Elemental Phosphorus as the cause of waterfowl mortality in an Alaskan salt marsh

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Abstract

The yearly death of an estimated 1000-2000 migrating dabbling ducks (*Anas* sp.) and 10-50 swans (*Cygnus* sp.) has been documented for the last ten years in Eagle River Flats, an estuarine salt marsh near Anchorage, Alaska. This marsh has been used over the past four decades for artillery training by the U.S. Army. The evidence presented here strongly supports the hypothesis that feeding waterfowl are ingesting small particles of the highly toxic incendiary munition white phosphorus (P_4) stored in the bottom anoxic sediments of shallow salt marsh ponds. Farm-reared mallards dosed with P_4 showed nearly identical behavioral symptoms to those of wild ducks that became sick in Eagle River Flats. P_4 does not occur in nature but was found in both the sediments where dabbling ducks and swans feed and in the gizzards of all 19 carcasses collected in Eagle River Flats.

Massive die-offs of waterfowl are uncommon but have occurred in the past due to avian diseases (1), pesticides such as organo-phosphates (2), oil spills (1,3), and lead shot in the sediments of shallow ponds in which waterfowl feed (4). Since 1980, an estimated 1000-2000 waterfowl deaths have been observed each year at Eagle River Flats, a 1000-ha estuarine salt marsh complex on Cook Inlet near Anchorage, Alaska (Figure 1). This salt marsh has been used since 1949 as a primary impact area for artillery training by the military at Ft. Richardson. Eagle River Flats is inhabited by waterfowl primarily during spring and fall migrations when the deaths have been observed. Avian disease, cholinesterase inhibition by pesticides, predation, trauma, or direct injury from explosions and metal fragments have been excluded as the primary cause of death. Poisoning from lead, other heavy metals, or well-known organic toxicants such as the insecticide DDT and polychlorinated biphenyls (PCBs) were also ruled out (5,6).

In the spring of 1990, we investigated the possibility that munitions compounds fired into the salt marsh are the cause of the mortality. Dabbling ducks including pintails (*Anas acuta*), mallards (*A. platyrhynchos*) , and green-winged teal (*A. carolinensis*), as well as trumpeter swans (*Cygnus buccinator*) and tundra swans (*C. columbianus*), are the most common species found dead. These waterfowl species feed mainly in the bottom sediments, unlike other unaffected species such as geese that are common in Eagle River Flats during the migrations (6). For this reason, we hypothesized that the toxin was located in the bottom sediments of the shallow salt marsh ponds in which the dabbling ducks feed. Of the various munitions compounds that have been fired into Eagle River Flats, the smoke-producing incendiary, white or elemental phosphorus (P_4), became a prime suspect. White phosphorus is known to be highly toxic to waterfowl (7) and other animals including humans (8). Release of colloidal P_4

from an arsenal (Pine Bluffs, Arkansas) (9) and a white phosphorus manufacturing plant (Long Harbour, Newfoundland) (10) into fresh and marine waters respectively caused massive fish kills.

P_4 does not exist in nature since it spontaneously oxidizes in the presence of oxygen to form phosphorus oxides. When applied to well-drained upland soils, P_4 has been shown to oxidize and even provide a source of phosphate to growing plants (11). However, when smoke rounds introduce P_4 into a salt marsh environment with anaerobic sediments (12) and standing water, incomplete combustion and storage in the sediments is possible. We conducted both field and laboratory studies of waterfowl and sediments from Eagle River Flats and made an assessment of the toxicity of P4 in the laboratory.

During the September 1990 migration in Eagle River Flats, detailed observations of feeding ducks were made from a blind erected in a shallow salt marsh pond where large numbers of duck carcasses had been found in the past. During 49 hours of observations, we observed eight green-wing teal and one pintail duck violently convulse and subsequently die, each after four to six hours of their arrival at the pond. The first obvious signs of poisoning in each duck was rapid head shaking and repeated drinking. This behavior alternated with periods of lethargy during which the eyes were closed. These ducks also sought shelter in tall vegetation and could be readily approached. The next stage of poisoning was backward arching of the neck and head swaying while the bird swam in very tight circles. Finally, each duck convulsed, with its wings fully extended and its head arched backwards and tail up so that the head and tail nearly touched over the back. Most of the convulsing ducks would repeatedly somersault in the water and become entangled in vegetation. These signs of distress by poisoned ducks attracted numerous eagles and gulls, predators that were observed to preferentially prey on ducks dslaplaying the symptoms of P4

poisoning. Currently no data exists to show that such predation may result in deleterious effects on these avian predators or scavengers. Lipid-soluble toxins such as DDT are known to pass from prey to predator (14) and lead poisoning of eagles has occurred as a result of swallowing lead shot embedded in the flesh of their prey (1).

The behavioral symptoms of dying wild ducks from the salt marsh were compared to those of ducks dosed in the laboratory with the suspected toxicant P_4. Six adult farm-reared mallards were each gavaged with 12 mg/kg body weight of P_4 dissolved in 5 ml of oil (tricaprylin). The dosage was based on an earlier study of P_4 toxicity in ducks (7) and was known to be lethal within a few hours. Each duck was dosed separately and returned to a room containing a shallow pool, food and other untreated ducks. All laboratory procedures followed the guidelines of the institutional animal use committee.

The symptomatic behavior of mallards dosed with P_4 was similar to the behavior of wild ducks dying in Eagle River Flats. These behaviors are similar to the field symptoms reported for ducks dying from avian cholera (9). Following P_4 administration, normal activities were observed, including wing flapping, preening, drinking, bathing and frequent movement from the pool to the floor. Within one to two hours, violent head shakes with an open beak occurred, followed by more normal behavior with mild head shakes. Four to five hours after P_4 administration each duck showed uncontrollable head-shaking with an open beak and constant drinking followed by lethargy with the head placed under the edge of the pool and the eyes closed. Finally, convulsions of varying magnitude occurred ; these involved extension of the wings and arching of the head and neck over the back. Upon observation of convulsions, each duck was anesthetized with 45 mg of ketamine and killed by exsanguination and the various tissues were prepared for P_4 analysis.

Tissues were collected from both the wild and the farm-reared treated ducks. The tissues of wild birds that were observed to die in Eagle River Flats were stored in small glass vials and frozen in dry ice. In addition, carcasses of several ducks and eight tundra swans found dead in Eagle River Flats were collected, frozen and shipped to the laboratory for tissue analysis. During September, five green-winged teal were trapped in Susitna Flats, another Cook Inlet salt marsh 40 km from Eagle River Flats (Fig. 1) The tissues from these ducks were used as controls. In the laboratory, tissue samples were cut into small pieces and blended with degassed water in a nitrogen atmosphere. The tissue homogenate was extracted with isooctane by shaking for 12 hours and centrifuged for one hour to separate the organic phase from the aqueous phase. An aliquot of the isooctane phase was analyzed by gas chromatography–mass spectrometry (GCMS) to obtain positive identification of P_4 in these tissue samples. Quantitative measurement of P_4 in the tissue and sediment samples was made using gas chromatography with flame photometric detection (GC-FPD) following procedures similar to those described by Addison and Ackman (13).

P_4 was initially confirmed by GCMS in the gizzard contents of three ducks (a green winged teal, a pintail and a mallard), and one tundra swan found dead in Eagle River Flats (Fig. 2). Subsequent analyses by GC-FPD showed that the gizzard contents of all 19 waterfowl carcasses (8 swans and 11 ducks) collected in Eagle River Flats contained P_4 in widely varying amounts (Table 1). The mass of P_4 in the gizzard contents varied from a low value of 0.01 µg in a green-winged teal up to 3 mg in one mallard duck and 11 mg in a tundra swan. Clearly all of these waterfowl ingested P_4. In addition, virtually all tissues including the fat, skin and livers of these birds contained detectable concentrations of P4 (Table 1) indicating that the compound had been absorbed from the digestive

tract. Of these tissues, the fat of both wild and farm-reared ducks contained the highest P_4 concentrations as would be expected of a lipid-soluble chemical. In contrast, none of the five green-winged teal from the Susitna Flats contained P_4 in either the gizzard contents or their body fat.

All domestic mallards that were dosed orally with P4 contained P_4 in the tissues (Table 1). The highest concentrations of P_4 were detected in body fat and were generally higher than the concentrations in fat from wild birds. The mechanism by which P_4 kills waterfowl is not known.

Salt marsh sediments were collected from the shallow ponds where wild ducks were observed to feed and die. Two types of samples were collected. During the fall migration, the top 5 cm of sediments were collected at each sampling point. The depth of the overlying water as well as the pH and redox potential of the saturated sediment sample were measured. During the winter, frozen sediment cores were collected through the ice using an ice core auger. About 40 fall and 10 winter sediment samples were obtained from the shallow pond.

Ten to 20 grams of each sediment sample were placed in isooctane to extract the P_4 and were shaken for about 12 hours and analyzed by both GCMS and GC-FPD. P4 was determined by GC-FPD in six of the 20 sediment samples obtained from the shallow ponds where waterfowl were observed to become sick. Concentrations varied from 10.2 µg/g wet weight down to 0.0025 µg/g. The presence of P_4 was confirmed by GCMS in the sample with 10.2 µg/g P_4 (Fig. 2). Concentrations varied greatly in sediment subsamples from the same sample, so it is difficult to define concentrations.

Because P_4 is insoluble in water and because sediment-feeding waterfowl are poisoned, we assume that P_4 is ingested as a particulate. This assumption is supported by the great variability in P_4 concentrations between subsamples

from both the gizzard contents and the sediments. To identify particulate P_4 in the sediments, samples that tested positive for P_4 by GC-FPD were placed in a dispersing agent and then washed through a 0.150-mm mesh sieve. The material left in the sieve was placed in water and examined under a stereomicroscope for particulate P_4. Examination of five sediment samples revealed particulate P_4 in two of these samples. The P_4 particles were waxy, transparent yellow and very irregular in shape with rough surfaces and they smoked when cut and exposed to air. Particle sizes ranged from 0.14 to 1 mm in diameter. These sand-sized particles are probably easily distinguished and selected by sediment-feeding waterbirds from the silt and clay-sized (0.0002 to 0.005 mm) particles that make up the salt marsh sediments. P_4 particles were also isolated from the gizzard of a mallard carcass from Eagle River Flats in which over 3 mg of P_4 was measured with the GC. Because the P_4 particles vary greatly in size and distribution within the salt marsh sediments, not all of the dabbling ducks and swans that feed in this salt marsh become sick and die.

All evidence indicates that the incendiary and smoke-producing P_4, as a particulate in the sediments, is responsible for the death of waterfowl in Eagle River Flats: a) P_4 is highly toxic to waterfowl at ingestion levels of 3- to 5-mg/duck, b) farm-reared adult mallards, dosed with P_4, showed almost identical behavorial symptoms to those of wild ducks observed to become sick and die in Eagle River Flats, c) P_4 was detected by gas chromatography in the gizzard contents and fat of all 11 dabbling ducks and 8 tundra swan carcasses collected in Eagle River Flats but in none of five healthy teal collected in a nearby salt marsh, d) P_4 was similarly detected in several sediment samples from the bottom of the pond in which ducks feed and were observed to become sick and e) sand-sized particles of P_4 (0.015-1.0 mm) were isolated from some of these sediment samples as well as from the gizzard of one duck. Although P_4 does not

occur naturally and it spontaneously oxidizes when exposed to air, the flooded and anaerobic salt marsh sediments apparently prevent combustion and promote storage of P_4 particles.

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Figure Legends

Figure 1. Map of the Upper Cook Inlet area in southcentral Alaska (Inset) showing the location of Eagle River Flats and other estuarine salt marshes used by migrating waterfowl.

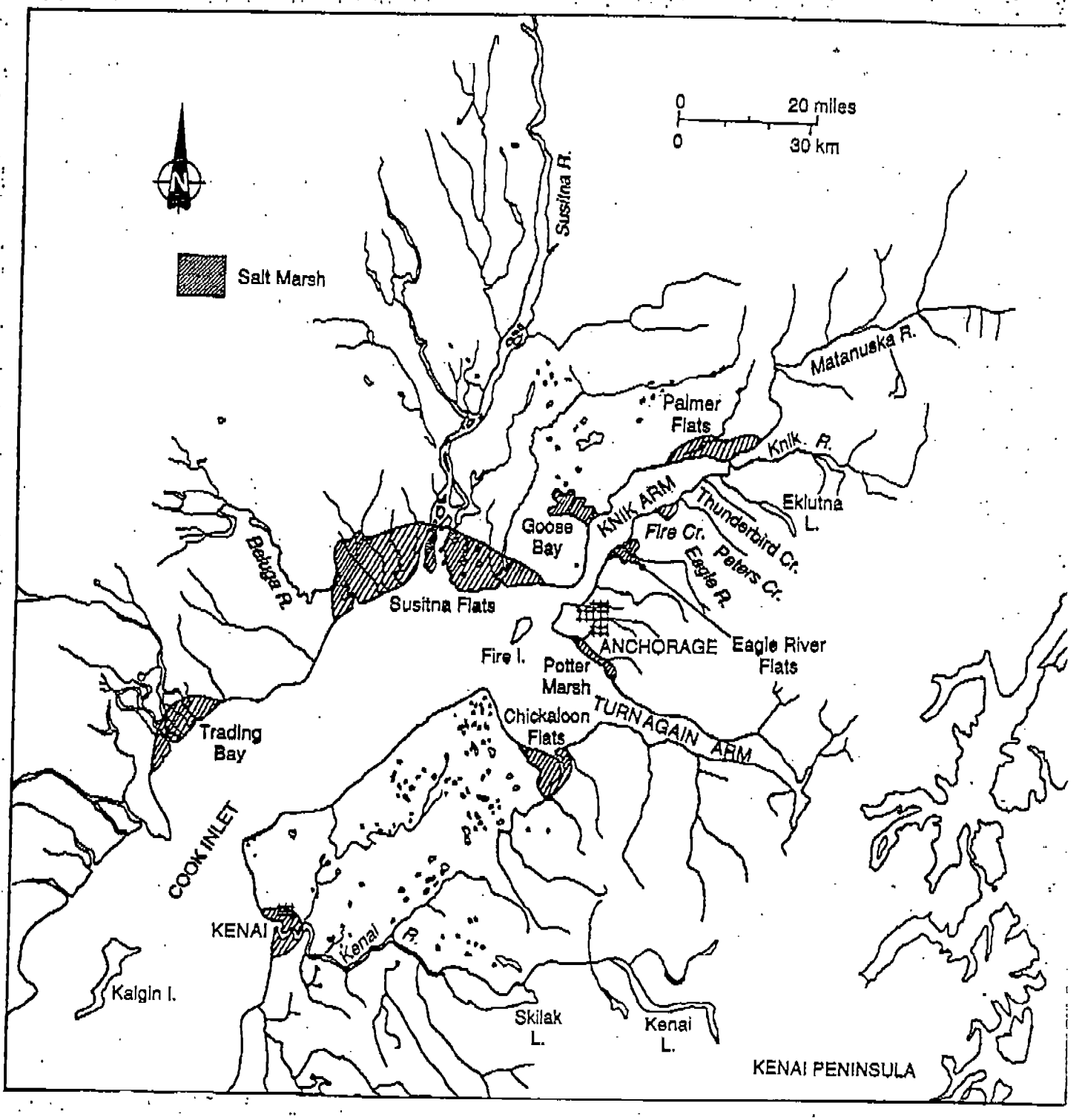
Figure 2. Mass spectra for a white phosphorus (P_4) standard, an Eagle River Flats salt marsh sediment sample and the gizzard contents of a pintail duck carcass collected in Eagle River Flats. The mass spectrum of white phosphorus has a large peak at mass 124, the molecular weight of P_4. This molecule also fragments into P, P_2 and P_3, which have masses of 31, 62 and 93 respectively.

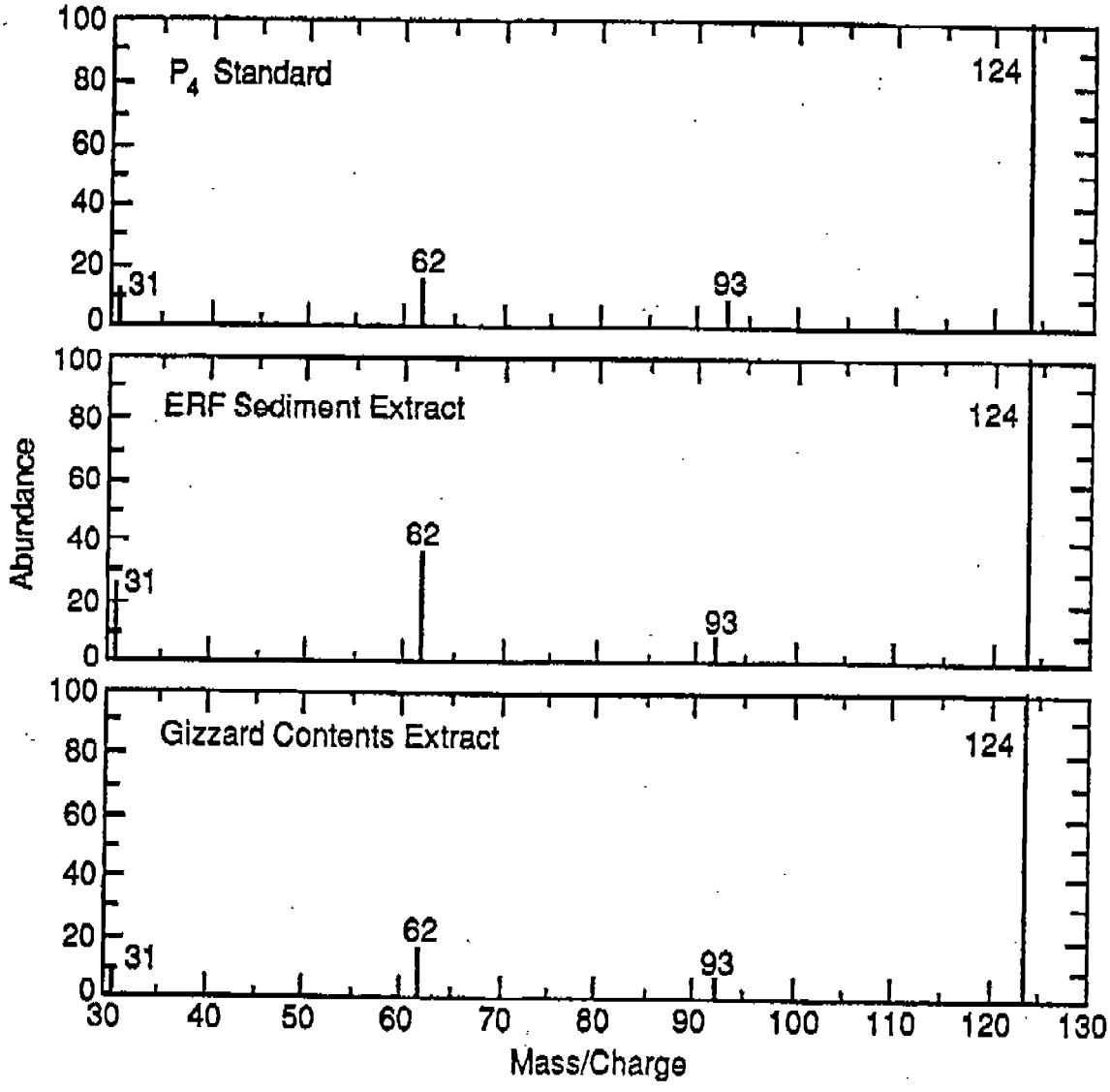
Table 1. P_4 concentrations in tissues from wild duck and swan carcasses collected in the Eagle River Flats salt marsh, from "control" green-winged teal in Susitna Flats and from adult mallards dosed with 12 mg P_4 per kg body weight in the laboratory.

TISSUE/ ORGAN	WILD SWANS (ug/g)	N*	WILD DUCKS (ug/g)	N	CONTROL DUCKS (ug/g)	N	FARM-REARED MALLARDS (ug/g)	N
Gizzard Contents								
Mean(SD)	52(95)	8	304(943)	11	0.00	5	ND†	
Range	0.02-207		0.08-3144					
Fat								
Mean(SD)	0.67(0.99)	7	0.21(0.20)	5	0.00	5	1.98(1.23)	6
Range	0.10-2.90		0.00-0.43				0.39-3.52	
Skin								
Mean (SD)	0.06(0.07)	3	0.07(.06)	4	ND		1.29(0.61)	5
Range	0.01-0.14		0.03-0.13				0.59-2.23	
Liver								
Mean (SD)	ND		0.05(0.06)	5	ND		0.25(.25)	6
Range			0.00-0.14				0.01-0.68	

* N=Number of birds analyzed

†ND=Not determined


N
Salt Marsh
0 20 miles
0 30 km
Susitna R.
Matanuska R.
Palmer Flats
Knik R.
Eklutna L.
Knik Arm
Thunderbird Cr.
Fire Cr.
Peters Cr.
Eagle R.
Goose Bay
Beluga R.
Susitna Flats
Fire I.
ANCHORAGE
Eagle River Flats
Potter Marsh
Chickaloon Flats
Turnagain Arm
Trading Bay
Cook Inlet
KENAI
Kenai R.
Kalgin I.
Skilak L.
Kenai L.
KENAI PENINSULA


P4 Standard
ERF Sediment Extract
Gizzard Contents Extract
Abundance
Mass/Charge
31
62
93
124
0
20
40
60
80
100
30
40
50
60
70
80
90
100
110
120
130