



# Reduced vitamin A (retinol) levels indicate radionuclide exposure in Streaked Shearwaters (*Calonectris leucomelas*) following the 2011 Fukushima nuclear accident



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## ABSTRACT

The Fukushima nuclear accident in 2011 released significant amounts of radionuclides into the marine environment. Exposure to radiation reduces levels of antioxidants such as carotenoids and vitamins A and E within exposed individuals. Such reductions can cause teratogenic or mutagenetic effects leading to reduced reproductive viability and fitness. Reduced antioxidant levels therefore may be used as an indicator of radionuclide contamination and to infer individual or population level impacts; however, the taxa-specific responses of marine organisms, such as seabirds, are poorly understood. As top predators, seabirds are ideal bio-indicators of the prevalence of contaminants and pollutants in marine ecosystems. At-sea foraging distributions of Streaked Shearwaters (*Calonectris leucomelas*) from Mikura Island (MKR), Japan during the post egg-laying period coincide with the Fukushima nuclear plume while the breeding colony on Birou Island (BRU) lies outside the affected zone. We examined the physiological responses of Streaked Shearwater chicks at MKR and BRU to possible radiation exposure during the 2011 breeding season, four to seven months after the Fukushima nuclear accident. Fledgling mass did not differ between islands but fledglings from MKR displayed significantly reduced vitamin A levels. Available information suggests these depletions most likely result from radiation exposure due to the Fukushima nuclear accident, implying that the risk of radionuclide contamination is considerably elevated for Streaked Shearwaters on MKR, where more than 60% of the world's population breeds. While additional negative impacts are expected due to delayed effects of radionuclide transport via biomagnification in the food chain, this study highlights the potential immediate and worrisome consequences of the Fukushima nuclear accident for marine wildlife.

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## 1. Introduction

Significant amounts of radionuclide were released into the terrestrial and marine environment from the Fukushima Daiichi Nuclear Power Plant (FDNPP; 37°25' N, 141°01' E) following the

Great Tohoku Earthquake on March 11, 2011 (TEPCO, 2011). Extensive areas of the terrestrial ecosystem were polluted (Kinoshita et al., 2011) and radionuclides quickly dispersed into the western North Pacific Ocean, including the Kuroshio–Oyashio transition area (Honda et al., 2012).

Following exposure to radioactive contamination, the absorption of ionizing radiation by living cells can directly disrupt atomic structure and alter chemical and biological properties. It can also act indirectly via the radiolysis of water, which generates reactive oxygen species (ROS). ROS are known to initiate and propagate free radical chain reactions that cause oxidative stress and are therefore potentially highly damaging to cells (Riley, 1994; Hall and Giaccia, 2012). Such damage needs to be repaired in conjunction with the production of anti-oxidative compounds that will neutralize

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free radicals and reduce oxidative stress. Enzymatic antioxidants (e.g. superoxide dismutase, catalase, glutathione peroxidase), and non enzymatic antioxidants (e.g. glutathione, vitamins, ascorbate, betacarotene, and uric acid) (Storey, 1996) or antioxidant defense system, are used in cell defense, in maintaining cell homeostasis and in the repair of DNA (Sies, 1993; Doyotte et al., 1997; Collins and Horvathova, 2001). The activation of antioxidant systems may reflect a specific response to pollutants (Doyotte et al., 1997). Antioxidant levels within radionuclide exposed individuals become depleted allowing ROS to develop (Lliakis, 1991; Bonisoli-Alquati et al., 2010). Antioxidant depletion, which was recorded following the Chernobyl nuclear accident in 1986, has been suggested to be responsible for reduced individual fitness (e.g. reduced reproductive viability and probability of survival), as well as teratogenic (tissue disruption during development) and mutagenetic (DNA mutation) effects (Ellegren et al., 1997; Møller and Mousseau, 2003, 2011; Møller et al., 2005a,b).

Results of a recent modeling study by Smith et al. (2012) suggest that anti-oxidative mechanisms in birds are able to cope with radiation at levels observed after the Chernobyl and Fukushima nuclear accidents. However, declines in the abundance of terrestrial birds, insects, spiders and mammals after both accidents contradict these modeling outcomes (Møller and Mousseau, 2007a,b, 2011; Møller et al., 2013). Further, a recent study by Hiyama et al. (2012) reports that the Fukushima nuclear accident caused physiological and genetic damage to common lycaenid butterflies (*Zizeeria maha*) in the terrestrial ecosystems adjacent to the Fukushima Daiichi Nuclear Power Plant (FDNPP). While the severity or range of exposure may differ, it is certain that many marine organisms were exposed to radiation within the Fukushima plume and that this may have serious consequences for long-term population viability. However, to our knowledge, no ecological study has examined the immediate- or short-term effects of radiation exposure on oxidative stress and antioxidant levels in natural marine systems.

Radionuclide concentration factors (CF) have been shown to increase in upper trophic levels following the outflow of man-made radionuclides from the Chernobyl nuclear accident and from nuclear waste dumping into the marine environment (Fisher et al., 1999; Heldal et al., 2003; IAEA, 2004). This implies that trophic transfer and biomagnification within food webs are key determinants of radionuclide pathways in marine systems. Fisher et al. (1999) reported that seabirds displayed the highest levels of radiocaesium CF of the apex marine predators examined. The authors concluded that this was likely because seabirds encounter pollutants while in contact with water during prey capture and their physiology prevents the release of accumulated contaminants through desorption (i.e. hypoosmotic regulation). The high levels of radiocaesium were thought to reflect terrestrial origins. Once introduced in the marine environment, radiocaesium can persist at shallow depths for around a decade (Young et al., 1975).

Due to their global distribution and broad sampling of the marine environment, seabirds are ideal bio-indicators of the prevalence and biomagnification of contaminants (e.g. Jarman et al., 1996; Fisk et al., 2001; Braune et al., 2002) and marine ecosystem health (e.g. Burger and Gochfeld, 2004; Frederiksen et al., 2007; Piatt et al., 2007). Streaked Shearwaters (*Calonectris leucomelas*) are trans-equatorial migrants that breed on offshore islands around the Japanese Archipelago and Korea (Oka, 2004; Yamamoto et al., 2010). The majority of the Streaked Shearwater population forages within the Fukushima nuclear plume during incubation, and possibly the chick provisioning period (Yamamoto et al., 2011; Honda et al., 2012). Importantly, 24% of the fishes surveyed between March and June 2011 in the marine ecosystem adjacent to the FDNPP, exceeded

the radiocaesium safety standard for food (100 Bq/kg) determined by the Japanese government (Fisheries Agency, 2013). This implies that shearwaters foraging these areas likely consumed radionuclide-contaminated fishes during the 2011-breeding season. The exposure of Streaked Shearwaters to radionuclide contamination has the potential to negatively impact breeding success and the continued viability of this top marine predator. To address these issues, we investigated the physiological responses of Streaked Shearwater chicks (body condition and antioxidant levels) to indirect radionuclide exposure as a result of the Fukushima nuclear accident, our overall aim being to assess the level of radionuclide uptake and its potential longer-term impacts.

## 2. Methods

### 2.1. Study sites

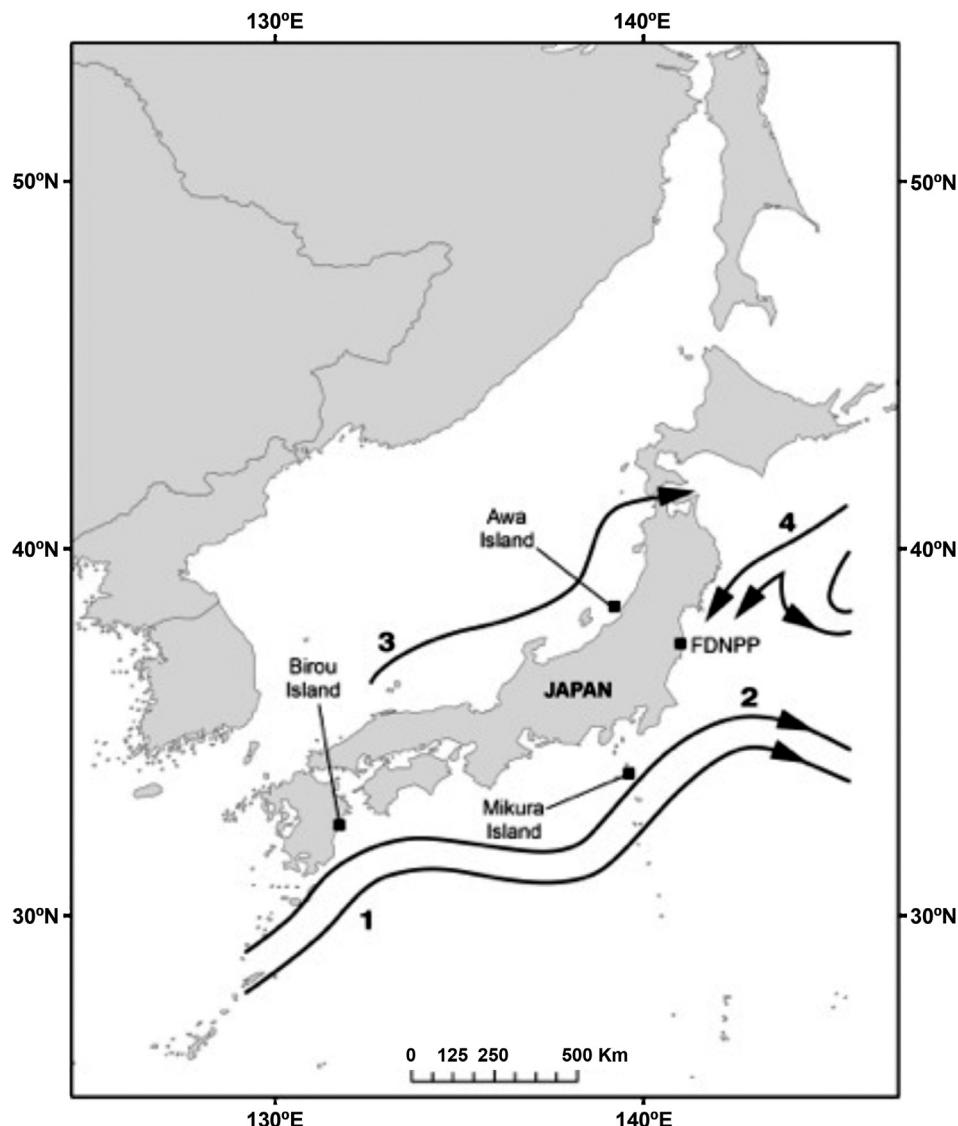
Fieldwork was conducted between August and November 2011 on Mikura (MKR; 33°52' N, 139°35' E), Birou (BRU; 32° 27' N, 131° 43' E), and Awa (AWA; 38°26' N, 139°13' E) Islands (Fig. 1). MKR is situated approximately 220 km south of Tokyo within the Fukushima nuclear plume. MKR is home to the world's largest breeding population of Streaked Shearwaters; an estimated 1.75–3.5 million individuals (RCTMG, 1980), accounting for 68–81% of the global population (Oka, 2004). BRU is located southwest of mainland Japan in Miyazaki Prefecture in an area considered less likely to have been affected by the accident (Honda et al., 2012). Streaked Shearwater population estimates for BRU are not currently available. Both MKR and BRU are located in the Kuroshio region which is characterised by low biological productivity and nutrient-poor surface water (Qiu, 2001). AWA is situated in the northwest Sea of Japan in the Tsushima warm current region. This island is home to approximately 84,000 individuals (Yamamoto, 2011).

### 2.2. Background radiation levels at study areas

The background radiation levels of Miyazaki Prefecture on October 31, 2011 were obtained from information disclosed by the Ministry of Education, Culture, Sport, Science and Technology in Japan (MEXT). The measurements were taken in  $\mu\text{Gy/h}$ , and values were expressed 1  $\mu\text{Gy/h}$  equivalent to 1  $\mu\text{Sv/h}$ . The background radiation-monitoring site in Miyazaki Prefecture is located approximately 76 km south of BRU. The background radiation levels of MKR were recorded during November 14–16, 2011 using a radiological monitor RD-0806 (Toshiba Ampex). Values were expressed in Counter Per Minute (CPM). To compare the background radiation level to the recommended radiation limit determined by the Japanese government (1 mSv/year; 0.23  $\mu\text{Sv/h}$ ; MOE, 2011), CPM was converted to  $\mu\text{Sv/h}$  where 120 CPM is approximately equivalent to 1  $\mu\text{Sv/h}$  as the instrument was calibrated to radiocaesium.

### 2.3. Sample collection

To accurately assess vitamin A levels, chick weights and blood samples were collected on MKR (November 14–16) and BRU (October 31) as close to fledging as possible and during the chick fasting period when adult feeding of chicks had ceased for the season (Mejia and Arroyave, 1983). Birds were captured by hand and weighed with an electronic balance ( $\pm 0.5$  g). External morphological measurements (e.g. bill length) were collected from chicks at MKR ( $\pm 0.5$  mm). Adult weights were also collected on AWA between August and November 2011. For each chick, approximately 0.5 ml of whole blood was collected from the tarsal vein.



**Fig. 1.** Location of the Fukushima nuclear power plant and Streaked Shearwater colonies sampled for this study and currents surrounding the Japanese archipelago. 1. Kuroshio Current, 2. Kuroshio Extension, 3. Tsushima Current, 4. Oyashio Current (Qiu, 2001).

Blood samples for retinol analysis were stored at room temperature (16–20 °C) for approximately 12 h in the field, centrifuged at 4 °C to separate plasma from red blood cells, and frozen as soon as logistically possible at approximately –20 to –25 °C. Blood samples were shielded with aluminium foil to prevent light exposure during storage.

Vitamin A was chosen as the most appropriate index of antioxidant level as it is derived from dietary intake, the antioxidant properties and activities of retinol are well established in various experimental studies (Das, 1989; Keys and Zimmerman, 1999; Tesoriere et al., 1993, 1997), and it is a sensitive physiological index of contaminant exposure (Champoux et al., 2002). In addition, vitamin A is an essential fat-soluble compound for many functions such as growth, reproduction, and cell division and differentiation, which act as a barrier against infection (Combs, 2008). Blood samples were collected after dark and therefore, exposure to light (a major factor in vitamin A degradation) was minimal. Additionally, samples were shipped together, and therefore conditions they were exposed to were very similar. Preservation method and storage duration are not thought to significantly influence vitamin A degradation (Mejia and Arroyave, 1983; Driskell et al., 1985).

#### 2.4. Chick condition

Chicks from BRU were weighed two weeks earlier than those at MKR, at a time that coincided with the pre-fledging, weight recession period when chicks were exploring outside or leaving their burrows. This behaviour is consistent with that previously reported from MKR where chicks have been observed exiting the burrow approximately 9 days after parental provisioning had ceased (Oka et al., 2002). This same study reported a decline in chick body mass by date and age that closely fitted exponential relationships ( $r^2 = 0.94$ ,  $r^2 = 0.96$ , for date and age respectively). These regression equations predict a 30% decline in chick body mass over the three weeks from the end of October to mid November on MKR (Oka et al., 2002). The current study collected data from BRU (control) at the end of October and from MKR in mid-November. Therefore, to enable comparison between chicks on MKR and BRU at an equivalent stage of the breeding cycle, chick weights on BRU were reduced by 30% as per the predicted exponential decline. Chick weights at fledging have been shown not to differ from adult or sub-adult body mass measured in the summer or autumn months (Oka et al., 2002). Therefore, adult weights recorded during the summer and

autumn (mid-August to early-October) of 2011 on AWA were used to assess the relative physical condition of chicks from MKR and BRU in late-October to mid-November 2011.

### 2.5. Antioxidant and vitamin A levels

In order to measure levels of vitamin A in Streaked Shearwater fledglings, plasma retinol was assayed at the SRS laboratory in Japan. Frozen samples were thawed at room temperature prior to analysis. Extraction procedures were as follows: methanol and dibutylhydroxytoluene (BHT) were added to the plasma for deproteinisation. Samples were then centrifuged, and supernatants separated. Chromatographic separation was performed by reversed phase high-performance liquid chromatography (HPLC). Standard dilution series were used to generate the calibration curve to determine the concentration of vitamin A. Detection after separation on an Intersil ODS column (GL Science Inc., Tokyo, Japan) was carried out, from which chromatograms were extracted at Ex. 325 nm and Em. 480 nm.

### 2.6. Data analysis

Statistical tests were carried out using STATISTICA (version 10, StatSoft, Tulsa, OK, USA). Data were tested for normality and equality of variance, transformed where necessary using either log or square root transformations (Whitlock and Schlüter, 2008), or analysed using appropriate alternative tests. Data were tested and analyses performed with and without the presence of any obvious outlying data points. The addition or removal of these data did not change any results. Results are given for the full data set and presented as means  $\pm$  1 SE unless otherwise stated.

For each fledgling an index of body condition (body stores) adjusted for chick size was calculated as the residuals from the linear regression of chick weight versus bill length (cm). This regression was performed using chicks from MKR only, where external morphological measurements were collected. No significant relationship was observed between weight and bill length (Appendix A), indicating that chick weight alone could be used as a valid index of condition in further analysis.

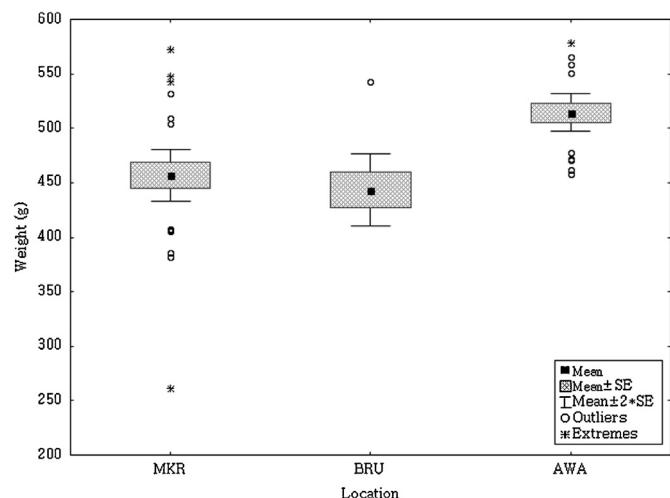
To investigate the physiological response of fledglings immediately following the Fukushima nuclear accident, mean chick-fledgling weights were compared between MKR ( $n=28$ ) and BRU ( $n=9$ ) using independent sample *t*-tests. A One-way ANOVA with Tukey's post-hoc analysis was used to compare the mean chick-fledgling weights at MKR and BRU with adult weight at AWA.

Mean vitamin A values were compared between MKR ( $n=12$ ) and BRU ( $n=9$ ) using an independent samples *t*-test with vitamin A included as a fixed factor. To test the statistical power with the relatively small sample size, a power analysis was carried out. Vitamin A supplementation is known to positively influence body condition (i.e. increase immunity, body mass and organ weight for liver, spleen, testis and intestine) in captive chickens (Abdalla et al., 2009). Therefore, simple linear regression was used to examine whether vitamin A levels influenced chick weight for MKR and BRU.

## 3. Results

### 3.1. Chick physical condition and vitamin A

A comparison of chick-fledgling weights between treatment (MKR;  $n=28$ ) and control (BRU;  $n=9$ ) sites showed no significant difference (MKR  $456.57 \pm 11.81$  g; BRU  $443.44 \pm 16.55$  g;  $t_{2,35} = 0.57$ ,  $p = 0.57$ ). A comparison of chick-fledgling weights with adult weights from AWA indicated a significant difference ( $F_{2,54} = 8.67$ ,  $p < 0.001$ ) with post-hoc analysis indicating that adult



**Fig. 2.** Mean fledgling weight on MKR ( $465.57 \pm 9.67$  g) and BRU ( $443.41 \pm 16.60$  g) and mean adult weight on AWA ( $514.50 \pm 8.82$  g). There was no significant difference in chick-fledgling weights. Weights of AWA adults were significantly higher compared with MKR and BRU chick-fledgling weights.

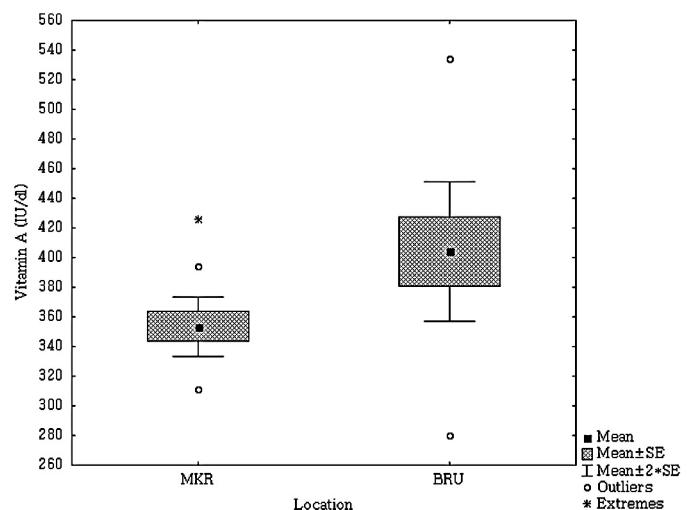
mean weights at AWA ( $514.50 \pm 8.82$  g;  $n=20$ ) were significantly higher than chick-fledgling weights at either MKR or BRU (Fig. 2).

### 3.2. Antioxidants and vitamin A

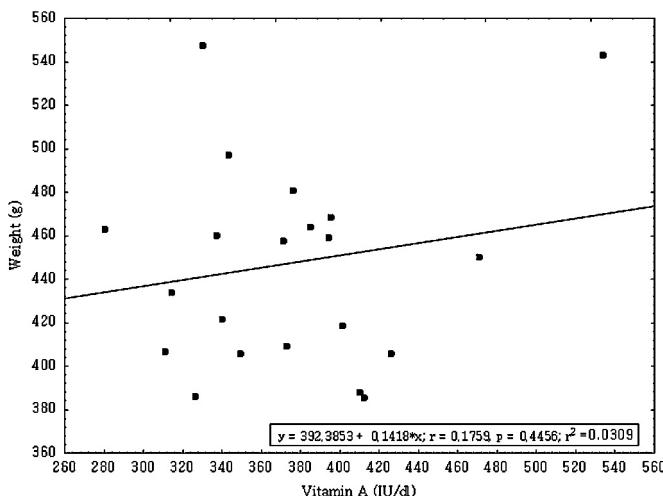
Mean vitamin A levels were found to be significantly higher in fledglings at BRU ( $404.11 \pm 71.01$  IU/dl;  $n=12$ ) as compared to MKR ( $353.41 \pm 34.81$  IU/dl;  $n=9$ ;  $t_{2,19} = -2.16$ ,  $p = 0.043$ ). Vitamin A levels in chicks at BRU varied considerably more than at MKR where levels were consistently low (Fig. 3). Importantly, very few chicks from MKR had vitamin A levels approaching the lowest levels recorded for birds from BRU (Fig. 3). There was no significant relationship between vitamin A concentration and fledgling mass in Streaked Shearwaters ( $F_{1,19} = 0.61$ ,  $p = 0.45$ , Fig. 4).

### 3.3. Background radiation level at study areas

The mean level of background radiation level in Miyazaki Prefecture was  $0.026 \pm 0.0002$   $\mu\text{Sv}/\text{h}$  ( $n=24$ ). The mean level of background radiation on MKR was  $17.26 \pm 0.50$  CPM ( $n=27$ ), which is equivalent to approximately  $0.143 \mu\text{Sv}/\text{h}$ . These values are both



**Fig. 3.** Significant difference in mean vitamin A values for chicks from MKR ( $353.41 \pm 34.81$  IU/dl) and BRU ( $404.11 \pm 71.01$  IU/dl).



**Fig. 4.** No relationship between vitamin A concentration and Streaked Shearwater fledging weights ( $F_{1,19} = 0.61$ ,  $p = 0.45$ ). This relationship explained approximately 3% of the total variation in fledgling weights.

below the radiation threshold of  $0.23 \mu\text{Sv}/\text{h}$  considered to be dangerous by the Government of Japan (MOE, 2011).

#### 4. Discussion and conclusion

##### 4.1. Chick physical responses

Chick development in birds is influenced by a number of parameters including meal mass, feeding frequency, adult provisioning patterns, and the distribution of food resources (Golet et al., 2000; Smithers et al., 2003; Takenaka et al., 2005). Characteristics of chick development (e.g. growth rate and fledging mass) have been directly linked to post-fledging survival and recruitment age (Croxall et al., 1988). Therefore, changes in development can be used as an index of oceanographic and environmental anomalies that may influence long-term reproductive success.

A previous study by Oka et al. (2002) indicated Streaked Shearwater fledgling mass approximates adult weight. In 2011, fledgling weights from MKR and BRU were significantly lower than AWA adult weights; no difference in fledgling mass was detected between MKR and BRU. Average fledgling mass from MKR in 2011 was also approximately 13% lower than reported for the same location in 1985 and 1990 (Oka et al., 2002). Together these findings suggest that in 2011, newly fledged chicks from both MKR and BRU were in relatively poor condition, both relative to previous years and compared with adults at AWA. This suggests that fledgling weight in Streaked Shearwaters varies significantly from year to year, both within and among islands. These findings also imply that in 2011, there was likely reduced food availability to chicks at both MKR and BRU islands and that reduced fledgling weight at MKR cannot be attributed solely to potential radionuclide exposure. A lack of weight change following radiation exposure is consistent with findings reported by Bonisoli-Alquati et al. (2010), in which body mass of Barn Swallows (*Hirundo rustica*) did not vary according to radiation levels.

##### 4.2. Antioxidant, vitamin A and radiation exposure

It is known that vitamin A requirements in chicks during development are high so as to prevent various deformities (e.g. leg weakness, leg and facial deformities, poor feathering and reduced weights) (McFarlane et al., 1931; Koutsos and Klasing, 2005). Consequently, vitamin A levels in birds vary naturally with age and

stage of development. For example, correlations between retinol concentration and stage of egg or embryo development have previously been reported in a number of bird species (Boily et al., 1994; Champoux et al., 2006; Miljeteig et al., 2012). Similarly, vitamin A levels in birds also vary with body condition. Captive birds fed higher levels of vitamin A having better overall health and condition (e.g. laying performance, immune maturation, development) (Koutsos and Klasing, 2005; Abdalla et al., 2009). Importantly, these are the only factors previously known to naturally affect vitamin A levels in birds. This suggests that the variation in vitamin A levels observed in chicks from BRU (the control site) must be primarily associated with natural variation due to both chick growth/development and body condition.

Natural variation in vitamin A levels is also expected in chicks on MKR due to differences in chick age and condition. Overall, chick age and body condition (i.e. body weight) at MKR and BRU were statistically equivalent. This implies that the magnitude and patterns of variation in vitamin A observed in chicks at the two locations should also be equivalent. However, this was not the case, with vitamin A levels in chicks from MKR were significantly lower and less variable than those observed at BRU. This finding implies that there is an additional factor or factors lowering the levels of vitamin A on MKR.

Vitamin A concentration in birds is not currently known to vary naturally with factors other than age and/or health condition. Specifically, vitamin A deficiency is not associated with low dietary intake (Champoux et al., 2006). For example, wild waterfowl feeding on grains and wheat, a diet thought to be low in vitamin A, maintain moderate to good body condition (e.g. body, liver and kidney weights, spleen size) and ample fat stores (Wobeser and Kost, 1992; Honour et al., 1995), while vitamin A supplementation in captive birds has no effect on body weight (Lin et al., 2002; Abdalla et al., 2009). This suggests the low levels of vitamin A seen in MKR chicks and differences between islands are not due to dietary deficiency at MKR, particularly as shearwaters forage on epipelagic fishes that contain high retinol concentrations as the source of vitamin A (Combs, 2008).

Instead, the findings of previous studies suggest that the vitamin A depletion found in chicks at MKR likely reflects a specific response to one or more pollutants. This is because plasma retinoid concentrations (vitamin A) in wild birds have repeatedly been shown to be negatively impacted by exposure to specific stressors such as organic, inorganic (Spear et al., 1986; Grasman et al., 1996; Champoux et al., 2006), or radionuclide (Møller et al., 2005b) contaminants.

Organochlorine xenobiotics such as polychlorinated biphenyls (PCBs) and dioxin-like compounds (e.g. coplanar PCB congeners) are known to interfere with the homeostasis of circulating and/or stored retinoids (e.g. Spear et al., 1986; Zile, 1992; Bishop et al., 1999; Rolland, 2000), and associations between concentrations of persistent organic pollutants and induction of antioxidant systems in birds are well documented (Champoux et al., 2006; Murvoll et al., 2006). In Herring Gulls (*Larus argentatus*), liver retinoid stores were inversely related to the extent of contamination (e.g. PCBs). Vitamin A stores in the liver of Herring Gulls remained moderately to severely depleted at some sites and was attributed to dietary availability of vitamin A and/or continuing effects of contaminants, which affected the ability to store vitamin A (Spear et al., 1986; Fox et al., 1998). The influence of radionuclide contamination on vitamin A stores in birds is also documented. Free-living migratory Barn Swallows breeding in radionuclide contaminated areas around Chernobyl displayed levels of vitamin A more than 50% lower than those in the control area (Møller et al., 2005b).

As with most species and associations, variability in species-specific responses of retinoids to xenobiotics is expected (Rolland, 2000). Consequently, plasma retinoids are regarded as a good

biomarker of exposure effects, and are sensitive enough to reflect local and regional variation in contaminants (Champoux et al., 2002). Pollutant types and levels are known to vary spatially and regionally across the Japanese coast, with contaminant concentrations (e.g. cadmium, total mercury, and PCBs) in sediments generally being higher in inner bays and coasts and decreasing with increasing distance from shore (MOE, 2009). Streaked Shearwaters are likely exposed to region-specific levels of toxic contaminants and pollutants in accordance with their foraging areas, as their at-sea distributions are colony-specific during the pre-laying to incubation period (Yamamoto et al., 2011, 2012).

Therefore, it is possible that chicks at MKR are exposed to higher levels of pollution compared to BRU due to the colony being located offshore of metropolitan areas. However, contaminated hotspots (e.g. butyltin and phenyltin) were also detected in sediments in the offshore Pacific Ocean adjacent to Sikoku and Kii (MOE, 2009), where the foraging ranges of adults from both MKR and BRU colonies are expected to overlap. Although high PCB concentrations were most frequently found in plastic pellets from Tokyo and Osaka Bay, only low concentrations have been found both in the Tohoku (MKR shearwaters foraging areas during the incubation/chick-rearing period) and Pacific Ocean side of Sikoku (BRU shearwaters expected foraging areas) (Endo et al., 2005). Overall these findings suggest it is likely shearwater populations on both MKR and BRU are equally exposed to relatively low contaminant stress from these types of pollution, and depleted levels of vitamin A at MKR relative to those at BRU are not due to differences in this type of exposure.

Adult shearwaters on MKR likely feed chicks from offshore or pelagic sources, specifically the Kuroshio–Oyashio transition area – within the Fukushima nuclear plume, during the chick-rearing period (Yamamoto et al., 2011, 2012). They also forage primarily on epipelagic fishes (Matsumoto et al., 2012). Fishes in these same areas have been shown to be radionuclide-contaminated as a consequence of the Fukushima nuclear accident (Fisheries Agency, 2013). Consumption of contaminated food source could have resulted in depleted levels of vitamin A from Streaked Shearwaters at MKR. A relationship between consumption of contaminated fishes and decreased levels of plasma thyroid hormone and retinol in Common seals (*Phoca vitulina*) in an experimental study has been reported previously (Reijnders, 1986).

Alternatively, it may also be possible reduced vitamin A levels displayed in chicks on MKR may be attributed to shearwaters prey carrying lower levels of vitamin A as a consequence of exposure to contaminants. Whether the cause of the reduced vitamin A is due to direct exposure (chicks being exposed to higher levels of radionuclide contaminants via food source) or indirect exposure (prey carrying lower levels of vitamin A as a consequence of contamination) is not known. Previous studies have documented fishes both in field and laboratory experience elevated oxidative stress induced by the production of ROS when exposed to xenobiotic contaminants (Winston, 1991; Rolland, 2000; Almeida et al., 2002) and waterborne radioisotope (Kochhann et al., 2009). Both direct and indirect route of exposure is possible and could result in similar outcomes; however, shearwaters prey need to be investigated in order to identify precise route of exposure and cause for mechanisms of reduced level of vitamin A.

Combined with our findings, this implies that reduced vitamin A levels observed at MKR are, at least in part, associated with indirect exposure to radionuclides through contaminated food sources. These interpretations are consistent with a previous study by Møller et al. (2005b), which reported lower vitamin A levels in birds resulting from exposure to radionuclide contaminated areas. As chicks remain in burrows that are about 1–2 m lengths (Jida et al., 1987; Lee and Yoo, 2002) and their colonies are located some distance from the FDNPP, direct exposure to radionuclides is unlikely.

Low levels of background radiation at both study sites support this conclusion.

In summary, our findings suggest that birds from the world's largest Streaked Shearwater colony experienced significant exposure to radionuclide-contaminants during 2011. The long-term persistence of radionuclides at shallow depths (Young et al., 1975) also means that Streaked Shearwaters will likely continue be subjected to long-term, chronic exposure to radionuclide-contaminated water during the breeding season in years to come. Both the level of ongoing exposure and the potential long-term consequences of this exposure are unknown. However, since antioxidants are essential compounds for energetically demanding activities such as the first year migration (Costantini et al., 2007), initial and continuing exposure may pose a major threat to fledgling survival and ultimately the viability of this population. Moreover, the delayed and chronic effects of radiation exposure on adults are cause for additional concern as seabirds are often slow to recover from perturbations as a result of deferred reproduction, low fecundity, and reliance on high adult survivorship to maintain populations (Pianka, 1970; Ricklefs, 1990). For such species, even small amounts of adult mortality substantially influence population viability (Begon et al., 2006).

Further studies involving increased sampling and sample size over multiple years with direct measurement of radionuclide concentration within organisms, and assessing multiple biomarkers are needed to investigate radionuclide uptake and transfer through the food chain in this system, the relationship between oxidative stress, antioxidant defences, oxidative damage and exposure to radionuclide-contamination. Dietary vitamin A availability also needs to be controlled in order to interpret the retinoid alterations as a consequence of exposure to radionuclide contaminants in seabird.

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## Appendix A.

(a) Relationships weight (g) against beak length (cm) within MKR indicated there is no relationship between the two variables ( $t_{1,26} = 0.732, p = 0.47$ ). The beak length explained only 2% of the variations in weights. (b) Residuals calculated from the linear regression of weight on beak lengths (cm) from MKR were plotted against vitamin A levels. There was no significant difference between residuals ( $F_{1,10} = 0.086, p = 0.77$ ). Vitamin A levels only explained 0.86% of the variation in residuals.

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