Investigation into the long-finned pilot whale mass stranding event, Pittenweem, Fife, 2nd September 2012

Final report to Marine Scotland, Scottish Government, September 2014

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Table of Contents

ABSTRACT 4
1. BACKGROUND TO THE SCOTTISH MARINE ANIMAL STRANDING SCHEME 5
2. LONG-FINNED PILOT WHALE STRANDINGS IN SCOTLAND 5
3. PREVIOUS MASS STRANDINGS 8
Uist near stranding 27th October 2010, Donegal mass stranding 6th November 2010 8
Uist stranding/near mass stranding 21st May 2011 8
4. DURNESS MASS STRANDING 22/07/2011 (M168/11) 9
5. FORTH AND TAY REGION CETACEAN STRANDINGS, AUGUST TO SEPTEMBER 2012 10
6. OTHER UK STRANDINGS: 11
7. PITTENWEEN MASS STRANDING (M280/12) OVERVIEW 11
8. NECROPSIES 13
Post mortem findings 14
Bacteriology results 14
9. EAR ANALYSIS FOR ACOUSTIC TRAUMA 20
10. EARTHQUAKE ACTIVITY 22
11. SEISMIC SOURCES 23
12. SHIPPING AND NAVAL ACTIVITY 26
13. NATURAL PREDATORS 26
14. DOMOIC ACID TOXICOLOGY ANALYSIS 27
15. MORBILLIVIRUS 28
16. METAL CONTAMINANT ANALYSIS 29
17. TEETH AGEING, REPRODUCTIVE ASSESSMENT 30
18. PERSISTENT ORGANIC POLLUTANTS 30
19. WEATHER AND TIDAL FACTORS 31
20. CONCLUSION 32
21. DISCUSSION 32
22. RECOMMENDATIONS 33
23. ACKNOWLEDGEMENTS 34

REFERENCES 35

APPENDIX 2: ACTIVITIES LICENSED BY MARINE SCOTLAND IN FIRTH OF FORTH POTENTIALLY ACTIVE DURING AUGUST AND SEPTEMBER 2012 38

APPENDIX 3: DECC LICENCED ACTIVITIES ON 2ND SEPTEMBER 2012 40

FIGURE 1: DISTRIBUTION MAP OF LONG-FINNED PILOT WHALE SIGHTINGS (REID, EVANS, & NORTHRIDGE, 2003) 6
FIGURE 2: MASS STRANDINGS 1992-2012 6
FIGURE 3: DENSITY OF PILOT WHALE STRANDINGS 1992-2012. THE HOTSPOT IN EAST SCOTLAND IS LARGELY ATTRIBUTABLE TO THE 2012 MSE DESCRIBED IN THIS REPORT 7
FIGURE 4: PILOT WHALE MASS STRANDINGS 1992-2012 7
FIGURE 5: LOCATIONS OF NOTABLE STRANDINGS AND SUBSEQUENT POD SIGHTINGS 10
FIGURE 6: DETAIL OF M280/12 STRANDING SITE SHOWING LOCATION OF INDIVIDUAL ANIMALS. INSET SHOWS WIDER SCALE WITH YELLOW BAR REPRESENTING 5 NM. 12
FIGURE 7: MSE RESCUE ATTEMPT (PHOTO SMRU) 12
FIGURE 8: REFLOAT ON RISING TIDE (PHOTO SMRU) 13
FIGURE 9: STAFF FROM SMRU AND MOREDUN ASSISTING WITH NECROPSIES CARRIED OUT IN NEIGHBOURING FIELD. 14
FIGURE 10: SCANNING ELECTRON MICROSCOPE (SEM) IMAGES OF THE RIGHT COCHLEA OF THE INDIVIDUAL M280.1/12 A: COCLEAR APEX, =LOW FREQUENCY CODING. B: APICAL TURN=HIGHER FREQUENCY CODING. YELLOW ASTERISKS REPRESENT SCARRING OHC=OUTER HAIR CELLS 21
FIGURE 11: EARTHQUAKE EPICENTRE 30/08/2012 22
FIGURE 12: AREAS LICENCED FOR SEISMIC ACTIVITY 2012/13 24
FIGURE 13: REGIONS OF ACTIVE LICENCES FOR SEISMIC ACTIVITY AUGUST/SEPTEMBER 2012 25

Figure 14: AIS screen grab showing typical shipping activity in the Firth of Forth (http://www.marinetraffic.com/ais/ ... 26
Figure 15: Distribution of Killer Whale, Long-finned Pilot Whale and Northern Bottlenose Whale sightings 1992-2010 (Anderwald et al., 2010) ... 27
Figure 16: Relationship between hepatic mercury levels and age ... 29
Figure 17: Weather from proximal meteorological station (Leuchars air base) for period Wed 29th August to Monday 3rd September (via http://www.wunderground.com) Yellow line marks beginning of MSE ... 31
Figure 18: Tides 1-3rd September. Yellow line marks beginning of MSE ... 31

Table 1: Summary of pathological findings ... 19
Table 2: Domoic acid levels in sampled cases ... 28
Table 3: Morphometrics and age summary ... 37
Table 4: Activities licensed by Marine Scotland ... 39
Table 5: DECC licenced activities active on 02/09/2012 ... 47
Table 6: Number of samples archived (1,2 = number of samples taken, x sample not taken) ... 48

Cetacean mass stranding events (MSEs) elicit much interest from both the public and scientific community but the underlying reasons largely remain a mystery. Live stranding events and more specifically mass live stranding events are extreme situations in which public safety, animal welfare and conservation science issues have to be managed with a clear perception of priorities. Thorough investigation of these events usually requires the consideration of a number of natural and anthropogenic factors. In 2011 and 2012 two large mass strandings of long-finned pilot whales (*Globicephala melas*) occurred in Scotland. This report outlines the diagnostic and investigative pathways followed to investigate any potential causal or contributory factors for the 2012 mass stranding. This work was co-ordinated by the Scottish Marine Animal Stranding Scheme (SMASS) and undertaken using funding allocated by Marine Scotland.

On Sunday 2nd September 2012 a group of approximately 35 animals were reported as stranded or attempting to strand on the rocky coastline between Pittenweem and Anstruther, Fife. A large rescue and refloat attempt was launched and ten animals were refloated on the following tide. Twenty-one animals were either found dead by the rescue teams or died during the refloat. The carcases were recovered to an adjacent field and necropsied by veterinary pathologists and biologists from the SMASS, UK Cetacean Stranding Investigation Programme (CSIP), the Sea Mammal Research Unit (SMRU) and Moredun Research Institute (MRI). The proximal cause of death in all cases was live stranding and cases exhibited typical pathology of this syndrome, including hyperthermia, myositis and water aspiration. Assessment of the disease burden indicated many animals in the group were not in optimum health. Whilst this was not severe enough to account for a stranding, it may have had bearing on the pod's behaviour and location. Samples were collected according to standard protocols and investigations into potential trigger factors for the MSE were undertaken. The investigation included detailed pathological examination to quantify overall disease burden and a number of additional diagnostic tests. This included microbiology, histopathology, morbillivirus (RT-PCR), quantitative analyses for algal toxins (domoic acid) and heavy metals concentration. External triggers, such as unusual climatic conditions and influences of underwater noise were also investigated. A request was made to the Department of Energy and Climate Change (DECC) and the UK Ministry of Defence (MOD) to establish the temporo-spatial distribution of military and civilian sources of underwater noise preceding the MSE.

Gross and histopathological investigation of twenty-one animals did not find any indication of specific disease processes which would account for the stranding although several animals were in poor general health. Toxin burden from harmful algal blooms (HAB) and heavy metal contaminant burden were also insufficiently elevated to be likely causal agents for the stranding.

Ears were extracted from most specimens, of which six were selected for analysis based on minimum autolysis condition. These were examined under electron microscopy and no evidence of acoustic trauma was found at the base of the cochlea in any of the six cases. Five of six cases were non-diagnostic at the cochlea apex due to autolysis. One animal, where the ears had been extracted and fixed within 3 hours of death, was however diagnostic for the whole cochlear structure. In this case it was possible to identify significant

pathology at the cochlea apex. Lesions were identified in the outer hair cells which would be compatible with acoustic trauma and could affect hearing in the very low frequencies. The chronicity of this lesion did not define this to be a contributory factor to the stranding, but does serve to suggest at least one of these animals may have been acoustically 'blind' at some frequencies due to historic noise exposure.

1. Background to the Scottish Marine Animal Stranding Scheme

The Scottish Marine Animal Stranding Scheme (SMASS) has been monitoring and investigating stranded cetaceans since 1992. Working as part of the UK CSIP (Cetacean Stranding Investigation Programme), and in collaboration with scientists from the Sea Mammal Research Unit, Moredun Research Institute and the University of Aberdeen, the scheme aims to monitor the health of, and threats to, many of Scotland's marine animal species. Investigation of a stranded animal can yield substantial information about the health and life history of the individual and, with careful inference, insight into the population as a whole. Analysis of trends in the number and location of stranded animals assists with identifying new or emerging threats and examination of animals at post-mortem provides useful data about the health, ecology and conservation issues of these species. SMASS is part of Scotland's Rural College and is based in Inverness, Scotland. More information about the scheme can be found here:

http://www.sruc.ac.uk/info/120150/scottish_marine_animal_stranding_scheme

2. Long-finned pilot whale strandings in Scotland

A mass stranding is defined as two or more animals found together excluding cow/calf pairs. Long-finned pilot whales are the species most prone to mass strand in the UK. Since 1913, there have been 31 long-finned pilot whale MSEs in the UK with an average of 24 individuals at each event. A near mass stranding can be defined as a group of animals close to shore exhibiting behaviour consistent with an attempt to strand, but prevented from becoming beached by human intervention or topography. In general, mass strandings are unusual. Figure 2 shows the total number of animals recorded as part of MSE's since 1992. There has been a notable increase in the frequency and magnitude of long-finned pilot whale mass strandings since 2010. Figure 3 is a map showing the density of long-finned pilot whale strandings in Scotland since 1992. Hotspots for single strandings of this species are the Western Isles (n=74), North West Scotland (n=26), Orkney (n=20) and Shetland (n=22) which is explicable given this is the land closest to the normal shelf-edge foraging zones for this species. It can be seen that the location of the 2012 MSE in Fife (Figure 4) is neither close to the typical shelf-edge sightings (Figure 1) nor in a location typical for recent strandings of this species (Figure 3), however is worth noting that the largest UK *G.melas* MSE on record occurred off the SE coast of Scotland, where 148 individuals were reported stranded in May 1950 in East Lothian (Natural History Museum data).

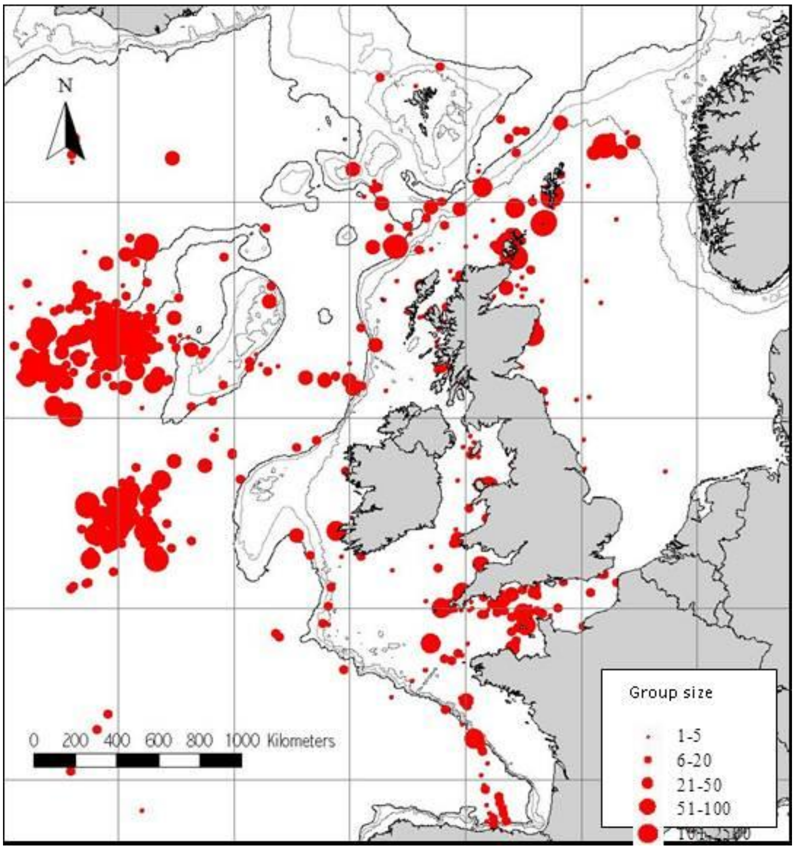

Figure 1: Distribution map of long-finned pilot whale sightings (Reid, Evans, & Northridge, 2003)

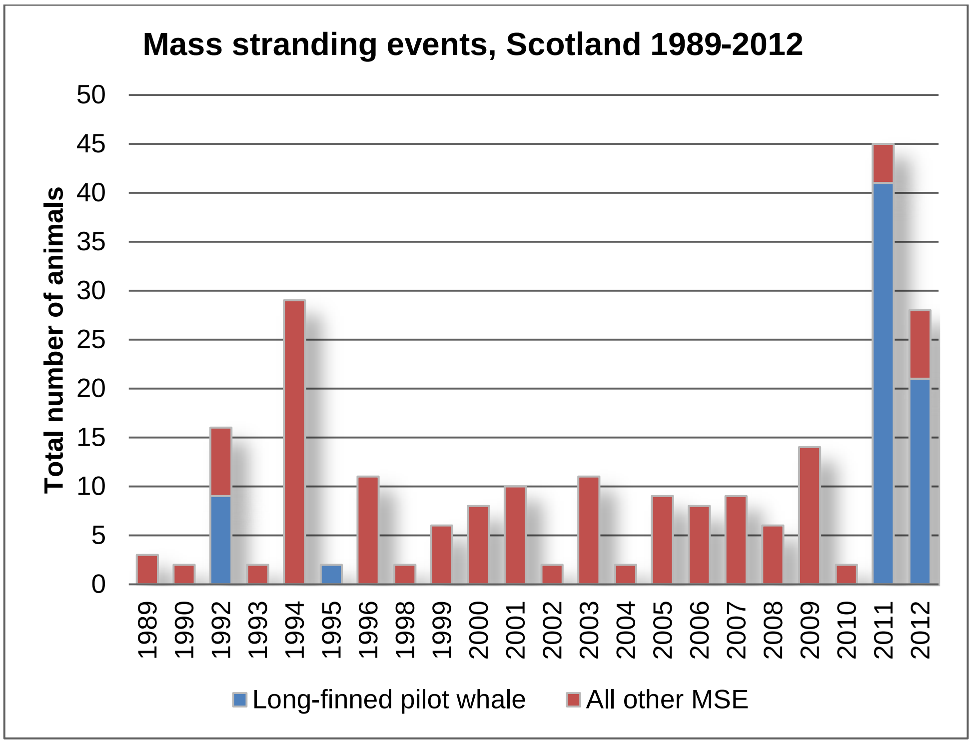

Figure 2: Mass strandings 1992-2012

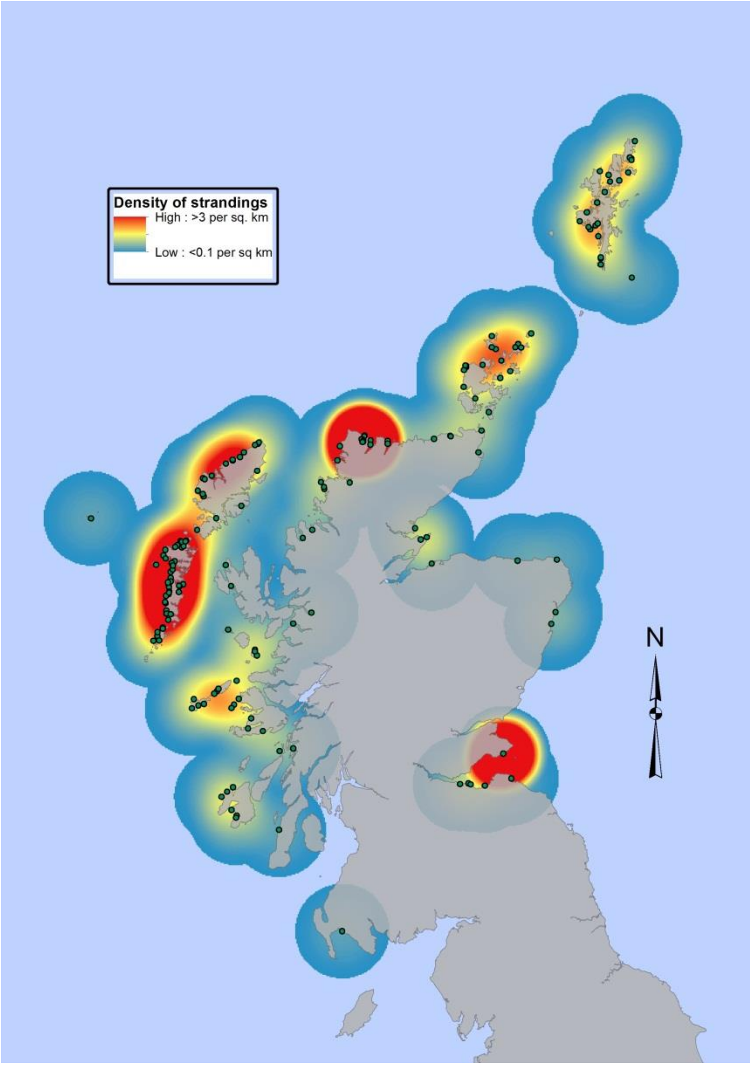

Figure 3: Density of pilot whale strandings 1992-2012. The hotspot in east Scotland is largely attributable to the 2012 MSE described in this report

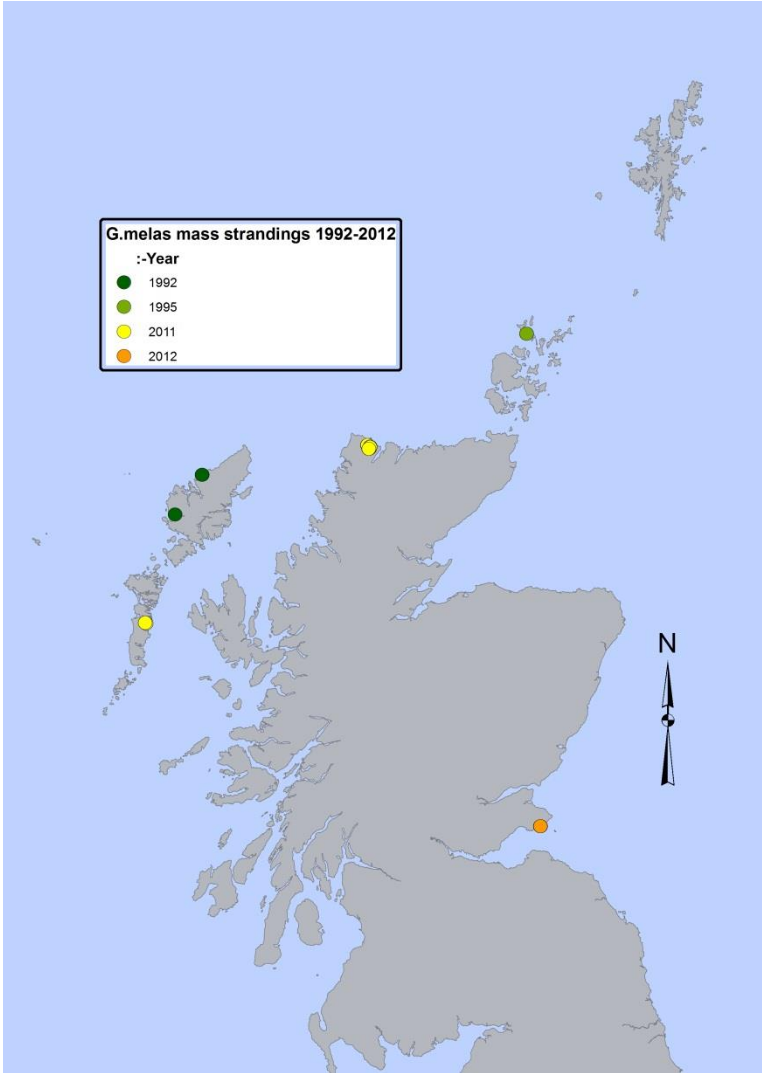

Figure 4: Pilot whale mass strandings 1992-2012

3. Previous mass strandings

Between 1992 and 2009 there were three recorded mass strandings of long finned pilot whales in Scotland (Figure 4): two MSE's comprising 2 and 7 animals respectively on the Western isles in 1992 and a single MSE involving two animals in Orkney in 1995. In the past two years there have been four other recorded pilot whale mass or near-mass strandings.

Uist near stranding 27th October 2010, Donegal mass stranding 6th November 2010

Loch Carnan, South Uist, GR NF 835 429. Approximately 40 long-finned pilot whales were seen very close to the rocky shore, packed in a tight group, milling and spyhopping. They were herded out to sea using small boats, however 10 days later (6th November 2010) 33 long-finned pilot whales mass stranded on Rutland island, Donegal in the Republic of Ireland. This was a remote, relatively inaccessible location however the Irish Whale and Dolphin Group (IWDG) was able to confirm the mass stranding of 33 pilot whales in total, all which were found dead (http://www.iwdg.ie/article.asp?id=2422). Necropsies are not routinely carried out in Ireland, and without financial support from the Irish Government it was not possible for a CSIP team to allocate UK funds to this work, so these cases were not examined. Photographic identification of dorsal fin images confirmed some animals were those which left Uist the previous week.

Uist stranding/near mass stranding 21st May 2011

Approximatly six months later, in precisely the same region of Loch Carnan, South Uist, approximately 50 long-finned pilot whales were noted spyhopping. Many animals demonstrated head lacerations indicative of recent trauma, most likely from rock abrasions. No attempt was made to herd this pod out to sea. Two carcases were found, one recoverable for necropsy which showed a thin animal but without any significant pathology. After two days the rest of the group left the area. No subsequent re-sighting in region was noted, and no confirmed carcases were reported elsewhere.

At both of these near stranding events, investigations were conducted by the charities British Divers Marine Life Rescue (BDMLR) and Whale and Dolphin Conservation (WDC) to identify any concurrent anthropogenic activity which may have led to the animals congregating in Loch Carnan. The Royal Navy stated the only vessel in the region of the first Uist stranding was 50nm away (HMS Ramsay) and another MOD source stated that no royal Naval units were operating within 50 miles of the Irish stranding site and no sonar units within 100nm. It was also stated that the vessel in nearest proximity to the first sighting at Uist was a minehunter vessel deploying low frequency sonar. The lack of necropsies meant it was not possible to investigate other possible causes for these near mass stranding or the mass stranding itself in Ireland a week or so later. Loch Carnan itself does not appear topographically or bathymetrically unusual to the rest of the island, so it was not clear why two near mass strandings both occurred in this particular inlet. A subsea cable crosses Loch Carnan from an 11MW Oil-fired power station and consideration was given to the possible impact on cetacean navigation from any electromagnetic interference (Klinowska, 1985; Walker, Kirschvink, Ahmed, & Dizon, 1992) however no definitive cause was identified which would plausibly explain either of these stranding events.

4. Durness mass stranding 22/07/2011 (M168/11)

On 22nd July 2011 approximately 70 long-finned pilot whales entered the Kyle of Durness, a shallow tidal inlet bordering Cape Wrath, Northern Scotland (58°34'52"N 4°48'23"W). As the tide receded at least 39 animals stranded, of which approximatly 20 were sucessfully refloated. A rapid response from local people and strandings response teams enabled this successful refloat of a large proportion of the stranded animals on the following tide. Nineteen animals were known to have died during the MSE. Sixteen animals, comprising eight males and eight females were recovered for post-mortem examination

One adult male, M168.5/11 had a large, purulent abscess in the left scapulohumeral joint. *Brucella ceti* was isolated in mixed culture from this material. The pathology appeared severe enough to impair use of the joint. The animal was however in normal body condition, suggesting it was able to successfully forage.

All other biological indicators suggested the animals in the group were healthy, in good body condition and not suffering from any significant infectious, metabolic or toxic burden.

An underwater munitions disposal exercise was in operation the previous day where several 1000lb bombs underwent sucessive controlled explosions close to the entrance to the Kyle. There was no evidence of barotrauma detected on gross necropsy that would be consistent with the direct physical effect of energy release from these underwater explosions. Due to the rapid autolysis of hair cells, the impact of acoustic trauma and hearing derangement could not be reliably assessed.

The investigation was therefore able to rule out several potential causes and, based on the spatiotemporal correlation with the munitions disposal exercise, acoustic disturbance from these underwater explosions and navigational error were hypothesised to be the most likely factors with potential to cause the MSE. It should be noted however that due to the nature of the data avaliable, the evidence for this was circumstantial rather than definitive.

5. Forth and Tay region cetacean strandings, August to September 2012

Figure 5 shows the location of strandings in the Forth and Tay region in August and September 2012. On 14th August a Sowerby's beaked whale (*Mesoplodon bidens)* was reported stranded at Culross, Fife, considerably upstream of the Forth bridges. The following day a second Sowerby's beaked whale was discovered on the other side of the Firth of Forth at Bo'ness. Both of these carcases were examined by pathologists from SMASS. The animals were juvenile and sub-adult females, both in thin body condition with no evidence of recent feeding. Gross and histopathological examination of these cases did not find any evidence of disease or trauma and concluded that death was most likely due to live stranding as a result of navigation into a narrow, tidal estuary from which it was difficult to exit.

On 1st September a minke whale (*Balaenoptera acutorostrata*) was reported at Redcastle, Arbroath as a suspect entanglement case. Subsequent investigations however could not locate this carcase and no further information was available.

On 12th September a sei whale *(Balaenoptera borealis)* was seen swimming close to shore off St Andrews and was later found dead on a beach at Eliot near Arbroath. A full necropsy was carried out on site. The animal, a 12.75m young adult male, had moderate chronic multi-focal gastric parasitism and abscess formation caused by the Acanthocephalan parasite, *Bolbosoma turbinella* in the intestine. Parasitic gasteroenteritis is the most likely cause of the live stranding of this animal.

Figure 5: Locations of notable strandings and subsequent pod sightings

6. Other UK strandings:

Data from the UK CSIP showed an increase in both sei whale and Sowerby's beaked whale strandings throughout the UK in August and September 2012

A sei whale (*Balaenoptera borealis*) stranded in Druridge Bay in Northumberland, England on 26th September (SW2012/413). This animal was found to be in moderate-poor nutritional condition. Similar parasites were noted in the intestinal tract, but with a markedly lower burden than that found in the Arbroath stranding. The cause of death was given as live stranding.

Seven Sowerby's beaked whales (*Mesoplodon bidens)* stranded around the UK coast between June and October 2012. Six of these stranded in a two day window between 14th and 16th August - one in the Bristol Channel, next to the Severn Bridge and the others along the UK North Sea coast (two in Scotland and three in England). The Sowerby's beaked whales which stranded at Aust in the Bristol Channel were, as in the Forth cases, live strandings; photos of the condition of the other three along the North Sea coast of England also indicated that they had been live strandings. Finally, a seventh Sowerby's beaked whale live stranded and was euthanised at Bridlington on 30th September.

7. Pittenween mass stranding (M280/12) overview

On the morning of Sunday 2nd September 2012 a group of long finned pilot whales (*Globicephala melas*) was reported to have live stranded on the rocky shore between the Fife villages of Pittenweem and Anstruther (Figure 6, 56.2151N, 2.718W). A response coordinated by BDMLR managed to refloat ten animals on the high tide around 5pm. Twenty-one animals were either found dead by the rescue teams or died during the refloat. Of these, four carcases were towed from the stranding site to a neighbouring beach to enable easier refloat of the live cases (Figure 6). The remaining seventeen were recovered for necropsy from the stranding beach.

Over the following week up to 30 long-finned pilot whales were sighted as far up the Forth as the Kincardine Bridge. Two more animals were found dead on 8th and 10th September but the rest of the pod appeared to safely navigate back out to deeper waters.

No animals were euthanised due to a lack of suitable drugs on site at the time. SRUC vets administered a sedative dose of barbiturate solution to a live but terminally moribund juvenile found alive on the falling tide for welfare reasons.

The area is not an obvious site for a mass stranding with no topographical features characteristically associated with natural 'whale traps', such as shallow or sinusoidal bays or lagoons. The foreshore at Pittenweem comprises a wide, relatively flat region of Carboniferous sandstone which is wholly exposed at low water. These reefs were expansive enough to have trapped any cetaceans close to shore as the tide dropped. Whilst this offers an explanation for the mechanism of stranding, there is no wider scale topographical feature which would explain why animals were close to the shore along this section of coastline.

Figure 6: Detail of M280/12 stranding Site showing location of individual animals. Inset shows wider scale with yellow bar representing 5 NM.

Figure 7: MSE rescue attempt (Photo SMRU)

Figure 8: Refloat on rising tide (photo SMRU)

None of the refloated animals were tagged or photographically identified during the refloat therefore reliable assessment of subsequent re-strands was not possible in this case. Estimates from morphometrics data however suggest at least eight of the refloated animals did not immediately re-strand in the vicinity. An adult female pilot whale (M281/12) live stranded the following day at Leith docks although it is not clear if this was an individual involved in the Pittenweem stranding. Over the subsequent 5 days, a group of approximately 25 animals was sighted swimming against the prevailing tidal streams in the Forth as far up river as the Kincardine Bridge. Despite this being a tidal area with significant maritime activity, no strandings were reported in this region. Six days after the main MSE stranding, another calf was found stranded at Pittenweem (M288/12). Necropsy examination indicated this latest carcase had been alive for several days after the main MSE but it is not clear if this was one of the refloated animals. A final dead animal was spotted in the Firth of Forth on 10th September, however this was too autolysed for meaningful necropsy.

A timeline of the rescue outlined by BDMLR can be accessed here:

http://www.bdmlr.org.uk/index.php?mact=News,cntnt01,detail,0&cntnt01articleid=908&cntnt01returnid=96

8. Necropsies

In total twenty-one animals were recovered for necropsy examination (M280.1 to M280.21/12). Teams from the CSIP, SMRU and MRI assisted with the gross pathology and a full diagnostic necropsy was undertaken on site on 3rd and 4th September (Figure 6). The animal which live stranded in Leith docks (M281/12) was secured and access permissions granted for a necropsy on 5th September. A complete suite of samples was taken (Appendix 3) in addition to histopathology. Particular care was taken to extract the ears from several animals to enable subsequent histologically examination of the coclear ultra-structure.

Figure 9: Staff from SMRU and Moredun assisting with necropsies carried out in neighbouring field.

Post mortem findings

A detailed synopsis of gross and histological findings is given in Table 1. Autolysis was a feature in several of the cases and it is possible this masked relevant pathology. Five of the nine adult females examined were lactating and a number of dependant calves were in the group. Only one animal examined had any recent ingesta in the stomachs, indicating most of the animals had not recently fed. Aside from pathology consistent with live stranding, cases examined did not present evidence of a single infectious, metabolic or physical process which could explain the stranding. However, most animals exhibited a mild to moderate disease burden, chronic active in nature and generally considered to be parasitic in origin. In addition three animals exhibited a mild to moderate encephalitis whose underlying cause was not definitively established but considered most likely to be due to parasitic larval migrans. Whilst none of these disease processes were severe enough to definitively account for a stranding, they do indicate many animals in the group were not in optimum health and this may have had bearing on their behaviour and location.

Bacteriology results

Bacteriological examination was carried out on all 21 pilot whales involved in the mass stranding event (MSE) and the two pilot whales that stranded subsequently to this event. Additionally bacteriological examination was also carried out on the two Sowerby's beaked whales that stranded on 15th August and the sei whale that stranded in 14th September. Tissue samples or swabs of selected tissues, including liver, kidney, lung and brain, were taken aseptically for bacteriological examination using aerobic, anaerobic and capnophilic incubation and standardised methods (Jepson 2006). Swabs or tissues were inoculated directly onto either Columbia blood agar base (Oxoid, CM331) with 5% sheep blood, MacConkey agar (Oxoid CM0007) and Farrell's agar (Farrell 1974) and incubated aerobically, anaerobically or capnophilically at 37°C for up to 14 days. Cultures of these tissues from all animals produced mixed growths of a variety of organisms including; *Photobacterium damselae, Edwardsiella tarda, Edwardsiella hoishinae, Vibrio sp., E.coli,, Streptococcus sp.* and *Aeromonas sp.* These are isolates frequently found in cetaceans and do not appear to be associated with particular disease or specific pathology. Consequently none of these isolates are considered significant pathogens in mixed growth. Specific cultures for *Brucella ceti,* which in contrast has been associated with the live stranding of various cetacean species (Foster et al., 2002) were set up on various tissues including brain from all animals. All of these were negative.

ID	Sex	Age (years)	Age Group	Cause of Death	Overall summary	Mean Blubber thickness (mm)	Disease index	Evidence of recent feeding?
M280.01/12	F	2.5	sub-adult	Live Stranding	Individual refloated and died in the water. Mild meningioencepalitis, possibly parasitic in origin, mainly affecting cerebrum. Not generalised, severe or acute enough to indicate neurological disease. Moderate verminous pneumonia	30	Mild	No
M280.02/12	F	26	adult	Live Stranding	No significant lesions, minimal parasitic burden	38	None	No
M280.03/12	F	17	adult	Live Stranding	Head and melon abrasions. Lactating. Some partially digested squid, No significant gross lesions. Mild verminous pneumonia, Enlarged adrenal cortex in comparison to medulla; no obvious reason for chronic stressors	40	Mild	Yes
M280.04/12	F	NA	adult	Live Stranding	Adult lactating female. Estimate dead >24hours, moderate autolysis. Scant nematodes. Reflux bile. Scant parasite burden, gastritis, mild, secondary to parasitism. No bacteria isolated	37	Mild	No
M280.05/12	F	20	adult	Live Stranding	Adult lactating female. Large amount of ventral bruising. Recently given birth based on haemorrhagic tarry uterine lining. Liver gassed up. Moderate autolysis. White fibrous material over heart. Mild hepatic lipidosis, not unusual due to lactation. Stomach trematodes and mild inflammation, orange brown pigment in kidney, possibly myoglobin. No significant reproductive tract abnormalities on histology. Single parasitic cyst in skeletal muscle. No parasite lesions noted in brain.	38	Mild	No
M280.06/12	F	NA	juvenile	Live Stranding	Female calf, refloated animal. Teeth just starting foetal folds still visible. Mild multifocal lesions in brain, probably stranding related. Subtle, nonspecific visceral pathology.	22	Mild	No
M280.07/12	F	NA	juvenile	Live Stranding	Female calf, foetal folds still visible Early mild to moderate parasitic infiltration, possibly due to early visceral larval migrans and mild verminous pneumonia	27	Mild	No

M280.08/12	F	NA	juvenile	Live Stranding	Calf foetal folds still visible teeth starting to show. Green watery faeces. Large coronary vessels over left side of heart. Early mild parasitic infiltration, possibly due to early visceral larval migrans	26	Mild	No
M280.09/12	M	NA	subadult	Live Stranding	Sub-adult male ventral bruising, fluid filled cyst, likely parasitic.. Parasitic profile in lungs indicative moderate to severe chronic active verminous pneumonia.	36	Moderate	No
M280.10/12	F	4	subadult	Live Stranding	Fish skulls in stomach, recent feeding. Focal mild chronic meningioencepalitis, typical of other cases in this stranding. Probably infectious origin No eosinophils so not straightforward parasitic larval migrans. no notable visceral pathology	37	Mild	No
M280.11/12	F	29	adult	Live Stranding	Lactating female probably with calf but also pregnant. Foetus stored in formalin (28cm length). Mild meningioencepalitis, mild verminous pneumonia. Small late first trimester foetus, little likely energetic costs. Not significant enough pathology to explain stranding	40	Mild	No
M280.12/12	F	3	subadult	Live Stranding	Non-pregnant female. Moderate autolysis of liver and kidney. Developed thymus. Massive pulmonary associated lymph nodes. Focal necrosis in brain (moderate to severe chronic multifocal lympho-granulomatous predominantly polioencephalitis and a mild to moderate chronic multifocal lymphocytic meningitis) large perivascular cuffs, indicative of parasitic larval migrans in brain, Thymus appeared normal. Mild visceral pathology associated with parasite lesion. Intralesional parasite larvae in lymph nodes.	34	Moderate	No
M280.13/12	F	9	adult	Live Stranding	Adult nematode present in lungs. Possible recently pregnant, no significant bacteria, moderate chronic-active multi-focal verminous pneumonia.	38	Mild	No
M280.14/12	M	2.5	subadult	Live Stranding	Abrasions on dorsal and pectoral fins. Similar mild meningioencepalitis to other cases, could be parasitic exacerbated by stranding. Mild visceral pathology, verminous pneumonia.	32	Mild	No

M280.15/12	F	36	adult	Live Stranding	Abrasions on pectoral and dorsal fins. Not lactating, large number of embryonated parasite eggs in mammary gland. No significant bacteriology, mild verminous pneumonia, mature animal, background parasite burden. Substantial myocardial fibrosis	41	Mild	No
M280.16/12	M	4	subadult	Live Stranding, encephalitis	Male testes 14cm in length, head trauma quite gassy Neuronal necrosis with large perivascular cuffs and multifocal encephalitis extending throughout entire brain. No eosinophils. Atypical pattern to other cases could be stranding related. Not a clear parasitic or infectious profile. Could have been clinically ill. Uncertain origin but may be due to parasite larval migration. Severity is greater and the distribution far more extensive in this case compared to others in the MSE as they affect all major regions of the brain and not just the cerebrum.	36	Moderate to severe	No
M280.17/12	M	2	subadult	Live Stranding	Sub-adult male found alive after refloat had taken place wedged in amongst rocks and no chance of refloat. 140ml of barbiturate into right sub-lumber musculature as sedative. Tetraphyllidean cyst present. Mild lymphocytic/ plasmacytic meningioencepalitis with multifocal, nonspecific cell lines in brain. Could be related to prolonged stranding. Stranding related skeletal muscle damage. Mild verminous pneumonia.	37	Mild	No
M280.18/12	F	25	adult	Live Stranding	This female adult long finned pilot whale was in good-moderate condition. No squid beaks and only minimal numbers of fish bones were found within the gastro-intestinal tract suggesting that the animal may not have fed for a while prior to death. No significant disease processes were found on gross post mortem examination that could be considered to have predisposed the animal to stranding alive. There was some evidence of a focal pneumonia, possibly verminous, but the preservation of the tissues precludes certainty. The lipofuscin in the neurones is suggestive of an aged animal.	33	Mild	No
M280.19/12	M	15	adult	Live Stranding	Mild verminous pneumonia present, not severe enough account for the cause of death or live-stranding.	29	Mild	No

M280.20/12	F	14	adult	Live Stranding	Background non significant bacteriology. Lactating, large number of parasite eggs in mammary tissue and associated inflammation. Suspect crassicauda.	32	Mild	No
M280.21/12	M	16	adult	Live Stranding	This adult was in good-moderate condition. No prey contents were found within the gastro-intestinal tract suggesting that the animal may not have fed for a while prior to death. No significant disease processes were found on gross post mortem examination that could be considered to have predisposed the animal to stranding alive. No overt pathology. Background parasitism. Possible bile stasis	34	Mild	No
M281/12	F	NA	adult	(Meningo) encephalitis	The lesion in the brain is highly suggestive of a parasitic larval migrans despite no parasite profile being found. The accumulation of material in the skeletal muscle it the most severe we have encountered. Focal brain lesions, could explain clinical neurological signs seen in animal in Leith Docks prior to death. Severe chronic focal necrotic encephalitis with extension into the adjacent meninges and severe chronic widespread accumulation of complex polysaccha. Aged animal. Myocardial fibrosis. Possibly parasitic due to eosinophils in brain. Probably sub-acute, at least 2 days old. On balance it is not considered that this pathology can wholly be explained by a previous stranding.	30	Moderate to severe	No
M288/12	F	NA	juvenile	Live Stranding	Inflammatory cells within active lymph nodes, moderate chronic verminous pneumonia. Fatty change in liver but this finding nonspecific due to fatty diet of this age	19	Mild	No
M295/12	F	NA	adult	Not Examined	Dead stranded animal not examined due to advanced autolysis.	NA	NA	NA

Table 1: Summary of pathological findings

9. Ear analysis for acoustic trauma

Investigation of the 2011 pilot whale MSE in the Kyle of Durness suggested acoustic trauma could be a potential cause of the MSE. Definitive assessment was not possible due to sample autolysis, so for this MSE extracting the ears from carcases was prioritised in an attempt to reliably assess evidence for acoustic trauma. The ears can be processed and examined in order to assess any damage to the physiological structures responsible for sound transduction cells which, if present, would indicate the animal had impaired hearing. This is hypothesised as a causal factor in cetacean strandings and investigation in this case would be valid. It is however a relatively costly procedure and success depends on the effective fixation of the ears post-mortem. The cellular structures under analysis are very susceptible to autolysis and if fixation is delayed or ineffective, the results can be equivocal. This work is carried out in collaboration with the Laboratory of Applied Bioacoustics in Barcelona who have significant experience of this procedure.

The complete report for this work is attached with this document; however a summary and conclusions are listed here.

A representative subset of 6 ear samples was considered for analysis from the total of 29 ears extracted and fixed with buffered formalin between 18 and 22 hours post-mortem, except individual M280.1/12 that was fixed within 3 hours post-mortem. Specifically, they were 2 juveniles, 2 sub adults and 2 adults. The periotic bone surrounding the cochlea was decalcified using RDO®, a rapid decalcifier based on hydrochloric acid. Subsequently, the cochleas were dissected, dehydrated in increasing series of ethanol, critical point dried with CO_2 and gold-palladium sputtered. The samples were observed using the scanning electron microscope (SEM) of the Institute of Marine Sciences - CSIC (Hitachi S-3500N).

With the exception of individual M280.1/12, all samples generally presented an advanced decomposition status, being more noticeable in adult specimens.

The analysis of the outer hair cells (in individual M280.1/12) and the indirect analysis of the imprints of their stereocilia on the tectorial membrane (in individuals M280.1/12, M280.6/12, M280.7/12, M280.12/12) in the basal portion of the cochlea concluded that there was no evidence of hearing loss in the high frequencies. For individual M280.1/12, the cochlea presented lesions 380 µm along the apex. The formation of scars in this area as a result of outer hair cell disappearance is compatible with an acoustic trauma, leading to a hearing loss in the very low frequencies. Dating the scar formation, and hence the overexposure to sound, is difficult as scar formation depends on both the magnitude and duration of the original lesion and the more severe is the damage, the faster is the scar evolution. In this case the observed scarring involves the inner pillar cells of the ear and would be consistent with quite severe damage.

Figure 10 shows scanning electron microscope (SEM) images of the right cochlea of the individual M280.1/12. The upper image, (A) shows the organ of Corti of the apex, with a few outer hair cells (OHCs) and an area with scars (highlighted with asterisks) as a result of OHC disappearance. In contrast, the lower image, B, shows three rows of intact OHCs in the lower apical turn. According to an estimated cochlear frequency map for this species (LAB unpublished data), the zone of scarring, extending 380 µm from the apex, corresponds to a loss of signal for frequencies below approximately 200 Hz. Work assessing the audiogram of a pilot whale suggest 200Hz is at the lower limit of their hearing (Pacini et al., 2010) but these frequency components do form part of the lower-end of long-finned pilot whales' pulsed calls acoustic energy spectra (Nemiroff & Whitehead, 2009). Extrapolations as to hearing range based on the theoretical cochlear frequency map should be used with caution, however, until data from more long-finned pilot whale audiograms are available (IFAW data, in preparation).

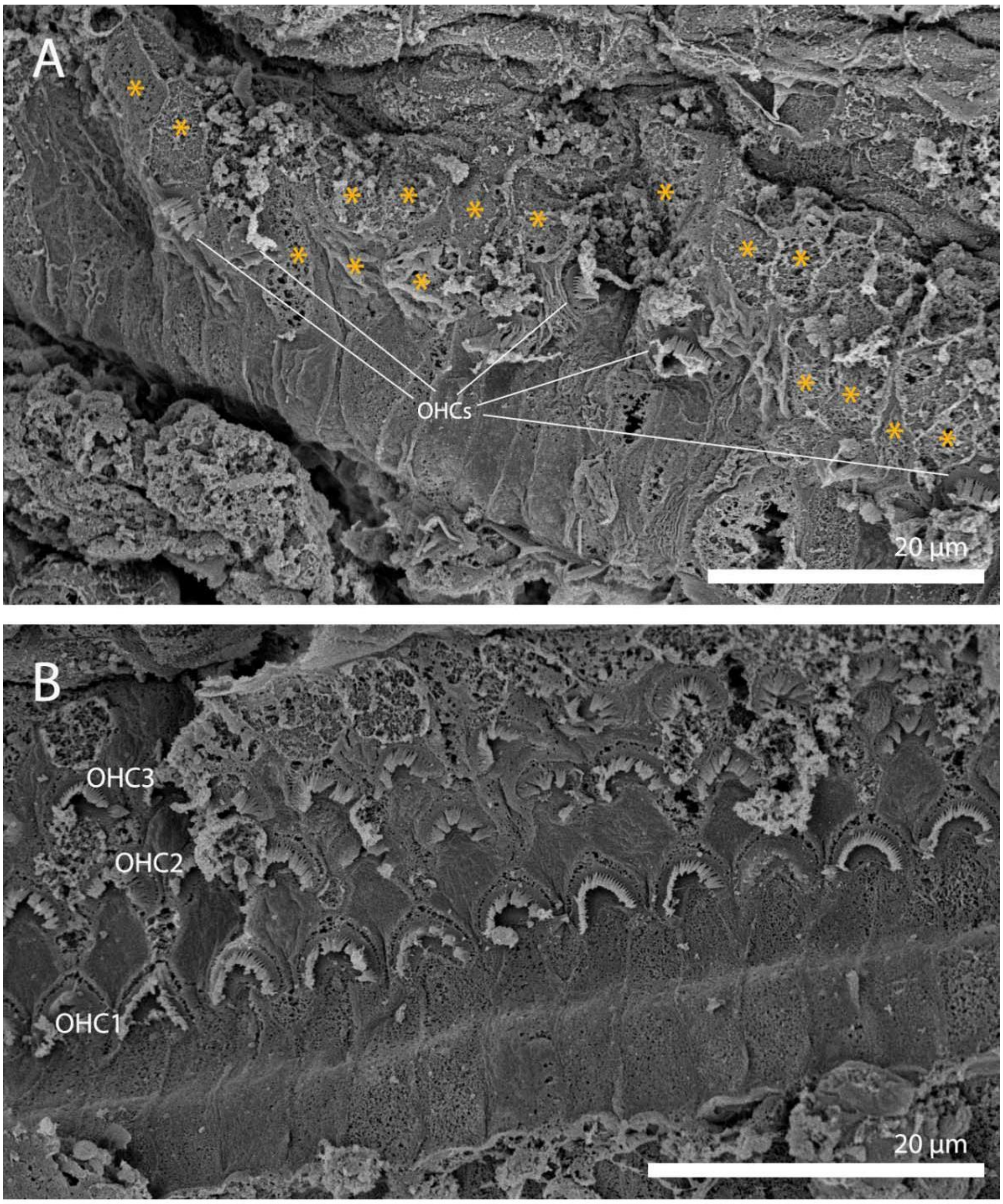

Figure 10: Scanning electron microscope (SEM) images of the right cochlea of the individual M280.1/12 A: coclear apex, =low frequency coding. B: apical turn=higher frequency coding. Yellow asterisks represent scarring OHC=Outer hair cells

The lesions observed in individual M280.1/12 are consistent with exposure to low frequency sound, which LAB extrapolated to be approximately 130 Hz. Other potential causes include presbycusis (age related hearing loss) or exposure to ototoxic drugs (Fausti et al., 1992). Both these processes are unlikely however as teeth analysis estimated this animal to be only 2.5 years old and no medication was administered to this animal prior to death. Furthermore, both these processes predominantly affect high frequency regions which were unaffected in this case. It was not possible with this technique to accurately determine the chronicity of the scarring, so the influence of this particular pathology on the stranding event, is unclear.

This is a promising method for assessing hearing loss in stranded cetaceans. It suggests that this single animal could have been over exposed to a sound source of compatible frequency characteristics during its lifetime. This would be consistent with both anthropogenic and naturally occuring noise generating events (e.g. pile-driving, geo-physical, seismic surveys, detonation of explosives or tectonic and volcanic activity) Whilst this does not confirm acoustic exposure as having been a contributory factor to the stranding event, it does provide evidence that historical sound exposure may have compromised the hearing of this animal to some degree.

10. Earthquake activity

There was an undersea earthquake measuring 6.8 magnitude on 30th August at 13:43 UTC. The epicentre location was at 71.461°N, 10.919°W in the Jan Mayen Island Region, approximately 1100 miles from Pittenweem (Figure 11). This region has many low magnitude earthquakes but this is the only significant one within a relevant time span of the MSE.

Figure 11: Earthquake epicentre 30/08/2012

11. Seismic sources

A request was submitted under the Freedom of Information Act to DECC (Department of Energy and Climate Change) for activity logs of any civilian activities likely to produce underwater sound for the period 13th August to 9th September 2012, in specific any vessels licenced to undertake seismic surveys during this period. The areas of interest were waters to the east of Cape Wrath clockwise around the coast to the English border. Data supplied by DECC and Marine Scotland outlined licences held, but not activity logs. These data are tabulated in Appendix 2 & 3. DECC do not hold activity logs for the survey vessels as they are not a requirement of the survey consent, but, where available, survey close out forms were supplied. Any licensed operator has to submit a close out form within 12 weeks following completion of a survey. Figure 12 shows the licensing status of blocks for September 2012 and Figure 13 shows the specific regions on 2nd September with active licences for seismic surveys.

The current licencing conditions do not require operators to detail when, within the time window of the licence, the survey work specifically took place. As some licence windows are several months in duration, this makes it difficult to establish actual activity during a particular period. Surveys which did not return close-out forms are not shown on these maps.

The closest seismic vessel was operating between 50 and 130 nautical miles from Pittenweem on Sunday 2nd September. The marine mammal observer on the vessel stated they had been in transit at the time of the MSE, however concerns were raised by BDMLR that their planned testing line could run across an area where the whales would go if they headed straight out to sea. Following discussions with BDMLR, the charterer of this vessel agreed to delay the testing until midnight on Tuesday 5th September.

As can be seen Figure 12 and Figure 13, whilst there were several potential sources of seismic surveys in the North Sea in the period prior to the stranding, there is no activity with sufficient spatiotemporal overlap to the stranding event to be reasonably considered to be more influential than any other.

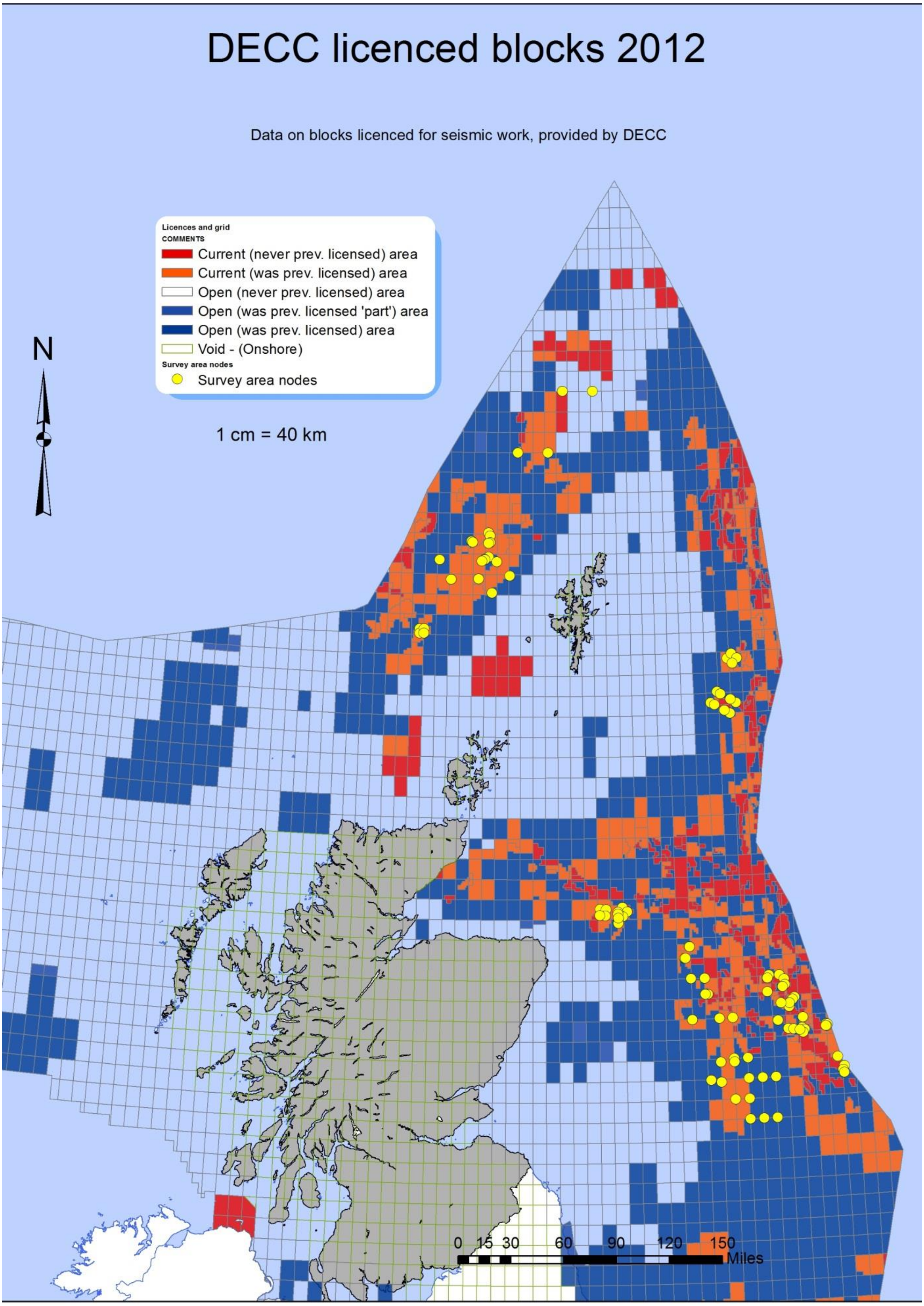

Figure 12: Areas licenced for seismic activity 2012/13

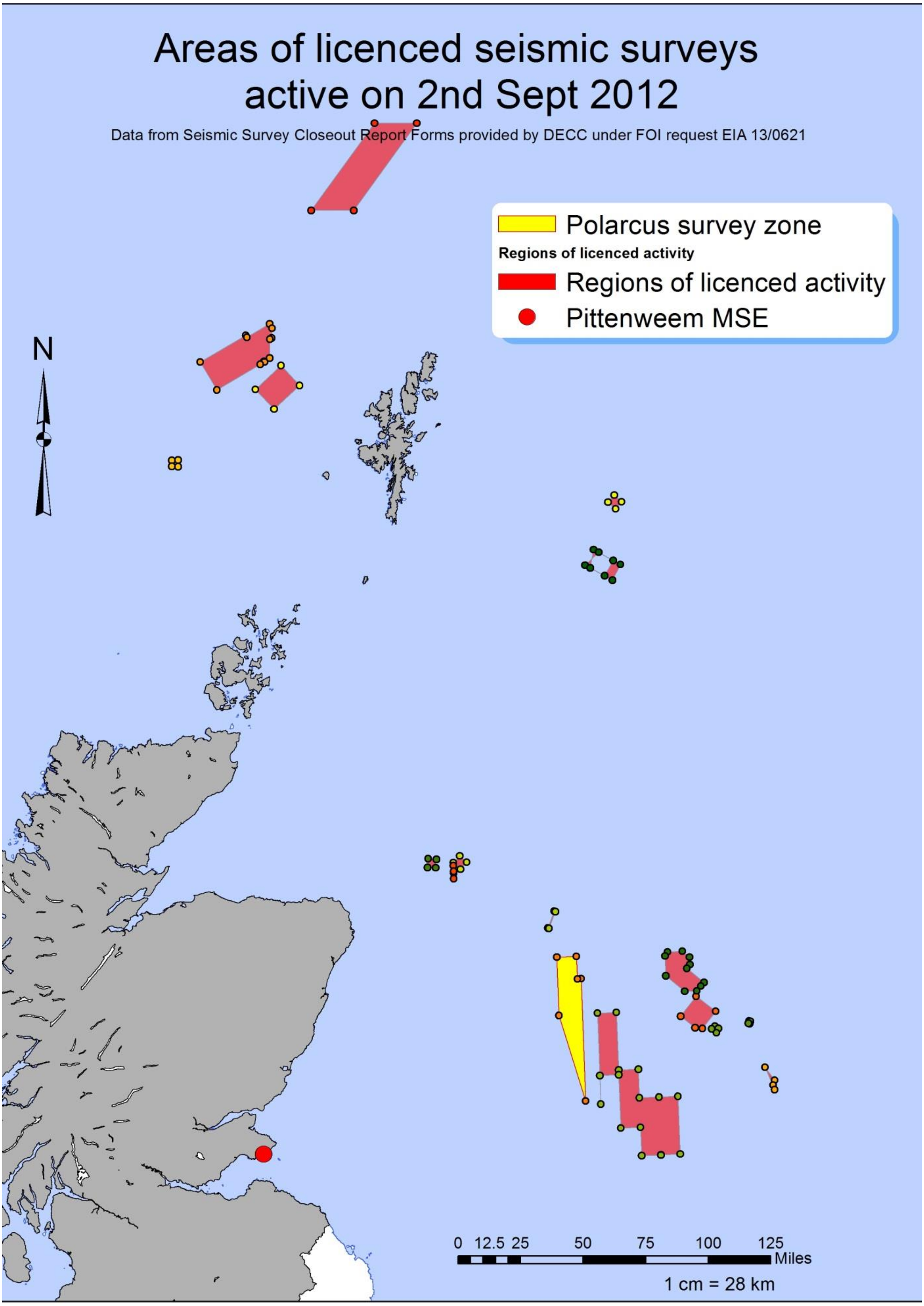

Figure 13: Regions of active licences for seismic activity August/September 2012

12. Shipping and naval activity

Pittenweem borders the northern approach to the Firth of Forth and is consequently a busy area for shipping using the Edinburgh and Fife ports. Over the preceding 48 hours of the MSE there was no unusually heavy activity based on data received from vessels broadcasting with an Automatic Identification System (AIS) transponder (Figure 14).

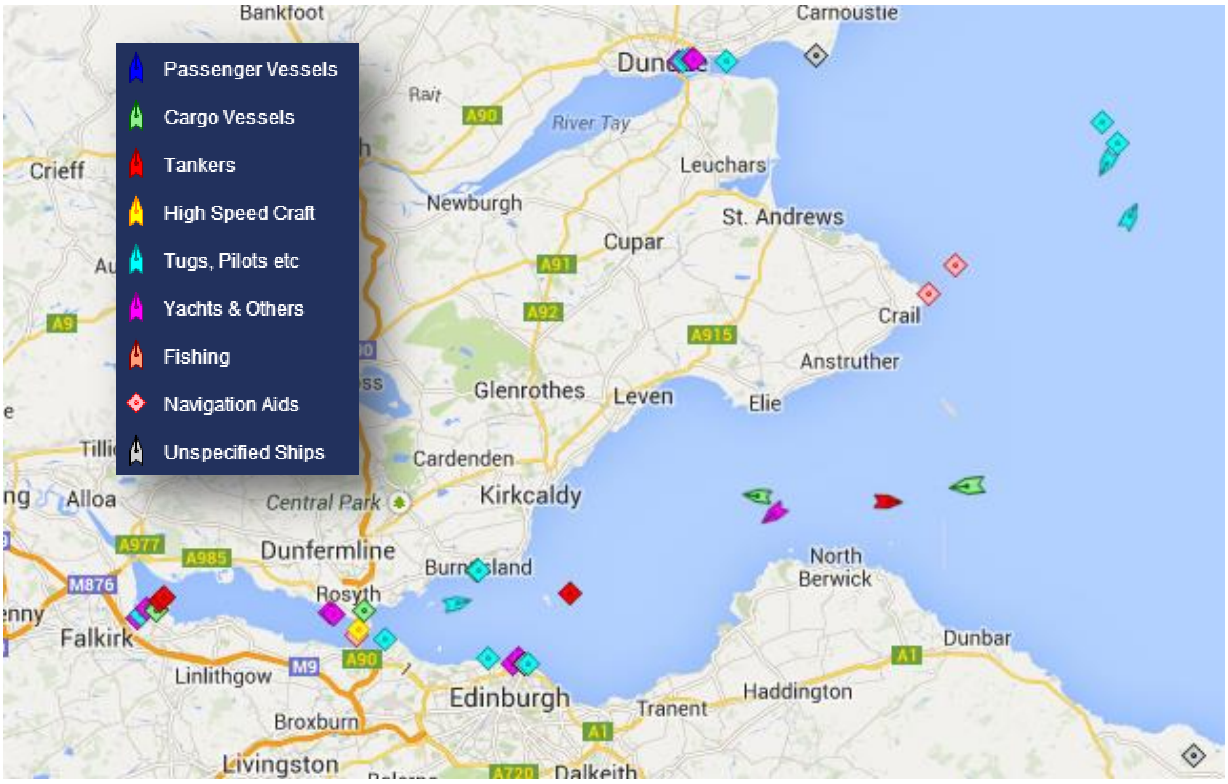

Figure 14: AIS screen grab showing typical shipping activity in the Firth of Forth (http://www.marinetraffic.com/ais/

A request was made to the Ministry of Defence (MoD) for any concurrent naval activity or operations likely to generate significant underwater sound for the two week period prior to the stranding. They stated no activity was ongoing during this period and for one day before and two days after there were no surface vessels moving out of the Crombie base in the Firth of Forth. Based on this information, there is no indication that military activities were influential on the Pittenweem stranding.

13. Natural predators

Seawatch maintain a database of cetacean sightings around the coast. There is little evidence in the literature to suggest that a group of long-finned pilot whales could be affected by natural predators to the extent that they would strand. There was no record of killer whale (*Orcinus orca*) sightings in the immediate area but they have been sighted from the east coast in the past (Figure 15). Whilst the potential influence of killer whales on this stranding event cannot be excluded, from the information available it is not considered a probable factor.

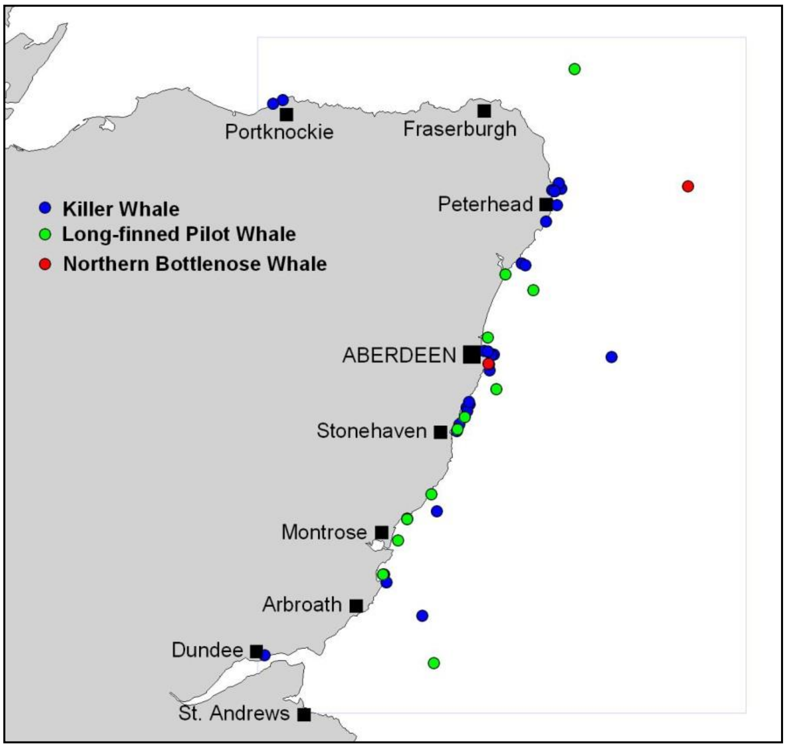

Figure 15: Distribution of Killer Whale, Long-finned Pilot Whale and Northern Bottlenose Whale sightings 1992-2010 (Anderwald et al., 2010)

14. Domoic acid toxicology analysis

Harmful algae are phytoplankton that produce toxins at certain times in their life cycle. These toxins are well recognised as causing severe health impacts in humans and animals. Among marine mammals, domoic acid (DA), a neurotoxin produced by the diatom *Pseudo-nitzschia spp,* has been recorded to cause mortality events since 1998 particularly in California sea lions (*Zalophus californianus*) (Scholin et al., 2000). The impact of cetacean exposure to DA is still unclear, although there is concern exposure may have detrimental effects at the level of both individual health and population and through reproductive failure (Brodie et al., 2006; Goldstein et al., 2008). In Scotland limited research has been conducted on DA and marine mammals, but the toxic diatoms are regularly found in Scottish waters, and DA has been documented in Scottish harbour seals (Hall & Frame, 2010). DA have been detected in fish, crustaceans and cephalopods (K. A. Lefebvre, Silver, Coale, & Tjeerdema, 2002) and these species act as trophic transfer of DA. Pilot whales (*Globicephala melas*) are known to feed on cephalopods and levels as high as 241 700 ng / g have been reported in the digestive glands of this prey species (Costa, Rosa, Duarte-Silva, Brotas, & Sampayo, 2005). Therefore screening for DA was appropriate.

A direct competitive enzyme linked immunosorbent assay (ELISA) (ASP assay kits, Biosense, Norway) was used to determine biotoxin concentration in long-finned pilot whale tissue. This assay has been widely used to detect domoic acid in various matrices including shellfish tissue, urine and faeces from marine mammals (Kleivdal, Kristiansen, & Nilsen, 2007; Kathi A. Lefebvre et al., 1999). The samples were analysed at the SMRU, University of St. Andrews. Long-finned pilot whale kidney, liver, stomach contents or faeces (4g) were homogenized in a 1:4 dilution of 50 % methanol and centrifuged at 3000 x g for 10 min. Supernatants were retained for the ELISA method and further diluted to 1:200. The faecal samples were collected for cleanup though a Solid Phase Extraction (SPE), using Strong Anion Exchange (SAX) columns (Supelco, UK) following the method by K.A Lefebvre 1999. Urine was not diluted for the ELISA method and was used directly. All samples were tested in duplicate for the ELISA method.

The LOD (limit of detection) for the direct competitive ELISA method used to measure concentrations of DA in marine mammal excreta has been set to >4 ng/ml DA in urine and >20 ng/g DA in faecal extracts. Of the 21 pilot whales stranded in Pittenweem, 13 urine samples were analysed for DA where four (22.2 %) were greater than the limit of quantification (LOQ) for DA concentration, the highest sample tested had 40.7 ng/ml DA. Eight faecal samples were analysed for DA where two (25.0 %) were > LOQ, the highest faecal extract tested were 79.8 ng/g. The remaining samples were <LOD ranging from 0.45-3.63 ng/mL in urine and 3.36-13.8 ng/g in faecal extracts (Table 2: Domoic acid levels in sampled cases).

Acute DA toxicosis occurs via rapid ingestion of a large amount of DA. Symptoms such as seizures, coma and death can occur. These results do not indicate acute high level DA toxicosis and can thus be ruled out as a cause of the stranding, although a low level of DA exposure was found. Accumulation of toxin from potential vector prey for these pelagic offshore feeders may be indicating offshore blooms that are not currently being monitored, as all monitoring is inshore in Scotland. Although the majority of these samples were negative, this is the first time DA has been detected in pilot whales in Scotland, and shows the importance of understanding the role DA has in the food chain. Future research should focus on offshore monitoring of phytoplankton species, their abundance and the importance of vector prey items consumed by pilot whales, other cetaceans and marine mammals in general. It is therefore concluded that exposure to DA was unlikely to be a contributory cause of this MSE.

DA ng/ml	M280.11/12	M280.10/12	M280.9/12	M280.1/12	M280.17/12	M280.6/12	M280.15/12	M280.16/12	M280.12/12	M280.14/12	M280.8/14	M280.13/12	M280.3/12	M280.2/12	M280.4/12	M280.7/12
Faeces	79.8	46.5	13.5			4.6		3.4				0.0			0.0	0.0
Urine		40.7	5.3	6.8	4.7		3.6	0.0	1.1	1.1	0.5	0.6	0.0	0.0	0.0	

Table 2: Domoic acid levels in sampled cases

15. Morbillivirus

Distemper, caused by cetacean morbillivirus, is a known cause of mortality in cetaceans. An epizootic in a group of animals would have a significant impact on health. A common dolphin mass mortality event in 1994 in the Black Sea was linked to cetacean morbillivirus infection and it was therefore a differential in this mass stranding event.

Total RNA was extracted from sections of frozen (-80°C) lung (n=16) samples and the presence of morbilliviral RNA was tested using reverse transcriptase polymerase chain reaction targeting the conserved N terminus of the morbillivirus N gene (Raga & Banyard, 2008). All reactions were conducted in duplicate.

No lesions consistent with distemper were found in any of the cases and no evidence of morbillivirus nucleic acid was detected in any of the screened samples. Distemper was therefore ruled out as a contributory factor in this MSE.

16. Metal contaminant analysis

Frozen samples were freeze dried and homogenized. For total metal analysis the samples were subjected to total digestion in nitric acid whereas for mercury speciation, samples were solubilized in tetramethylammonium hydroxide. A range of metals were analysed however only values for mercury and selenium were studied in detail

Results from mercury speciation analysis showed the highest concentrations of $MeHg^+$ species accumulating in the liver followed muscle and kidney. However, the highest methylated fractions of total mercury were found in the muscle, on average 81%. Moreover, lowest methylated fractions were found in the liver. This was consistent with the demethylation properties of liver as suggested in previous studies. Bioaccumulative properties of mercury are demonstrated by high correlation between hepatic mercury and age with R2=0.71 (Figure 16). No difference in accumulation trend was detected between female and male groups.

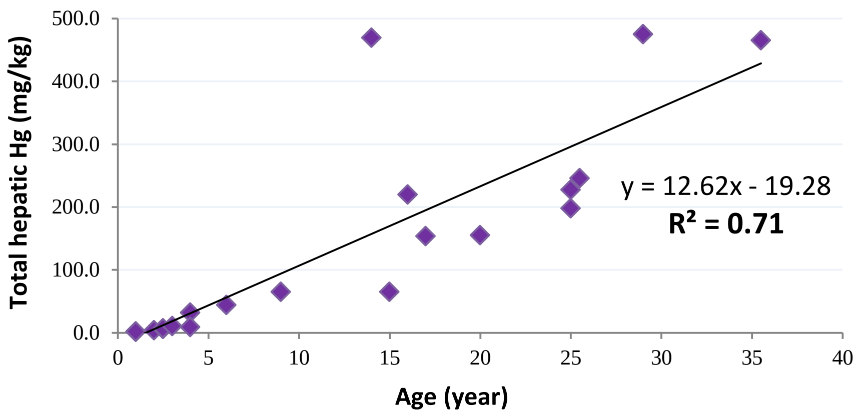

Figure 16: Relationship between hepatic mercury levels and age

Muscle was found to contain the lowest selenium concentration, with an increase in the kidney and liver of the adult whales. The highest selenium concentrations in juveniles were found in the kidney. Hg:Se molar ratio of the adult whales was, less than 1 in the adult liver and kidney and between 0.3 and 3.27 in the muscle tissues. The molar ration in juveniles was less than 1 in all studied tissues. High correlation between mercury and selenium in the liver was found, suggesting strong interaction between these elements, possibly through $MeHg^+$ demethylation.

Levels and ratios of trace elements in pilot whales are not yet fully understood. Research is ongoing using samples derived from this and previous mass stranding events. For the 2012 stranding, however, there is no indication of specific toxicosis in individuals or at a group level which would explain the mass stranding.

17. Teeth ageing, reproductive assessment

Age was estimated for each individual using methods adapted from Lockyer (1993). Five teeth were collected from the mid-section of the left mandible of each sampled individual and preserved in 10% buffered formalin. The least worn/damaged and least curved tooth was selected for age determination from each individual and rinsed in water overnight. All gum tissue was carefully removed using a scalpel without scraping or causing damage the tooth.

A 2-3 mm thick longitudinal section encompassing the centre of the tooth was taken using a Dremel Rotary tool with a sandstone attachment. Sections were decalcified using the commercial decalcifying agent *Rapid Decalcifier* (RDO©) until they were slightly pliable. Once decalcified, the teeth were rinsed thoroughly in water for at least 8 hours.

Sections of 25 µm thickness were cut using a cryostat set at -12°C. Sections were stained with Mayer's haematoxylin (modified by Grue) and 'blued' in a weak ammonia solution. The best sections (those cut through the centre point of the crown and pulp cavity) were selected and mounted on glass slides using DPX. Age was estimated by counting growth layer groups (GLGs) in the dentine of the tooth sections, using a binocular microscope (x 10-50 magnification). Duplicate age estimates were obtained, by two independent readers, without reference to biological data. If the age estimates obtained by the two readers differed by more than 1 year, readings were repeated. If the increments were difficult to count, both readers discussed the interpretation and either reached an agreed age or judged the tooth to be unreadable.

Results from teeth aging can be found in Table 3 & Appendix 1

18. Persistent organic pollutants

Based on results from the 2011 Kyle of Durness mass stranding investigation, persistent organic contaminants testing was not undertaken on this sample set on account of the relatively high cost and prior belief that levels were likely to be low.

19. Weather and tidal factors

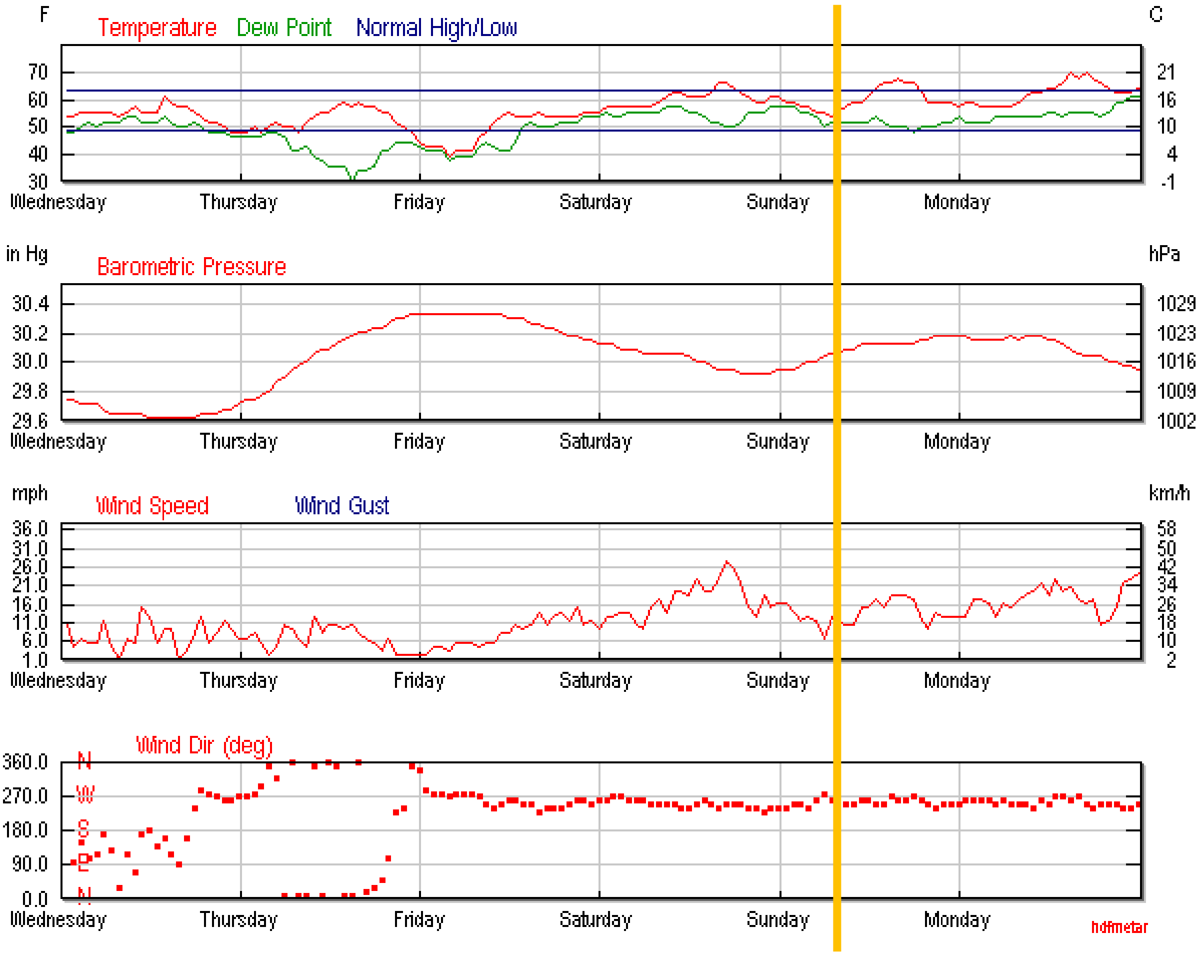

Figure 17: Weather from proximal meteorological station (Leuchars air base) for period Wed 29th August to Monday 3rd September (via http://www.wunderground.com) Yellow line marks beginning of MSE

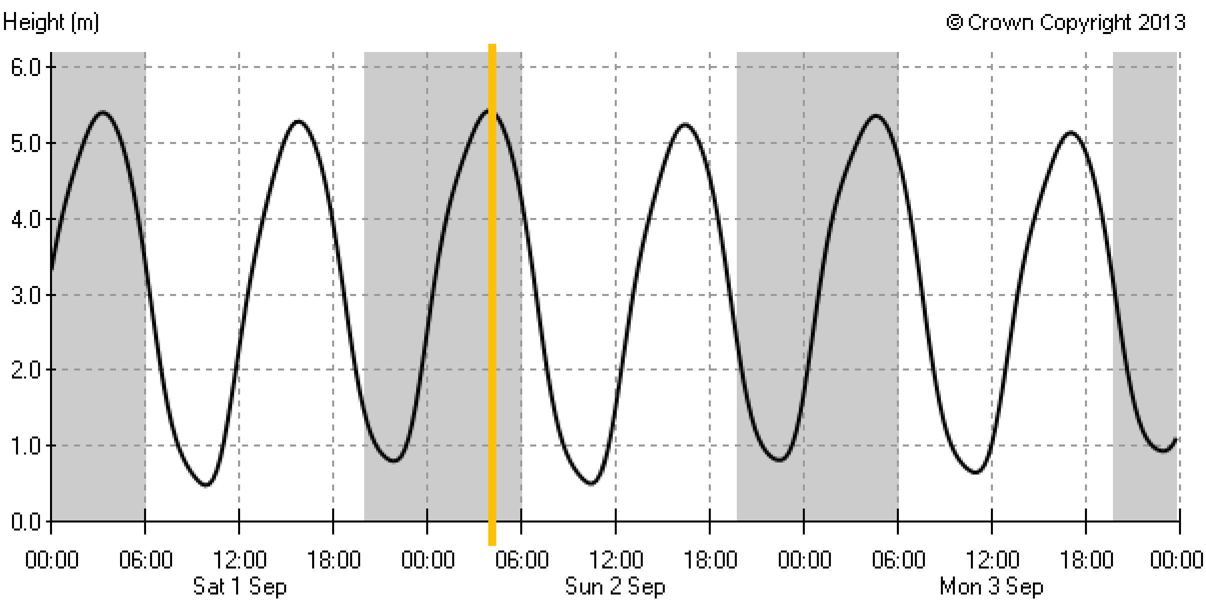

Figure 18: Tides 1-3rd September. Yellow line marks beginning of MSE

No severe weather events were recorded in the locality during the previous week. Wind was recorded as westerly, average 18km/h, decreasing to force 3-4 the day prior to the stranding. Good visibility. Tidal range was large for this area, with a spring high of 5.4m at 03:17 on 2nd September 2012. This extreme tidal range combined with the topography of the stranding beach would have resulted in any animals close to shore rapidly becoming stranded on the falling tide (Figure 18).

20. Conclusion

This work followed and developed on an investigative protocol used for the 2011 long-finned pilot whale MSE in the Kyle of Durness. The investigation assessed a range of factors both natural and anthropogenic with the potential to cause the mass stranding and identified no conclusive single reason which would reasonably account for the stranding event.

The Firths on the east coast of Scotland are not considered typical foraging areas for long-finned pilot whales, although their presence in these regions is not unusual. In the week following the Pittenweem MSE a pod of pilot whales was observed in the upper reaches of the Forth Estuary, however the group eventually made their way back out to sea without any subsequent mass strandings. Proximity to the coast does not mean the animals are at direct risk of stranding, although certain features can significantly increase the stranding hazard. No obvious topographical features were evident which would have channelled animals into shallow water, although the combination of shallow reefs and high tidal range at the stranding site would explain why any animals close to shore as the tide dropped would be at risk of stranding. Additionally, the stranding group comprised a high proportion of calves and sub-adults so it is plausible the actions of the group were dictated by navigational errors of a few individual group members.

Pathological examination showed a number of animals exhibited low grade pathology, in isolation unlikely to lead to the stranding but probably sufficient to act as an internal stressor. Only the ears from one animal, M280.1/12, could be recovered in time to be diagnostic for effective auditory analysis. This one case showed attenuation and loss of hair cells in the very low frequencies (less than 200Hz). This is at the very low end of hearing frequencies for long-finned pilot whales, so the behavioural impact of hearing loss in this range is unclear. The chronicity of this lesion did not define this to be a contributory factor to the stranding, but does serve to suggest at least one of these animals may have been acoustically 'blind' at some frequencies due to historic noise exposure. Equally, a number of potential sources of underwater noise were identified but no specific event could be identified which could reasonably be considered to have contributed to the mass stranding event.

21. Discussion

Public and media interest following an event as dramatic as a cetacean mass stranding event raises an expectation to establish the ‘cause’ for the incident. This is understandable, and investigation requires thorough examination of a wide range of potential causal factors with the aim of establishing, where possible, those which are plausible and for which there is evidence. In many cases the information available does not permit definitive conclusions to be drawn and the significance of a factor or factors has to be carefully interpreted. Whilst it is important not to attribute definitive causation to spatiotemporal correlation, many acoustic or behavioural triggers do not leave diagnostic lesions which can be detected after the event, and therefore 'proof' of either causation or exclusion is difficult.

There were indications of physiological compromise, from both the gross and histological examination and specific scanning electron microscopy analysis of the ear, and it could be speculated that this pathology may have had some bearing on the animals' behaviour or abilities. Acoustic damage to the ear (cochlea), specifically the hair cells responsible for sound transduction, has been one of the main hypotheses in the past 20-30 years for mechanisms in acoustically-induced MSEs. It is very hard to demonstrate pathologically because, due to the rapid autolysis of hair cells, ears have to be extracted and fixed within a few hours of death. Outwith this time window the pathology associated with acoustic trauma becomes indistinguishable from that of autolysis. Additionally, decalcification, imaging and interpretation of the cochlear structures require a high level of technical expertise not always available.

In this case histological assessment of ears did, however, provide valuable information on the potential role of sound exposure on cetacean hearing and these findings represent the first time physical lesions, consistent with chronic sound exposure, have been identified in mass stranding cases. Whilst a result based on n=1 cannot be conclusive it does suggest that this approach to hearing analysis is worthwhile and may be the best option to elucidate the effects of noise exposure on these populations in future stranding events.

22. Recommendations

- Using the experience of the 2011 and 2012 MSE's, develop protocols to be used during future mass stranding events to optimise the diagnostic capacity of any subsequent investigations. Emphasis should be case triage and methodologies to acquire time critical data or samples
- Closer input from members of the live animal rescue teams would assist with acquiring essential morphometric and behavioural data from any animals refloated or euthanised as part of any rescue attempt.
- In busy parts of the ocean, establishing normal, background, sound intensity levels is arguably a necessary precursor before evaluating the significance of a specific event. It is understood there is currently work to develop a noise register based on, amongst other sources, the activity logs of anthropogenic activities with the potential to generate underwater noise. This register would be of great help in future MSE's if it were able to contain data at a sufficiently high temporal and spatial resolution.
- Consideration should be given to the specific impact of significant levels of underwater sound in coastal locations and the potential effect of a two stage process, whereby anthropogenic sounds predispose a group to become confused and subsequently stranded in unfamiliar coastal landscape. Deployment of passive acoustic monitoring equipment could help assess the presence of echolocating odontocetes in key areas.

23. Acknowledgements

This investigation was co-ordinated by staff from the Scottish Marine Animal Stranding Scheme and the Cetacean Stranding Investigation Programme. Logistical, technical and scientific input to this investigation was also supplied by a large number of individuals, groups and organisations. Their collective time, knowledge and expertise were essential for this work to be undertaken and the authors would like to extend their thanks to all involved in this investigation. Particular thanks are due to the staff and students of SMRU who assisted with the necropsy, WDC staff and volunteers, Martin Smith of Forth & Tay Marine Ltd and the Fife countryside rangers who organised the logistics for carcase disposal. Extensive photographs and shipping data were provided by Stephen Marsh from British Divers Marine Life Rescue (BDMLR) and medics trained and coordinated by BDMLR ensured the successful refloat of many of the animals. Thanks to Rod Jones from the Fleet Maritime Environmental Policy centre for collating MoD activity logs and to Kim Willoughby at the Moredun Research Institute for expediting viral screening of the samples. Specific thanks are due to Elaine Tait for input and advice on the structure of the report. Other valuable comments on the draft text were supplied Kate Brookes, Harriet Auty Mark Tasker and Alicia Coupe.

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Appendix 1: Morphometric and age summary

M reference SAC	Sex	Final age (Years)	Age Group	Length (PM) (cm)	Girth (cm)	Dorsal Blubber (mm)	Lateral Blubber (mm)	Ventral Blubber (mm)	Heart Weight (g)
M280.01/12	F	2.5	subadult	291	176	34	26	30	
M280.02/12	F	26	adult	420	238	45	31	37	
M280.03/12	F	17	adult	389	230	51	34	35	
M280.04/12	F	NA	adult	420	244	44	29	39	4000
M280.05/12	F	20	adult	411	246	50	29	36	
M280.06/12	F	NA	juvenile	192	129	30	16	20	
M280.07/12	F	NA	juvenile	191	129	31	25	26	
M280.08/12	F	NA	juvenile	194	138	32	23	23	
M280.09/12	M	NA	subadult	333	216	48	31	29	3250
M280.10/12	F	4	subadult	291	200	38	33	39	2500
M280.11/12	F	29	adult	445	250	58	33	30	
M280.12/12	F	3	subadult	315	192	42	26	34	2500
M280.13/12	F	9	adult	360	226	50	29	35	
M280.14/12	M	2.5	subadult	296	182	39	27	29	
M280.15/12	F	36	adult	462	262	50	33	40	
M280.16/12	M	4	subadult	318	192	42	30	35	
M280.17/12	M	2	subadult	287	186	45	31	35	
M280.18/12	F	25	adult	440	250	40	29	30	
M280.19/12	M	15	adult	444	260	33	25	29	
M280.20/12	F	14	adult	435	266	34	30	32	
M280.21/12	M	16	adult	538	258	33	36	32	

Table 3: Morphometrics and age summary

Appendix 2: Activities licensed by Marine Scotland in firth of forth potentially active during August and September 2012

Activity	Licensee	Type	Location	Licence Number	Activity from	Activity to
SURVEY WORK	Repsol Nuevas Energias	Geotechnical Survey	Firth of Forth	N/A exempt	21/08/2012	Not given
	Repsol Nuevas Energias	Geophysical Survey	Firth of Forth	N/A exempt	21/08/2012	Not given
	SeaGreen Wind Energy Ltd	Scientific Instrument Deployment	Firth of Forth	04348/11/0	11/11/2011	31/08/2012
	Neart na Gaoithe	Geotechnical Survey	Outer Firth of Tay	N/A exempt	01/08/2012	01/10/2012
	EMU Ltd	AWAC Deployment	Leith	04397/12/0	Not given	30-Sep-12
	Babcock Engineering Services	Benthic Sampling	Rosyth	04566/12/0	Not given	03-Sep-13
DREDGING	Forth Ports	Dredging/Sea Disposal	Rosyth	04053/11/0	Not given	16-May-14
	Forth Ports	Dredging/Sea Disposal	Grangemouth	03995/12/0 - 4836	Not given	10-Oct-13
	Forth Ports	Dredging/Sea Disposal	Leith	04052/11/0	Not given	07-May-14
	Forth Ports	Dredging/Sea disposal	Kirkcaldy	04457/12/0	Not given	30-Apr-15
	Babcock	Dredging/Sea disposal	Rosyth	04477/12/0	Not given	13-Aug-13
	Scottish Enterprise	Dredging/Sea disposal	Methil	04550/12/0	Not given	13-Aug-13
	British Waterways Scotland	Plough Dredging	Forth-Clyde Canal	04544/12/0	Not given	14-Aug-13
	Royal Forth Yacht Club	Plough Dredging	Granton	04375/12/0	Not given	25-Jun-15

	Grangemouth Yacht Club	Plough Dredging	Grangemouth Old Harbour	04334/12/0	Not given	22-Feb-15
CONSTRUCTION	Grahams Construction	Remedial works to Quay wall	Fife Energy Park, Methil	04415/12/0	Not given	05-Mar-13
	City of Edinburgh Council	Repairs to gabion groyne	Portobello	04404/12/0	Not given	30-Apr-13
	Henry Abrams & Sons Ltd	Temporary mooring installation	Firth of Forth	04204/11/0	Not given	14-Aug-17
	RNLI	Slipway extension	Burntisland	04311/11/0	Not given	30-Apr-13
	Network Rail	Coastal defence repairs	Aberdour to Burntisland	04373/12/0	Not given	20-May-13
	Keir Scotland	Outfall construction	Methil Docks	04276/11/0	Not given	14-Sep-12

Table 4: Activities licensed by Marine Scotland

Appendix 3: DECC licenced activities on 2nd September 2012

DECC Ref	Operator	Name	Location (Quad/Block)	Survey Type	Form Received	Proposed Start Date	Proposed End Date	Duration of Survey (Days)	Consent Issued
2723	TGS-NOPEC Geophysical Company (UK) Limited	NP123D0001	14/30, 15/16-18, 15/21-23, 15/26-28, 20/5 & 21/1-3	3D Regional	07/02/2012	10/04/2012	30/10/2012	95	16/04/2012
2737	Ithaca	Stella	30/6a	2D Site Survey	24/02/2012	07/03/2012	30/09/2012	2	13/03/2012
2738	Ithaca	Stella	30/6a & 30/6c	Seabed Survey	24/02/2012	07/03/2012	30/09/2012	1.5	Notification Only 29/02/2012
2739	Polarcus MC Ltd	PS12000Q21	21/23&24 & 21/27-29	3D Regional	28/02/2012	15/03/2012	31/10/2012	100	23/03/2012
2746	PGS Exploration (UK) Ltd	PP123DGHBR	210/22-55, 210/27-30, 211/20, 211/26&27, 2/2-5, 2/8-10, 3/1&2, 3/6-8	3D Regional	02/03/2012	20/03/2012	30/09/2012	37	05/04/2012
2748	BP Exploration Operating Company Ltd	North Uist	213/25c	VSP	08/03/2012	01/05/2012	30/09/2012	1	03/04/2012
2749	Total	Longmorn	205/4, 205/5, 205/8, 205/9, 205/10, 205/13, 205/14, 205/15, 206/1, 206/2, 206/6, 206/11	3D Regional	08/03/2012	01/04/2012	31/10/2012	64	02/04/2012
2750	BP Exploration Operating Company Ltd	Clair	206/7, 206/8, 206/9, 206/11, 206/12, 206/13	2D Site	09/03/2012	01/05/2012	30/09/2012	45	04/04/2012

2753	Nexen	Buzzard	20/01s	VSP	12/03/2012	01/05/2012	31/10/2012	1	04/05/2012
2757	BP Exploration Operating Company Ltd	Clair	206/6, 206/7a, 206/7b, 206/8, 206/11a, 206/11b, 206/11c, 206/12a, 206/12b, 206/13a, 206/13b	3D OBC/OBS & 3D Multi-component 4C	15/03/2012	01/06/2012	30/09/2012	77	12/04/2012
2772	Total E&P Nederland B.V.	J36175	44/29, 44/30, 49/04, 49/05, 49/10	44/29, 44/30, 49/04, 49/05, 49/10	30/03/2012	15/04/2012	31/12/2012	150-210	24/05/2012
2776	TGS-NOPEC Geophysical Company (UK) Limited	NP123D0002 (EB12)	208/9&10, 208/13-15, 208/20, 209/1-14, 209/16, 210/1, 218/28-30, 219/26	3D Regional	02/04/2012	16/05/2012	30/10/2012	95	09/05/2012
2778	Ithaca	Stella	30/6a & 30/6c	Seabed Survey	07/04/2012	10/04/2012	30/09/2012	1.5	Notification Only 10/04/2012
2779	RWE Dea UK	Breagh	42/13a	VSP	04/04/2012	01/07/2012	31/10/2012	2	02/04/2012
2790	BP	Marnock	22/24	2D Site Survey	20/04/2012	01/05/2012	30/09/2012	3	10/05/2012
2791	BP	Bruce	9/8 & 9/9	2D Site Survey	20/04/2012	01/05/2012	30/09/2012	4	10/05/2012
2793	BP	Mungo	22/20 & 23/16	2D Site Survey	20/04/2012	01/05/2012	30/09/2012	3	10/05/2012
2794	BP	Keith	9/8, 9/9, 9/13 & 9/14	2D Site Survey	20/04/2012	01/05/2012	30/09/2012	3	10/05/2012
2796	BP	Machar	23/16-3	n/a	24/04/2012	01/05/2012	30/09/2012	3	Notification Only 10/05/2012

2798	CGGVeritas Services (UK) LTD	VC123DW051	208/6&7, 208/11&12, 208/16&17, 208/21, 213/14&15, 213/18-20, 213/22-25, 213/27-30 & 214/6-29	3D Regional Survey	25/04/2012	01/06/2012	10/10/2012	60	Cancelled - 22/05/2012
2801	Centrica	North Rhyl	113/27b	VSP	27/04/2012	10/08/2012	30/09/2012	1	14/05/2012
2806	TGS-NOPEC Geophysical Company (UK) Limited	ETOW12	210/5, 219/25,30,220/16, 21/22, 26/27	Regional Survey	30/04/2012	15/06/2012	30/10/2012	50	01/06/2012
2809	BP	Cyrus	16/28	2D Site Survey	03/05/2012	01/06/2012	30/09/2012	7	25/05/2012
2812	Shell U.K. Limited	UK Sector, SNS	Numerous	Acoustic annual pipeline survey	11/05/2012	21/05/2012	21/07/2102	30	Notification Only 11/05/2012
2815	PGS Exploration (UK) Ltd	PP123DGBYL	Q8/10,15,20,25, Q9/1-24	3D Regional Survey	16/05/2012	15/06/2012	30/09/2012	75	25/06/2012
2818	Dana	Pharos	22/4e	VSP	23/05/2012	01/08/2012	31/12/2012	2	22/06/2012
2823	Centrica	Greater York	42/26-28, 47/1-3, 47/7-8	3D Reservoir Survey	28/05/2012	25/06/2012	30/11/2012	80	cancelled
2824	Nexen	Bardolph	20/07	VSP	28/05/2012	01/07/2012	31/12/2012	0.5-1	28/06/2012
2825	Nexen	Blackbird	20/02a	VSP	29/05/2012	01/07/2012	31/12/2012	0.5-1	22/06/2012

2826	Dolphin Limited	Geophysical	Q29 CNS	29/6, 29/11, 29/16, 29/21, 29/26, 37/1, 29/7, 29/12, 29/17, 29/22, 29/27, 37/2, 29/8, 29/13, 29/18, 29/23, 29/28, 37/3, 29/9, 29/14, 29/19, 29/24, 29/29, 37/4, 29/10, 29/15, 29/20, 29/25, 29/30, 37/5, 30/16, 30/21, 30/26, 30/27, 38/2, 30/18, 30/23, 30/28, 38/3	3D Regional Survey	30/05/2012	08/06/2012	31/10/2012	145	12/06/2012
2829	Nexen		Griffon	19/05b	VSP Survey	05/06/2012	02/08/2012	31/12/2012	0.5-1	28/06/2012
2830	Total		Elgin	22/30c, 29/4d, 29/5b, 29/5c	4D Reservoir	04/06/2012	01/07/2012	30/11/2012	90	11/07/2012
2832	Total		Martin Linge	3/9, 3/10, 3/14, 3/15, 3/18, 3/19, 3/20, 29/6, 29/9, 30/4, 30/5, 30/7, 30/8	3D Reservoir Survey	08/06/2012	01/08/2012	01/11/2012	90	18/07/2012
2833	Enquest		Cairngorm	16/2b	2D Site	08/06/2012	01/07/2012	30/10/2012	7	29/06/2012
2834	Enquest		Juniper	3/11a	2D Site	08/06/2012	01/07/2012	30/10/2012	7	09/07/2012
2835	Enquest		Scolty, Crathes & Torphins	21/1, 21/7, 21/8, 21/9, 21/10, 21/12, 21/13 & 21/18	2D Site	08/06/2012	01/07/2012	30/10/2012	34	Cancelled DECC ref 2852 now refers
2837	Enquest		Marram	3/11a	2D	08/06/2012	01/07/2012	30/10/2012	7	23/07/2012
2838	Enquest		Caterpillar	3/11a	2D	08/06/2012	01/07/2012	30/10/2012	7	19/07/2012

2841	Statoil (UK) Limited	Mariner	9/11a, 9/11b, 9/12c	3D Regional Survey	18/06/2012	20/07/2012	15/09/2012	30	24/07/2012
2844	Nautical Petroleum PLC	Kraken South	9/2b, 9/2c,9/7b & 9/1a	2D Site Survey	20/06/2012	08/07/2012	30/10/2012	7	13/07/2012
2845	Nautical Petroleum PLC	Kraken West	9/2b & 9/1a	2D Site Survey	20/06/2012	08/07/2012	30/10/2012	7	13/07/2012
2847	BG	Thunderer	30/2a	2D Site Survey	21/06/2012	19/07/2012	30/09/2012	6	12/08/2012
2848	Shell	Scoter	22/30aF2, 22/30aF1, 22/30a - 22/30e, 22/30b, 23/21, 22/25a, 22/25aF3, 22/25aF2, 23/26d, 23/26dF1, 23/26c, 23/26e & 23/26a	4D Reservoir Survey	22/06/2012	15/07/2012	15/11/2012	35	17/07/2012
2850	Nautical Petroleum PLC	Kraken Head	9/2b, 9/1a, 9/3c, 9/2a & 3/27a	2D Site Survey	26/06/2012	08/07/2012	30/10/2012	7	19/07/2012
2851	Shell	ETAP	22/18c, 22/19a, 22/19c, 22/23a, 22/23c, 22/23e, 22/24a, 22/24b, 22/24c, 22/24d, 22/24e, 22/24f, 22/25a, 22/25aF2, 22/25aF3, 22/25c, 22/25f, 22/29a, 22/29c, 22/30a, 22/30aF1, 22/30aF2, 22/30e, 22/30f, 22/30g, 23/26cF1, 23/26a, 23/26CF1, 23/26d, 23/26e, 23/26aF1	4D Reservoir	26/06/2012	20/07/2012	15/11/2012	60	24/07/2012

2852	Enquest	Solty-Crathes-Torphins	21/1a, 21/1b, 21/1c, 21/6b, 21/7b, 21/8c, 21/8a, 21/12b, 21/13c, 21/13b, 21/9b, 21/9a, 21/10a, 21/10c, 21/18a	2D site survey	26/06/2012	07/07/2012	30/10/2012	34	06/07/2012
2853	GDF Suez E&P UK Ltd	Cygnus	44/11 & 44/12	2D Site Survey	28/06/2012	20/07/2012	30/10/2012	7	19/07/2012
2854	GDF Suez E&P UK Ltd	Marconi	30/1f & 30/1c	2D Site Survey	02/07/2012	28/07/2012	30/10/2012	6	27/08/2012
2855	EnQuest	Goosander	21/12c, 21/12d, 21/12a	Pipeline Route Survey	04/07/2012	24/07/2012	30/10/2012	7	06/08/2012
2857	EOG Resources (UK) Ltd	CNS	21/12b	Analogue Survey	05/07/2012	06/07/2012	06/10/2012	3	Notification Only 05/07/2012
2859	GDF Suez E&P UK Ltd	Jaqui	30/13	2D Site survey	06/07/2012	30/07/2012	30/10/2012	5	16/08/2012
2860	Statoil	ST12003	213/16, 17, 21, 22, 23, 26, 27 & 212/1	3D Regional Survey	09/07/2012	08/08/2012	15/09/2012	27	16/08/2012
2863	Shell	Gannet	21/25 & 21/30	Gannet	17/07/2012	02/08/2012	28/10/2012	5	16/08/2012
2864	GDF Suez E&P UK Ltd	Cygnus	44/11 & 44/12	Cygnus	18/07/2012	05/08/2012	30/10/2012	6	08/08/2012
2865	BG Group	Jackdaw	30/2 & 30/3	Jackdaw	18/07/2012	11/08/2012	30/10/2012	6	10/08/2012
2866	Shell U.K. Limited	Bittern	21/25, 28/05 & 29/1	Bittern	18/07/2012	02/08/2012	28/10/2012	2	Notification Only 19/07/2012
2867	Chevron	Rosebank	213/26, 213/27, 213/28, 205/01 & 205/02	Rosebank	19/07/2012	15/08/2012	15/10/2012	62	20/08/2012

2868	OMV	OM12EM0001	204/9b, 10b, 13a, 14a, 14b, 14d, 15, 19b, 19c	OM12EM0001	20/07/2012	01/09/2012	30/09/2012	10	Notification Only 06/09/2012
2869	Taqa Bratani Ltd	Cormorant	211/21 & 211/26	Cormorant	20/07/2012	21/08/2012	30/10/2012	8	27/08/2012
2870	TGS-NOPEC Geophysical Company (UK) Limited	NP123D0004	208/1-4, 6-8, 214/5b, 5c, 9a, 10a, 217/26-30	NP123D0004	24/07/2012	15/08/2012	30/09/2012	60	10/08/2012
2871	Apache	AA123D0002	21/10	AA123D0002	25/07/2012	01/09/2012	31/10/2012	3	04/09/2012
2872	Centrica	Rose	47/15a, 47/15b & 47/10d	Rose	26/07/2012	01/08/2012	15/09/2012	2	Notification Only 26/07/2012
2873	Shell	Leman	49/26a	Leman	27/07/2012	07/08/2012	20/09/2012	1	09/08/2012
2874	Dana	Western Isles Project	210/24	Shallow Drilling	31/07/2012	20/08/2012	10/09/2012	8	17/08/2012
2880	Hydrocarbon Resources Ltd	Rhyl	113/27b	Geophysical Borehole Survey	06/08/2012	13/08/2012	13/09/2012	2	16/08/2012
2881	Shell	Commander	22/30a	2D Site Survey	06/08/2012	22/08/2012	28/10/2012	3	27/08/2012
2885	EMGS ASA	EMGS1206	217/9, 217/14, 217/10, 217/15, 218/6, 218/11b, 218/12a, 218/13, 218/18, 218/19, 218/20, 219/16, 219/21, 219/17, 219/22, 219/23, 219/24, 219/39 & 219/30	3D CSEM Survey	06/08/2012	01/09/2012	01/11/2012	14	31/08/2012
2888	BG Group	White Bear	22/4	22/08/2012	08/08/2012	22/08/2012	02/10/2012	4	14/09/2012

2891	E.On E&P UK Ltd	Johnston	43/27a	25/08/2012	14/08/2012	25/08/2012	01/10/2012	1	Notification only 14/08/2012
2892 (was 2730)	Maersk	Brochel	15/18a	VSP	17/07/2012	20/08/2012	30/10/2012	2	15/08/2012
2893	Shell	Inde	49/24a	2D Site Survey	16/08/2012	26/08/2012	28/10/2012	7	Notification only 20/08/2012
2896	Nexen	Lily	20/1	Magnetic survey with sampling	22/08/2012	28/08/2012	05/11/2012	5	Notification Only 30/08/2012
2901	Nexen	Ivy	21/02a, 03a, 03b & 03f	Magnetic Survey with sampling	24/08/2012	28/08/2012	05/11/2012	5	Notification Only 30/08/2012
2902	EnQuest	Kildrummy	15/12 & 15/17	VSP Survey	30/08/2012	30/08/2012	28/02/2013	2	24/09/2012

Table 5: DECC licenced activities active on 02/09/2012

			Life history		Skeletal archive	Toxicology									Bacteriology								
ID	Age	sex	Teeth	Stomach cont.	Scapula	Skin	Muscle	Blubber	Liver	Kidney	Lung	Blood	Faeces	Urine	Milk	Liver	Kidney	Lung	Spleen	SI	Heart	MLN	Blood	Brain
M280.1	**subadult**	**m**	1	x	1	2	2	2	2	2	2	1	1	1	x	x	x	x	x	x	x	x	1	x
M280.2	**adult**	**f**	1	1	x	2	2	2	2	2	2	1	1	1	x	1	1	1	1	1	x	1	1	1
M280.3	**adult**	**f**	1	1	x	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	x	1	1	1
M280.4	**adult**	**m**	x	1	x	2	2	2	2	2	2	1	x	1	1	1	1	1	x	1	x	1	1	x
M280.5	**adult**	**f**	1	1	x	2	2	2	2	2	2	x	x	x	x	1	1	1	1	1	1	x	x	x
M280.6	**calf**	**f**	1	x	x	2	2	2	2	2	2	1	1	x	x	1	1	1	1	1	1	1	1	x
M280.7	**calf**	**f**	1	x	x	2	2	2	2	2	2	x	1	x	x	1	1	1	x	1	1	x	x	x
M280.8	**calf**	**f**	1	x	x	2	2	2	2	2	2	1	1	1	x	1	1	1	x	1	1	1	1	x
M280.9	**subadult**	**m**	x	x	x	2	2	2	2	2	2	1	1	1	x	1	1	1	x	x	x	x	1	x
M280.10	**subadult**	**m**	1	1	x	2	2	2	2	2	2	1	1	1	x	1	1	1	1	1	x	x	1	1
M280.11	**adult**	**f**	1	x	1	2	2	2	2	2	2	x	1	x	x	1	1	1	1	1	1	x	x	x
M280.12	**subadult**	**f**	1	x	x	2	2	2	2	2	2	1	1	1	x	1	1	1	1	1	1	x	1	x
M280.13	**adult**	**f**	1	x	x	2	2	2	2	2	2	x	x	1	1	1	1	1	1	x	1	x	x	x
M280.14	**subadult**	**m**	1	1	x	2	2	2	2	2	2	1	1	1	x	1	1	1	1	1	x	1	1	1
M280.15	**adult**	**f**	1	x	x	2	2	2	2	2	2	1	1	x	x	1	1	1	1	1	x	1	1	x
M280.16	**subadult**	**m**	1	x	x	2	2	2	2	2	2	x	1	1	x	1	1	1	1	1	1	1	x	x
M280.17	**subadult**	**m**	1	1	x	2	2	2	2	2	2	x	1	1	x	1	1	1	1	1	1	x	x	x
M280.18	**adult**	**f**	1	1	x	3	3	3	2	2	2	1	1	x	x	1	1	1	x	1	x	1	1	x
M280.19	**adult**	**m**	1	x	x	3	3	3	2	2	2	1	1	x	x	1	1	1	x	x	x	x	1	x
M280.20	**adult**	**f**	1	x	x	3	3	3	2	2	2	1	1	x	x	1	1	1	x	x	x	x	1	x
M280.21	**adult**	**m**	1	x	x	3	3	3	x	2	2	x	1	x	x	1	1	1	x	x	x	x	x	x
M281/12	**adult**	**f**	1	x	x	2	2	2	2	2	2	1	1	1	x	1	1	1	1	1	x	x	1	1
Total			**16**	**8**	**2**	**46**	**46**	**46**	**40**	**42**	**42**	**14**	**18**	**12**	**3**	**20**	**20**	**20**	**12**	**15**	**9**	**9**	**14**	**4**

Table 6: Number of samples archived (1,2 = number of samples taken, x sample not taken)