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Fatal Paralytic Shellfish Poisoning in Kittlitz's Murrelet (*Brachyramphus brevirostris*) Nestlings, Alaska, USA

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ABSTRACT: Paralytic shellfish poisoning (PSP) is an acute toxic illness in humans resulting from ingestion of shellfish contaminated with a suite of neurotoxins (saxitoxins) produced by marine dinoflagellates, most commonly in the genus *Alexandrium*. Poisoning also has been sporadically suspected and, less often, documented in marine wildlife, often in association with an outbreak in humans. Kittlitz's Murrelet (*Brachyramphus brevirostris*) is a small, rare seabird of the Northern Pacific with a declining population. From 2008 to 2012, as part of a breeding ecology study, multiple Kittlitz's Murrelet nests on Kodiak Island, Alaska, were monitored by remote cameras. During the 2011 and 2012 breeding seasons, nestlings from several sites died during mild weather conditions. Remote camera observations revealed that the nestlings died shortly after consuming sand lance (*Ammodytes hexapterus*), a fish species known to biomagnify saxitoxin. High levels of saxitoxin were subsequently documented in crop content in 87% of nestling carcasses. Marine bird deaths from PSP may be underreported.

Key words: Avian, harmful algal bloom, Kittlitz's Murrelet, paralytic shellfish poisoning, saxitoxin.

Harmful algal blooms (HABs), often referred to as "red tides," are defined as episodic or persistent blooms of toxin-producing algae. Well-known negative effects of HABs include sickness or death in humans and reduction of aquaculture harvests and tourism (Hallegraeff 2003; Etheridge 2010). Paralytic shellfish poisoning (PSP) is the common term for toxic illness in humans resulting from consumption of shellfish during red tides. In Alaska, PSP occurs during blooms of marine dinoflagellates, typically *Alexandrium* sp., that produce multiple neurotoxins, the most potent of which is saxitoxin (STX) (RaLonde 1996). Saxitoxin binds to and blocks sodium channels in nerve cell membranes, stopping the flow of nerve impulses. Untreated, severe cases in humans may result in fatal respiratory paralysis.

Harmful algal blooms can affect ecosystem health via the transfer of toxins to higher trophic levels through food chains. Shellfish accumulate STX through direct ingestion of toxic algae (Shumway 1990). Shellfish are generally resistant to the acutely lethal effects of STX, but recent studies suggest the possibility of sublethal or chronic effects, including immune system modulation and tumorigenesis (Landsberg 1996; Nunez-Acuna et al. 2013). Zooplankton also ingest toxic algae and, in turn, may be ingested by organisms at higher trophic levels (Doucette et al. 2005). Fish kills occur because of consumption of toxic zooplankton, and marine mammal mortalities occur because of consumption of toxic fish (Landsberg 2002).

Mortality of wild birds due to STX exposure has rarely been documented, but there have been suspect cases in piscivorous seabirds in Washington State and in England (Shumway et al. 2003). In these reports, mass mortalities occurred in seabirds during red tide events that also caused outbreaks of illness in humans. Two PSP events resulting in mortality of seabirds have been documented in nesting Common Terns (*Sterna hirundo*) on Cape

Cod, Massachusetts, USA (Nisbet 1983; National Wildlife Health Center [NWHC] unpubl. data). In both cases, the terns had ingested sand lance (*Ammodytes* sp.) containing high levels of STX.

Kittlitz's Murrelet (KIMU) (*Brachyramphus brevirostris*), a member of the Alcidae family, is a diving seabird that ranges from southeastern Alaska, USA, to the eastern coast of Russia. It feeds on small planktivorous fish and euphausiids in the neritic zone (Hatch 2011). Kittlitz's Murrelet is one of the rarest seabirds in the North Pacific, and population monitoring suggests significant declines in some local populations (Kuletz et al. 2011; Piatt et al. 2011). Known causes of mortality or loss of productivity include oil spills and gillnet bycatch (Wynne et al. 1992; van Vliet and McAllister 1994), but these factors do not entirely explain the population declines.

A study to measure demographic parameters and investigate behavioral aspects of KIMU breeding ecology on Kodiak National Wildlife Refuge, Kodiak Island, Alaska, USA revealed extremely low nest survival, with only 18 of 74 monitored nests fledging young. Nests consist of a scrape in the rocky soil where a single egg is laid. Nest failure was primarily due to depredation (Lawonn 2012); however, 19% of nestling mortality was unexplained. Between 2008 and 2011, only three chicks had been found dead on the nest during this study, and in two of these cases exposure to severe weather was suspected as the cause of death. In 2011, only four of the 22 nests found on the study site successfully fledged chicks. Eight nestlings were found dead on nests at varying ages from 3 to 24 days after hatch, with death occurring over a span of several weeks between 24 June and 23 July. Seven of the eight dead chicks were being monitored by motion-sensitive infrared cameras placed at the nest, and photographs indicated that feeding rates were regularly 3.9–4.8 fish per day, and chicks consumed prey within hours of death. The field sites were remote and lacked access to electricity; thus, chick carcasses were preserved in 70% ethanol. Of the eight mortalities, two chicks were scavenged and decomposed and were not collected.

Because of the large number of chick deaths in 2011, a propane freezer was stationed at the study site in 2012; three chick carcasses were collected and frozen in 2012. All three chicks died on the nest at healthy weights (45–50 g) and during a period of relatively mild weather conditions, including higher temperatures and less precipitation than is typical for this region. Unlike 2011, the three chicks were approximately 4 days posthatch and deaths occurred over a narrow window of time (27 June–4 July). Motion-sensitive infrared cameras recorded the parents feeding fish to two of the three chicks 1–3 hr prior to death (the camera on the third chick malfunctioned).

Six chick carcasses preserved in 70% ethanol were sent to the US Geological Survey's NWHC in Madison, Wisconsin, USA, to determine cause of death in 2011. Examination was limited to gross necropsy and histopathologic examination of tissues because of preservation method. Chicks were in good nutritional condition. Four chicks had abundant sand lance in their crops and gizzards. Ventricular nematode infestations with mild mucosal invasion were seen in five of the chicks; in one, intravascular nematodes were present in the lung and associated with bacterial sepsis. Upper gastrointestinal contents and selected organs from each chick were saved frozen.

Necropsy examinations were performed on three frozen 4-day-old chicks submitted in 2012. All chicks were in good nutritional condition and had large quantities of sand lance in the crop and gizzard. No significant gross or histopathologic lesions were observed. Attempts at aerobic bacterial culture of lung and liver resulted in no growth. No viruses were isolated from brain or lung. Liver, heart, kidney,

TABLE 1. Concentrations of saxitoxin (ng/g) detected in recently ingested fish (upper gastrointestinal contents) and in tissues from acutely dead Kittlitz's Murrelet (*Brachyramphus brevirostris*) chicks, Kodiak Island, Alaska, USA, 2011 and 2012. Upper gastrointestinal contents from 2011 chicks were preserved in ethanol and therefore saxitoxin concentrations measured are likely to be artificially low.

	Saxitoxin (ng/g)		
Age (days)/date of death	Upper gastrointestinal content[a]	Liver[b]	Kidney[b]
7/24 June 2011	42.2	N/A	N/A
7/30 June 2011	12.6	N/A	N/A
3/30 June 2011	bdl	N/A	N/A
10/11 July 2011	58.4	N/A	N/A
11/11 July 2011	7.6	N/A	N/A
4/27 June 2012	52.4	56.3	27.9
4/28 June 2012	216	106.4	N/A
4/4 July 2012	210.3	69	N/A

[a] bdl = below detectable limits.

[b] N/A = not available.

lung, and gastrointestinal tract were examined for parasites; a few pieces of degraded nematodes were found in the upper gastrointestinal tract of one chick; all other tissues were negative.

Upper gastrointestinal content, liver, and kidney samples (where available) from five 2011 chicks and three 2012 chicks were sent to the National Oceanic and Atmospheric Administration's Northwest Fisheries Science Center, Wildlife Algal-Toxin Research and Response Network, for analysis. Preservation methods and inadequate samples precluded one of the six chicks from 2011 from being analyzed. Samples were extracted in 80% ethanol in a 1/4 (wt/wt) ratio, and homogenized for 60 sec using an Omni ES homogenizer (Omni International, Kennesaw, Georgia, USA). Homogenates were centrifuged in a Sorvall RC 5C Plus (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) for 15 min at 10,000 × G. Supernatants were removed and filtered through a 0.45-μm syringe filter and kept refrigerated for 2 hr until analysis. One or more samples from each animal were analyzed for STX using enzyme-linked immunosorbent assay. Matrix testing was done for each type of sample material, and minimum dilution levels were set for each sample type, to avoid false positive (or negative) results.

Of the eight KIMU chick carcasses analyzed, seven were positive for STX (Table 1). High concentrations of STX were detected in the upper gastrointestinal contents and livers of all three chicks collected in 2012. Of the five chicks tested from 2011, four were positive for STX, but at levels lower than those found in the 2012 chicks. Concentrations of STX in sand lance ingested by KIMU nestlings ranged from 7.6 to 58.4 ng/g in 2011 and from 52.4 to 216 ng/g in 2012. The 2012 levels are comparable to those measured in sand lance consumed by terns found dead in Massachusetts in 2005, which ranged from 160 to 8,550 ng/g, with most values in the 200–300 ng/g range (NWHC, unpubl. data). Concentrations in ingesta from 2011 chicks likely were falsely depressed because of their preservation in ethanol; ethanol is used in the extraction of STX from tissues. Toxic effects resulting from STX vary significantly across taxonomic groups, and the LD_{50} for avian species is not established. For comparison purposes, the intraperitoneal LD_{50} for STX in mice is 10 μg/kg (Landsberg 2002). Paralytic shellfish poisoning caused up to 21% of nestling mortality in KIMU on Kodiak Island in 2011 and 2012. The impact of PSP in marine bird populations may be more

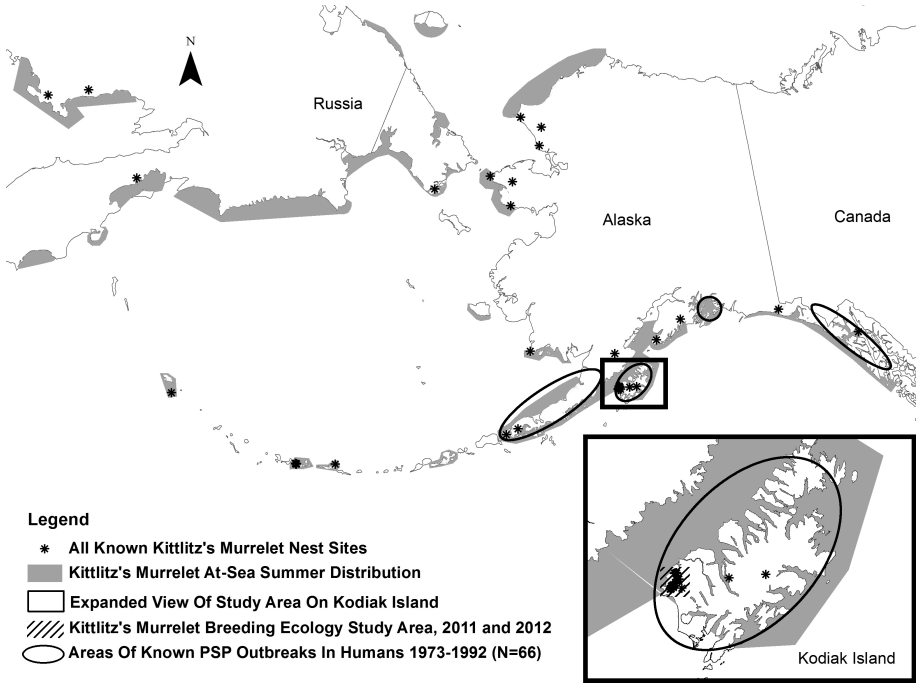

FIGURE 1. Spatial overlap between known Kittlitz's Murrelet (*Brachyramphus brevirostris*) nest sites and their summer range (J.P. unpubl.; Kuletz et al. 2003) with locations of known paralytic shellfish poisoning (PSP) outbreaks in humans (RaLonde 1996). Known outbreaks of paralytic shellfish poisoning in humans occurred mainly in May, June, and July, which is peak breeding time for Kittlitz's Murrelets.

severe than previously recognized. Globally, HABs are increasing in frequency and duration, in part because of climate change (Dale et al. 2006), although predictions of the local effects of climate change on phytoplankton communities are complex (Hallegraeff 2010). An epidemiologic analysis of human PSP cases in Alaska occurring between 1973 and 1994 found that most cases occurred during May, June, and July, which is also peak nesting season for KIMU (RaLonde 1996), and outbreak locations were within foraging distance of known KIMU nest sites (Fig. 1). The Centers for Disease Control and Prevention in Atlanta, Georgia, USA, identified 21 human cases of PSP in southeast Alaska during May–June 2011, a significant increase over the $\leq$10 cases reported annually since 1998. Given the temporal and spatial overlap, similar impacts to KIMU reproductive output may be occurring in other locations where KIMU nests and known PSP outbreaks in humans occur. Because the trophic level of seabirds is high in marine food webs, their monitoring may provide an excellent early warning system for changes in phytoplankton communities.

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