APPENDIX C

MARINE FLORA AND FAUNA

Overview of Osmoregulation in Fish

Bony fish (teleosts) are osmoregulators i.e. they control the ionic concentration of their internal body fluids within fairly narrow limits despite a potentially much wider range of external ion concentrations. In full seawater with an osmotic concentration of ~1000 mosM/kg adult fish tend to maintain their osmotic concentration around 300-350 mOsmoles, which is equivalent to an external osmotic concentration of estuarine salinity of 11 or 12psu. Larvae on the other hand, while also osmoregulating, tend to be less efficient in general than adults, with wider internal osmotic concentrations depending on their stage of development. Their smaller size and therefore greater surface area to volume ratio is an important reason for this difference. Marine fish are said to be hypo-osmotic regulators in full seawater or outer estuarine conditions, while freshwater fish and those marine fish, which can penetrate into inner estuaries are said to be hyperosmotic regulators because their internal osmotic concentration is higher than that of the external medium.

In the marine environment the tendency will be for salts to diffuse from the external environment into the fish because of the higher external salt concentration and for water to move by osmosis in the other direction across the gills (in particular) or the skin. To counteract this marine fish (adults as well as larvae) drink water to replace lost water and also actively excrete salts that have come in via diffusion, in drinking and in food. The main site of salt excretion in the adult is via the gills and to a lesser extent the kidneys and the gut. The specific cells involved, ionocytes, in particular MCR's (mitochondrial rich cells) also know as chloride cells, which are present in high concentrations in the gill are adapted for ion excretion. In the larvae, ion-excreting cells tend to be spread around the body and only become concentrated in the gills as these begin to develop.

In elasmobranches (sharks, skates and rays), the blood salt content is similar to that of marine teleosts, however the osmotic concentration is much higher, in fact just higher than seawater making them hyperosmotic to seawater. This is achieved mainly by retaining high concentrations of urea (a by product of protein metabolism) in the blood and to a lesser extent [1]TMAO an organic compound. This situation means that sharks do not need to drink to maintain water balance because their blood is almost isosmotic with seawater but instead excrete salts, which diffuse in or are ingested with their food. The main site of salt excretion is the rectal gland but the gills and the kidneys are also involved.

Fish, which can penetrate into estuaries and therefore tolerate a range of salinities are termed euryhaline while marine fish which cannot enter estuaries or are confined to the outer areas are said to be stenohaline. Many of the important commercial species in and around the Irish Sea (cod, herring, sprat, plaice, sole) are euryhaline to some extent. Others such as bass and flounder are strongly euryhaline. Elasmobranchs generally have fewer euryhaline species, although species such as the common dogfish, *Scyliorhinus canicula* are moderately euryhaline. It is true of most euryhaline organisms (animal and plant) that their salinity tolerance tends to be extended at both ends of the salinity spectrum i.e. at upper as well as lower salinities.

[1] TMAO = Trimethylamine Oxide

Osmoregulation, i.e. the mechanism by which fish control their ionic and osmotic balance has to develop at a very early stage, i.e. in the egg embryo and larvae if the species is to survive. Furthermore, salinity is also known to affect the success rate of fertilisation of fish eggs by sperm. These effects are present in all fish but will vary depending on the specific biology of each. For example Baltic herring (*Clupea harengus membras*) and Pacific Herring *C. pallesai* have optimum fertilizations success at 8psu and 16psu respectively because both either live permanently in conditions of reduced salinity (Baltic Herring) or spawn in such areas (Pacific herring). Atlantic herring (*C. harengus*) in contrast has a higher and broader range of salinity over which successful fertilisation takes place (~20-50psu), reflecting the broader range of salinities in which it spawns (Holliday and Blaxter, 1960). It is clear therefore that the optimum salinity for a given species will vary depending on its general biology and even within a given species may vary depending on its stage of development.

Detailed assessment of the osmoregulatory capacity and salinity tolerance of fish species is limited to a fairly small number of species, including euryhaline, anadromous, catadromous and marine species (e.g. flounder, bass, salmon, eel, cod etc.). Moreover, the maximum salinity at which these species are tested is usually full seawater, i.e. anything from 32psu to 35 psu depending on the study, unless specialised species which occur in naturally hypersaline environments are be researched. A small number of studies have extended the testing range a bit above normal seawater salinities even when examining species which don't naturally occur in hypersaline environments and these can throw light on how fish may react at the higher salinities predicted around the Islandmagee outfall. However, the mechanisms that fish use to osmoregulate at 22psu and 34 psu for example, will be the same used to osmoregulate at 36, 37 or 38psu also. Several fish species have been shown to osmoregulate in a wider range of salinities than they would normally encounter in nature, for example yolk-sac larvae of Atlantic herring (*C. harengus*) were seen to osmoregulate in salinities from 11psu to 48psu, while yolk sac larvae of plaice (*Pleuronectes platessa*) osmoregulated in the range 5psu to 65psu with the efficiency of regulation improving in older metamorphosed larvae (Holliday and Blaxter, 1960; Holliday, 1965; Holliday and Jones 1967 quoted in Varsamos *et al.,* (2005)

Clearly, osmoregulation outside a species normal range will require more energetic effort because ion-excretion and the other physiological responses associated with osmoregulation all require energy and the farther from that optimum range the greater the energy likely to be required. Nevertheless, the increased salinities associated with the proposed Islandmagee brine discharge within the nearfield (100-200m) around the outfall (namely 36-38) are relatively minor compared to the upper salinities ranges quoted above for the herring and plaice larvae so that any extra energy requirements associated with osmoregulation are likely to be very modest. Furthermore, fish eggs and larvae are largely controlled by the movement of the general water body and a recent modelling study in the Irish Sea has shown that eggs and larvae can be carried on average 20 to 80km from their spawning grounds (depending on the location of the latter) before they settle (in the case of demersal species) or shoal (in the case of pelagic species such as sprat) and in some cases the distances can be much greater i.e.> 100-300km (van der Molen *et. al,.* 2007). It is unlikely therefore that any given group of fish eggs and larvae are going to be present in waters of significantly elevated salinity (i.e. close in to the outfall), for any extended period, which will reduce the risk of adverse impacts to these generally more sensitive life stages. Furthermore an extensive survey of fish eggs and larvae in the North Sea and the Irish Sea (Conway *et al.,* 1997) showed that most of the common [2]species encountered, although distributed throughout much of the water column sampled, were nevertheless concentrated heavily in the upper layers with density generally diminishing in a pronounced manner with depth. Sandeel larvae were an exception to this trend as they were more or less evenly distributed down the water column. This means that, on average, fish eggs and larvae have a better chance of avoiding the higher salinity concentrations from the proposed diffuser because the density of the plume will keep more concentrated salinities closer to the bottom. It is also worth noting that the diffuser location has not been identified as being a spawning ground for important economic or ecological species nor have commercial fish spawning grounds been reported to occur within the mixing zone.

In the wider near to medium field area the rise in salinity predicted by the model is very low i.e. less than 0.3psu with a maximum salinity in the far-field of less than 34.25psu where 34.20psu is the chosen background salinity for the model. To put these figures into context, Gowen *et al.,* (1998) in a survey in August 1996, extending from the North Channel south of Islandmagee, as far as the north west coast of Donegal showed that near-surface salinities in the North Channel which averaged around 34.3psu began to rise in an east to west direction beginning off the north Antrim coast rising to 34.9psu off Malin Head and to 35.35psu off the NW coast of Donegal. Thus higher salinity water is a common feature of the sea area not far to the north of the study site and these same waters hold commercial stocks of the same species of fish and shellfish taken in the Irish Sea and the south coast including for example cod, haddock, plaice, herring and brown crab (Marine Institute, 2007).

Sandeels

Sandeel were specifically mentioned by the RSPB as a species, which may be of particular importance as a prey item for tern colonies in the region. In a study on the lesser sandeel (*Ammodytes marinus*) in waters around the Shetlands (Wright *et al.,* 2000) modelled the preferences of this species based on extensive sampling in areas where they occurred. They showed that this species like other sandeel species had a preference for sandy sediments, and in particular coarse sand areas (<2mm particle size) with strong currents, typical of a rippled bottom and with a silt content of less than 2%. The species were absent from sediment with silt >10% and declined in the 2-10% silt range. Fine gravels including shell fragments could also be present in sandeel habitat but coarse gravels or pebbles tended to be avoided. Sandeels burrow in these sediments and emerge during the day to forage. It is expected that the areas around the proposed brine diffuser off Islandmagee are dominated by sediments that are coarser, often far coarser, than those preferred by sandeel. The closest concentration of suitable sand is in the shallow waters of Portmuck Bay, which is more than 1km from the outfall and subject to only marginal increases in salinity, and well within the range of the known tolerance of *A. marinus*, and almost certainly of other members of the genus also. Thus, while small pockets of sandeel can't be ruled out from the area, a

[2] The most common larvae caught in plankton hauls in this survey were: sprat (44%), dab (14.5%), sandeel (10.6%), dragonet (5.2%), whiting (5%), butterfish (2.8%) and cod (2%).

large concentration of these prey fish is unlikely to occur. The species may forage in the area but they tend to forage over the sediment they inhabit (Macer, 1966 and Reay 1970 quoted in Wright *et al.*, 2000).

The salinity tolerance of sandeels doesn't appear to have been specifically reported in the literature however they may be mildly euryhaline because in the case of *Sandeel (Ammodytes spp.)* they are reported to form part of the diet of grey seals in the Baltic Sea (Lundström *et al.,* 2010), where salinities are lower than in the North Sea (Sandeel diet of eels). In a recent study of the specie's preferences in the North Sea, van der Kooij *et al.,* (2008), showed that across the Dogger Bank *A. marinus* was present in salinities ranging from 34.76 to 35.18psu showing a slight preference for stations with higher salinities (34.9-35.0psu). The brine dispersal model for Islandmagee show that outside the immediate mixing zone of the plume, that the salinity will be well below these levels and well within the tolerance range of sandeel and indeed all fish species occurring in the area.

The possibility that some fish will avoid the immediate area of the diffuser within a few tens of metres particularly bottom species such as gobies, dragonets and wrasse cannot be ruled out but this would constitute only a minor negative impact considering the very small area involved. Also, the likelihood is that fish will enter periodically into the area of higher salinity, e.g. when pursuing prey or foraging. In avoidance experiments using juvenile sea bream (*Pagrus major*) acclimated to 35psu in artificial seawater, no avoidance was detected at or below 40psu, with increasing degrees of avoidance with increasing salinity, i.e. as the salinity increased above 40psu the time spent in the higher salinity water grew shorter than the time spent in normal salinity water (Iso *et. al.,* 1994).

Baltic Salinities vs Islandmagee

Some fisheries associations (ANIFPO) have suggested that difficulties in the Baltic fisheries, which they attribute to a small decline in salinity could be reproduced in the case of Islandmagee. There are obvious differences however between the two situations;

- Changes in the Baltic fish stocks are not due to salinity alone but to a complex mix of overfishing and unfavourable environmental factors (in the case of cod) as well as changes to salinity and temperature, all of which drive complex food web outcomes, which have been positive for some species (sprat) but negative for others e.g. herring and cod. The Baltic situation differs significantly from that at Islandmagee, however.

- The changes in the Baltic are over a much larger scale, whereas the predicted increase in the salinity at Islandmagee over 0.05 psu above background is confined to a very limited sea area of about 1km by 4km at worst.

- The changes that occur in the Baltic are generally on the scale of decades, and cover a salinity range of about 0.4psu (i.e. -0.2 to +0.2 psu) about the norm (at 80m depth) and a temperature range of 0.8°C (i.e. -0.4°C to +0.4°C) (during spring at 30m depth) (Möllmann *et al.,* 2005) both of which are significantly greater than predicted for Islandmagee outside an area of a few hundred metres square around the outfall.

- The poorest commercial fishery outcomes in the Baltic were associated with declining salinity, whereas in the case of the Islandmagee project a slight rise in salinity is predicted.

Primary Productivity

There is a suggestion from a third party representation that the brine discharge might impact on phytoplankton. The waters around Islandmagee are likely to hold a diverse assemblage of phytoplankton species (see McKinney *et al.,* 1997 and Gowen *et al.,* 1998) and while the salinity tolerances and preferences of some of these or related species are known, that seems to be more the exception than the rule. Despite this there are some broad facts known about the influence of salinity on phytoplankton depending on their usual habitats, which can help in assessing possible impacts. In a seminal piece of research to examine salinity tolerance, Brand (1984) grew 46 marine phytoplankton isolates in six salinities (0, 5, 15, 25, 33, and 45 psu) and showed that typical estuarine species have the widest salinity tolerance being able to grow from low estuarine salinities up to the 45psu, while oceanic species had the narrowest tolerance range, with coastal species, which are probably the dominant component of the Islandmagee phytoplankton community, having an intermediate range of tolerance. Of the 13 coastal species that Brand tested, all of which would have naturally occurred in salinities of 33psu, only one didn't reproduce at 45psu, one grew better at 45psu than at 33psu while the remaining eleven isolates grew at a reduced rate at 45psu compared to 33psu, on average 1.22 cell divisions per day compared to 1.51. It is important to point out however that the salinity steps employed by Brand were very wide, in this case 12psu (between 33psu and 45psu). However, at Islandmagee, such large steps in salinity are only predicted to be present in the first few meters around the diffuser. Moreover, in the area outside the first few tens of metres, the salinity differences will be so slight (fractions of 1 psu) it is seems unlikely that there will be any significant reduction in phytoplankton growth anywhere as a result. It is possible, that there may be subtle shifts in dominance among different species with some becoming more abundant than others, for example *Skeletonema costatum*, a common species in the Irish Sea at times (McKinney *et al.*, 1997), which is known to compete well in fluctuating salinity (Rijstenbil, 1988). Again, however, given that the increases in salinity predicted by the model in the medium and far field areas are so marginal, compared for example to the large semi-diurnal changes in salinity that would occur in an estuary and where distinct phytoplankton assemblages can be detected along a salinity gradient (Dijkman and Kromkamp, 2006), such changes may not be detectable against a background of natural temporal and spatial variation in the phytoplankton community due to strong tidal mixing along this open coast. In conclusion, primary productivity at Islandmagee is very unlikely to be significantly affected by the predicted increase in salinity.

Impacts on Scallop, Crab, Lobster and Shrimp.

Several commercial fishermen's organisations have voiced concerns about the possible impact of the proposed discharge on scallop and crustacean fisheries. The impacts of increased salinities on these groups were dealt with extensively in the ES and will not be reiterated in detail here. We can say that the early larval stages in all cases are generally the most sensitive when exposed to osmotic stress. However, these effects are only likely to begin to take place at salinity levels in the 36-40psu range, below these concentrations, little if any impacts would be anticipated and even at these salinities a certain minimum exposure time would be required to result in an effect (several hours or even days). The model predicts that the area around the outfall experiencing salinities in excess of 36psu is about 100m in diameter therefore any adverse impacts are expected to be restricted to a zone of less than this around the outfall and as such won't be significant for any of these commercial species. Adults and juveniles on the seabed may simply avoid this inner zone. In the case of plankton, exposure in this zone is likely to be limited because (i) of tidal movements pushing them past this area and (ii) because these salinities will rarely if ever be experienced in the middle or surface layers of the water column, thus avoiding all the larva situated at these levels.

Impurities in the Brine

Several parties have commented on the possible impact of impurities in the brine. The project is awaiting this planning permission so as to be allowed to construct a site from which to drill the salt deposits to obtain cores of the salt for detailed chemical analysis. This analysis will be used to confirm the modelling assumptions which will then be used as part of the (separate) consenting process under the Water Order. Nevertheless, our knowledge of the composition of salt deposits from the same geological strata may cast some light on what can be expected. The Larne deposit is from the Permian era and is considered to be of the same type as that which has been leached to form caverns at Aldbrough. That salt had very low levels of heavy metals and the same is expected to be true of the Larne deposit. **Table B1** lists the maximum reported heavy metal levels for the Aldbrough deposit.

Table B1: Reported Maximum Non-Salt Component Levels in the Aldbrough Salt Deposit.

Metal	Concentration in Brine $\mu g\ l^{-1}$
Arsenic	<5
Boron	<20
Cadmium	<2
Chromium	<2
Copper	<12
Lead	<25
Mercury	<0.25
Nickel	<5
Zinc	<20

To put these levels into perspective we can compare them with Environmental Quality Standards (EQS) for marine waters in the UK set in response to the EC Dangerous Substances Directive (76/464/EEC) as well as the results of water quality monitoring in UK estuaries between 1999 and 2001 (Table 2 – Cefas, 2004). They can also be compared (in the same table) with the background ('control') concentrations (BRC's) in non-impacted parts of UK estuaries. **Table B2** gives a summary of the metal concentrations in filtered waters, 1999 to 2001.

Table B2: Summary of Metals Concentrations in Filtered Waters, 1999 to 2001 in UK Estuaries (Cefas, 2004).

Metal	BRC, µg l^{-1}	EQS, µg l^{-1}	Range of median concentrations across sites, µg l^{-1}	Sites Assessed
Arsenic	-	25	1.1-3.0	9
Boron	-	7,000	700-4,089	8
Cadmium	0.004-0.025	2.5	0.012-0.25	17
Chromium	0.09-0.12	15	0.157-1.5	8
Copper	0.05 –0.36	5	0.738-4.73	18
Lead	0.005-0.02	25	0.086-5.98	17
Mercury	0.0001-0.0005	0.3	0.003-0.011	12
Nickel	0.16 –0.26	30	0.345-3.05	18
Zinc	0.03 -0.45	40	1.26-26.2	20

BCR = Background Reference Concentration (in this case for UK estuarine waters away from discharges)

EQS = Environmental Quality Standard

Finally **Table B3** lists the dilution in the seawater of the hypothetical brine concentrations that would be required to attain (i) the EQS, (ii) the lower BRC and (iii) the upper BRC. It can be seen that in all cases the the maximum reported brine concentration for Aldbrough is well below the EQS. In the case of copper this level would be achieved almost immediately on discharge from the diffuser. To reach background levels reported for these metals (BRC's) would require significantly more dilution especially in the case of lead and mercury. However, it is important to point out that in the case of lead in particular, it will disappear from solution very quickly as it rapidly binds to particulate matter in the water column. More importantly however, the background levels listed are far below levels reported in the scientific literature as likely to have any biological impact on marine life – such levels are more in line with the EQS values quoted.

Table B3: Dilution Ratio in Seawater Required for Hypothetical Brine to Reach Marine EQS and BRC's Quoted in Table B2

Metal	Dilution of Brine Required to reach EQS	Dilution Required to reach Lower BRC	Dilution Required to reach Upper BRC
Arsenic	0	-	-
Cadmium	0	500	80
Chromium	0	22	17
Copper	2.4	240	33
Lead	0	5000	1250
Mercury	0	2500	500
Nickel	0	31	19
Zinc	0	667	44

Another feature of the brine is the fact that it will probably have different proportions of its main constituent ions, i.e. chloride, sodium, sulphate, potassium, calcium, and magnesium, than seawater which may in turn affect the toxicity of the brine compared to seawater.

In laboratory toxicity testing of gas cavern brine in Portugal, (Quintino *et al.,* 2008), researchers noted that the brine, had a higher toxicity than seawater at the equivalent salinity. This may relate to the composition of its major ions and or its trace metals. That study noted than when the brine was actually discharging (to an exposed sandy beach), its concentrations rapidly fell below those concentrations, which were noted in the lab to be required to have adverse impacts on the test organisms. This is also expected to be the case at Islandmagee and so no material adverse impacts are anticipated.

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