



Parasitology / Full Paper

Postmortem and helminthological examination of seabirds killed by oil spilled at Ishikari, Hokkaido, Japan, in November 2004

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Summary

Postmortems and helminthological examinations were performed on beached seabirds killed by oil spilled from a grounded freighter at Ishikari, Hokkaido, Japan in November 13, 2004. The carcasses were covered with crude oil, but they had adequate subcutaneous fat levels. Gross pathological findings consisting of gastric ulcers, pulmonary edema, enlarged spleens and blackish liquid contents in the digestive tracts suggested that they had a rapid progression to death caused by a loss of ascending force, hypothermia and dehydration. Although the visceral organs had degenerated, no direct evidence of mortality caused by ingesting oil was observed. However, extensive acute inflammatory reactions caused by large numbers of mature and immature nematodes (*Contraecaecum rudolphii*) deeply penetrating the gastric walls was observed in two of the birds. Helminthological investigations were conducted on 21 birds from six species, namely: *Phalacrocorax capillatus*, *Aythya marila*, *Cerorhinca monocerata*, *Synthliboramphus antiquus*, *Aethia cristatella*, and *Brachyramphus perdix*. Thirteen helminth species were obtained and identified, including eight nematodes (*Eucoleus contortus*, *Baruscapillaria mergi*, *B. rudolphii*, *Amidostomum acutum*, *C. rudolphii*, *Tetrameres fissispina*, *Cosmocephalus obvelatus* and *Stegophorus stercorarii*), three trematodes (*Aporchis* sp., *Hyptiasmus* sp. and *Renicola* sp.), one cestode (*Diorchis nyrocae*) and one acanthocephalan species (*Andracantha phalacrocoracis*). Of these, *B. rudolphii* from *S. antiquus*, *C. rudolphii* and *Renicola* sp. from *A. cristatella*, and *C. obvelatus* and *Renicola* sp. from *B. perdix* were first host records. Additionally, *B. rudolphii* was the first geographical record of this species in Japan.

Key Words: helminths, oil spill, postmortem, seabirds

Introduction

In November 13, 2004, a freighter named “Marine Osaka” ran aground in the breakwater and broke open. About 200 bkl of bunker C oil spilled into Ishikari Bay, New Port, Hokkaido, Japan. Roughly 35 seabirds, including dead and injured birds, were found in the oil spill. Most of them were either rescued or taken for scientific research from the oil-affected beaches between 16 and 20 November 2004. Seabirds are affected directly and/or indirectly by oil pollution. These effects include immunodeficiency, thermoregulatory failure and reproductive dysfunction [10,13,16-19, 21,24,28,38]. Seabirds, especially neritic and coastal divers such as murrelets, cormorants and diving ducks, are among the most vulnerable species to oiling [19,21,28,38]. In addition, there are many seabirds, including several endangered species, breeding and wintering around Hokkaido [32]. Taking the lifestyle and recent habitat reduction of these seabirds in Japan into consideration, it seems necessary to clarify the cause of death, impact on seabirds and other biological victims, and the extent of detrimental human activity on seabird populations [13,19,28]. In conservation projects concerning wild birds, it is essential to determine any disease-causing agents, such as parasites, to assess the health risks and to avoid disease outbreaks [3,15]. Therefore, we conducted postmortems and helminthological surveys to construct base data for the conservation of seabirds and prevention of parasitic disease outbreaks.

Materials and Methods

Twenty-five oiled seabird carcasses were collected at the beach between Hamamasu (Chiyoshibetsu, Poro, Kunnbetsu, Kawashimo, Kashiwagi and Bishabetsu) and Ishikari (Estuary of Ishikari River) (Fig. 1). These included the Greater scaup *Aythya marila* (Linnaeus, 1761) (n=1), Japanese cormorant *Phalacrocorax capillatus* (Temminck & Schlegel, 1850) (n=1), Slaty-backed gull *Larus schistisagus* Stejneger, 1884 (n=1), Long-billed murrelet *Brachyramphus perdix* (Gmelin, 1789) (n=2), Ancient murrelet *Synthliboramphus antiquus* (Gmelin, 1789) (n=15), Crested auklet *Aethia cristatella* (Pallas, 1769) (n=2), Rhinoceros auklet *Cerorhinca monocerata* (Pallas, 1811) (n=1) and unidentified Alcidae gen. sp. (n=2). All specimens were taken to the Wild Animal Medical Center (WAMC) of Rakuno Gakuen University, Japan. Despite two unidentified Alcidae gen. sp. carcasses having been heavily preyed upon (only wings and the breast bone remained), they were identified

as *S. antiquus* based on their mitochondrial cytochrome b gene sequence, obtained using the PCR-RFLP method [36]. Although eight alcid birds (probably *S. antiquus*) were also found, they could not be recovered. Two individuals, one Red-throated loon *Gavia stellata* (Pontoppidan, 1763) and one *S. antiquus*, were rescued alive and taken to a local veterinarian to be washed and treated. However, they died a few days later from multiple organ failure due to crude oil ingestion. This was confirmed by postmortem (Dr. Saito, S. pers. comm.).

Before postmortems were performed, the carcasses from WAMC were numbered and tested for Avian Influenza Virus antibodies using the rapid diagnostic device Quick-S Influenza [A, B] (Denka Seiken) (Table 1). The birds' nutritional statuses were classified according to their subcutaneous fat levels (+: sparse, ++: thin fat covering, +++: thick fat covering). Their oil contamination level was classified according to the percentage of their body surface covered by oil (-: 0%, +: 1~30%, ++: 31~80%, +++: over 80%) (Table 1).

Helminthological investigations were performed on 21 of the 25 seabirds' remaining visceral organs (*A. marila* (n=1), *P. capillatus* (n=1), *B. perdix* (n=2), *S. antiquus* (n=14), *A. cristatella* (n=2), and *C. monocerata* (n=1)). The organs were examined under a binocular microscope, and about 5 g of pectoral muscle tissue from each individual was examined for *Trichinella* larvae using thick glass plates. Helminths obtained from the carcasses were preserved and fixed in a 70% ethanol solution. Nematodes and acanthocephalans were stained in a lacto-phenol solution, and trematodes and cestodes were stained with an acetocarmin solution for microscopic identification. Morphological and biometric data were recorded using a camera lucida (OLYMPUS Model DP20). All helminth specimens are preserved at the WAMC.

Results

The seabirds had oil adhering to their body surface, especially their abdominal area. About 80% of them were heavily contaminated (Fig. 2, Table 1) and had sufficient subcutaneous fat, except for *C. monocerata*. The gross pathological findings of the visceral organs are summarized and include gastric ulcers, blackish liquid contents in the intestinal mucosa, pulmonary edema, enlarged spleens, and stasis of the hearts (Table 2). Gastric ulcers ranged in diameter from 0.5–1.5 cm and were associated with nematodes. They were found in the proventriculus of eight individuals of *S.*

antiquus and one of *A. cristatella* (Table 2, Fig. 3). Numerous yellow punctate erosions, approximately 2 mm in diameter, were also noted around the periphery of the gastric ulcers. These appeared to be induced by nematodes. Adult and immature *Contraecum* sp. were consistently found tightly attached to the ulcers, and the burden of nematodes in the stomach was recorded as being up to a hundred nematodes per bird. Acute gastric ulcers consisted of extensive depressions in the mucosa with numerous hemorrhagic foci located on the edges and in the deeper parts of the lesions.

Thirteen helminth species, including eight nematode, three trematode, one cestode and one acanthocephalan species were obtained and identified. The presence, infection site, and number of each helminth species was recorded (Table 3). All individuals that were examined were found to be infected with at least one helminth species, but no *Trichinella* larvae were observed. Their morphological characters are described below.

Three of the nematode species were identified as capillariids due to their thin, filiform body; divided esophagus and the glandular ones surrounded by longitudinally attenuated rows of moniliform cells (stichocytes); one spicule including a long tubular spicule sheath; and eggs with a plug at each pole [9,26]. Two genera, *Eucoleus* and *Baruscapillaria*, were identified by the characteristic caudal end of the males. *Eucoleus* has two small rounded dorsolateral processes with a small papilla on each, pseudobursa closely surrounded by a cuticle linking the processes with a reduced dorsal cuticle membrane, and an armed spicular sheath [7,26]. One species was identified as *E. contortus* (Creplin, 1839) because it parasitizes the esophagus of aquatic birds and has a spiked section on the spicular sheath, which is 0.29–0.60 mm in length [7]. *Baruscapillaria*, however, has pre-bursal lateral alae absent and is supported on either side by one lateroventral process—each bearing one papilla—a well-sclerotized spicule, and an unarmed spicular sheath [8, 26]. Two species were identified. One obtained from the small intestine of *A. marila* has been identified as *B. mergi* (Madsen, 1945) by its vulvar appendage, which has a rounded projection, and a spicule 0.7–1.3 mm in length [8]. The other species, from the small intestine of *S. antiquus*, resembles *B. carbonis* (Dubinin and Dubinina, 1940), but was identified as *B. rudolphii* Moravec, Scholz and Nasincova, 1993 owing to a reduced male bursa composed of two lateral lobes, a spicule length of

0.9–1.3 mm (1.25 mm in the present specimen), and the absence of a vulva appendage in the females [8,14]. The trichostrongylids, classified as such by a club-shaped esophagus and a cuticular bursa supported by a number of rays at the end of the male, were obtained from the gizzard of *A. marila* and have been identified as *Amidostomum acutum* (Lundahl, 1848) by a shallow buccal capsule with thin walls and one triangular tooth with a sharp tip on the bottom, trilobate male caudal bursa, and a spicule divided into three branches [9]. Two species of ascaridids belonging the genus *Contraecum* were obtained from the digestive tracts of *S. antiquus*, *C. monocerata*, *A. cristatella*, *B. perdix* and *P. capillatus*, and identified by their thick cuticle, mouth surrounded by three well-developed lips, and a ventriculus with an intestinal cecum situated between the esophagus and intestine [9]. They have been identified as *C. rudolphii* Hartwich, 1964, by the morphology of their lip pulp in the anterior parts, which forms two lobes, each divided into a rounded lateral and a rounded medial lobe; interlabia with distinctly bifurcated tips reaching four fifths of the length of the lips; and the spicules 4–10 mm in length [9]. Three spirurids were found from the digestive tracts of *A. marila*, *B. perdix* and *C. monocerata*. They were identified by their mouths surrounded by two lateral lips; a pharynx present; and the male having a caudal wing [9,11]. One of them, obtained from the proventriculus of *A. marila*, was identified as *Tetrameres fissispina* (Diesing, 1861) by the longitudinal rows of cuticular spines on the male body surface, lateral cuticular appendage on the head end and by the length of the spicule [9]. The others belong to the family *Acuariidae* because their anterior body has cuticular cordons beginning on the side of their lips [11]. One of them, which was taken from the esophagus of *B. perdix*, was identified as *Cosmocephalus obvelatus* (Creplin, 1825) because of its widened anterior body with cordons forming a characteristic loop and a scalloped inner border [9,11]. The other, obtained from the small intestine of *C. monocerata*, was identified as *Stegophorus stercorarii* (Leonov, Sergeeva and Tsimbalyuk, 1966), by each head end having a helmet-like structure composed of two lateral membranes, 24–30 denticles on the helmet-like structure, and a large spicule without striations [9,11]. Three trematode species were found. The first, from the small intestine of *S. antiquus*, was identified as *Aporchis* sp. by an oral sucker surrounded by a single row of spines on the cephalic collar, the ventral

cylinder of the cephalic collar being absent, the cirrus sac extending beyond the posterior edge of the ventral sucker, and the vitellaria extending anteriorly to be level with their testis [34]. The second species, from the trachea of *A. marila*, was identified as *Hyptiasmus* sp. because it lacked intestinal diverticula, the gonads were aligned longitudinally, and the ovary deviated towards the side of the anterior testis [34]. Another, obtained from the kidneys of *S. antiquus*, *A. cristatella*, *B. perdix* and *P. capillatus*, was identified as *Renicola* sp. because it parasitized the renal system, the long, loop uterus extended into both left and right halves of the body, the testis were posterior to the ovary, and the cirrus sac was absent [12,34]. Because the specimens of *Aporchis* sp., *Hyptiasmus* sp. and *Renicola* sp. were severely degraded, stained blackish, or immature, we could not identify them definitively. Also, the genus *Renicola* has been reported to have at least 50 species, but we could not name them specifically as the characteristic form criteria could not be observed in these gravid specimens [12].

Cestodes obtained from the small intestine of *A. marila* belong to the family Hymenolepididae because the scolex has four suckers and a rostellum armed with a single crown of hooks [33]. The specimens were identified as *Diorchis nyrocae* (Yamaguti, 1935) by the 10 hooks, each 24–27 µm in length, on their rostellum crown, two testis measuring 16–22 µm x 18–36 µm, an isolated ovary in each mature proglottis, and the cirrus pouch being elongated, club-shaped, and about one fourth the length of the proglottis breadth [33,39].

One acanthocephalan species was obtained from the small intestine of *P. capillatus* and classified as a member of the family Polymorphidae due to a short neck, the anterior part of the pyriform body armed with cuticular spines, and an oval-shaped proboscis armed with hooks [1,40]. It was further identified as *Andracantha phalacrocoracis* (Yamaguti, 1939) because the proboscis was arranged in 16 rows, each with 10–13 hooks [40].

Discussion

With the exception of one, *C. monocerata*, the seabirds examined in this survey were heavily contaminated by crude oil, especially on the abdominal surface of their bodies. If the birds had died a long time after exposure to the oil, we would expect them to be quite thin [19,27,28,30]. However, almost all of the seabirds examined still retained stored subcutaneous fat. Also, the birds' bodies were very wet, and pul-

monary edema and acute gastric ulcers were observed in most of the birds. Oiling of a bird's plumage reduces its waterproofing properties and leads to increased heat loss. Birds with oiled plumage must increase their metabolic heat production and energy expenditure to conserve their body temperature [13,17,18]. If the oiled birds reside in water, their heat loss may exceed their maximum capacity for heat production, resulting in hypothermia [17,18]. The metabolic rates of oiled birds are higher and the body temperatures lower than those of non-oiled birds [17-19,27]. Because pulmonary edema can also be caused by disease, it is not clear whether the birds developed the edema from drowning or disease because of the lungs endured the effect of freezing and thawing the corpse to remain strong [19].

Enteritis, dissociation of intestinal mucosa and immunodeficiency are effects of crude oil ingestion [10,19,38]. Acute gastric ulcers and penetration of the gastric wall by several nematodes were observed in 52.3% of examined birds (table 2). Liu and Edward [25] reported gastric ulcers associated with *Contraecium* spp. in the Stellar Sea Lion *Eumetopias jubatus* and White Pelican *Pelecanus erythrorhynchos*, and extensive inflammatory reaction caused by nematodes penetrating deep into the gastric wall. In this case, the ulcers were acute and had nematodes present. The birds had pale musculature and blood was present in the lumen of the proventriculus (Fig. 3). This suggests that the seabirds were weakened after ingesting the oil, making it easy for nematodes to penetrate the gastric mucosa [10,19]. Several postmortems of non-oiled alcid birds have been conducted in WAMC, with no observations of gastric ulcers [Yoshino et al. unpublished].

The gut contents of the examined birds included mainly food, intestinal mucosa, blood, or body fluids. A blackish liquid was found in the intestines of some of the seabirds, including two of *S. antiquus*, one of *B. perdix*, and one of *C. monocerata* (Fig. 4). This suggests that most of the seabirds we examined swallowed a small amount of oil, and that they died before being affected by the ingestion of the oil. However, these findings might be a result of acute nutritional problems, a state of anemia caused by gastrointestinal bleeding, increased viscosity of the blood due to fluid lost to the gastrointestinal tract, or acute cardiovascular failure caused by ingesting oil [19,21,29].

In several cases, inflammatory lesions and/or the precipitation of uric acids has been observed in the kid-

neys of oiled seabirds [10,24,37]. Although many trematodes and their eggs were observed in the renal parenchyma and tubular, no evidence of an inflammatory response was found.

Taking observations of the seabirds from this survey into consideration, it is proposed that most of the birds ingested large amounts of oil, which caused digestive, metabolic and circulatory system defects [16-18,24]. It further suggests that the birds deteriorated rapidly and were killed by the oil adhering to their feathers, causing them to lose water repellency and buoyancy, and resulting in them dying after a steep decrease in temperature.

In addition, some of the carcasses were severely preyed upon, particularly *L. schistisagus* and two individuals of *S. antiquus*. This suggests that there may be secondary oil pollution affecting other animals such as crows, gulls, raptors and mammals. Seabirds contaminated by crude oil have been found in the stomach of a dead Steller's sea eagle *Haliaeetus pelagicus* (Pallas, 1811) in 2006 at Shiretoko Peninsula [23]. Thus, oil pollution not only affects seabirds (directly and/or indirectly), but it also affects the surrounding ecosystem, which includes predators and scavengers.

There are several reports of parasitic helminths found in seabirds from Japan (Table 4). These include 13 nematodes (*E. contortus*, *B. mergi*, *Trichuris* sp., *Syngamus* sp., *A. acutum*, *C. rudolphii*, *C. microcephalum* Rudolphi, 1809, *Porrocaecum pharacrocacis* Yamaguti, 1941, *Procammallanus* sp., *T. fisispina*, *Paracuaria adunca* (Creplin, 1831), *S. stercorarii*, *Streptocara crassicauda* (Creplin, 1829)), 10 trematodes (*Aporchis* sp., *Acanthoparyphium kurogamo* Yamaguti, 1939, *A. marilae* Yamaguti, 1934, *A. spinulosum* suzugamo Yamaguti, 1939, *Echinoparyphium pharacrocacis* Yamaguti, 1939, *Echinostoma revolutum* (Froelich, 1802), *Paramonostomum* (*Paramonostomum*) *bucephalae* (Yamaguti, 1935), *Paryphostomum radiatum* (Dujardin, 1845), *Renicola* sp., *Trichobilharzia* sp.), two cestodes (*D. nyrocae*, *Drepanidotaenia nyrocae* (Yamaguti, 1935)) and an acanthocephalan (*A. phalacrocoracis*) [2,20,22,35,41,42]. Of the helminths, *B. rudolphii* from *S. antiquus*, *C. rudolphii* and *Renicola* sp. from *A. cristatella*, and *C. obveratus* and *Renicola* sp. from *B. perdix* are first host records. It is also the first geographical record of *B. rudolphii* in Japan.

Members of the nematode genus *Amidostomum* are regarded as highly pathogenic agents in waterfowls (mainly Anatidae) and acanthocephalans, including

the genus *Andracantha*, are regarded as pathogens causing severe, and sometimes fatal, enteritis in various wild and captive birds [3,5,15]. The acuariid nematodes (*C. obvelatus* and *S. stercorarii*) are cosmopolitan and nearly cosmopolitan in distribution, and are pathogens that cause severe esophagitis and ventriculitis; sometimes resulting in fatal cases in wild and domestic birds [5,6,15]. The renal trematode, *Renicola* sp., was previously reported as a possible cause of renal fibrosis and inflammation of the renal tubular as a result of its body and eggs creating a blockage [37]. Furthermore, the family Cyclocoelidae, including the genus *Hyptiasmus*, has been reported as a pathogenic agent that is sometimes fatal in wild and captive birds [5,31]. *Hyptiasmus* sp. has been recorded from Whooper Swan, *Cygnus cygnus*, and *H. arcuatus* has been recorded from Bufflehead, *Bucephala albeola*, and Smew, *Mergus albellus*, both in Japan [4,35].

Although no evidence of pathogenicity or mortality could be directly attributed to helminth infections in this survey, with the exception of *C. rudolphii* in the gastric ulcers of some of oiled birds, the occurrence and intensity of the helminths were higher in oiled alcid birds than in non-oiled ones subject to postmortem in WAMC [Yoshino et al. unpublished]. It appears that birds subjected to stress (e.g., captured, experiencing deteriorating habitat) are more susceptible to parasitic infection than those from intact wild populations [13,15]. Several helminthiasis, including fatal cases caused by these helminthes, were reported in the wild and captive birds [5,15,25,31]. Because some highly pathogenic helminths were identified from the seabirds in Japan, special attention to these parasitic infections is required when considering their conservation.

Acknowledgements

The present survey was supported in part by the Strategic Research Foundation at Private Universities (2013-2017) and by the grants-in-Aid for Scientific Research (Kakenhi-Kiban C 26460513) from the Ministry of the Education, Science and Culture of Japan. We are grateful for donation of the seabird samples provided by the 1st Regional Coast Guard Headquarters of Japan Coast Guard, Maritime Disaster Prevention Center, Institute of Geological Survey of Hokkaido and Natural Environmental Division of Hokkaido Prefecture. Thanks are also due to Fujimaki, Y., Hamada, S., Kawasaki, Y., Kawasaki, S., Kurosawa, N., Morita, T., Saito, S., Saito, K., Shiraki,

S., Takeda, T., Yokohata, Y. and Watanabe, Y. for their kind advice and expertise during this study, and to Aizawa, K., Sato, N., Ishii, K. and Ishii, S. for their assistance during postmortems and sampling.

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石狩湾重油流出事故において死亡した海鳥類の剖検所見および寄生蠕虫類検査

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和文摘要

2004年11月13日、北海道石狩市の石狩湾新港にて転覆した貨物船マリン・オオサカ号から流出した重油により死亡し、浜益町から石狩川河口にかけて漂着した海鳥7種25個体について、剖検および寄生虫検索を実施した。死体は重油が多量に付着していたが、皮下脂肪は十分に蓄えられていた。主だった剖検所見は、胃潰瘍、肺水腫、脾腫、腸管内の黒色液状異物であり、これらの所見から、海鳥は重油の付着による急激な体温低下、浮力喪失などにより死亡したと考えられた。各臓器には、直接死因と関係したと考えられるような所見は見られなかったが、2個体で、胃潰瘍部に多数の線虫幼虫 (*Contraecum* 属) が刺さり、急性の炎症反応が見られたものがあった。また、臓器の残存していた6種21個体の寄生蠕虫類を検索したところ、線虫8種 (*Eucoelus contortus*, *Baruscapillaria mergi*, *B. rudolphii*, *Contraecum rudolphii*, *Amidostomum acutum*, *Tetrameres fissispina*, *Cosmocephalus obvelatus*, *Stegophorus stercorarii*)、吸虫3種 (*Aporchis* sp., *Hyptiasmus* sp., *Renicola* sp.)、条虫1種 (*Diorchis nyrocae*)、鉤頭虫1種 (*Andracantha phalacrocoracis*) の計13種が検出された。これらのうち、ウミスズメからの *B. rudolphii*、エトロフウミスズメからの *C. rudolphii* と *Renicola* sp.、マダラウミスズメからの *C. obvelatus* と *Renicola* sp. は新宿主記録であり、*B. rudolphii* は国内初記録であった。

キーワード：蠕虫類、油汚染、剖検、海鳥

Table 1: Oiled seabirds collected in 2004 at Ishikari, Hokkaido

AS ^{*1}	Species	Sex	Age	Coll. date	Coll. Loc.	AIV ^{*2}	Fat covering ^{*3}	Oil adhesion on body surface ^{*4}
5004	<i>Cerorhinca monocerata</i>	F	A	11/18	Chiyoshibetsu	-	+	+++
5005	<i>Aethia cristatella</i>	M	A	11/18	Chiyoshibetsu	-	+++	+++
5006	<i>Synthliboramphus antiquus</i>	M	A	11/18	Chiyoshibetsu	-	+++	+++
5007	Alcidae gen. sp. ^{*6}	U	U	11/18	Chiyoshibetsu	×	Not classifiable	Not classifiable
5008	<i>Aethia cristatella</i>	M	A	11/18	Chiyoshibetsu	-	+++	+++
5009	<i>Phalacrocorax capillatus</i>	M	A	11/18	Poro	-	+++	+++
5010	<i>Larus schistisagus</i>	U	J	11/18	Poro	×	Not classifiable	+++
5011	<i>Synthliboramphus antiquus</i>	M	A	11/18	Kunnebetsu	-	+++	+++
5012	<i>Brachyramphus perdix</i>	F	A	11/18	Kunnebetsu	-	++	+++
5013	<i>Synthliboramphus antiquus</i>	F	A	11/18	Kunnebetsu	-	+++	+++
5014	<i>Synthliboramphus antiquus</i>	F	A	11/18	Kunnebetsu	-	+++	+++
5015	<i>Aythya marila</i>	F	A	11/18	Kunnebetsu	-	+++	+++
5016	<i>Synthliboramphus antiquus</i>	F	A	11/17	Kashiwagi	-	+++	++
5017	<i>Synthliboramphus antiquus</i>	M	A	11/17	Bishyabetsu	-	+++	++
5018	<i>Synthliboramphus antiquus</i>	M	A	11/17	Estuary of Ishikari River	-	+++	_*5
5019	<i>Synthliboramphus antiquus</i>	M	A	11/18	Kawashimo	-	+++	+++
5020	<i>Synthliboramphus antiquus</i>	F	A	11/18	Kawashimo	-	+++	+++
5021	<i>Synthliboramphus antiquus</i>	M	A	11/18	Kawashimo	-	+++	+++
5022	<i>Synthliboramphus antiquus</i>	M	A	11/18	Kawashimo	-	+++	+++
5023	<i>Synthliboramphus antiquus</i>	F	A	11/18	Kawashimo	-	+++	+++
5024	Alcidae gen. sp. ^{*6}	U	U	11/18	Kawashimo	×	Not classifiable	Not classifiable
5025	<i>Synthliboramphus antiquus</i>	M	A	11/19	Poro	-	+++	+++
5026	<i>Synthliboramphus antiquus</i>	M	A	11/19	Poro	-	+++	+++
5027	<i>Synthliboramphus antiquus</i>	M	A	11/19	Poro	-	+++	+
5028	<i>Brachyramphus perdix</i>	M	A	11/19	Poro	×	+++	+++

*1. Specimen serial number of WAMC

*2. Rapid diagnostic test of Avian Influenza Virus

*3. amount of subcutaneous fat; +: only sparse fat, ++: fat covering entire thin, +++: thick fat covering entire

*4. oil adhering score of body surface; -: 0%, +: 0~30%, ++: 30~80%, +++: over 80%

*5. Washed by rescue team before came from WAMC

*6. Heavily preyed and remain wings and breast bone, could not identified for species

Table 2. Occurrence of various postmortem findings in oiled seabirds

Species	Necropsy findings (observed / examined)				
	Gastric ulcer	Pulmonary edema	Blackish liquid contents in digestive tract	Hemorrhage in digestive tract	Enlarged spleen
<i>Synthliboramphus antiquus</i>	10/14	12/14	2/14	3/14	1/14
<i>Aethia cristatella</i>	1/2	-	-	-	1/2
<i>Brachyramphus perdix</i>	-	2/2	1/2	1/2	-
<i>Cerorhinca monocerata</i>	-	1/1	1/1	1/1	-
<i>Phalacrocorax capillatus</i>	-	-	-	-	-
<i>Aythya marila</i>	-	1/1	-	1/1	-

Table 3. Helminths obtained from oiled seabirds

Parasitic helminths	Site	Host	Occurrence		Intensity		
			Infected	%	mean	SE	range
Nematoda							
Enoplida							
Trichinelloidea							
Trichuridae							
<i>Eucoelus contortus</i>	oe	<i>Aythya marila</i>	1/1	100	2	-	2
<i>Baruscapillaria mergi</i>	si	<i>Aythya marila</i>	1/1	100	5	-	5
<i>Baruscapillaria rudolphii</i>	si	<i>Synthliboramphus antiquus</i>	1/14	7.14	2	-	2
Strongylida							
Trichostrongyloidea							
Amidostomatiidae							
<i>Amidostomum acutum</i>	gi	<i>Aythya marila</i>	1/1	100	55	-	55
Ascaridida							
Ascaridoidea							
Anisakiidae							
<i>Contraecaecum rudolphii</i>	pr, gi, si	<i>Synthliboramphus antiquus</i>	14/14	100	72.21	22.56	5-346
	pr, gi, si	<i>Aethia cristatella</i>	2/2	100	72.5	68.5	4-141
	pr	<i>Cerorhinca monocerata</i>	1/1	100	4	-	4
	gi	<i>Brachyramphus perdix</i>	1/2	50	1	-	1
	pr, gi, si	<i>Phalacrocorax capillatus</i>	1/1	100	194	-	194
Spirurida							
Habronematoidea							
Tetrameridae							
<i>Tetraneres fissispina</i>	pr	<i>Aythya marila</i>	1/1	100	60	-	60
Acuariiidea							
Acuariidae							
<i>Cosmocephalus obveratus</i>	oe	<i>Brachyramphus perdix</i>	1/2	50	3	-	3
<i>Stegophorus stercorarii</i>	gi	<i>Cerorhinca monocerata</i>	1/1	100	1	-	1
Trematoda							
Strigeitida							
Cyclocoelidae							
<i>Hyptiasmus</i> sp.	tr	<i>Aythya marila</i>	1/1	100	3	-	3
Echinostomatiida							
Echinostomatiidae							
<i>Aporchis</i> sp.	si	<i>Synthliboramphus antiquus</i>	1/14	7.14	4	-	4
Rencolida							
Rencoliidae							
<i>Rencola</i> sp.	si, ki	<i>Synthliboramphus antiquus</i>	7/14	50	231.29	113.2	1-708
	ki	<i>Aethia cristatella</i>	2/2	100	5	3	2-8
	ki	<i>Brachyramphus perdix</i>	2/2	100	7.5	6.5	1-14
	ki	<i>Phalacrocorax capillatus</i>	1/1	100	15	-	15
Cestoda							
Cyclophyllidea							
Hymenolepididae							
<i>Diorchis myrocae</i>	si	<i>Aythya marila</i>	1/1	100	6	-	6
Acanthocephala							
Polymorphida							
Polymorphidae							
<i>Andracantha phalacrocoracis</i>	si	<i>Phalacrocorax capillatus</i>	1/1	100	1	-	1

*Abbreviation of sites; oe: oesophagus, pr: proventriculus, gi: gizzards, si: small intestine, tr: trachea, ki: kidney

Table 4: Helminths previously reported from seabirds examined in present survey in Japan

Seabirds	Helminths	Groups*	Locality (Pref.)	References
<i>Synthliboramphus antiquus</i>	<i>Contracaecum rudolphii</i>	N	Ishikawa	[41]
	<i>Paracuaria adunca</i>	N	Ishikawa	[41]
	<i>Stegophorus stercorarii</i>	N	Ishikawa	[41]
	<i>Aporchis</i> sp.	T	Ishikawa	[41]
	<i>Renicola</i> sp.	T	Ishikawa	[41]
<i>Brachyramphus perdix</i>	<i>Contracaecum rudolphii</i>	N	Ishikawa	[41]
<i>Cerorhinca monocerata</i>	<i>Contracaecum rudolphii</i>	N	Yamagata to Fukui	[41]
	<i>Streptocara crassicauda</i>	N	Niigata	[41]
	<i>Syngamus</i> sp.	N	Niigata	[41]
	<i>Aporchis</i> sp.	T	Niigata and Hokkaido	[2][41]
	<i>Renicola</i> sp.	T	Ishikawa	[41]
<i>Aythya marila</i>	<i>Eucoleus contortus</i>	N	Hokkaido	[42]
	<i>Baruscapillaria mergi</i>	N	Hokkaido	[42]
	<i>Amidostomum acutum</i>	N	Hokkaido	[42]
	<i>Tetrameres fissispina</i>	N	Hokkaido	[42]
	<i>Acanthoparyphium kurogamo</i>	T	Yamaguchi	[35]
	<i>Acanthoparyphium marillae</i>	T	Shiga	[35]
	<i>Acanthoparyphium spinulosum suzugamo</i>	T	Aichi and Yamaguchi	[35]
<i>Phalacrocorax capillatus</i>	<i>Echinostoma revolutum</i>	T	Kanto District	[35]
	<i>Paramonostomum (Paramonostomum) bucephalae</i>	T	Yamaguchi	[35]
	<i>Trichobilharzia</i> sp.	T	Aichi	[35]
	<i>Diorchis nyrocae</i>	C	Shiga and Kanto District	[35]
	<i>Drepanidotaenia nyrocae</i>	C	Shiga	[35]
	<i>Contacaecum microcephalum</i>	N	Kanto District	[35]
	<i>Contracaecum spiculigerum</i> = <i>Contracaecum rudolphii</i>	N	Mie and Kanto District	[35]
<i>Porrocaecum phalacrocoracis</i>	N	Mie	[35]	
<i>Phalacrocorax capillatus</i>	<i>Procamallanus</i> sp.	N	Kanto District	[35]
	<i>Trichuris</i> sp.	N	Kanto District	[20]
	<i>Echinoparyphium phalacrocoracis</i>	T	Japan	[35]
	<i>Paryphostomum radiatum</i>	T	Mie	[35]
	<i>Retevitellus phalacrocoracis</i>	T	Oita	[22]
	Stigeidae gen. sp.	T	Hokkaido	[2]
	<i>Andracantha phalacrocoracis</i>	A	Oita	[22]

*Abbreviation of Groups; N: Nematoda, T: Trematoda, C: Cestoda, A: Acanthocephala

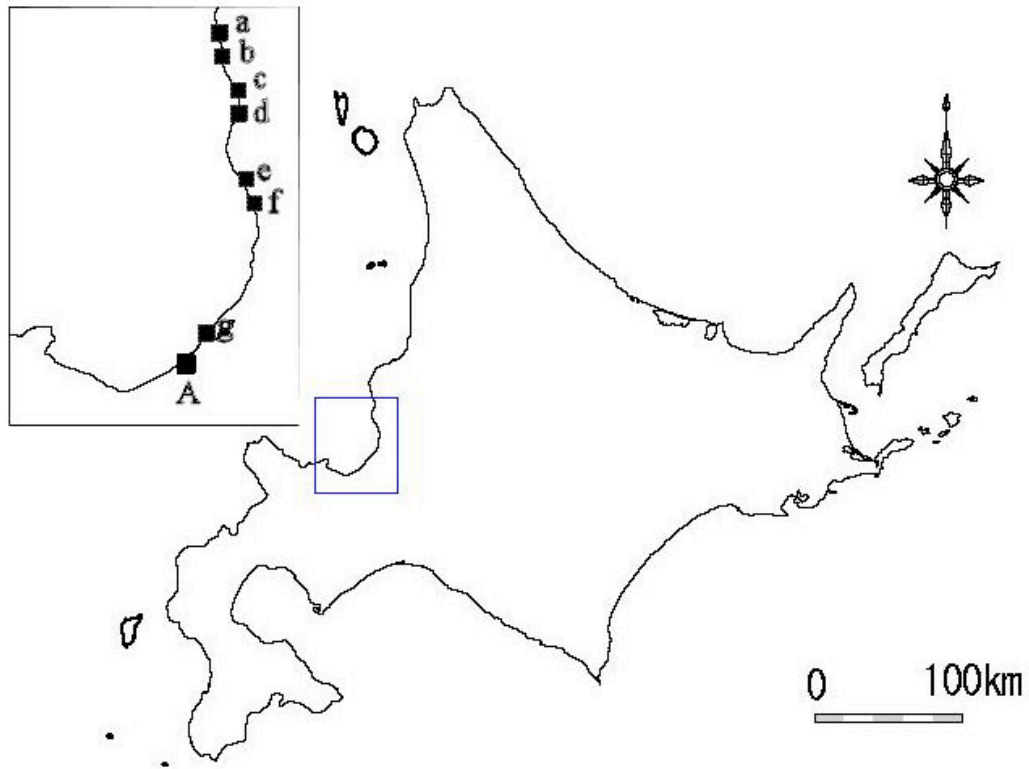


Fig. 1 Locations where the freighter overturned and oiled seabirds were collected (A: Ishikari Bay New Port, a: Chiyoshibetsu, b: Poro, c: Kunnbetsu, d: Kawashimo, e: Kashiwagi, f: Bishyabetsu, g: Estuary of Ishikari River)



Fig. 2 Synthliboramphus antiquus heavily oil adhered



Fig. 3 Gastric ulcer in the proventriculus of *Synthliboramphus antiquus*, and several nematodes piercing into the gastric wall



Fig. 4 Blackish liquid contents in the small intestine of *Cerorhinca monocerata*