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Estimating and mitigating perinatal mortality in the endangered Saimaa ringed seal (*Phoca hispida saimensis*) in a changing climate

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The ringed seal (*Phoca hispida*) is dependent on sufficient ice and snow cover for a breeding habitat. Therefore, climate change has a negative effect on pup survival. We developed methods to estimate perinatal mortality and to mitigate the effects of mild winters on the critically endangered subspecies (*P. h. saimensis*). Underwater surveys were used for collecting pup carcasses and placentas. Lanugo pup mortality was 13.5% and *Brucella* sp. was not found in the placentas. Camera traps showed sporadic human and medium-sized carnivore activity in the breeding habitat. Although carnivore activity was most intensive at lair sites, no penetration of the lairs was observed. We developed a method to mitigate the effects of poor snow cover by piling up snowdrifts (n = 117) at potential lair sites. Seals subsequently occupied 62% of these snow drifts. We suggest that the methods developed in this study should be implemented to conserve the Saimaa ringed seal.

Introduction

As an ice and snow associated species, the ringed seal (*Phoca hispida*) highlights the direct negative impacts of climate change in the arctic and subarctic regions. Breeding habitat degradation associated with warming winters is a growing threat to ringed seals whose breeding success is dependent on ice and snow (Kelly 2001, Meier *et al*. 2004, Stirling & Smith 2004, Laidre *et al*. 2008, Kovacs *et al*. 2012, Sundqvist *et al*. 2012, Trukhanova 2013). Lack of adequate snow cover and earlier snowmelt may destroy protective subnivean lairs and expose pups to adverse weather, increased predation, (Kelly 2001, Stirling & Smith 2004, Hezel *et al*. 2012, Kovacs *et al*. 2012) and human disturbance (Ministry of the Environment 2011). It has been suggested that these severe impacts are seen first in southern ringed seal populations (Meier *et al*. 2004). The impact of global climate change on the critically endangered (Kovacs *et al*. 2012) endemic ringed seal subspecies (*P. h. saimensis*) living in Lake Saimaa in Finland is of immediate concern due to its small population size, specific breeding habitat requirements, and southern landlocked lacustrine habitat (Sipilä 1990, Kovacs *et al*. 2012).

The ice cover of Lake Saimaa normally persists from late November to the beginning of May, and pups are born from February to March (Helle *et al*. 1984), when snow cover is at its thickest. Ringed seals give birth in a subnivean snow lair, and a typical breeding habitat is landfast ice with snowdrifts formed along pressure ridges (e.g. Smith & Stirling 1975). However, in Lake Saimaa, ice cover is formed continuously and pressure ridges do not occur. Therefore, sufficiently deep, wind-deposited snowdrifts are formed only by the shorelines of islands where ringed seals dig their lairs (Sipilä 2003). Saimaa ringed seals exhibit high breeding-site fidelity (Helle *et al*. 1984, Sipilä 1990) making it easy to predict lair locations. The location of lairs by shorelines brings the seals in close contact with terrestrial predators and humans (Helle *et al*. 1984, Sipilä 1990).

The main threats to the Saimaa ringed seal population are reduced survival of juveniles, primarily due to incidental by-catch, and mild winters with inadequate snow cover for lairs (Sipilä 1990, 2003, Kovacs *et al*. 2012, Niemi *et al*. 2012, 2013). Population size estimates are based on annual lair censuses (Sipilä 1990, Sipilä *et al*. 1990), and population trends and birth rates are well known. By-catch, a critical mortality factor for Saimaa ringed seals, has been discussed in many contexts (Ranta *et al*. 1996, Kokko *et al*. 1998, Sipilä 2003, Niemi *et al*. 2012, 2013, Auttila *et al*. 2014), whereas the magnitude and causes of perinatal mortality has received comparably little attention. This is surprising because of the significant impact mild winters and predators are thought to have on the survival of lanugo pups. Indeed, local people express distrust of population size estimates (Bell *et al*. 2008) and suggest that predators, such as red fox (*Vulpes vulpes*), wolf (*Canis lupus*), brown bear (*Ursus arctos*) and lynx (*Lynx lynx*), kill the pups and that juveniles vanish because of natural causes rather than due to entanglement in fishing gear.

In the present study, we developed methods for monitoring and mitigating the perinatal mortality of the Saimaa ringed seal to understand the species' response to warm winters and to develop new conservation strategies for this endangered population. To achieve these goals (1) camera traps were used to monitor the amount of human-induced disturbances and the magnitude of predator activity during the breeding season; (2) underwater surveys at birth lair sites were conducted to examine the perinatal mortality rate and (3) the occurrence of *Brucella* sp., which may cause reproductive disorders such as placentitis and/or abortion, also in marine mammals (Miller *et al*. 1999, Rhyan *et al*. 2001, Foster *et al*. 2002), was examined in the placentas discovered during underwater surveys. We focused our bacteriological examination on *Brucella* sp., because of its known role in reproduction failure of many mammal species (Godfroid 2002).

A method to artificially enhance snow conditions at the Saimaa ringed seal's lair sites was developed to improve pup survival during mild winters.

Material and methods

This study was conducted in Lake Saimaa (61°05´–62°36´N, 27°15´–30°00´E) in Finland. The lake is inhabited by the Saimaa ringed seal, whose population size is around 300 individuals, and ca. 60 pups born annually (Metsähallitus 2013). This freshwater lake (4400 km^2) is about 180 km long and 140 km wide, with approximately 14 000 islands. The ice and snow cover was sufficient for seals to construct lairs during their breeding seasons examined in this study (Finnish Meteorological Institute 2013; *see* also Oiva database, Finnish Environment Agency, at https://wwwp2.ymparisto.fi/scripts/oiva.asp [in Finnish]).

Human disturbances and predator activity in winter were estimated by placing game camera traps (Scout Guard Camo 550VB) at potential lair sites of Saimaa ringed seals in two main breeding areas (Haukivesi and Pihlajavesi basins) in 2010–2013. The cameras have a passive infra-red (PIR) sensor (operational distance 2–10 m, but with large objects like snowmobiles up to ~30 m) and an infra-red flash (up to 12 m), which facilitates image acquisition in the dark without a noticeable flash. Cameras collected images in response to motion (high sense level) by taking three photos. After a ten-second trigger interval the cameras were set to react to motion again. All images were saved with a time and date stamp. The majority of cameras were installed shortly after the lake froze in December–January and they were removed in April–May. After the lairs at the camera sites were verified during the annual lair census in late April, data were divided into two categories: (1) at lair site and (2) at site without lair during the study year.

Underwater surveys to examine perinatal mortality (including stillborn pups and pups that lived less than a month) covered all birth lair sites detected during the annual lair censuses in 2011–2013 in Lake Saimaa. Immediately following ice break-up in early spring, we investigated the lakebed around the lair location (from a distance of 10 m to 100 m from the exact location) by wading, snorkeling, and/or scuba diving (maximum depth at the sites was < 20 m). Pup carcasses and placentas discovered and collected during these investigations were stored at –20 °C. Autopsies of the carcasses were conducted by Finnish Food Safety Authority Evira.

Placenta samples from the years 2011–2012 (n = 57) were examined for the presence of *Brucella* sp. bacterium by bacteriological methods. Five of the placentas belonged to dead pups. All placenta samples were dissected, mixed with approximately 2.5 ml of sterile phosphate buffered saline (PBS) pH 6.8 ± 0.2, macerated and inoculated onto Farrell's selective solid agar media containing *Brucella* agar with 5% bovine serum and antibiotics (Polymixin B sulfate 2500 IU l^{-1}, Bacitracin 12 500 IU l^{-1}, Natamycin 25 mg l^{-1}, Nalidixin acid 2.5 mg l^{-1}, Nystatin 50 000 IU l^{-1} and Vancomycin 10 mg l^{-1}). The samples were incubated for ten days at 37 °C in aerobic conditions in 5% CO_2 atmosphere, and were controlled for growth of *Brucella* every second day for up to ten days. Smears from macerated samples were also stained with stamp-staining for presence of *Brucella*. Placentas were also used in genetic analyses (M. Valtonen unpubl. data).

Our investigation into improving snow conditions at lair sites was conducted in the main breeding areas in Haukivesi and Pihlajavesi basins from 2010 to 2012. Man-made snowdrifts, designed to mimic wind-drifted snow, were constructed no later than 10 February in accordance with a permit from the local Center for Economic Development, Transport and the Environment (permit number: ESA-2009-L-375-254). Snow cover thickness at the drift sites before the addition of man-made drifts was on average 19 cm (range 2–49 cm), and ice cover thickness was 19 cm (7–34 cm). In the areas where snow was collected, snow cover was 12 cm (2–34 cm) thick. The completed man-made drifts were on average 82 cm (range: 64–110 cm) high, 802 cm (230–1730 cm) long (parallel to the shoreline) and 345 cm (130–550 cm) wide (Fig. 1). It took around 30 minutes (13–74 min) to pile up a snowdrift, with approximately four people collecting snow from the ice using shovels and pushers. After the first study year, we confirmed that the water depth was ≥ 70 cm under all of the completed man-made drifts. The efficacy of the man-made snowdrifts was examined the following April during the annual lair census. We measured the width and length of the lairs in the man-made snowdrifts, and the presence of lanugo hair was evaluated to categorize each site as a birth lair or a haulout lair.

Results

Altogether, 202 camera traps were placed at potential lair sites to monitor the breeding habitat and 87% of the cameras continued to operate throughout the study season (total 17 276 trapping days). Subnivean structures (9 birth and 60 haulout lairs) made by seals occurred at 39% of the camera sites. During this four-winter study, 18% of the cameras recorded humans and 39% recorded carnivores. The proportion of cameras recording humans fluctuated across the study years, whereas carnivore activity remained rather stable (Fig. 2). The number of cameras recording carnivores was significantly higher (Fisher's exact two-sided test p = 0.018) at the lair sites than at other sites, but human activity was similar at both sites (Fisher's exact two-sided test p = 0.312).

During the study winters, the cameras (n = 175) recorded 55 visits (i.e. separate observations) of humans and 125 of carnivores. The visits were

Fig. 1. A man-made snowdrift piled up at the breeding habitat of the Saimaa ringed seal. The drifts were on average 82 cm high, 802 cm long (parallel to shoreline) and 345 cm wide.

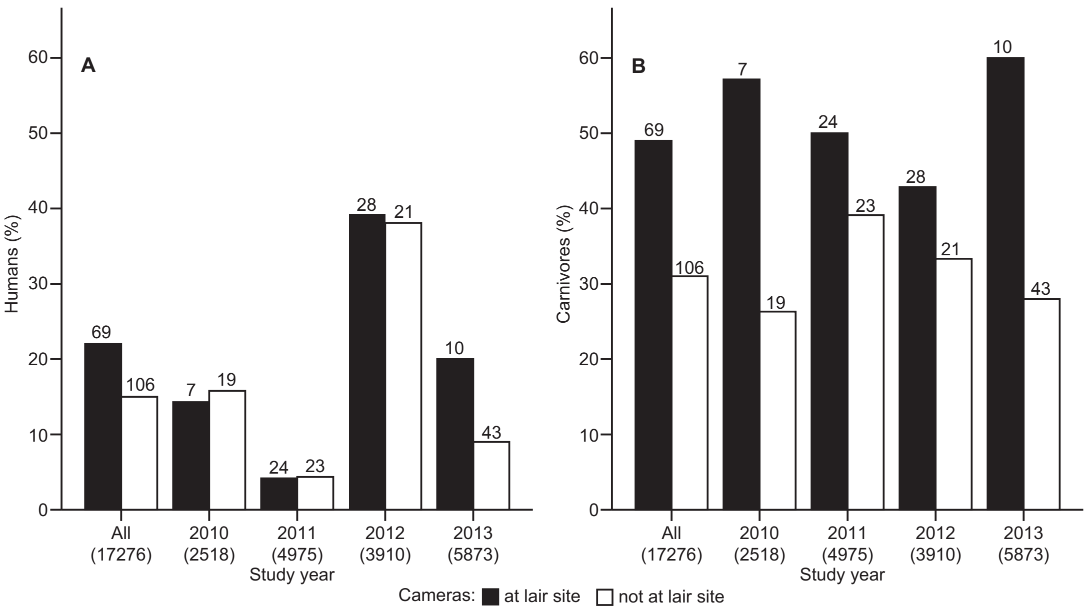

Fig. 2. Disturbance monitoring at the seal's breeding areas in Lake Saimaa. Proportions (%) of camera traps that recorded (**A**) human, and (**B**) carnivore (most commonly red fox) activity during winter. Numbers of the cameras are shown above the bars and trapping days below the study years.

mostly sporadic; only 4% and 8% (respectively) of the cameras recorded visits more than twice. About half (55%) of both human and carnivore visits were at the lair sites. Carnivores were not observed to penetrate the lairs; they usually travelled along the shoreline and bypassed the lairs. The red fox was the most common (68% of observations) among medium-sized carnivores, followed by the raccoon dog (*Nyctereutes procyonoides*, 13%), otter (*Lutra lutra*, 8%) and unidentified species (10%). There was one observation of a lynx, and a few other mammals (*Lepus* spp.

and *Alces alces*) and birds (*Larus* spp. and *Corvus* spp.) were also occasionally recorded. Observations of animals and humans in the breeding areas were concentrated in March and April each year.

We used underwater surveys to examine a total of 190 (165 birth and 25 haulout) lair sites to estimate perinatal mortality. In total, six pup carcasses (Table 1) and 76 placentas were found at birth-lair sites. In addition, three pup carcasses and three placentas were found at four of the sites that were identified in the census as haulout lairs. When the results of the underwater surveys were combined with the lair census and with public observation data on born and dead seal pups (Metsähallitus 2013), the average perinatal mortality rate was 13.5% (Table 1). However, mortality rate ranged between 5.0% and 17.9% over the three years of our study. Results of the bacteriological examination for the presence of *Brucella* sp. were negative for all placenta samples ($n = 57$).

Overall, 117 man-made snowdrifts were formed (Table 2), 44% of them at regularly-used birth-lair sites, and the rest at sites where lairs had not been detected earlier. Subnivean structures made by the seals were found in 62% of the man-made snowdrifts. There were 3 birth lairs, 62 haulout lairs (Table 2), and 7 breathing holes. All three of the birth lairs were in snowdrifts situated at regularly used birth lair sites. Based on the underwater surveys, pups born in the man-made snowdrift lairs survived beyond the suckling period. The portion of lairs located in man-made snowdrifts increased from 32% to 67% over the three years of this study. The haulout lairs ($n = 56$) in the man-made snowdrifts were on average (± SD) 166 ± 52 cm wide and 232 ± 109 cm long.

Table 1. Summary of born and dead pups in Lake Saimaa. Underwater surveys were conducted in early May at the lair sites discovered in the lair censuses in April. Observations by the public were performed during the early open-water season.

Observation method	Born			Dead		
	2011	2012	2013	2011	2012	2013
Lair census[a]	52	58	60	3	2	4
Underwater survey	3	0	1	5	0	4
Public observation[a]	1	2	1	2	1	3
Total	56	60	62	10	3	11
Pup mortality (%)				17.9	5.0	17.7

[a] Metsähallitus 2013.

Table 2. Improving the breeding habitat of the seals in Lake Saimaa. Number of man-made snowdrifts and number of lairs in those drifts at regularly used birth lair sites (sites type A), and at sites where lairs had not been detected earlier (sites type B). Percentage of lairs in the man-made snowdrifts during the study years are shown.

		Sites type A	Sites type B	Total
Man-made snowdrifts	2010	13	18	31
	2011	18	22	40
	2012	20	26	46
	Total	51	66	117
Lairs in man-made snowdrifts	2010	7	3	10
	2011	12[a]	12	24
	2012	14[b]	17	31
	Total	33	32	65
	Percentage	65	48	56

[a] one-birth lair, [b] two-birth lair.

Discussion

Our camera monitoring indicated that predators are not a considerable threat to Saimaa ringed seal pups during winter when there is a sufficient amount of ice and snow. Although the carnivores (especially red fox) visited lair sites more often than other sites, there was no evidence that they penetrated the lairs or attacked the pups. However, a few days of rain may thin or even collapse the roof of the lair, and carnivores (including the domestic dog *C. familiaris*) may be able to break in and catch a small pup (Stirling & Smith 2004). In addition to polar bears (*Ursus maritimus*) and arctic foxes (*Alopex lagopus*) (which do not occur in Lake Saimaa), predation and scavenging on Arctic ringed seals by red foxes, wolves, gulls and ravens have been reported, especially during exceptional weather conditions (Lydersen & Gjertz 1986, Lydersen & Smith 1989, Smith *et al*. 1991, Stirling & Smith 2004). In Lake Ladoga, where ringed seals (*P. h. ladogensis*) also use shorelines for lair habitat, red fox and wolf have been shown to mark and penetrate the lairs, but evidence of kills has not been reported (Kunnasranta *et al*. 2001). There is only one observation of a newborn pup that was supposedly killed by dogs or foxes in Lake Saimaa (Sipilä 2003). However, a changing climate may increase the role of predation as a cause of mortality due to thinning of snow roofs or the complete lack of subnivean lairs.

Little is known about the effects of human disturbance on seal breeding success, but it has been suggested that Saimaa ringed seals avoid lair sites near the mainland or towns (Sipilä 1990). Human activity near breeding sites may disturb nursing, which may hamper seal pup growth (Engelhard *et al*. 2002). In addition, lanugo pups without the thermal protection of subcutaneous blubber are prone to hypothermia if wetted (Smith *et al*. 1991), and if pups are thermally stressed, they allocate less energy to growth. Furthermore, low weight at weaning increases seal pup mortality (Craig & Ragen 1999, Hall *et al*. 2001, Sipilä 2003). The Saimaa ringed seals' main breeding areas are used also for outdoor activities, but our study indicated that human visits at lair sites are mostly sporadic. Regardless, human-induced disturbance may have a negative effect on breeding success; for example, one of our cameras recorded a snowmobile running over a lair and collapsing it. Variables such as the amount of powder snow and water on the ice probably explain some of the inter-annual variation in the amount of human activity observed in this study.

Underwater surveys confirmed the results of the lair census and proved useful in estimating the perinatal mortality rate of the Saimaa ringed seal. Our results show that a variable proportion of pup mortality remains undetected, and that some birth lairs are incorrectly determined as haulout lairs in the censuses. This is also the first study to estimate the natural lanugo pup mortality of ringed seals, when predation does not exist. Average mortality (13.5%) was higher than that previously suggested for the Saimaa ringed seal, although it varied across the study years. Based on long-term lair census data, it has been estimated that lanugo pup mortality is ca. 8% during normal winters (Sipilä 2003). Pup mortality, however, reached near 30% during some years in the 1980–1990s, when there were large fluctuations in the water level during winters (Sipilä 2003) and in 2006–2007 when snow cover for the lairs was insufficient (Ministry of the Environment 2011). It has been shown (Ranta *et al*. 1996) that if water level changes are greater than 20 cm during the seal's breeding season (1 Jan.–30 Mar.) it will have a detrimental effect on pup survival. The water level in Lake Saimaa dropped ca. 30 cm during the breeding season in 2013 (*see* Oiva database, Finnish Environment Agency, at https://wwwp2.ymparisto.fi/scripts/oiva.asp [in Finnish]), which may explain some of the observed mortality (17.7%). However, mortality was also rather high in 2011 (17.9%), when the water level change was only ca. 12 cm (*see* Oiva database, Finnish Environment Agency, at https://wwwp2.ymparisto.fi/scripts/oiva.asp [in Finnish]). During the study years, ice and snow cover were sufficient for breeding (Finnish Meteorological Institute 2013; *see* also Oiva database, Finnish Environment Agency, at https://wwwp2.ymparisto.fi/scripts/oiva.asp [in Finnish]).

The causes of perinatal mortality of Saimaa ringed seals are still not fully understood. During our three-year study, a total of 24 pup carcasses were discovered; three of which were stillborn, 13 had survived for less than a month, and the rest were decayed to such an extent that autopsy was not possible (Metsähallitus 2013). Based on autopsy reports and our camera observations, we suggest that predation does not play a significant role in pup mortality. The role of infection remains largely unknown, but there was no evidence of *Brucella* sp. in Saimaa ringed seals during this study. However, this bacterium has previously been isolated from several seal species (Nymo *et al*. 2011), including ringed seals (Forbes *et al*. 2000). In addition, serological evidences of *Brucella* sp. infections in ringed seals have been reported (Nielsen *et al*. 1996, Tryland *et al*. 1999, Nielsen *et al*. 2001). It is notable that the genetic diversity of the Saimaa ringed seal is extremely low compared with that of other ringed seals (Palo *et al*. 2003, Valtonen *et al*. 2012) and although no evidence of inbreeding depression has yet been detected, we cannot ignore the potential impacts of genetic factors on pup mortality.

We report here the first usage of an artificially-improved breeding habitat for ringed seals. Saimaa ringed seals accepted man-made snowdrifts as lair sites, even when given the choice between wind-drifted and man-made snowdrifts. Despite the strong lair site fidelity of Saimaa ringed seals (Helle *et al*. 1984, Sipilä 1990), the seals accepted over half of the man-made drifts that were piled up at a location that had not previously been used as a lair site (Table 2). Thus, it seems that man-made snowdrifts may be used to attract the wintering seals to more suitable sites, e.g. areas with less human-induced disturbance. The acceptance of man-made snowdrifts increased over the course of the three-year study, despite the fact that snow cover was sufficient for lair construction each year. Ensuring that there was sufficient water depth (> 70 cm) under the drift sites after the first study year probably contributed to the increase (from 32% to 60%) in acceptance of man-made drifts. The size of haulout lairs in man-made snowdrifts was approximately similar to lairs in wind drifted snowdrifts (Sipilä 1990, Smith *et al*. 1991, Furgal *et al*. 1996, Kunnasranta *et al*. 2001), which demonstrates that the size of the piled snowdrifts was adequate for the lairs. To be suitable for a birth lair, the man-made snowdrifts should be approximately 1 m high, 3–6 m wide and 8–15 m long. Our study showed that a few-centimetre-thick snow is sufficient to construct man-made snowdrift, and that ice about 10 cm thick can bear the weight of the drift.

Due to poor snow cover, the man-made snowdrift method developed in this study was implemented as a conservation act in 2014. About 240 snowdrifts were piled up in Lake Saimaa just before the seals' breeding season, and over 90% of the pups discovered in the lair census were born in the man-made snowdrifts (Metsähallitus 2014).

Conclusion

Novel methods to estimate and mitigate perinatal mortality for the critically endangered Saimaa ringed seal in a changing climate were developed in this study. Due to the high lair site fidelity of these seals (Helle *et al*. 1984, Sipilä 1990) both game camera traps and man-made snowdrifts were easy to set. Underwater surveys were an effective method to verify the estimates of natality and pup mortality, and to collect seal tissue samples. We suggest that the methods developed in this study should be implemented to conserve the Saimaa ringed seal. More efficient monitoring of southern ringed seal populations provides information, which can also be used to predict the effects of climate change on other ringed seal populations.

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