



Seasonal variability in vulnerability for Cassin's auklets (*Ptychoramphus aleuticus*) exposed to microplastic pollution in the Canadian Pacific region



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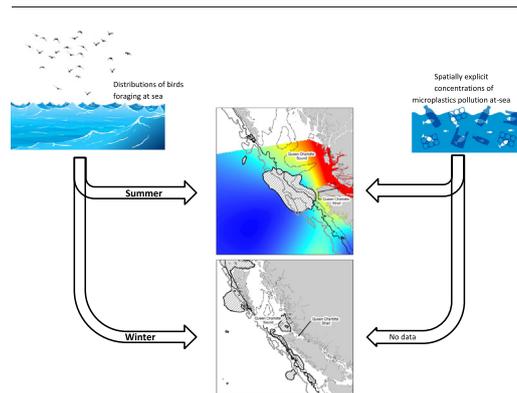
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HIGHLIGHTS

- A novel exposure model to assess possible impact from marine microplastics.
- Exposure to plastic pollution varies between seasons for Cassin's Auklet.
- Oceanography plays an important role in determining exposure.
- Exposure to microplastic concentrations quantified at population level

GRAPHICAL ABSTRACT



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ABSTRACT

Marine plastic pollution is an emerging global conservation challenge, potentially impacting organisms at all trophic levels. However, currently it is unclear to what extent plastic pollution is impacting marine organisms at the population, species or multispecies level. In this study, we explore seasonal exposure (i.e., vulnerability) of Cassin's Auklet (*Ptychoramphus aleuticus*) to plastic pollution with exposure models during boreal summer and winter seasons. Based on these models, we infer exposure at the population level for this species, in the Canadian Pacific region where approximately 75% of the global population of this species breeds. The models quantify plastic exposure by determining seasonal core foraging areas and plastic concentrations found in those same areas. Core foraging areas were determined using a Generalized Additive Model based on at-sea observation data

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(collected year round: 1990–2010) and 50% Home Range Kernels based on aerial telemetry data (May and June 1999–2001). Plastic concentrations within these core areas were interpolated based on seawater microplastic concentrations from the summer of 2012. We found that during the boreal summer, Cassin's Auklets were exposed to relatively low concentrations of plastics. During the winter, auklet distribution shifted towards the coast where plastic concentrations are considerably higher. Model derived seasonal variability in exposure was consistent with necropsy results from bird carcasses recovered during the winter of 2014, and from a multiyear study on chick provisioning during the summer. Local oceanography likely plays a role in determining seasonal shifts in both marine bird as well microplastic concentrations, and hence exposure. As well, individual sensitivity (i.e., dose-dependent effect) may vary with annual cycles. Currently, research is focusing on determining how sensitive individual birds are to microplastic concentrations, and our models will help translate sensitivity found at the individual level to potential impacts at population or species level.

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1. Introduction

Identified as a top emerging conservation challenge by the United Nations Environmental Programme in 2011 (Provencher et al., 2014; UNEP, 2011), plastic pollution is now considered a globally ubiquitous (Barnes et al., 2009; Thompson et al., 2009; van Sebille et al., 2015) and pervasive (Borrelle et al., 2017; Wilcox et al., 2015) threat to marine ecosystems. Plastic is highly persistent, resists chemical breakdown, accumulates in marine habitats, and breaks apart into smaller pieces (Barnes et al., 2009). These factors make plastic pollution generally extremely difficult to remove from oceanic habitats (Jambeck et al., 2015). As well, accumulation rates of plastic in the world's oceans have been linked strongly to global production rates (Jambeck et al., 2015). Since commercialization began in the 1930s and 1940s, plastic production has increased by at least 5% per year (Andrady and Neal, 2009), particularly during the last four decades when rates increased 660% overall between 1975 (47 million tonnes; PlasticsEurope, 2013) and 2014 (311 million tonnes; PlasticsEurope, 2013). Of the 270 million tonnes of global plastic production in 2010, Jambeck et al. (2015) estimated approximately 4.8 to 12.7 million tonnes end up in the world's oceans, where an unknown proportion is redistributed globally by large scale oceanic currents (van Sebille et al., 2015).

Impacts on marine organisms from plastic pollution exposure can range from physical (entanglement, smothering, and blockage of airway or gastrointestinal tract) to a broad array of potential toxicological effects (such as oxidative damage, carcinogenic properties, endocrine disruption and organ damage from leaching of constituent or sorbed toxic chemicals) (Desforges et al., 2015; Gilardi et al., 2010; Jacobsen et al., 2010; Pierce et al., 2004; Rochman et al., 2013; Wright et al., 2013). To date, at least 557 marine species have been reported to have ingested plastic or to have been entangled including all known species of sea turtle, 66% of all marine mammals, and 50% of all seabird species (Kühn et al., 2015). Although impacts are broad and extensive, it has been difficult to translate these documented effects into ecologically relevant impacts at higher level biological organization such as populations, species, or species assemblages (Koelmans et al., 2014; Rochman et al., 2016a; Rochman et al., 2016b). Further research is required to better understand the mechanistic effects of plastic at the individual level, such as improving our understanding of the relationship between dose and effect, and how this relationship may vary across a wider range of taxa. Apparently this research gap currently is being addressed (GESAMP, 2016; Avery-Gomm et al., 2018), and it would be useful to be able to translate dose-dependent effects, if found at the individual level, to higher levels of biological organization.

Determining where and when birds are exposed to elevated levels of plastic can lead to valuable insights on potential impacts from exposure. Clarifying the degree animals are exposed to a pollutant is the first step to a better understanding of population level effects of exposure (Nisbet, 1994). This is particularly true if it can be shown that significant proportions of populations, species or species assemblages are exposed to elevated plastic concentrations. As well, identifying areas where birds

are most exposed can inform management and mitigation efforts, particularly when plastic sources in these areas can be identified and mitigated locally (e.g., sewage outfall). However, determining these population-level responses of a species to environmental challenges requires consideration of potential impacts throughout the full annual cycle of that organism (Marra et al., 2015). Vulnerability (i.e., degree of exposure; sensu Zacharias and Gregor, 2005) to plastic pollution, or any other stressor, can vary temporally as well as spatially as both species distributions and plastic concentration can vary among locations, seasons, and across years. Sensitivity (i.e., dose-dependent effect or impact from stressor) can also vary with annual cycle events within an organism. For example, birds may be more sensitive to ingesting plastic during the winter season when food resources are normally low. Spatially-explicit risk assessment models (Fox et al., 2016; Lieske et al., 2014) estimate population level impacts from exposure to a stressor such as plastic pollution by integrating both vulnerability and sensitivity of an organism to that stressor. Unfortunately, sufficient information quantifying species sensitivity to plastic exposure is not currently available to parameterize a full risk assessment model, and for this reason it is only possible to explore spatial and temporal variability in exposure (i.e., exposure model) at this stage.

To assess risk of exposure to microplastic (<5 mm) ingestion and how this may vary seasonally, we use Cassin's Auklet (*Ptychoramphus aleuticus*) distributions based on aerial telemetry and at sea survey data, and estimate co-occurrence with seawater microplastic concentrations reported by Desforges et al. (2014). Our first objective was to determine whether Cassin's Auklet core use areas (i.e., presumably areas where they are likely foraging) are located where microplastics are concentrated in the Pacific Region of Canada. Our second objective was to determine whether or not exposure to microplastics vary among seasons (boreal summer: May 1 to September 30; boreal winter: 1 October to April 30). We compare our seasonal variability with quantities and characteristics of microplastics found in breeding Cassin's Auklet gular pouches as reported by Hipfner et al. (2017), and necropsy results (Floren and Shugart, 2017; and results presented here) from carcasses collected during the mass mortality event of 2014 (Jones et al., 2018; Jones et al., 2017). We discuss how an improved understanding of sensitivity to plastic exposure (dose-dependent effect to exposure) is critical for a full spatially explicit risk model, and how sensitivity may vary with seasonal conditions and annual cycle of the auklets. Finally, we discuss how our model can help translate sensitivity at the individual level to potential impacts at the population or species level.

2. Materials and methods

2.1. Study area and oceanography

The study area is located in the Pacific Canada's Exclusive Economic Zone (EEZ) and is divided into three significant oceanographic zones based on bathymetry; the continental shelf ("shelf": coastal areas with depths of 200 m or less), the shelf break and slope ("shelf break and

slope”: 200–2000 m), and the pelagic zone (“pelagic”: depths >2000 m) which also includes shallower areas around seamounts (Fig. 1). Oceanographically much of the study area is characterized as the Transition Zone where the North Pacific Current bifurcates into the Gulf of Alaska Current flowing northward, and the southward flowing California Current (Favorite et al., 1971; Ware and McFarlane, 1989). Wind-driven upwelling is the dominant feature of the California Current System (Huyer, 1983) and can be an important oceanographic feature occurring along the shelf break and slope off the west coast of Vancouver Island. Upwelling in general brings nutrient rich water to the surface from 100 to 300 m depth, and is driven by favorable winds that typically occur during the summer (Thomson, 1981). This feature is often associated with elevated oceanic productivity attracting a number of foraging seabird species (Springer et al., 1996; Studwell et al., 2017) including Cassin's Auklet (Ainley et al., 2011). Wind-driven upwelling is increasingly seasonal (i.e., occurs only during the summer) moving northward through the California Current System. West of Triangle Island, wind-driven upwelling occurs weakly during a couple of months in the summer typically starting around June and ending in September (Wolf et al., 2009, also see Bakun Index for 51 degrees north (National Oceanic and Atmospheric Administration or NOAA, 2018)).

The temperature and salinity data collected at a mooring installed approximately 40 km northwest of Triangle Island (Fig. 1) show a rich spectrum of variability between 40 m and 280 m (Fig. 2 water depth 288 m) that defies simple explanation. The dominant feature is the vertical mixing caused by fall storms. The mixing of warm water from the surface causes temperatures to increase at 40 m in October and the ocean becomes isothermal to 100 m in December. This isothermal layer deepens to 150 m from January to April. Temperatures decline to a minimum in January, and in May the 40 m temperature starts to increase while the deeper temperatures decline. The salinities also show the signature of vertical mixing in the fall. In April, the 40 m salinity starts to decrease and the 150 m starts to increase (the opposite of

temperature). The decreasing temperatures at depth in May and the increasing salinities from July to October at 40 m and 100 m may be the signature of upwelling. If these seasonal changes in temperature and salinity indicate upwelling, it does not appear to be driven by the local winds, as the Bakun Index at this latitude does not generally achieve a sustained positive value until June or July (NOAA, 2018).

2.2. Cassin's auklets and conservation status

Cassin's Auklet is a small seabird in the Family Alcidae. Their global population is estimated at 3.57 million breeding individuals, of which about 75% breed in BC. The majority (approximately 1.09 million birds, and possibly more based on the most recent survey) of these birds breed on Triangle Island within the Scott Islands, an archipelago northwest of Vancouver Island, at the northern edge of the California Current System (COSEWIC, 2014). The Cassin's Auklet is listed as Near Threatened by the International Union for Conservation of Nature (IUCN, 2018); and has been assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2014).

Environment and Climate Change Canada established permanent monitoring plots on Triangle Island in 1989 which are revisited every 5 years to ascertain population trends (see Rodway and Lemon, 2011 for details on seabird monitoring programme). At Triangle Island, the population is estimated to have declined by 2.5% per year between 1989 and 2009 (Rodway and Lemon, 2011). However, with the most recent survey, rates of decline dropped from 2.5% to 1.6% per year overall, suggesting that there may have been a rebound between 2009 and 2014 ($\beta = -0.016$, SE = 0.004, $t = -4.23$, $P = 0.0001$; L. Wilson, unpublished data). Nevertheless, similar population level declines have been noted on the colony at the Farallon Islands in California (Lee et al., 2007; Warzybok and Bradley, 2011). Reduced food availability during warm water years is believed to be the main cause of the population declines in the California Current System (Lee et al., 2007). Ocean

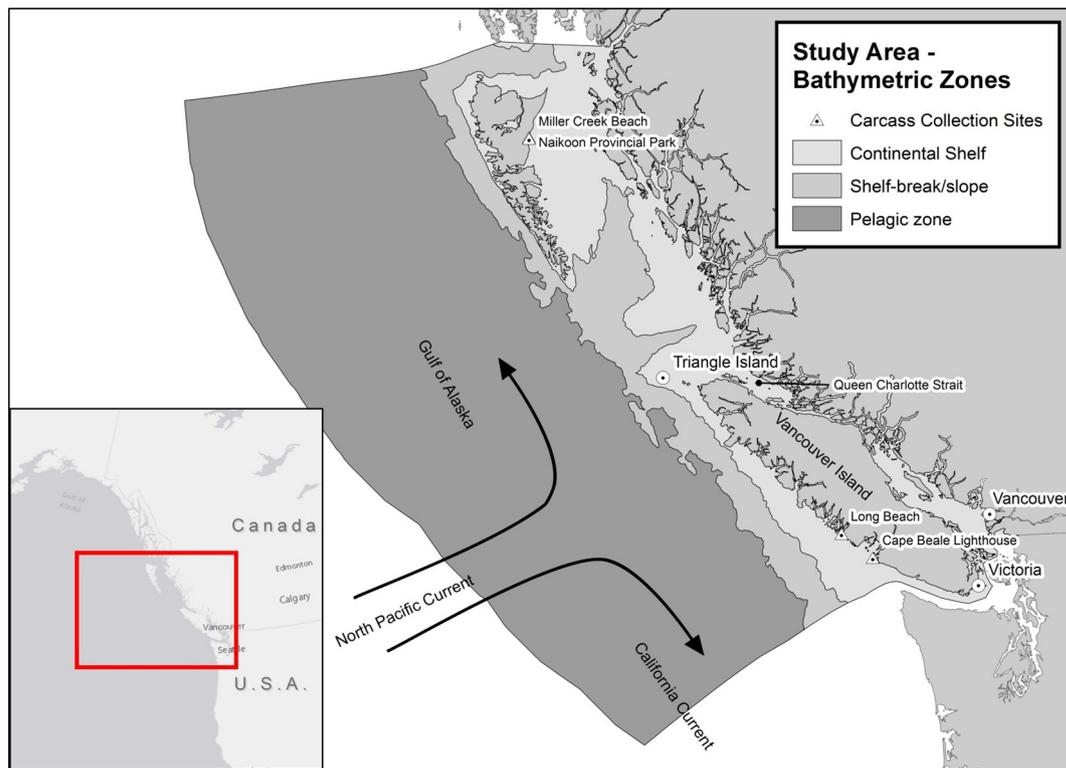


Fig. 1. Study area with location of collection sites for Cassin's Auklets collected in the winter of 2014. The study area (Canadian Exclusive Economic Zone (EEZ) is divided into three zones based on bathymetry that differ oceanographically; the continental shelf (“shelf”) which includes the shelf break (200 m or less), the shelf break and slope (“shelf break and slope”: 200–2000 m), and the pelagic zone (“pelagic”: depths >2000 m, but does include seamounts with depths of <2000 m). Included are major ocean circulation currents: the North Pacific, Gulf of Alaska and California Currents. Credits for inset map: Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community.

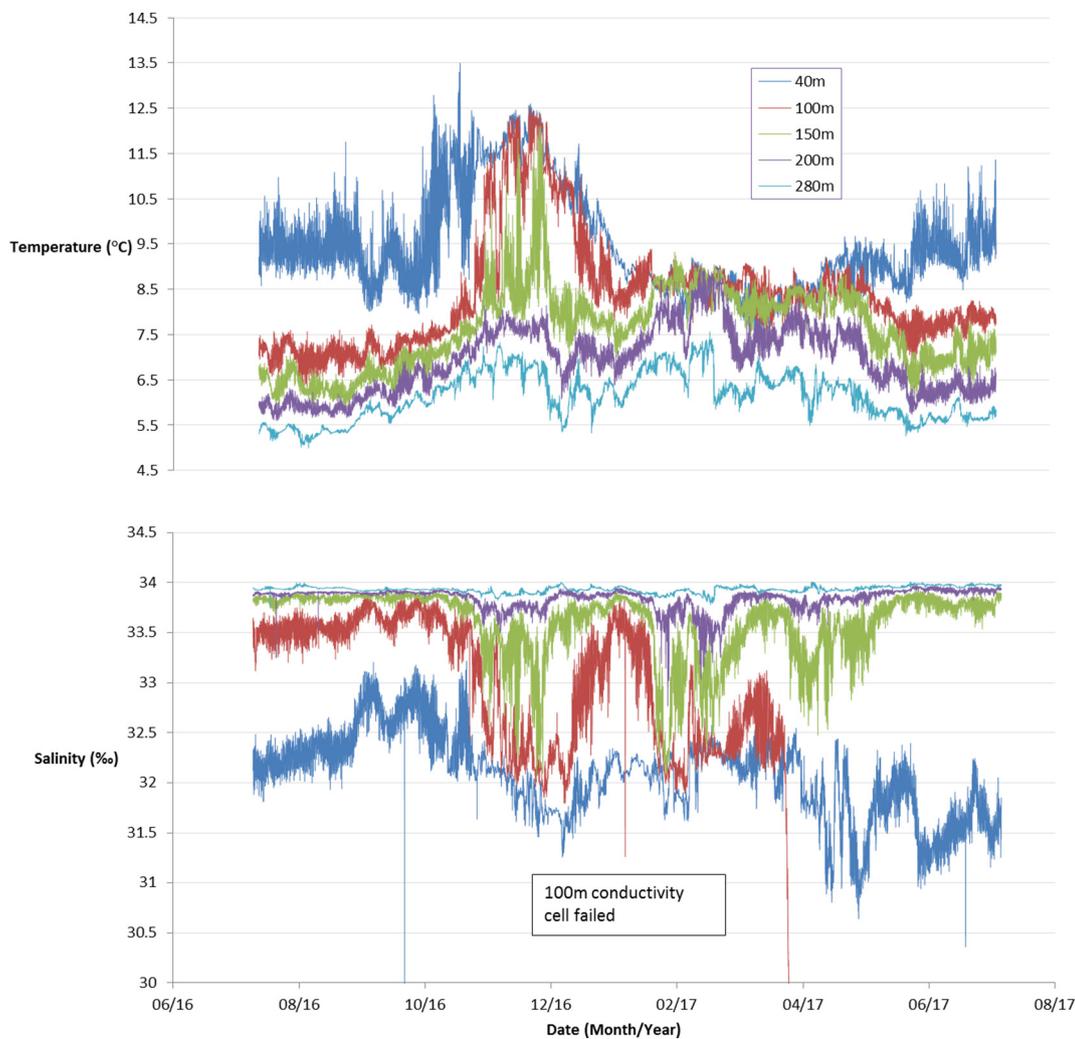


Fig. 2. Temperature (upper panel in degrees Centigrade) and Salinity (lower panel in parts per thousand) data collected from oceanography mooring approximately 40 km northwest of Triangle Island (see Fig. 1 for location) from June 2016 to August 2017. Mooring serviced and data accessed once a year in August.

conditions, associated with warmer marine waters that accompany El Niño events and alternating phases of the Pacific Decadal Oscillation, have resulted in reduced productivity, and changes to zooplankton distributions and timing of peak abundances; all of which affect Cassin's Auklet reproductive performance and survival (COSEWIC, 2014; Morrison et al., 2011).

2.3. Exposure model – Boreal summer season

2.3.1. Cassin's auklet distribution

At-sea survey observation data for marine birds in the Canadian Pacific EEZ were collected by the Canadian Wildlife Service (Environment and Climate Change Canada, see Kenyon et al., 2009; Morgan et al., 1991 for details). The following analyses were based on at-sea survey observations pooled among months from May 1–September 30 (“boreal summer” encompassing the breeding season), and years 1990–2010 inclusive. A negative binomial generalized additive model (R Core Team, 2016) was used to interpolate and predict at-sea observations of Cassin's Auklet densities with two predictor variables: position (longitude and latitude as metres Easting and Northing in equal area BC Albers projection), and depth (in metres square root transformed to normalize statistical distribution). Depth data came from the ETOPO1 1 Arc-Minute Global Relief Model, which is accessible online through the National Oceanic and Atmospheric Administration (NOAA) (Amante and Eakins, 2009). These predictor variables were implemented in the

GAM as bivariate (latitude, longitude) and univariate (depth) smoothing functions using penalized regression splines with Restricted Maximum Likelihood to determine the optimal degree of smoothing for each variable. Likelihood ratio tests (Chi square distribution) were used to determine factor significance ($\alpha = 0.05$), and Akaike's Information Criteria (AIC) were used to inform model selection with respect to smoothing flexibility (i.e., the number of knots used – see package mgcv documentation for further details: R Core Team, 2016). Survey effort (km^2 of area observed during each transect) was included as an offset variable (i.e., not included as a smoothed variable) and was log transformed to comply with the negative binomial log-link function. Best fit GAM predictions were made using a grid with 1×1 km grid cells, mean depth per cell, and effort of 1 km^2 per cell, and predictions were divided into five classifications based on Jenks or natural breaks for visualization (Fig. 3). GAM predictions were also classified into quantiles and the upper 50% quantile was used to define core areas based on at-sea survey data (Figs. 4 and 5), which were comparable to core areas based on aerial telemetry data (see below).

Core areas for Cassin's Auklets breeding on Triangle Island (Fig. 3) and fitted with radio transmitters were also used to estimate exposure to microplastic concentrations during the boreal summer. These core areas (50% Kernel Home Ranges or KHR) were derived using kernel density estimators based on aerial telemetry data collected during May and June 1999–2001 (Boyd et al., 2008). Adams et al. (2004)

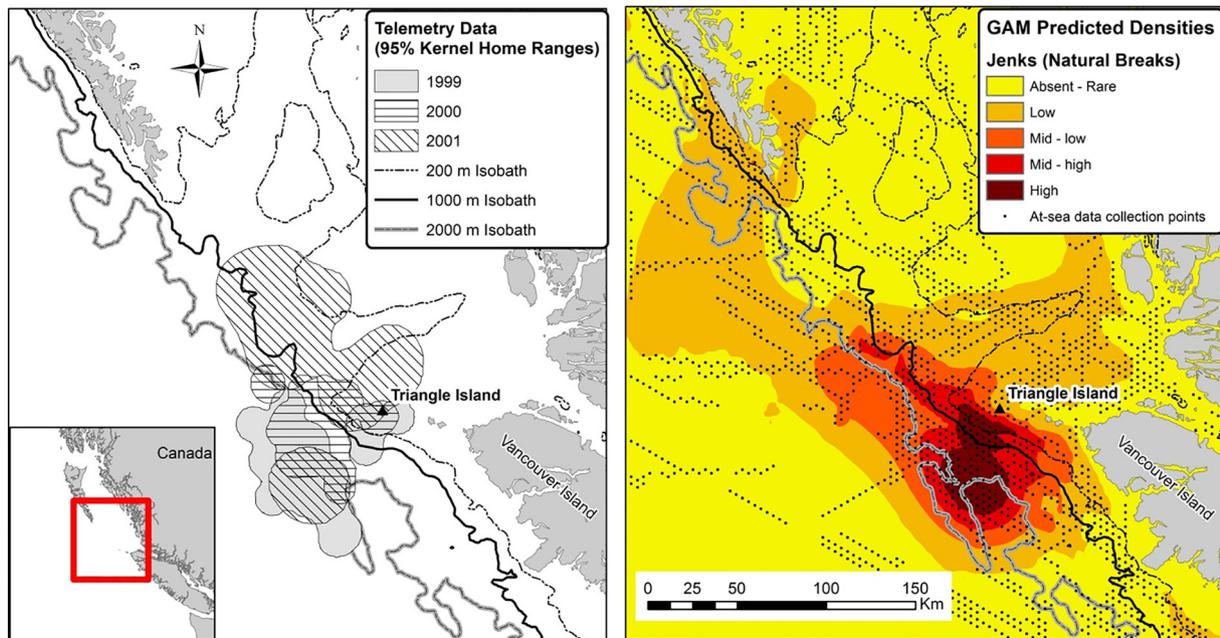


Fig. 3. 95% Kernel Home Ranges based on aerial telemetry data collected from Cassin's Auklets radio-tagged on their breeding colony on Triangle Island during 1999–2001 (left panel). Generalized Additive Model (GAM) predicted densities for Cassin's Auklet distributions based on at-sea survey data collected May–Sept. from 1990 to 2010 (right panel). At-sea data collection points indicate effort where transects were conducted during this period (right panel). Density categorization is Jenks (Natural Breaks).

used 95% KHR to delineate areas of “colony activity” and 50% KHR to delineate “core foraging areas”, however [Boyd et al. \(2008\)](#), make the point that they could not confirm that foraging was occurring when birds were located telemetrically. Here we define the 50% KHR as simply “core areas”.

2.3.2. Microplastic concentration distribution and Cassin's auklet exposure

Concentrations of microplastics in subsurface seawater (number of pieces/m³ seawater) were taken from [Desforges et al. \(2014\)](#) and represent sampling during the late summer of 2012 ([Fig. 4](#)). Only plastic

pieces <5 mm in size were quantified, and these included fibres and plastic fragments. Full precautions were taken to minimize contamination of samples during processing; see [Desforges et al. \(2014\)](#) for sampling and plastic analysis details. In this study, we assume that spatially explicit microplastic concentrations from [Desforges et al. \(2014\)](#) generally are representative of relative concentrations between summer and winter seasons and among years. The microplastic concentrations were compared to the core use areas as defined based on the at-sea survey (50% upper quantile for GAM predictions) and telemetry data (50% KHR).

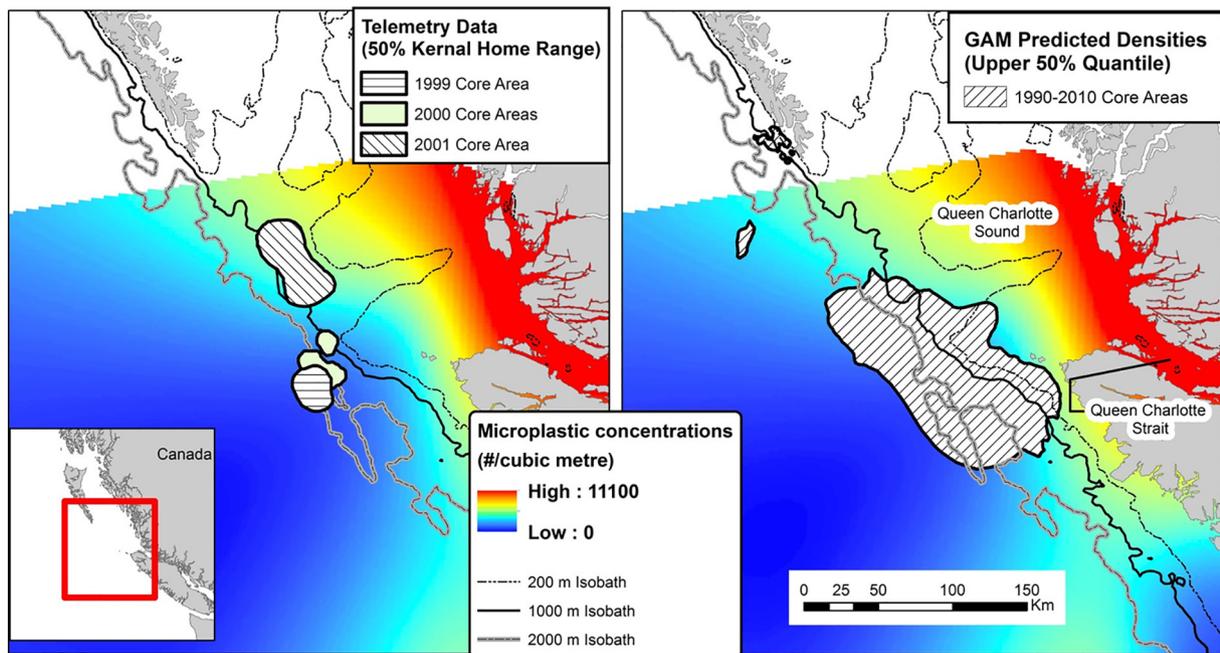


Fig. 4. Core areas of elevated at-sea densities of Cassin's Auklets and exposure to microplastic pollution during the summer season (May–Sept.). Core areas based on aerial telemetry data (50% KHRs) collected from Cassin's Auklets radio-tagged on their breeding colony on Triangle Island during 1999–2001 (left panel), and GAM predictions (upper 50% quantile) for Cassin's Auklet distributions based on at-sea survey data collected from 1990 to 2010 (right panel). Microplastics concentration modified from [Desforges et al. \(2014\)](#).

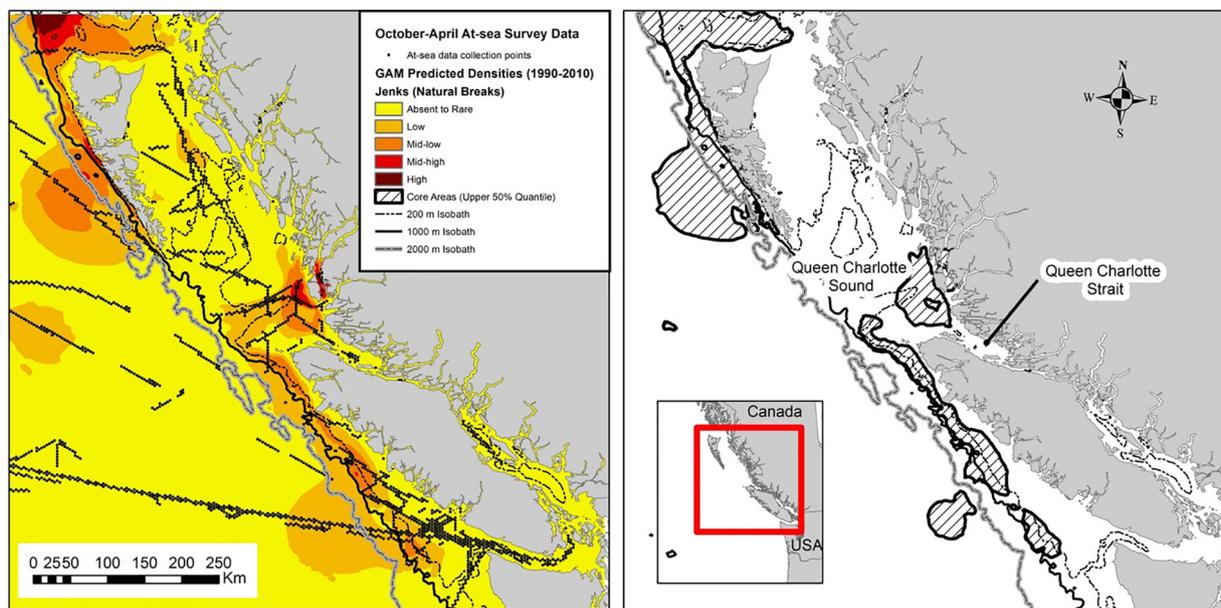


Fig. 5. Cassin's Auklet distribution during the winter season (Oct.–Apr.). Generalized additive model (GAM) predictions for Cassin's Auklet distributions based on at-sea survey data collected Oct.–Apr. from 1990 to 2010 (left panel). GAM predictions characterized using Jenks (Natural Breaks) categorization (left panel) and as core areas (upper 50% quantile) (right panel). At-sea data collection points indicate effort where transects were conducted during this period (right panel).

2.4. Exposure model – Boreal winter season

2.4.1. Cassin's auklet distribution, microplastic concentrations and Cassin's auklet exposure

Winter season distributions are entirely based on at-sea survey data as telemetry data were unavailable for this study. Core areas were defined using a generalized model as described above (Exposure Model – Boreal Summer Season, Cassin's Auklet Distribution), but based on at-sea survey data collected from October to April (1990–2010). These core areas were compared with microplastic concentrations as determined by Desforges et al. (2014).

2.4.2. Necropsies of recovered Cassin's auklet carcasses from the 2014 mass mortality event

2.4.2.1. Mortality event, specimen collection and processing. Following a series of severe storms off the west coast of North America from September to December 2014, 707 seabird carcasses were found on beaches of Vancouver Island and Haida Gwaii, British Columbia, Canada. Early storms (September–October) primarily impacted Common Murre (*Uria aalge*); whereas later storms (November–December) primarily impacted Cassin's Auklets. From December 12–26, 428 Cassin's Auklet carcasses were reported washed ashore (see Fig. 1: 178 on the west coast of Vancouver Island and 250 on the east coast of Haida Gwaii). A total of 85 carcasses were collected for post-mortem examination, with 65 birds from Vancouver Island (64 from Long Beach in Pacific Rim National Park Reserve, 1 from Cape Beale Lighthouse) and 19 birds from Haida Gwaii (16 from Miller Creek Beach, 3 from Naikoon Provincial Park) (Fig. 1).

All birds were frozen before being processed. Suspected final cause of death was determined by Dr. V. Bowes (co-author and avian pathologist veterinarian with the BC Government), and body condition was ranked as follows: 1 (emaciated), 2 (thin), 3 (fair), 4 (good/very good), and 5 (excellent) based on extent of muscle, and subcutaneous and visceral adipose stores. Sex and age were determined, and gular pouch, right wing chord (flat), right tarsus and culmen length were measured. Following that, the stomach (proventriculus and gizzard) was removed for content analysis to quantify plastic ingestion. The stomachs were opened lengthwise and rinsed with water using a

wash bottle over a 1 mm mesh sieve. Contents remaining in the sieve were transferred to a petri dish to be sorted and reviewed under both a compound and dissecting microscope. Because a dissecting microscope was used in this study, it is possible that small plastic pieces were missed that may have been picked up by the stereoscope used by Desforges et al. (2014). Plastics were visually identified and separated from non-plastic stomach contents and air dried for a minimum of seven days. The remaining contents that passed through the sieve were collected and stored. Precautions were taken to avoid cross-contamination of samples during processing including the use of non-plastic containing clothing (cotton lab coat), sealed and secure lab space (i.e., cleaned ventilation and no access for other personnel), and non-plastic containing metal and glass tools (i.e., petri dishes, funnels, and tweezers).

2.4.2.2. Quantification of plastic ingestion. Standardized necropsy methods (examining the stomachs of recovered carcasses) were used to characterize, quantify and report plastic ingestion (Provencher et al., 2017; Van Franeker et al., 2011). Our samples were assessed visually easily, and we opted not to use digestive methods such as potassium hydroxide, as these methods may affect results (Kühn et al., 2017). For each bird containing plastic ($n = 85$), the total number of plastic pieces along with the total dry mass of plastics was recorded using a Sartorius analytical scale (accurate to 0.0001 g). Each plastic piece was counted, weighed and categorized as either post-consumer 'user' plastic (i.e., fragment, sheet, thread, foam) or industrial plastic (symmetrical virgin plastic pellets). The dimensions of each plastic and non-plastic non-food item were measured and recorded (length, width and height or diameter) using digital calipers (accurate to 0.01 mm) and the dominant pigmentation for each piece was assigned to one of eight broad colour designations (off/white-clear, grey silver, black, blue-purple, green, orange-brown, red-pink, and yellow; Provencher et al., 2017). Unfortunately, we were unable to determine the types of polymers of plastic found in the necropsies (e.g., by Fourier-transform infrared spectroscopy).

We investigated whether age, sex or condition were significant predictors of whether Cassin's Auklets would ingest plastic (frequency of occurrence) or the amount of plastic they would ingest (total number, total mass). Statistical analyses based on necropsy data were done in R (R Core Team, 2016). We analyzed the frequency of occurrence of

plastic ingestion among birds using a Generalized Linear Models (GzLM) with age (adult, subadult and juvenile), sex, and condition index as predictors (binomial distribution with a logit link function; McCullagh and Nelder, 1989). These same predictors were used to examine the total number of plastics ingested by each bird using a GzLM with a negative binomial distribution with a log link function, to account for high occurrence of zero counts (O'Hara and Kotze, 2010). A General Linear Model was used to test for an effect of these predictors on the mass of plastic ingested. We ran post-hoc comparisons of fixed effects within these models using Wald's Chi-squared tests. We verified that our models were not overdispersed by ensuring that the ratio of the residual deviance over residual degrees of freedom was ~ 1 (Venables and Ripley, 2013), and evaluated model fit visually.

We report plastic metrics for the pooled sample ($N = 83$) and provide raw data in the supplementary materials. The arithmetic means (\pm SD) for number of ingested plastic and mass are reported for the full sample size, rather than the geometric mean, which is a more suitable metric for small sample sizes (Van Franeker et al., 2011). All effects were considered significant when $p < 0.05$.

3. Results

3.1. Exposure model – Boreal summer season

3.1.1. Cassin's auklet distribution

Based on at-sea survey data collected and pooled among the months May through September, and years 1990–2010, the best fit GAM included both the bivariate position variable (longitude, latitude: chi square = 1196.2, $p < 0.0001$) and depth (chi square = 65.1, $p < 0.0001$). The optimal number of knots was 125 for position and 20 for depth with a minimum AIC value of 13,686.3, which explained 64.4% of the model deviance.

Cassin's Auklet distributions or spatial patterns based on aerial telemetry data or at-sea survey data were similar, with a couple of key differences (Figs. 3 and 4). The 95% kernel home ranges (KHRs) based on aerial telemetry data collected in 2001 is not reflected in the GAM predicted distribution (Fig. 3), likely because there were no at-sea survey data collected in that area in 2001. Furthermore, at-sea surveys were not conducted in any year for most of the area identified as the 2001 core area (50% KHR: Fig. 4 – refer to Fig. 3 for at-sea survey data collection points). The GAM also predicted higher densities of Cassin's Auklets northwest of Triangle Island along the shelf break and slope and extending into the pelagic zone (just west of the 2000 m isobath) that was not completely reflected by the KHRs based on the telemetry data (Figs. 3 and 4).

3.1.2. Microplastic concentration distribution

Microplastic concentrations varied by several orders of magnitude across the study area. The highest concentrations occurred near mainland coast of BC, over the shelf and inland waters east and northeast of Vancouver Island, reaching levels upwards of 10,000 pieces/m³ seawater. Plastic concentrations decreased with increasing distance from the coast, with the lowest concentrations occurring in the pelagic zone (Table 1).

3.1.3. Exposure of Cassin's auklets to microplastic concentrations

Cassin's auklet exposure to microplastics appears to be relatively low during the summer season based on both aerial telemetry and at-sea observation data (Fig. 4). Core areas based on aerial telemetry data (50% KHRs) were located in areas with relatively low concentrations of microplastics, particular during the first two years (1999 and 2000) of the Boyd et al. (2008) study. As well, core areas based on at-sea survey data (upper 50% quantile) collected during the summer season were mostly over the shelf break and slope and west of the shelf break and slope (Table 2), in areas with relatively low concentrations of microplastics. Average concentrations in these core areas based on

Table 1

Mean (\pm standard deviation) and minimum-maximum plastic concentration by bathymetric zone (shelf, shelf break and slope, pelagic zone), and inside core areas of higher summer densities of Cassin's Auklets based on aerial telemetry (50% HR) and at-sea survey data (upper 50% quantile). Microplastic concentrations modified from Desforges et al. (2014) interpolated values, and aerial telemetry core areas modified from Boyd et al. (2008).

	Microplastic concentration (#/m ³ seawater)		
	Mean (\pm SD)	Minimum	Maximum
Entire study area	2217 (\pm 2486)	0	10,868
Shelf	4456 (\pm 2602)	645	10,868
Shelf break and slope	2354 (\pm 1309)	565	6618
Pelagic zone	1119 (\pm 928)	0	3530
Core areas			
50% KHR (aerial telemetry data)			
1999	722 (\pm 118)	520	994
2000	1082 (\pm 266)	677	1757
2001	3248 (\pm 394)	2328	4041
Upper 50% quantile (at-sea survey data)	1670 (\pm 712)	471	4038

both aerial telemetry and at-sea survey data were considerably lower than average concentrations for the entire microplastics study area (Table 1). Although Cassin's Auklet summer distribution varied among years, spatial shifts occurred mostly between the shelf break and slope and pelagic zones, and not into the shelf zone (Table 2) where microplastics are generally more concentrated. As well, spatial distributions likely vary with breeding phenology during the summer, but it is not clear at this point exactly how they vary.

3.2. Exposure model – Boreal winter season

3.2.1. Cassin's auklet distribution

The best fit GAM, based on at-sea survey data collected and pooled among the months October to April, and years 1990–2010, included both the bivariate position variable (longitude, latitude: chi square = 179.9, $p < 0.0001$) and depth (chi square = 27.4, $p = 0.0006$). The optimal number of knots was 135 for position and 20 for depth with a minimum AIC value of 2000.7, which explained 58.2% of the model deviance.

Although spatial coverage of surveys is not as complete as during the boreal summer season, GAM predicted densities based on data collected from October to April reveal a four-fold spatial expansion with increased core area size and a shift towards the coast and over the shelf (Table 2 and Fig. 5). As well, Cassin's Auklet distributions moved northward into Dixon Entrance and eastward to a coastal area just north of where Queen Charlotte Strait flows into Queen Charlotte Sound (Fig. 5).

Table 2

Proportion of core areas based on aerial telemetry data (50% KHRs) and at-sea survey (upper 50% quartile) in the three bathymetric zones. Aerial telemetry data were collected during the summer season only, while at-sea survey data were collected during both the summer and winter seasons.

		Proportion of Cassin's auklet distributions (%)			Total area (km ²)
		Shelf	Shelf break and slope	Pelagic zone	
50% KHR (aerial telemetry data)	1999	0	33.7	66.3	650
	2000	0.9	67.4	31.7	733
	2001	0	100	0	1723
Upper 50% quantile (at-sea survey data)	May–Sept.	13.3	53.8	32.9	10,739
	Oct.–Apr.	22.1	48.3	29.6	43,636

3.2.2. Necropsy results from Cassin's auklets collected in the 2014 winter

Of the 85 Cassin's Auklet carcasses recovered in December of 2014, the majority of birds were suspected to have died from drowning (the two exceptions were adults from Haida Gwaii which died of other traumatic injuries - one with a ruptured spleen and one with puncture wounds in neck and back suggestive of predation). All drowned birds also had gastrointestinal hemorrhage, interpreted as a sign of physiological stress. Necropsies revealed that the age ratio of recovered auklets was 59 adults; 14 sub-adults; 12 hatch-years; and the sex ratio was 43 males; 40 females, plus two unknown. Examination of the body condition revealed that 23 (27.1%) auklets were in poor condition, 60 (70.6%) were in fair body condition, and only two (2.4%) were in good body condition.

Plastic was found in the stomachs of 40% (35/85) of Cassin's Auklets. The average number of pieces of plastic ingested was 1.6 ± 6.8 (\pm standard deviation or SD) per bird (range 0–61, including birds with no plastic), and the average mass of plastic was 0.0085 ± 0.037 SD g (range 0–0.3291 g). Of the 136 pieces of plastic, only 15.4% were industrial plastics; the remaining plastics were fragments (72.1%), threads (6.6%), sheets (5.1%) or foam (0.7%). Ingested plastics were predominantly microplastics (1–5 mm, 86.6%) and mesoplastics (5–20 mm; 9.7%) with only 5 ingested macroplastics (>20 mm; 3.7%). The ingested plastics were dominated by off/white-clear (41.2%), followed by orange-brown (19.1%), black (14%), grey silver (13.2%), green (5.1%), blue-purple (4.4%), red-pink (2.2%) and yellow (0.7%).

For frequency of occurrence of plastic ingestion, there were no significant differences between age classes ($\chi^2_2 = 0.2863$, $p = 0.866$, $n = 86$), sex ($\chi^2_1 = 0.914$, $p = 0.334$, $n = 86$) or condition index ($\chi^2_1 = 1.922$, $p = 0.166$, $n = 86$) nor were there any significant interactions. Similarly, there were no significant differences between the number of pieces by age, sex or condition, and no interactions ($p > 0.05$, $n = 85$). This result was robust to the removal of an outlier (Cook's distance ~ 0.18 ID 13), which was a single bird that had ingested 61 pieces of plastic (four times as many as the next largest plastic load). Finally, there was no significant correlation between condition index and number of plastic pieces ingested (Pearson's correlation coefficient; $t = -0.14$, $df = 83$, $p = 0.88$), or mass of plastic ingested ($t = 0.05$, $df = 83$, $p = 0.96$).

4. Discussion

Our seasonal exposure models indicated that Cassin's Auklets are exposed to lower microplastic concentrations during the boreal summer (May through September) than during the winter (October through April), suggesting that microplastic ingestion rates are lower during the summer. Cassin's Auklet distributions tend to concentrate over the shelf break and slope (100–2000 m), extending into the pelagic zone (Fig. 4 and Table 2) during the summer season where they are exposed to lower microplastic concentrations (Table 1). Lower microplastic concentrations in these areas may be a result of upwelling (Desforges et al., 2014), or relaxed downwelling, as well as being located at greater distances from the coast (i.e., from terrestrial sources of plastic pollution). Conversely, Cassin's Auklets are exposed to higher plastic concentrations during the winter season because they exploit areas closer to shore with presumably higher plastic concentrations (Fig. 5 and Table 2). Microplastic concentrations have been shown to be much higher towards the coast during the late summer (Desforges et al., 2014), and we assume this pattern is consistent between seasons and among years. Clearly our approach to quantifying exposure would benefit from increased sampling of microplastics in our study region to comprehensively capture seasonal and annual variation.

Our exposure model predictions are consistent with incidence (proportion of individuals) and quantities of plastics found in gular samples from breeding bird and necropsy results from carcasses recovered during the winter season of 2014. Hipfner et al. (2017) report negligible amounts of plastic stored in the gular pouches of adults returning to

the breeding colony on Triangle Island. Our necropsy results indicate Cassin's Auklets either experience higher levels of exposure to microplastics (as suggested by our seasonal exposure model), or they specifically target them as food (or some combination of the two possibilities) during the late fall or early winter of 2014. As well, our results are strikingly consistent with those reported by Floren and Shugart (2017) with 41.5% (71/171) of birds necropsied found with plastic in their stomachs, suggesting that high microplastic incidence in wintering Cassin's Auklets was a northern California Current System phenomenon.

Plastic has been found in the gastrointestinal tracts of Cassin's Auklets collected on breeding colonies in the Gulf of Alaska Ecosystem to the north. Over three decades ago, Day (1980) reported incidence rates as high (40% for 10 birds collected from 1969 to 1977) as rates from recent necropsy results for birds collected during the winter 2014 mass mortality event. More recently, however, Robards et al. (1995) reported incidence rates of 11% (for 35 birds collected from 1988 to 1990). Based on these reports, it appears that Cassin's Auklets may be exposed to higher concentrations of plastic pollution in the Gulf of Alaska during the boreal summer than in our study area.

4.1. Seasonal and longer term oceanographic variability

4.1.1. Vulnerability

The dominant feature of the microplastic distribution is that concentrations are much higher towards the coast, than they are offshore. Although there may be transport in surface currents away from the coast during the summer (Borstad et al., 2011) there is no evidence that microplastics accumulate and concentrate in areas offshore. We assume that this general trend in spatial distribution of microplastic concentrations is consistent between seasons and among years, and that the change in distribution of Cassin's auklet is what drives most of the variability in exposure to microplastics for this species (i.e., the ecology of the system is the most important factor determining exposure). We speculate that seasonal oceanographic variability plays two critical roles in our exposure model by affecting: 1) the distribution and availability of Cassin's Auklets prey; and, 2) the spatial concentration of microplastics that may be relevant to Cassin's Auklet distributions. Seasonal changes in oceanography and vertical migration of primary copepod prey (Mackas et al., 1998) are likely responsible, at least to some extent, for the spatial redistribution of Cassin's Auklets between winter and summer seasons. Cassin's Auklets breeding on Triangle Island generally concentrate over the shelf break and slope where they are foraging primarily on neocalanoid copepods (Bertram et al., 2017). This was particularly true for 1999 and 2000 based on aerial telemetry data, and is generally true based on at-sea surveys conducted from 1990 to 2010 (Fig. 4). This spatial pattern of habitat use is consistent with movement data recently collected from chick-rearing birds on Triangle Island (Domalik et al., in revision). However, with the change to winter conditions, birds tend to move inshore away from the shelf break and slope and towards the shore (Fig. 5), to where microplastic concentrations are most likely higher. This seasonal shift towards the coast during the winter appears to be consistent throughout the non-breeding range for Cassin's Auklets (Studholme et al., in review).

Enrichment, transport and retention of nutrients and plankton are critical components enhancing oceanic productivity (Bakun, 1996). Microplastics likely behave similarly to nutrients and plankton in that they are transported in surface currents and concentrate in areas where contents of this flow are somehow retained oceanographically. This has been clearly shown where plastic transported in large basin scale wind driven currents accumulate in five major oceanic gyres (van Sebille, 2015). The transport and concentration of microplastics also is likely to occur with mesoscale oceanographic processes and features, which are much more strongly associated with marine birds and other marine predators (for example see Bost et al., 2009; Pinaud and Weimerskirch, 2005; Scales et al., 2014; Titmus and Hyrenbach, 2011),

hence making organisms exploiting these features particularly vulnerable to exposure.

As described earlier, our study area is characterized as a transition zone or area between the Gulf of California Ecosystem (upwelling domain) to the south and the Gulf of Alaska Ecosystem (downwelling domain) to the north (Favorite et al., 1971; Ware and McFarlane, 1989). Wind-driven upwelling occurs over the shelf break and slope as far north as Vancouver Island during the summer season when prevailing winds are from the northwest (Foreman et al., 2011; Thomson, 1981). Thomson and Ware (1996) used velocity data to show that upwelling on the Vancouver Island shelf is much stronger and lasts longer than indicated by the Bakun Upwelling Index (Bakun, 1975; NOAA, 2018). Based on data from 1989 to 1995, Thomson and Ware (1996) estimated that the upwelling season (summer) on the southern Vancouver Island shelf started between late April and late June (median date is May 20) and ended between late September and late November (mean October 19). This is much longer than estimates from along-shore wind data, but these dates are broadly consistent with the timing of significant transitions in the 40 m and 100 m temperature and salinity data, which are possible signatures of upwelling in May and vertical mixing in October and November (Fig. 2 and see Materials and Methods: Study Area and Oceanography). As well, enhanced upwelling can occur in association with certain types of shelf break canyons during upwelling favorable conditions (Allen et al., 2001; Freeland, 1982). Near the coast, upwelling-like behavior can also occur as a result of when the sustained winds that occur during the winter from the southeast slacken and the surface water that has built up along the coast must flow away from the coast (Borstad et al., 2011). This results in the rebounding of the isopycnals that have been depressed due to the increased sea level along the coast during the winter. Upwelling during the summer could serve to reduce local concentrations by bringing deeper and presumably cleaner water to the surface (Desforges et al., 2014), and there is recent evidence that Cassin's Auklets may be exploiting these features during the breeding season (Domalik et al., in revision).

Input of microplastics into our study area likely comes from two principal sources; 1) terrestrial outflow such as municipal wastewater (i.e., locally from BC and WA), and 2) the breakup of macroplastics (Andrady, 2011). It is well known that municipal wastewater is an important source of microplastics into marine and aquatic ecosystems (Mason et al., 2016; Napper and Thompson, 2016), which is consistent with increasing concentrations of microplastics towards the coast in our study region. It is less clear how the breakup of macroplastics contribute to microplastic concentrations in our study region. Coastal sources of macroplastics are probably important, but plastics from Asian sources are also known to transit the Pacific Ocean towards North America. The North Pacific Current entrains plastic pieces from well-known major sources (Jambeck et al., 2015; Lebreton et al., 2017), and transports these pieces across the Pacific Ocean to accumulate in the North Pacific Gyre (van Sebille et al., 2015), which acts as a mechanism concentrating plastic pieces. There is no clear mechanism that would concentrate plastic pieces transported in the North Pacific Current in coastal waters of North America (other than beaches potentially), which means it is unlikely that plastic input from Asia is a major source of microplastics in our study region. Nevertheless, clearly quantifying local versus global contributions is important, particularly when developing management strategies aimed at reducing plastic pollution.

Summer surface current outflow can have important implications for both the breeding success of Cassin's Auklets (Borstad et al., 2011) as well as the spatial distribution and concentration of microplastics at the surface. Surface water outflow likely transports microplastics resulting from local sources (municipal wastewater and outfalls in BC and WA) out over the shelf break and slope, potentially exposing Cassin's Auklets foraging there to increased concentrations. However, surface outflow could also function to disperse plastic pollution from local sources out into the larger pelagic zone, as well as preventing the

inflow of plastic transported in the North Pacific Current. Whereas, although we lack microplastic data in the boreal winter, we do know that net surface flow is towards the coast likely concentrating plastic pollution from both local terrestrial sources and possibly from the North Pacific Current, in areas where Cassin's Auklets tend to forage in BC waters. This possible elevated exposure to microplastic concentration occurs during a period in their annual cycles when auklet survival is likely challenged. Cassin's Auklets, like most seabird species, are sensitive to changes in adult survival, and may be regulated by events that occur during the winter season as suggested by Gaston (2003) for Thick-Billed Murres. Exposure to higher concentrations of microplastics during the winter season may exacerbate already challenging conditions for survival. However, it is important to emphasize that we found no evidence supporting a relationship between body condition and plastic quantity in our necropsy results (possibly due to sample size), suggesting that Cassin's Auklet sensitivity may not necessarily be directly dose-dependent. Instead, if there is a relationship between ingested plastic and mortality rates, it would likely be integrated into a more complicated relationship with other factors potentially affecting mortality in these birds.

4.1.2. Sensitivity

Species characterized by low annual reproductive output and high adult survival rates tend to be highly sensitive to stressors that affect survival rates (Gaston and Jones, 1998). Therefore, we speculate that sensitivity of seabirds to ingested plastic is likely to vary seasonally as a function of annual cycle events. In the north Pacific Ocean, the boreal winter can be energetically challenging and seabird survival can be impacted by various factors (see Piatt and Van Pelt, 1997 for a review) including large scale atmospheric forcing and oceanographic variability (Bertram et al., 2005; Jones et al., 2002; Jones et al., 2018; Morrison et al., 2011; Schreiber, 2001) or major storms (Bailey and Davenport, 1972). The energetically challenging nature of boreal winters was in evidence during the 2014 mass mortality event, which impacted thousands of Cassin's Auklet from British Columbia (BC), Canada to California, USA (Floren and Shugart, 2017; Jones et al., 2018). Most of the birds collected dead from beaches appeared to have died from starvation, and the implication is that birds may have targeted microplastics as food or were more sensitive to plastics in their stomachs during this period of apparent trophic stress, or some combination of both. Whether or not this is important remains to be seen, as plastic quantities are probably too low to result in an appreciable toxicological or physiological effect on their own.

Cassin's Auklets and other seabirds that spend their winter seasons in the Northeastern Pacific may have been particularly stressed during the 2014–15 winter season because of anomalously warm water detected throughout the study area (Bond et al., 2015; Peterson et al., 2015). There appears to be a strong positive correlation between Cassin's Auklet mortality, based on the beach-cast carcass encounter rate from beached bird surveys conducted in WA and OR by the Coastal Observation and Seabird Survey Team (COASST, 2018), and the dominance of the southern California Current species of copepods in coastal zooplankton communities (Jones et al., 2018). Indeed, this relationship between estimated Cassin's Auklet mortality and southern copepod biomass anomaly predicted accurately the mortality rates estimated during the 2014 warm water mass (Jones et al., 2018). The higher incidence of plastic found in Cassin's Auklets recovered from beached bird surveys conducted during this event suggest the possibility that birds were consuming plastic when regular prey availability was reduced, and when the birds were already experiencing trophic stress. However, it is important to note that further research is needed to better understand the relationship between food stress and plastic consumption in marine birds. In this case, we do not know if birds are intentionally targeting plastic pieces as food items, and if they do, we do not know whether or not they consume plastics preferentially when food is low. Furthermore, we do not know if there is an increased mortality in birds with these

levels of ingested plastic, because if there was, beach-cast bird carcasses would bias necropsy results in favour of individuals with ingested plastic.

5. Conclusion

We describe a modeling approach that assesses the vulnerability of an at-risk seabird, the Cassin's Auklet, to exposure to microplastics by quantifying their at-sea spatial and temporal overlap of with the spatial distribution of microplastic concentrations in BC; and, we describe how this vulnerability can change between boreal summer and winter seasons. We argue that oceanography plays a major role in determining the degree of exposure for Cassin's Auklet, particularly at the mesoscale level. Our focal species apparently do not exploit oceanographic features that concentrate microplastics during the summer. However, many marine species do exploit oceanographic features that would concentrate nutrients, plankton, as well as plastics making them potentially vulnerable to exposure to plastic pollution. This is a first step in a spatially explicit risk model that would also integrate bird sensitivity (i.e., impacts related to degree of exposure), similarly to what was done for a suite of marine bird species potentially exposed to oil pollution (Fox et al., 2016). However, as described in the introduction, there is little to no information on dose-dependent effects (i.e., relationship between amount of plastic and effect on bird exposed) at the individual level, unlike exposure to oil pollution. Perhaps the most important outcome is that we can estimate vulnerability at the study area population level meaning that if it is determined that Cassin's auklet are sensitive to microplastic exposure at the individual level, we should be able to predict the seasonal population-level impacts. Finally, we can identify regions, and potentially, sources of microplastic contamination that may present challenges to marine bird conservation, and address these challenges as effectively as possible.

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