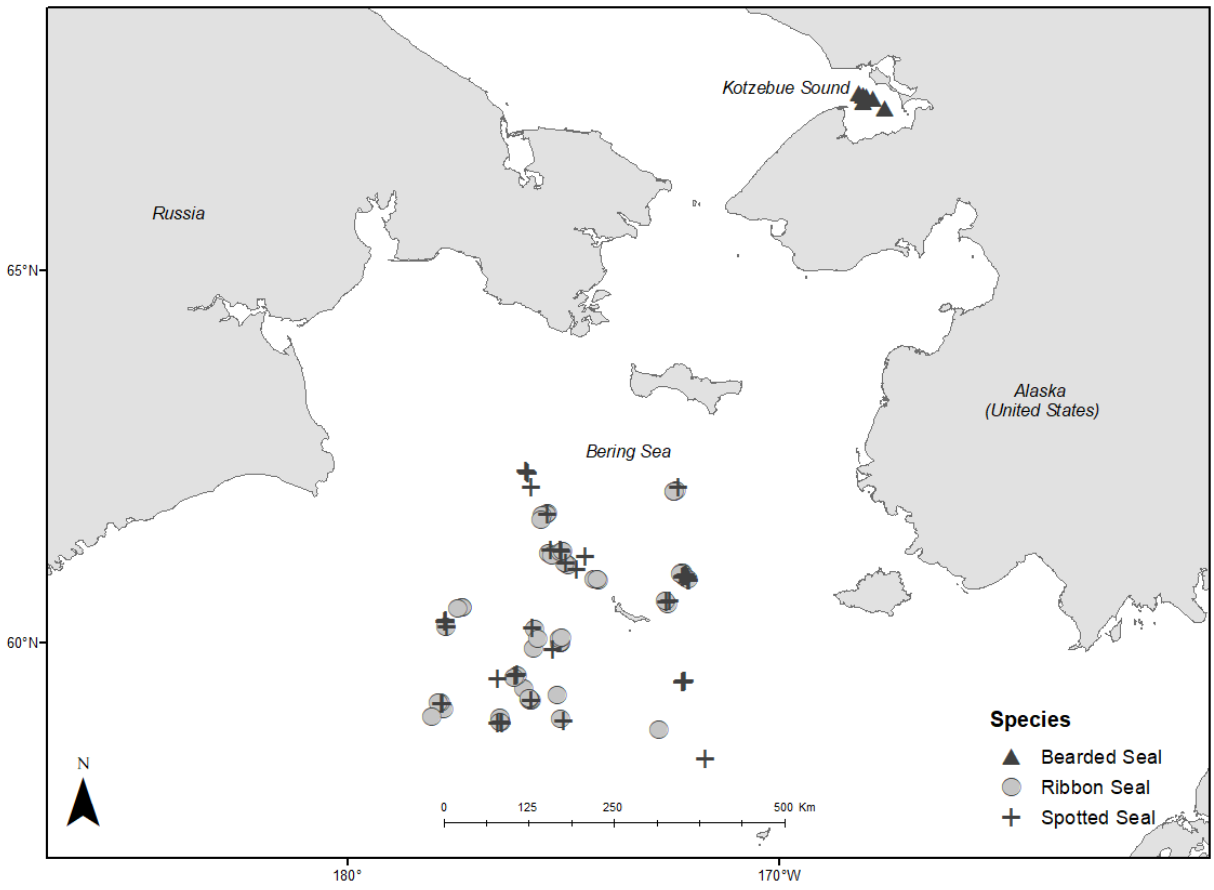

Table 1. Serology assays for antibodies to eight pathogens in ribbon, spotted, and bearded seals captured and sampled in the Bering Sea. The cut off values for positives and associated references are included.

Pathogen	**Serology test**	**Positive threshold**	**Dilution**	**Reference**
Phocine herpesvirus 1	Indirect enzyme-linked immunosorbent assay	≥ 10.00 U/ml	1:100	King et al. 2001
Leptospira spp.	Microscopic agglutination microtiter	> 1:100 titer	Screened at 1:100	Colagross-Schouten et al. 2002
Toxoplasma gondii Sarcocystis neurona	Indirect fluorescent antibody test	≥ 1:320	Screened at 1:100	Miller et al. 2002
Influenza A	Blocking-type enzyme-linked immunosorbent assay	S/N values < 0.50	1:100	Brown et al. 2009
Brucella spp.	Microagglutination test	≥ 1:160	Two-fold dilutions	Probert et al. 2004
Coxiella burnetii	Indirect fluorescent antibody test	≥ 1:128	Two-fold dilutions	Kersh et al. 2012
Phocine distemper virus	Microneutralization test	> 1:32	Two-fold dilutions	Cosby et al. 1983

Table 2. Percentages of ribbon, spotted, and bearded seals from the Bering Sea that tested positive for antibodies to eight pathogens, overall and by age class. Age classes are Adult (>4 years), Subadult (1-4 years), Young-of-the-Year (YOY; <1 year, weaned).

(a) Ribbon seals, % (No. positive/No. tested)				
Pathogen	**Overall**	**YOY**	**Subadult**	**Adult**
Phocine herpesvirus 1	9.5 (6/63)	0 (0/19)	6.7 (1/15)	17.2 (5/29)
Influenza A	4.8 (3/63)	10.5 (2/19)	0 (0/15)	3.4 (1/29)
***Brucella* spp.**	0 (0/54)	0 (0/15)	0 (0/12)	0 (0/27)
Phocine distemper virus	41.2 (21/51)	26.3 (5/19)	36.4 (4/11)	57.1 (12/21)
Coxiella burnetii	8 (2/25)	14.3 (1/7)	0 (0/7)	9.1 (1/11)
***Leptospira* spp.**	0 (0/57)	0 (0/15)	0 (0/13)	0 (0/29)
Toxoplasma gondii	0 (0/63)	0 (0/19)	0 (0/15)	0 (0/29)
Sarcocystis neurona	0 (0/63)	0 (0/19)	0 (0/15)	0 (0/29)
(b) Spotted seals, % (No. positive/No. tested)				
	Overall	**YOY**	**Subadult**	**Adult**
Phocine herpesvirus 1	76.6 (36/47)	70.4 (19/27)	78.6 (11/14)	100 (6/6)
Influenza A	10.6 (5/47)	11.1 (3/27)	0 (0/14)	33.3 (2/6)
***Brucella* spp.**	9.5 (4/42)	0 (0/27)	30 (3/10)	20 (1/5)
Phocine distemper virus	41.5 (17/41)	27.3 (6/22)	61.5 (8/13)	50 (3/6)
Coxiella burnetii	8.3 (2/24)	6.7 (1/15)	0 (0/5)	25 (1/4)
***Leptospira* spp.**	0 (0/47)	0 (0/27)	0 (0/14)	0 (0/6)
Toxoplasma gondii	0 (0/47)	0 (0/27)	0 (0/14)	0 (0/6)
Sarcocystis neurona	0 (0/47)	0 (0/27)	0 (0/14)	0 (0/6)
(c) Bearded seals, % (No. positive/No. tested)				
	Overall	**YOY**	**Subadult**	**Adult**
Phocine herpesvirus 1	33.3 (2/6)	(0/0)	20 (1/5)	100 (1/1)

Influenza A	0 (0/6)	(0/0)	0 (0/5)	0 (0/1)
***Brucella* spp.**	0 (0/4)	(0/0)	0 (0/3)	0 (0/1)
Phocine distemper virus	100 (3/3)	(0/0)	100 (2/2)	100 (1/1)
Coxiella burnetii	0 (0/4)	(0/0)	0 (0/4)	(0/0)
***Leptospira* spp.**	0 (0/6)	(0/0)	0 (0/5)	0 (0/1)
Toxoplasma gondii	0 (0/6)	(0/0)	0 (0/5)	0 (0/1)
Sarcocystis neurona	0 (0/6)	(0/0)	0 (0/5)	0 (0/1)