An Assessment of the Environmental Conditions required for Freshwater Pearl Mussels (*Margaritifera margaritifera*) and its Potential Viability in the River Annan, Scotland

Plate 1. Freshwater pearl mussels (Source: Scott, n.d. cited in Skinner *et al*., 2003: 1).

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ABSTRACT

The freshwater pearl mussel (*Margaritifera margaritifera*) is a critically endangered mollusc that is declining dramatically throughout its Holarctic range, particularly due to the decline of water quality. Scotland is a stronghold for the species, with at least half of the world's remaining sustainable population. This paper critically analyses key water chemistry and substrate conditions at 10 sites across the River Annan, in Southern Scotland and compares them to water quality objectives proposed for the species using box plots and plotting time series to determine the potential viability of the river for *M. margaritifera* populations, as they thrived in the past in the catchment. Key findings show that none of the locations meet all the water quality standards, with sites 6, 7, 8 and 9 being completely unsuitable for the species, with wide fluctuations and high overall pH, BOD, $CaCO_3$, suspended solids, conductivity, phosphate and nitrate levels and low or unnaturally high DO levels. The results indicate a general problem with nutrient enrichment, severely affecting the lower part of the catchment from pollution which can most likely be attributed to agricultural practices. Instead, sites 2, 3 and 5 have the most favourable environmental conditions. Nevertheless, further research is needed to fully understand the viability of the species. It is also necessary to examine other factors that could have adverse effects on *M. margaritifera* such as: status of host fish populations, the physical habitat and illegal pearl fishing. This study recommends working closely with land managers and implementing projects such as the Habitat Improvement Programme, as this could lead to the protection of an internationally protected species.

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LIST OF ABBREVIATIONS

FPM	-	Freshwater Pearl Mussel
RAT	-	River Annan Trust
BOD	-	Biochemical oxygen demand
DO	-	Dissolved oxygen
EU	-	European Union
SACs	-	Special Areas of Conservation
SNH	-	Scottish Natural Heritage

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1. INTRODUCTION AND REVIEW OF LITERATURE

1.1. Background

The freshwater pearl mussel (*Margaritifera margaritifera*) is a large, long-lived, bivalve mollusc of rivers and streams (Gaywood *et al*., 2016). It is one of the longest-lived invertebrates known, as individuals can live for over 100 years (Bauer, 1992) reaching up to 140 mm in length (JNCC, n.d.). *M. margaritifera* are dark brown to black in colour (SNH, n.d.) as seen in Plate 1 and

> "live buried or partly buried in coarse sand or fine gravel, often around boulders and other large rocks that help stabilize the river bed, in cool, clean, oligotrophic, fast-flowing and unpolluted rivers" (Skinner *et al.,* 2003: 4).

They feed by drawing in water through their exposed siphons and then filtering out minute organic particles (Skinner *et al*., 2003).

The freshwater pearl mussel (FPM) life cycle as seen in Plate 2 involves a larval, glochidial stage, living attached to the gills of juvenile fish from the salmonid family, which include the brown trout (*S. trutta*) and Atlantic salmon (*Salmo salar*); a juvenile stage, living interstitially in the river bed; and an adult stage, living as filter feeder (Young and Williams, 1984; Skinner *et al*., 2000; Bradley *et al*., 2012).

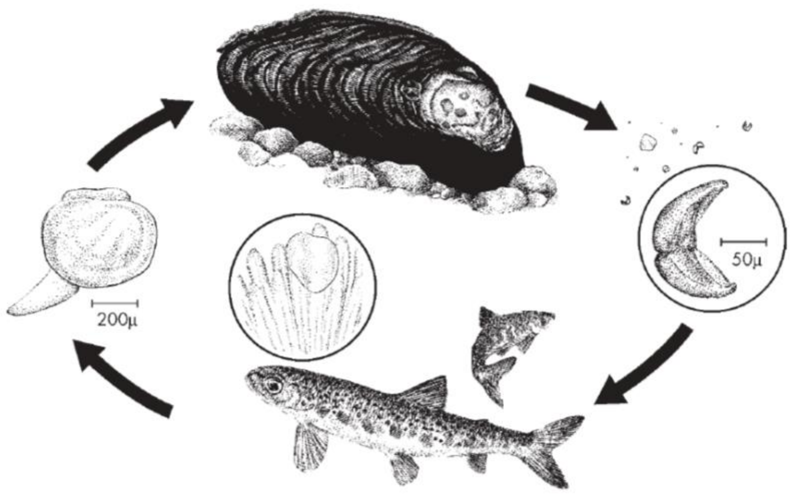

Plate 2. Freshwater pearl mussel life cycle (Source: Wroot, n.d. cited in Skinner *et al.*, 2003: 5).

In the summer, the adult mussel releases one to four million larvae that are parasitic known as glochidia, that attach themselves to the gills of the host fish which provide an oxygen-rich environment where they encyst and grow (Pearls in Peril, 2017). This association is not harmful for the host fish and facilitates mussel dispersal (Skinner *et al.,* 2000) since it enables the glochidia to recolonize upstream rivers and prevents them from being swept away downstream during floods (SNH, n.d.). However, only 4 glochidia in every million meet a suitable fish (SNH, n.d.) and the chances of survival are still limited. Once they drop off into the river bed the following spring to settle and grow, they must land in gravel or sand because if they land on silt or sludge, they will suffocate and die (Skinner *et al.*, 2003). It takes approximately 12 - 15 years before mussels become sexually mature and can start breeding and the process can start all over again (Pearls in Peril, 2016).

M. margaritifera are considered a keystone species (Geist, 2010) as adult mussels can filter about 50 litres of water per day (Zuiganov *et al*., 1994) and dense populations can change the physical structure of stream sediments, water clarity, light penetration, abundance of macrophytic plants and thus, their presence can greatly enhance the biodiversity and ecosystem functioning of the aquatic environment (Vaughn and Hakenkamp, 2001; Howard and Cuffey, 2006). In fact, they are considered an ideal target for conservation because they are an umbrella species, hence a wide range of other species benefit from management targeted at FPM's because they require high quality riverine habitat (Sime, 2007; Geist, 2010) and are a very important bio indicator of the general level of pollution (Bauer, 1988).

M. margaritifera has drawn a lot of attention in recent years due to its unique ecology, life cycle, ability to produce pearls, but most notably, its decline, which has left the species in danger of extinction (EPA Catchments Unit, 2009) due to the lack of juveniles as they have not recruited for decades in most rivers (Geist 2010; Moorkens *et al.,* 2017).

1.2. Status and Distribution

M. margaritifera is a Holarctic species that can be found in Northern Europe and in some areas in Spain, Portugal and France (Degerman *et al.,* 2009) as shown in Fig.1.

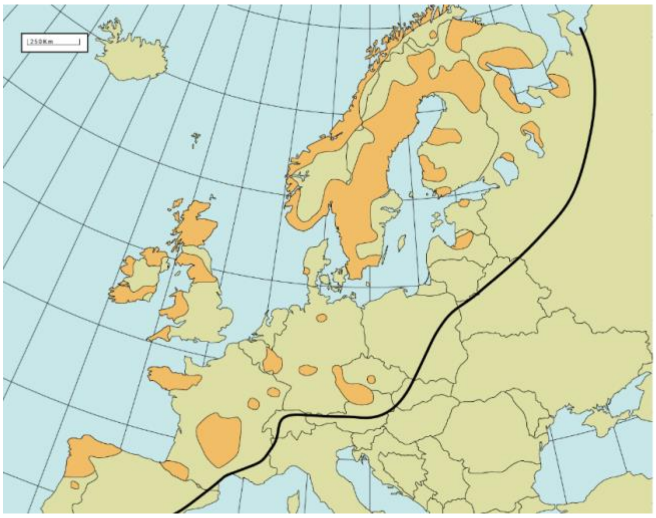

Fig. 1. Distribution of *M. margaritifera* in Europe (Source: Larsen, 2005 cited in Degerman *et al.,* 2009: 5).

However, the species is severely declining throughout its range (Kerney, 1975; Young and Williams, 1983; Bauer, 1986, 1988; Zuiganov *et al*., 1994; Baillie and Groombridge, 1996) with an estimated decline during the 20^{th} century of more than 90% in European populations (Bauer, 1988) and the situation continues to deteriorate (Araujo and Ramos, 2001), as a result of a range of factors: illegal pearl-fishing, pollution, siltation, river engineering and decreasing salmonid stocks (Langan *et al.,* 2007). Nonetheless, the decline of water quality has been identified as the principal cause for the international decline of *M. margaritifera* (Wells *et al*., 1983, CEN working group, 2014) and eutrophication noted as the main problem (Bauer, 1988) considering that FPM's are extremely susceptible to water pollution because they are filter feeders and they pass considerable amounts of water through their digestive system (SNH, n.d.).

UK distribution:

The distribution of pearl mussels in the UK can be seen in Fig. 2. which shows that most freshwater pearl populations are found in Scotland, mainly in the North and West, with scattered records of the species elsewhere. In fact, Scotland contains almost half of the world's known remaining viable populations of *M. margaritifera* (Young *et al*., 2001).

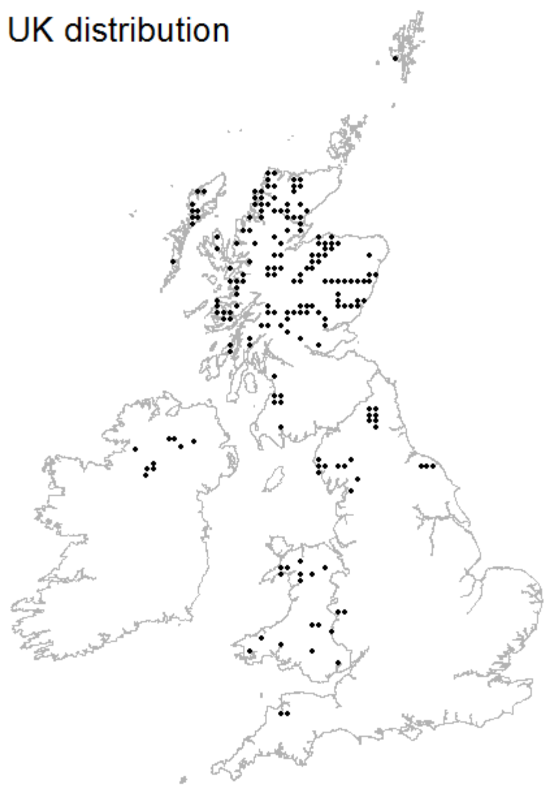

Fig. 2. UK distribution of freshwater pearl mussel (*M. Margaritifera)* (Source: JNCC, n.d.).

However, according to recent research conducted by SNH, *M. margaritifera* are now absent in 11 Scottish rivers where they were previously documented (Green, 2015). Water pollution has been identified as the main driver for the decline in many populations due to the increased nutrient levels from sources such as agriculture and sewage effluent, especially in lowland areas of Scotland where the species has nearly disappeared (SNH, n.d.).

1.3. Conservation

M. margaritifera are critically endangered (Cosgrove *et al*., 2000; Cuttelod *et al*., 2011). Consequently, they are protected by International, European and National legislation (Moorkens, 2011):

- Bern Convention, Appendix III.
- EU Habitats Directive, Annexes II and V.
- Wildlife and Countryside Act Schedule 5 (1981).

Overall, the prospects for *M. margaritifera* populations and their habitats, have been assessed as 'Bad' by the European Community Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora (JNCC, 2013). Consequently, all over the world there are new and ongoing conservation objectives for FPM's – legal protection, habitat restoration, infection of the host fish with glochidia and captive breeding programs (Henrikson *et al.,* 2009).

1.4. The River Annan Trust

The River Annan Trust (RAT) is a charitable organization based in Lockerbie that aims to improve and protect the environment of the River Annan and to educate the public on the importance of the river (River Annan Trust, n.d.-a).

RAT takes part in many conservation projects like invasive species control, riparian-tree planting and green bank protection. Now, a study has been requested by RAT to assess if environmental conditions are still suitable at the River Annan for *M. Margaritifera* populations, as pearl mussels thrived in the past in the Annan catchment (Chisholm *et al*., 2012).

1.4.1. The River Annan catchment

The RAT covers all the water that runs into the Solway between the West bank of the Sark and the West bank of the Lochar Water (See Fig. 3).

Fig. 3. Map of River Annan Catchment (Source: The River Annan Trust, 2010:13).

The River Annan catchment supports a wide range of species and habitats (River Annan Trust, n.d.-a). Nonetheless, over time, the river and its surrounding landscape have been heavily modified by human activities and there is a need for restoration of the river and the riparian habitat (River Annan Trust, n.d.-a). The Annan catchment is mainly dominated by agricultural land use, with approximately 30 % of the area being classified as such, around 70% being managed for this purpose and less than 1% accounting for urban areas (RADSFB, 2014).

1.4.2. Previous work relevant to this study

The River Annan population of FPM's is recorded in old pearl fishermen's diaries from the 1950s (River Annan Trust, n.d.-b). However, *M. margaritifera* populations were thought to be extinct in the Annan catchment as surveys undertaken by Scottish Natural Heritage in part of the river in 2006 failed to find any survivors (The River Annan Trust, n.d.-b). Nonetheless, in 2008, a single shell identified as *Margaritifera margaritifera* was found by an angler in the tributary of the Water of Milk (Chisholm *et al.,* 2012). Consequently, FPM surveys were carried out by RAT in 2013 and 4 more very old specimens that had recently deceased, were found. The map of the survey area with the locations of the shells can be seen in Fig. 4. Since then, cursory inspections have failed to find any more evidence, but it is highly unlikely that these were the last remaining individuals in the whole catchment (River Annan Trust, n.d.-b). For this reason, a study has been requested by RAT to assess if the environmental conditions are still suitable at the River Annan for *M. Margaritifera* populations.

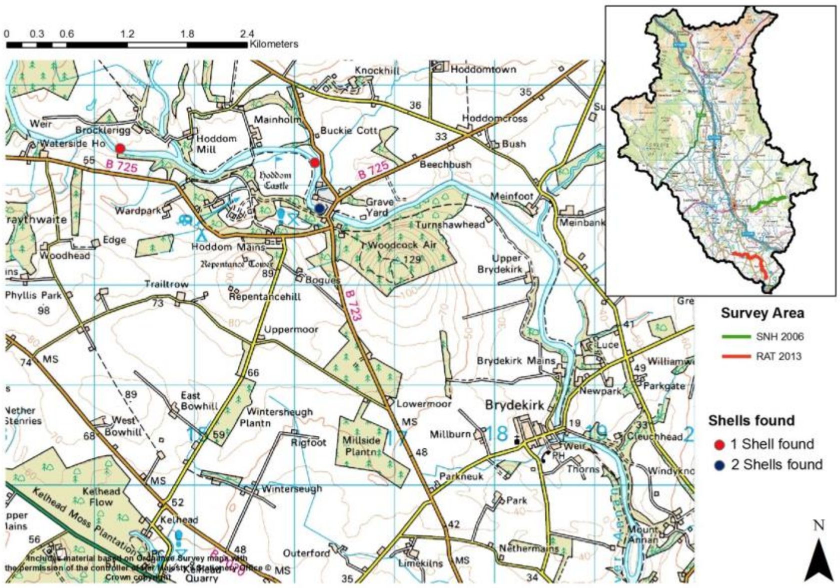

Fig 4. Map of survey area and locations where shells were found (Source: The River Annan Trust, n.d.-b: 2).

2. RESEARCH QUESTIONS AND OBJECTIVES

Research Questions:

1. What are the key water chemistry conditions required to sustain freshwater pearl mussels?
2. What are the key substrate qualities required to sustain a population of freshwater pearl mussels?
3. Do optimum water chemistry and substrate conditions currently exist within the River Annan?
4. What current land management approaches represent a threat for the successful colonization of freshwater pearl mussels?

Objectives:

The objective is to answer these research questions to understand if the water chemistry and substrate conditions that currently exist in the River Annan are suitable for *M. margaritifera* populations and provide future objectives for the management of the river system. Furthermore, these findings will be beneficial to RAT but also could lead to the protection and enhancement of populations of an internationally protected species.

3. METHODOLOGY

3.1. Procedure

- Source relevant studies and identify optimum water chemistry and substrate conditions for *M. margaritifera.*
- Collect and analyse data from SEPA on the current water quality of the River Annan examining mostly chemical parameters that are thought to be detrimental to *M. margaritifera* such as: pH, conductivity, nitrates and ortho-phosphate levels. Also, analyse the substrate requirements for the species, examining levels of suspended solids. Although data on other physical factors such as turbidity, temperature and preferred flow regimes will not be reviewed in this paper.
- Compare the water quality at different sites within the River Annan to water quality objectives for *M. margaritifera* (see Table 4).
- Identify viable sites for FPM populations taking into consideration water quality data.
- Identify risks and provide future objectives for the management of the river system.

3.2. Water quality parameters analysed

- DO (% saturation)
- Alkalinity as $CaCO_3$ (mg/L)
- BOD (ATU) (mg/L)
- pH
- Suspended sediments (mg/L)
- Nitrate as N (mg/L)
- TP as P (mg/L)
- Electrical conductivity (µS/cm at 25°C)

3.3. Data analysis

- Calculation of summary statistics for chemical species (mean, standard deviation, median, 25th and 75th percentiles, maximum and minimum values) using box plots to compare the water quality at different sites within the River Annan to proposed water quality objectives for *M. margaritifera* taken from an overview of key literature (see Table 4) to assess suitability of the sites.
- Plot time series for chemical variables to visually identify any potential seasonality and trends at different locations.

3.4. Study locations

- Water chemistry was assessed at 10 sampling locations on a monthly basis for almost 1 year from 14/01/2016 to 16/11/2016, resulting in approximately 12 samples per site. However, the time and date of the sampling vary and there are some monthly gaps in monitoring resulting in only 10 samples for some locations. An extract of the raw data can be seen in Appendix A with a link to the full data set.
- Site details, including sampling locations and descriptions are shown below in both Table 1 and Fig. 5.

Site number	Location code	Description	NGR
1	121093	River Annan, 500m u/s Evan Water	NT 09218 02891
2	121094	Evan Water at Beattock	NT 07802 02768
3	121095	Moffat Water u/s River Annan	NT 09582 02438
4	121096	River Annan at Johnstonebridge	NY 10106 91771
5	121097	Kinnel Water at Templand	NY 07863 86321
6	121100	River Annan at A709 Shillahill Br, Lockerbie	NY 10617 80681
7	121101	Water of Milk at Hoddom Mill	NY 14711 73484
8	121103	Mein Water at Meinfoot	NY 18542 72878
9	121105	River Annan at Brydekirk Gauging Station	NY 19011 70404
10	123241	Water of Ae d/s Elshieshields	NY 07829 85972

Table 1. Study locations (Source: adapted from SEPA, 2016).

Fig. 5. Locations of river water sampling points (in red) (Source: modified from Ordnance Survey, 2011).

4. RESULTS

4.1. Water quality requirements of FPM: literature review

The decline of water quality is the main reason for the international decline of *M. margaritifera* across its range (Wells *et al*., 1983; CEN working group, 2014) hence, this section compares and analyses different key studies (see Appendix B) and their recommended water quality standards for sustainable FPM populations, including key parameters such as: pH, conductivity, $CaCO_3$, BOD, dissolved O_2, nitrate and ortho-phosphate levels (see Table 2).
Table 2 shows that *M. margaritifera*

> "prefer oligotrophic conditions - poor in nutrients, pH 7.5 or less and with low overall conductivity" (Skinner *et al.*, 2003: 8).

Attribute	**Target (Bauer, 1988)**	**Target (Oliver, 2000)**	**Target (Cooksley and Blake, 2014)**	**Target (CEN working group, 2014)**	**Degerman *et al.* (2009)**	**Minimum Requirements (Moorkens, 2000)**
pH	≤ 7.5	6.5 – 7.2	6.5 – 7.5	6.2 – 7.3	≥ 6.2	6.3 - 8
Conductivity (µS/cm)	< 70	< 100	N/A	N/A		< 200
Calcium (mg/L)	2	< 10	N/A	N/A	N/A	N/A
BOD (mg/L)	1.4	< 1.3	< 1	< 1	N/A	< 3
DO (%)	N/A	90 - 110	100	100	N/A	< 100.0
Nitrate (mg/L)	< 0.5	< 1	< 1	< 0.002 - 0.5	< 0.125	< 1.7
Ortho-phosphate (mg/L)	< 0.03	< 0.03	Annual mean < 0.005 With no peaks > 0.06	Annual mean < 0.005 With no peaks > 0.06	Annual mean > 0.005 - 0.015	< 0.06

Table 2. Summary of suggested water quality targets for *M. margaritifera* by several scholars (Source: modified from Cooksley and Blake, 2014).

This highlights that *M. margaritifera* require clean, well-oxygenated water, free from pollution or turbidity (Langan *et al.,* 2007). These mussels are restricted to near natural, clean flowing waters (Moorkens, 2011) with a high Q-value as seen in Fig. 6. and stream orders 2, 3 and 4 (Moorkens, 2000) as they are vulnerable to even a minor degree of pollution (Skinner *et al.,* 2003). The juveniles are also far less tolerant than the adults (Hastie *et al.,* 2000) and high Ca, phosphate and BOD is linked with decreasing survival and establishment (Skinner *et al.,* 2003).

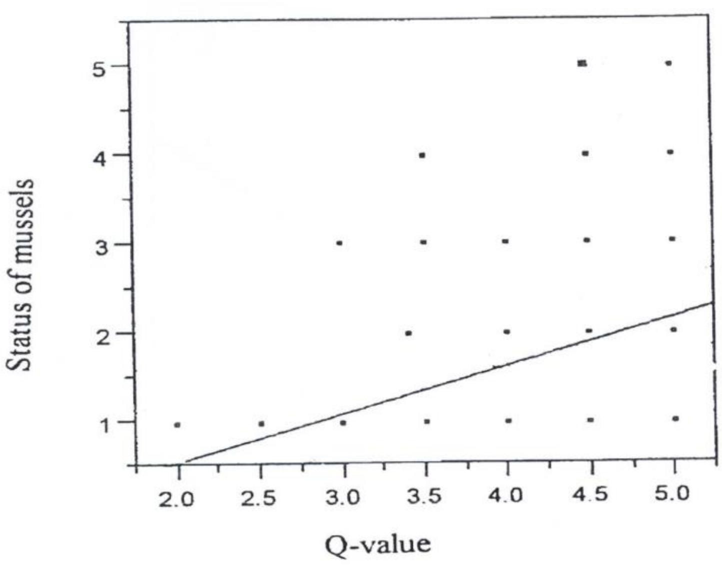

Fig. 6. Correlation of Q-value (Q1= poor quality, Q5= good quality) and the status of freshwater pearl mussels in rivers (1= no mussels, 5= large population with juveniles) (Source: Moorkens, 2000: 26).

4.1.1. pH

> "pH is a measure of the concentration of hydrogen ions in the water and hence the strength of acid present" (NIWA, 2016:1).

Most aquatic animals prefer a pH range of 6.5 - 8.0 as pH outside of this range reduces the biodiversity in the watercourse (EPA, 2012). This coincides with Moorken's (2000) pH range of 6.5 – 8 for *M. margaritifera* (see Table 2). However, there is a negative correlation between the status of FPM's and rising pH, with higher pH values having consistently lower numbers of mussels or no mussels (Fig. 7). So, although a small number of FPM populations have been found in Ireland in pH 7.5 (Lucey, 2006), in Norway in pH 7.7 (Larsen, 2005) and in Sweden at pH 7.7 in the river Harran (Degerman *et al*., 2009) these sightings do not necessarily specify if specimens where in good health (Moorkens, 2000).

Consequently, there is a consensus for pH to be circumneutral, ideally ranging from pH 6.5 - 7.5 for healthy, reproducing *M. margaritifera* populations (Cooksley and Blake, 2014).

pH values above 8 are moderately high and generally indicate intense photosynthetic activity by periphyton and macrophytes which is unsuitable for the species (EPA, 2012) as it can cause fluctuations in oxygen levels at the water-sediment interface (Moorkens *et al.,* 2017). Whereas, a low pH is also unfavourable because it can allow toxic elements in the water to become mobile and thus, it can be lethal for sensitive species like FPM's (EPA, 2012) and it is a well-documented threat to their salmonids hosts (CEN working group, 2014). Moreover, a lowering of pH directly impacts *M. margaritifera* through a slow, progressive destruction of their calcareous shell and genital organs which can cause infertility, as well by causing a significant acidosis of the mantle fluid (Vinogradov *et al.,* 1987; CEN working group, 2014).

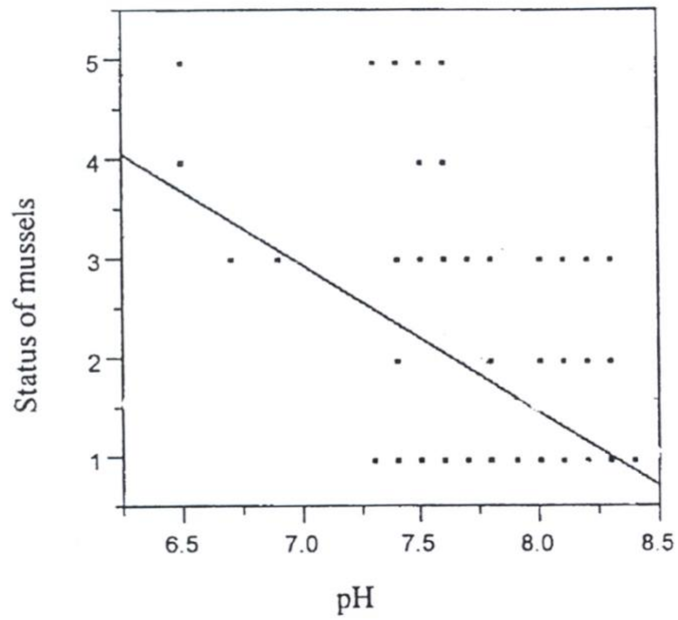

Fig. 7. Correlation of maximum pH with status of mussels in rivers (1= no mussels, 5= large population with juveniles) (Source: Moorkens, 2000: 27).

4.1.2. Conductivity

> "Conductivity is a measure of the total ionic strength of the water and can give an indication of the level of enrichment of a water body" (NIWA, 2016:1).

Although all streams contain some nutrients because of natural conditions and processes, excessive amounts trigger proliferations of algal growth which can in turn lead to considerable daily fluctuations in both pH and DO levels that can be harmful for FPM's (Environmental Protection Division, 2001; NIWA, 2016). Non-point sources of pollution and direct inputs of dissolved salts from urban and rural run-off containing dissolved solids from industry, sewage, agriculture and storm water lead to elevated levels of conductivity (CEN working group, 2014).

M. margaritifera require low overall conductivity as seen in Table 2 and Fig. 8. target levels below < 100 µS/cm are preferable and the status of FPM's in rivers decreases with increasing conductivity. In fact, ranges between 150 – 249 µS/cm are a sign of slightly enriched waters; 250 – 399 µS/cm moderately enriched waters; and > 400 µS/cm indicate heavily enriched waters with a very poor water quality rating (NIWA, 2016).

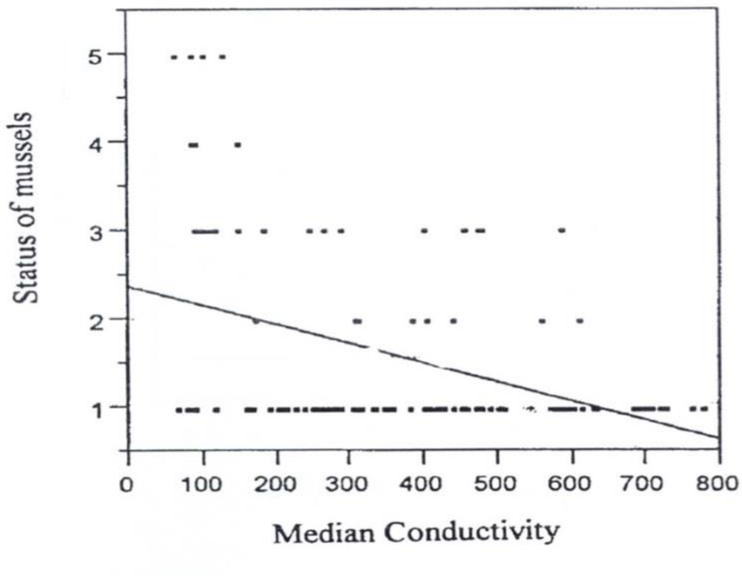

Fig. 8. Correlation of most recent annual median conductivity (µS/cm) with status of mussels in rivers (1= no mussels, 5= large population with juveniles) (Source: Moorkens, 2000: 26).

4.1.3. Calcium

> "Calcium is a natural component of surface water and its concentrations can be shaped by various factors, such as: the geological structure of a catchment area, soil class and type, plant cover, weather conditions, land relief, type and the intensity of the water supply" (Potasznik and Szymczyk, 2015: 1).

FPM sensitivity to calcium levels is poorly understood (Skinner *et al.,* 2003). Although a certain amount of calcium is essential for building their shell (Dolmen and Kleiven, 2009) and is crucial as a buffer systems against acidification of the watercourse and against acidosis within the animal (Dolmen and Kleiven, 2004), *M. Margaritifera* can be killed through direct toxic effects of artificially elevated calcium levels, such as from agricultural liming or through run-off from high-calcium sediment from quarrying (CEN working group, 2014). Also, where high calcium levels persist, FPM populations shift toward increased growth rates, so their optimum life history strategy of very slow growth and extensive reproductive opportunities is critically impaired (CEN working group, 2014). Nonetheless, there are few comprehensive studies that quantify Ca (mg/L) thresholds and considerable discrepancy amongst scholars (Cooksley and Blake, 2014). For example, early authors in this field emphasized the connection between the distribution of the FPM and bedrock poor in calcium (Boycott, 1936; Hendelberg, 1960; Bauer, 1992, 1998; Oliver, 2000). Yet, FPM populations have been recorded at sites with elevated calcium levels in Ireland, Finland and Norway (Chesney and Oliver, 1998; Young *et al*., 2001; Skinner *et al.,* 2003; Moorkens, 2000; Lucey, 2006) where the surrounding geology increases the calcium content well beyond the levels suggested by Bauer and Oliver (Table 2) (Skinner *et al*., 2003). Hence, the most up-to-date expert knowledge on water quality requirements for FPM's by CEN working group (2014) recommends that natural levels of calcium content for the river are required, as alkalinity should be as expected for the geology of the catchment.

4.1.4. Biochemical oxygen demand

Biochemical oxygen demand (BOD) is a measure of the amount of oxygen consumed by microorganisms in decomposing organic matter in river water (EPA, 2012). The greater the BOD, the more rapidly oxygen is being depleted in the river and the less that is available to higher forms of aquatic life like FPM's. Although the rate of oxygen consumption can be affected by several variables such as temperature and pH, an elevated BOD is usually caused by high levels of organic pollution that can be attributed to wastewater discharge from human activities or agricultural sources (EPA, 2012).

High BOD is linked to severe depletion of adult mussels and is extremely important for the survival and establishment of juvenile mussels since adequate oxygen levels are vital (Bauer, 1988; Young, 2005; CEN working group, 2014) and even small elevations in BOD can results in nutrient elevations and lead to filamentous algal growth (Moorkens *et al.,* 2017).

Although BOD values above 1.0 mg/L have been suggested for the species (Table 2), rivers with recruiting populations in the UK, Ireland and Spain have BOD levels consistently below 1.0 mg/L (CEN working group, 2014). Moreover, Bauer (1988) found that BOD levels above 1.4 mg/L are linked with poor juvenile survival in Central Europe, thus Moorkens (2000) < 3 mg/L target for FPM's is highly questionable and needs further clarification.

4.1.5. Dissolved oxygen

Dissolved oxygen (DO) is oxygen gas molecules present in water that are necessary to many forms of life including fish, invertebrates, bacteria and plants for respiration (Behar *et al*., 1997). DO levels vary depending on factors including water temperature, time of day, season, depth, altitude and rate of flow (Behar *et al*., 1997). For example, DO reaches its peak during the day whilst at night, it decreases as photosynthesis has stopped while oxygen consuming processes such as respiration, oxidation and respiration continue (Behar *et al*., 1997). DO in streams can be heavily modified by anthropogenic factors through the addition of oxygen consuming organic wastes like sewage, nutrients and chemicals (Behar *et al*., 1997). Overall, it is important that more oxygen is produced than consumed or else DO levels decline and sensitive animals may move away, weaken or die (EPA, 2012). Consistently high levels of DO indicate a healthy ecosystem (USGS, 2017) and are essential for FPM's, both in open water and in the juvenile interstitial habitat (CEN working group, 2014). As seen in Fig. 9 the status of mussels is greater at higher DO levels. Hence, DO levels should remain consistently near to 100% air saturation and never be subject to excessive fluctuations (CEN working group 2014) especially because anoxic conditions lead to the release of phosphate from sediment and the reduction of nitrate to nitrite, which is a potent neurotoxin for *M. margaritifera* (Cooksley and Blake, 2014).

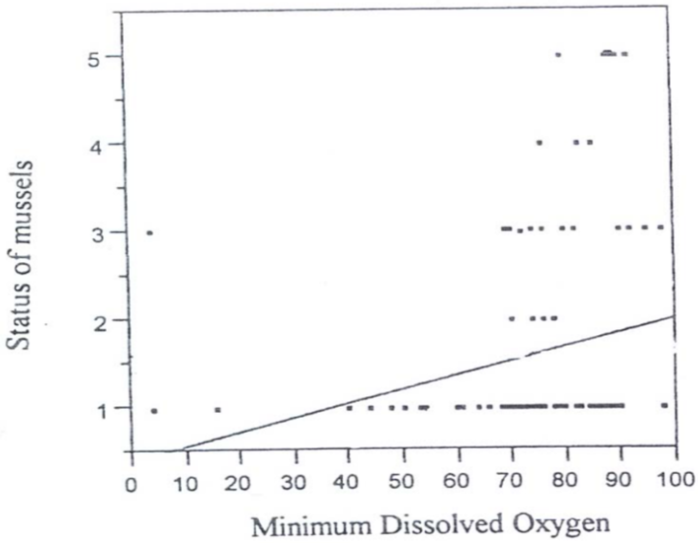

Fig. 9. Correlation of minimum dissolved oxygen with status of mussels in rivers (1= no mussels, 5= large population with juveniles) (Source: Moorkens, 2000: 28).

4.1.6. Nitrate

Nitrate is relatively common in freshwater aquatic ecosystems as it enters streams from natural sources like decomposing plants and animal waste, as well as human sources such as industrial sewage effluents or fertilizers and organic manures from agricultural land (Behar *et al*., 1997). However, excessive nitrogen inputs to surface waters may enhance productivity and can lead to eutrophication (CEN working group, 2014; Cooksley and Blake, 2014) depriving fish and invertebrates of available DO in the water (Behar *et al*., 1997). In fact, the probability of occurrence of all species is significantly reduced in reaches with elevated nitrate levels (Douda, 2010) and eutrophication is noted by Cosgrove *et al.* (2000) as the underlying reason for the global decline in FPM populations.

Therefore, *M. margaritifera* require nitrate levels that are natural for the catchment, with general concentrations in reproducing populations ranging from < 0.002 to 0.5 mg/L (CEN working group 2014). This target range corresponds to the recommended nitrate concentrations for other sensitive species, such as host fish like salmon with nitrate thresholds of 0.06 mg/L (Behar *et al*., 1997). Yet, Moorken's (2000) found *M. margaritifera* populations in Ireland at nitrate concentrations of 1.7 mg/L. However, the health of these populations was not considered, and this elevated nitrate threshold was largely contested (Young, 2005) as Bauer (1988) observed that adult FPM mortality rises with nitrate values above 1.5 mg/L and natural mortality levels were only recorded at locations where nitrate concentration was below 0.5 mg/L. In fact, Moorkens later modified the threshold for the species to 0.125 mg/L (Moorkens, 2006) which more closely resembles other targets for the species (Table 2).

4.1.7. Phosphorus

Phosphorus in small quantities is essential for plant growth and metabolic reactions in animals and plants (Behar *et al*., 1997). However, it is the nutrient in shortest supply in most fresh water ecosystems and despite it not being toxic to FPM's, it is directly linked to eutrophication which is the main cause of the decline in FPM populations (Cosgrove *et al*., 2000) due to:

> "increased organic sedimentation, colmation, oxygen depletion in the substrate, changes in fish communities and increased fluctuations in pH values" (CEN working group, 2014: 25).

The normal background ortho-phosphate level of 0.005 mg/L indicates favourable conditions for the maintenance of oligotrophic waters for sustainable FPM populations (Moorkens, 2006; Cooksley and Blake, 2014; CEN working group, 2014). However, sustaining low levels is fundamental, as even minor increases above natural background nutrient loads can lead to excessive filamentous algal growth and damage for aquatic ecosystems (Moorkens, 2011) and one large input can lead to an increased trophic status in the river on a long-term basis (EPA Catchments Unit, 2009). Sources of phosphate include animal wastes, sewage, detergent, fertilizer and disturbed land (Behar *et al*., 1997).

Table 2. specifies different targets for the species, with a suggested peak threshold limit of nitrate below 0.06 mg/L by Moorkens (2000), Cooksley and Blake (2014) and CEN working group (2014) and significantly lower annual levels below 0.005 mg/L, which coincide with Degerman et al. (2009).

4.1.8. Substrate requirements

FPM's require very specific substrate conditions most often associated with riffle areas and plane beds with a wide range of clast sizes (see Table 3) that provide stability, high exchange rates between free-flowing and interstitial water and a lack of infiltration of fine sediment that is essential for the different life stages (CEN working group, 2014).

In general, as seen in Plate 3. *M. margaritifera*

> "prefer small sand patches stabilized amongst large stones or boulders in fast-flowing rivers or streams" (Jung *et al.,* 2013: 923).

Plate 3. Typical substrate preference of *M. margaritifera* are large boulders and cobbles, with patches of sand in-between (Source: Scott, n.d. cited in Skinner *et al*., 2003: 10).

Category	**Size (mm)**
Tree root/other	Any
Silt	> 0.002 - 0.063
Sand	> 0.063 - 2.0
Gravel (fine)	> 2 - 6.63
Gravel (medium)	> 6.3 - 20
Gravel (coarse)	> 20 - 63
Cobble	>63 - 200
Boulder	>200 - 630
Large boulder	> 630
Bedrock	Exposed bedrock

Table 3. Clast sizes relevant to *Margaritifera* habitat (Source: CEN report, 2014: 18).

> "Adult mussels tend to live in dense beds at the tail-end of pools or in the moderate flow channels of river bends" (Langan *et al.,* 2007: 6),

whereas juveniles prefer the riffle areas in the river that provide a well-oxygenated and silt-free environment that is especially important in the post-settlement period when juveniles establish themselves in sediment (Skinner *et al.,* 2003). In fact, whereas adult *M. margaritifera* appear to be tolerant to silty conditions for a short-term, they are completely unsuitable for the more sensitive juvenile stages (Hastie *et al*., 2000; NIWA, 2016). This is because while adult FPM's are generally two thirds buried in the gravel or sand substrata, juveniles are totally submerged for the first couple of years (Cranbrook, 1976) so they require a high rate of exchange between the free water body and the interstitial water (Buddensiek *et al.,* 1993; Skinner *et al*., 2003) because if the interstitial spaces are clogged, the young mussels suffocate (Hastie *et al*, 2000). Hence, the substrate must be free of organic peat and detritus to ensure that the problem is not aggravated through a reduction in DO levels due to decomposition (Moorkens *et al*., 2017).

Thus, excessive sediment that can be carried into streams and rivers from erosion of unstable streambanks, construction sites, agricultural activities and urban runoff must be stopped (Environmental Protection Division, 2001) as high turbidity would adversely affect FPM's by siltation (Moorkens, 2000) and because the cloudiness would increase temperature and in turn, decrease DO levels (Machtinger, 2007). For this reason, climate change is also noted as a future menace to pearl mussel survival due to the potential increase of flash flood events that will increase high flow occurrence, result in greater annual run-off events and alter substrate (Black, 1996; Hastie *et al*., 2003b).

Consequently, concentrations of suspended solids in rivers with healthy, reproducing freshwater pearl populations are extremely low, with only minor peaks of very short duration occurring during periods of heavy rainfall or snowmelt (CEN working group, 2014; Cooksley and Blake, 2014). A level of 30 mg/L of suspended solids has been proposed as the limit of tolerance for adult pearl mussels, whereas persistent, long-term levels above 10 mg/L pose a serious threat (Valovirta, 1990; Valovirta and Yrjana, 1997; Skinner *et al.,* 2003; JNCC, 2005; Langan *et al*., 2007).

4.2. Other important requirements for FPM

M. margaritifera declines and lack of recruitment can also be attributed to other important factors such as changes in the physical and chemical conditions of their habitat caused by land management practices like overgrazing, pollution, threats to host-fish stocks and illegal pearl fishing aided by improved accessibility (JNCC, 2007). These factors also need to be taken into account as their interactions could have adverse impacts on the viability of FPM populations (JNCC, 2007).

4.2.1. Riparian habitat

The quality of the habitat on the river banks and surrounding land is also of great importance for FPM populations. Restoring meanders to straightened river channels will support a better habitat (Gaywood *et al.*, 2016) by promoting natural processes of sediment transport and vegetation colonization (Environmental Agency, n.d.). The presence of native trees and vegetation alongside the river can also benefit *M. margaritifera* as it increases bank stability and reduces the amount of silt and other pollutants that could otherwise enter the river and damage FPM's (Parrot and MacKenzie, 2000; Hastie *et al.,* 2003a). Also, riverside trees provide shade to reduce temperature fluctuations and thus, inhibit the growth of algal mats that could lead to eutrophication (Skinner *et al.,* 2003). Moreover, detritus and falling insects from overhanging trees provide food for adult pearl mussels as well as for their host fish (Pearls in Peril, 2017).

Instead, livestock farming near river banks leads to overgrazing, bank instability and soil erosion which can increase nitrate and ortho-phosphate levels and harm FPM's (Zuiganov *et al.,* 1994) through direct toxic effects and by increasing growth rates which will reduce life expectancy and, in turn lead to the loss of reproductive years (Bauer *et al*., 1991, Skinner *et al*., 2003).

4.2.2. Pollution

The most common types of pollution that threaten *M. margaritifera* are: siltation, oxygen deficiency, heavy metals, acidification and eutrophication due to increased ortho-phosphate and nitrate levels (Zuiganov *et al*., 1994) with juveniles being far less tolerant than adults (Hastie *et al.,* 2000).
Bauer (1988) noticed that reproducing populations of *M. margaritifera* could only occur when enrichment was not affecting its rivers with the best mussel populations being found at unfertilized, non-intensively farmed land sites and associated with stream orders 2, 3 and 4 (Moorkens, 2000) and a high Q-value as seen in Fig. 6.
In Scotland, FPM's have declined to near extinction, following siltation and raised nutrient status (Henrikson *et al*., 2009) largely due to the intensification of arable and livestock farming, afforestation in the uplands and to a lesser extent, effluent discharges from aquaculture and sewage disposal (Young *et al*., 2001).
Moreover, although there are few comprehensive studies on the effects of metals on FPM's in realistic field conditions (Young, 2005), Bauer (1988) observed that metal pollution must be absent for reproducing freshwater pearl populations, with Cu>Cd>Zn and Ni being acutely toxic in this order of toxicity (Naimo, 1995).

4.2.3. Host fish

Measures to conserve the long-term survival of FPM's must also include the main host fish: brown trout (*S. trutta*) and Atlantic salmon (*Salmo salar*) (Young and Williams, 1984), as they are essential for the larval, glochidial stage of the pearl mussel life cycle (Moorkens, 2011) seen in Plate 2. Thus, it is essential to ensure that there are no barriers to fish migration that would isolate pearl mussels and affect dispersal, such as waterfalls and dams (Gaywood *et al.*, 2016).
Although, host fish numbers do not need to be very high due to the natural adaptation of *M. margaritifiera* to live in rivers with very low food levels and productivity (Bauer *et al*., 1991; Popov and Ostrovsky, 2014), concerns have been raised with the severe decline of both fish species due to over-fishing and pollution which will inevitably threaten populations (Bauer, 1988; Chesney and Oliver, 1998; Cosgrove *et al*., 2000; Hastie and Cosgrove, 2001; Popov and Ostrovsky, 2014).

Figure 10 shows the decline in salmon and trout stocks to historically low levels in the River Annan, which could ultimately threaten the long-term survival of any remaining FPM populations in the catchment.

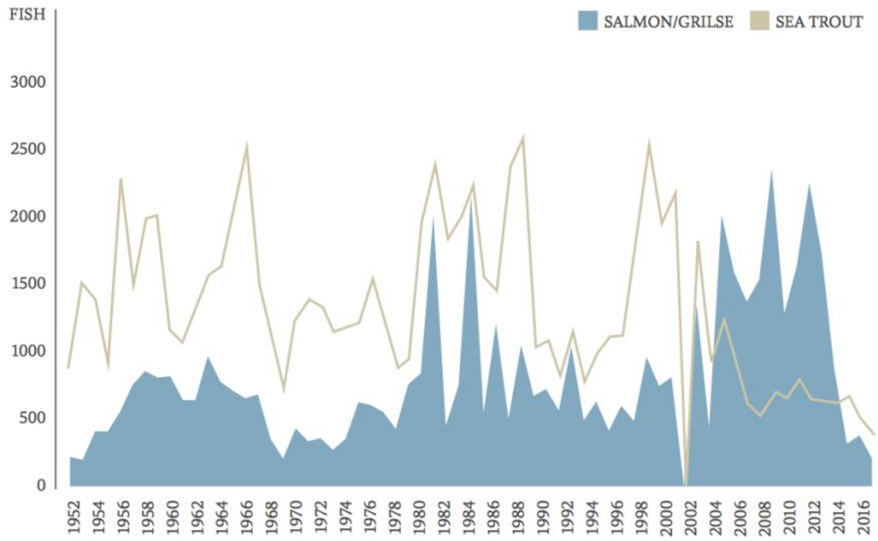

Fig. 10. Decline in salmon and trout stocks to historically low levels (Source: Fisheries Management Scotland, 2017: 35).

4.2.4. Illegal pearl fishing

Pearl fishing is illegal under EU law (Moorkens*,* 2011) and thus, numerous rivers throughout the EU have been designated as Special Areas of Conservation (SACs) (Moorkens *et al.,* 2017). In Scotland alone, there are 21 SACs and Sites of Special Scientific Interest with the FPM as the qualifying feature (Langan *et al*., 2007).

However, although *M. margaritifera* are protected in most countries with various levels of restriction, illegal fishing of mussels still takes place as the pearls have a very high market value. Historically pearl fishery was so highly prized that it has been referred to as the underlying motive for the invasion of Britain by the Romans (Johnston, 1850). Now, Scotland is unusual in being the only country in Europe where illegal pearl fishing is such a serious threat (Gaywood *et al.*, 2016) with considerable evidence of damage from criminal activities (Cosgrove *et al*., 2012). Hence, the exact location of FPM populations is kept a closely guarded secret by SNH and fisheries trusts (River Annan Trust, n.d.) as only a few pearl fishermen could have a damaging effect on the small populations remaining (Langan *et al*., 2007) since there is currently no sustainable way to extract the pearls (Moorkens 2004).

4.3. Water quality results for the River Annan

River water chemistry data from SEPA was available for 10 locations (Fig. 5) throughout the Annan catchment in 2016. Important water quality parameters that threaten FPM's were analysed and compared to proposed targets and thresholds (see Table 4) to assess the potential viability for *M. margaritifera* populations at these locations in the River Annan. The physio-chemical parameters analysed are: pH, conductivity, $CaCO_3$, BOD, DO, nitrate and ortho-phosphate levels and suspended solids.

Targets have been adapted from the literature review, considering several scholars such as Bauer (1988), Oliver (2000), CEN working group (2014) and Moorkens (2000) (see Table 2).

In this section, time series plots and boxplots enable comparison between the sites for each parameter. In red, the minimum requirements or threshold for the species is indicated, whereas the reference level target is shown in green.

Attribute	Target	Minimum requirement
pH	6.5 – 7.5	6.5 - 8
Conductivity (µS/cm)	< 100	< 200
$CaCO_3$ (mg/L)	< 120	< 120
Ca (mg/L)	< 10	< 10
BOD (mg/L)	< 1	< 3
DO (%)	100	90 - 110
Nitrate (mg/L)	< 0.5	< 1.25
Ortho-phosphate (mg/L)	< 0.005	< 0.06
Suspended solids (mg/L)	< 10	< 30

Table 4. Proposed targets for favourable water chemistry for *M. margaritifera* populations at the River Annan.

4.3.1. pH

- Figure 11 shows that all locations, with exception of site 7, have average pH values within the threshold for the species. However, peaks at sites 6, 8 and 9 exceed the favourable target range for FPM's.
- The highest pH value at site 7 reaches pH 8.41, greatly surpassing the threshold limit.
- Large fluctuations between minimum and maximum pH values can be observed at all sites.
- Locations 1, 3, 5 and 10 have the lowest average pH.
- Figure 12 shows a sharp rise in pH from February to March. Later, levels remain high from spring to late summer. From October to February there is a steady decline, with the lowest

pH values recorded in February for all sites except locations 5 and 10, where the pH increases slightly.

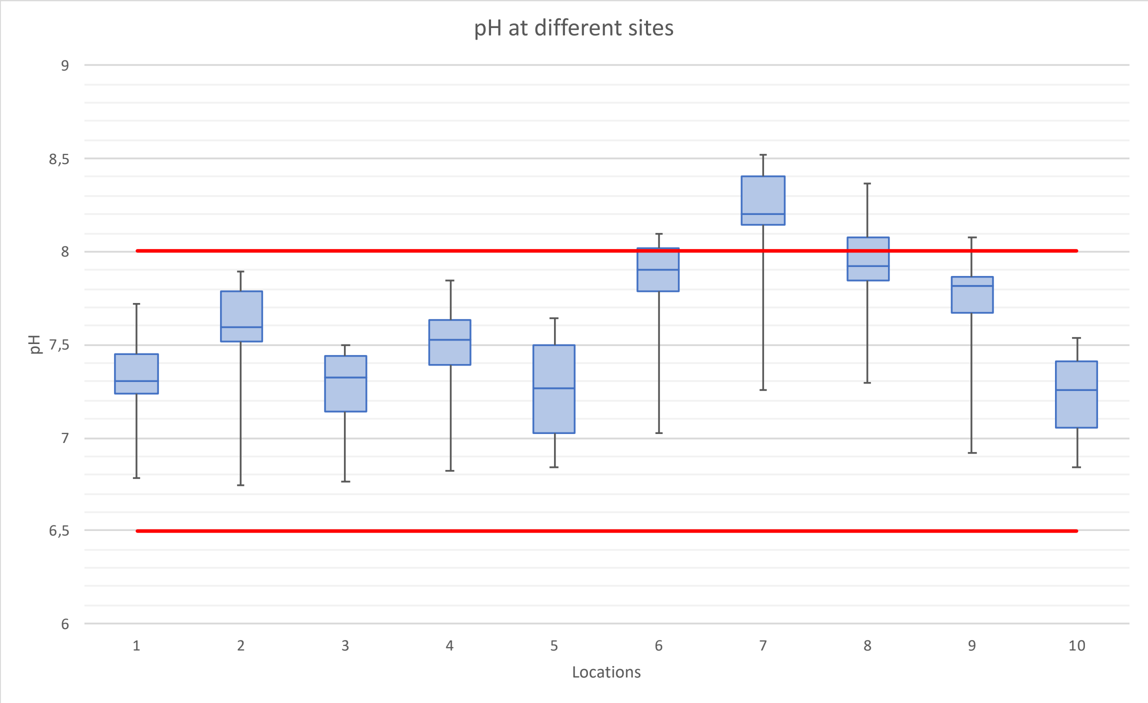

Fig. 11. pH at different sites (Source: data from SEPA, 2016).

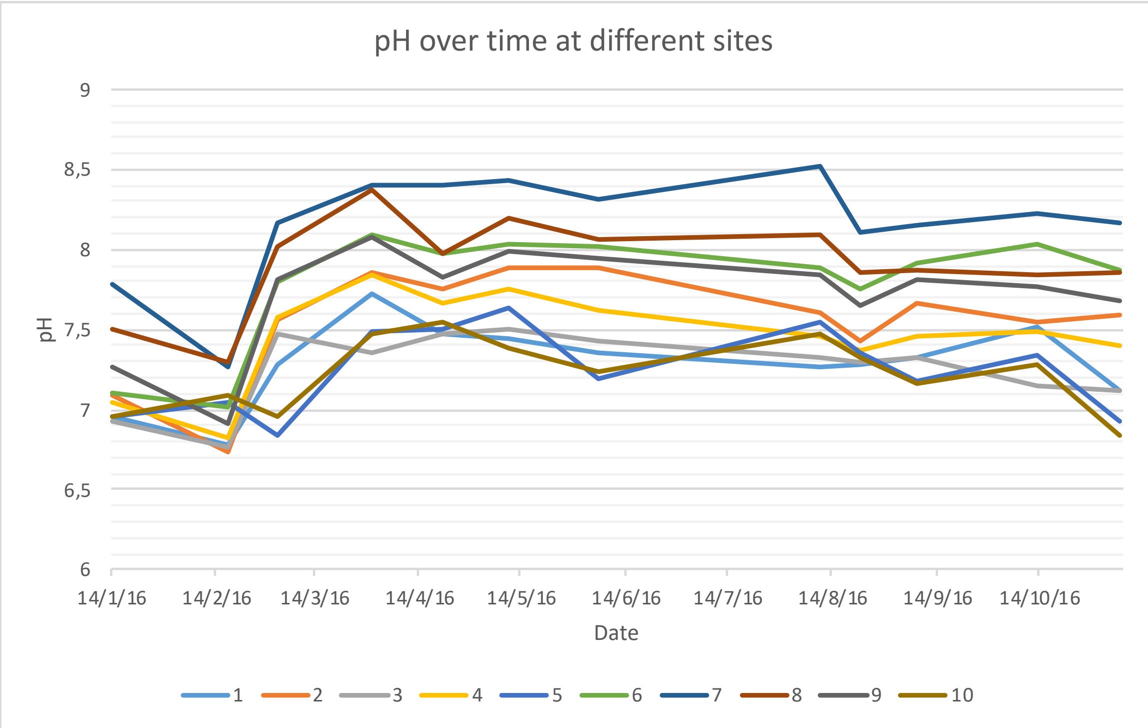

Fig. 12. pH over time at different sites (Source: data from SEPA, 2016).

4.3.2. Conductivity

- Figure 13 shows that excluding locations 6, 7, 8 and 9, all the sites meet the safe limit target for the species. Yet, only sites 1, 3, 5 and 10 meet the recommended objective.
- Average conductivity at site 8 of 321.167 µS/cm is nearly 2 times greater than the threshold limit.
- There are large fluctuations in conductivity levels at all sites, particularly at site 6, with an average conductivity of 232.558 µS/cm, but rising to 435 µS/cm and dropping to 95.7 µS/cm.
- Figure 14 shows that conductivity levels at most sites increase rapidly from February to March and then continue to rise steadily until April, followed by a slow decline until August – September, where there is a sharp drop. After, the conductivity levels gradually rise again until October, followed by a slow decrease until February where the lowest conductivity levels are recorded at all sites. The highest conductivity levels are generally recorded in the months of April and October, with the highest peak of 435 µS/cm at location 6 in October.
- Locations 5 and 10 do not follow the same general trend. Contrary to other sites, some of the lowest conductivity values are recorded in April, whereas at other locations this month has the highest values.

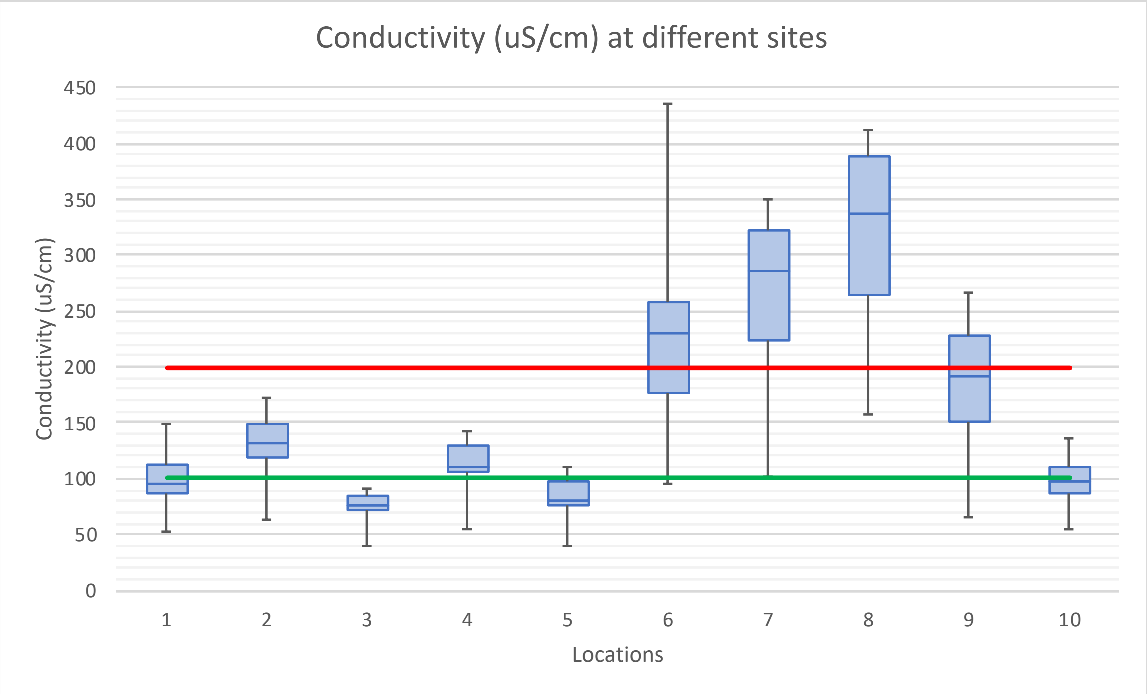

Fig 13. Conductivity (µS/cm) at different sites (Source: data from SEPA, 2016).

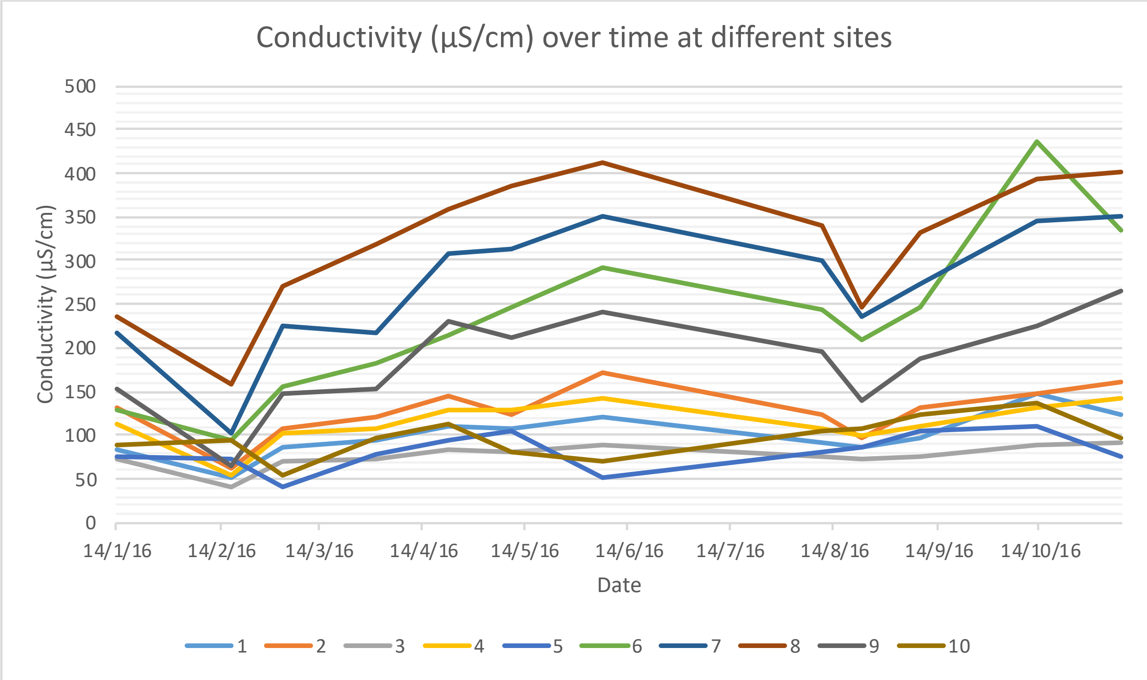

Fig. 14. Conductivity (µS/cm) over time at different sites (Source: data from SEPA, 2016).

4.3.3. Calcium

- Figure 15 shows that excluding locations 7 and 8, all sites have average $CaCO_3$ levels within threshold for the species.
- However, peak levels at location 6 also surpass the limit.
- Large variation between average $CaCO_3$ and lowest and highest levels for all locations. Largest fluctuation at location 6 - average concentration of 75.342 mg/L, lowest value of 21 (mg/L); and highest peak reaching 149 mg/L.
- The highest $CaCO_3$ level recorded rises to 153 (mg/L) at site 7.
- Sites 1, 3, 5 and 10 have the lowest average $CaCO_3$ levels.
- Figure 16 shows that most sites maintain roughly the same levels of $CaCO_3$ throughout the year, with higher levels from spring to the summer and the lowest levels in winter. Instead, site 6, 7, 8 and 9 follow this general trend but with steeper peaks and declines and substantially higher $CaCO_3$ levels overall.
- Figure 17 and 18 indicate a positive correlation between $CaCO_3$ (mg/L) and Ca (mg/L) levels.
- Figure 19 shows that at site 7 the Ca (mg/L) levels are substantially higher than at location 10, exceeding the threshold for the species at peak levels by over 5 times. Ca (mg/L) levels range from 12 to 54.4 mg/L with an average of 40.74 mg/L, whereas at site 10, Ca (mg/L) levels range from 3.92 to 12.5 mg/L, with an average of 9,11 mg/L which is within the favourable target range for the species.

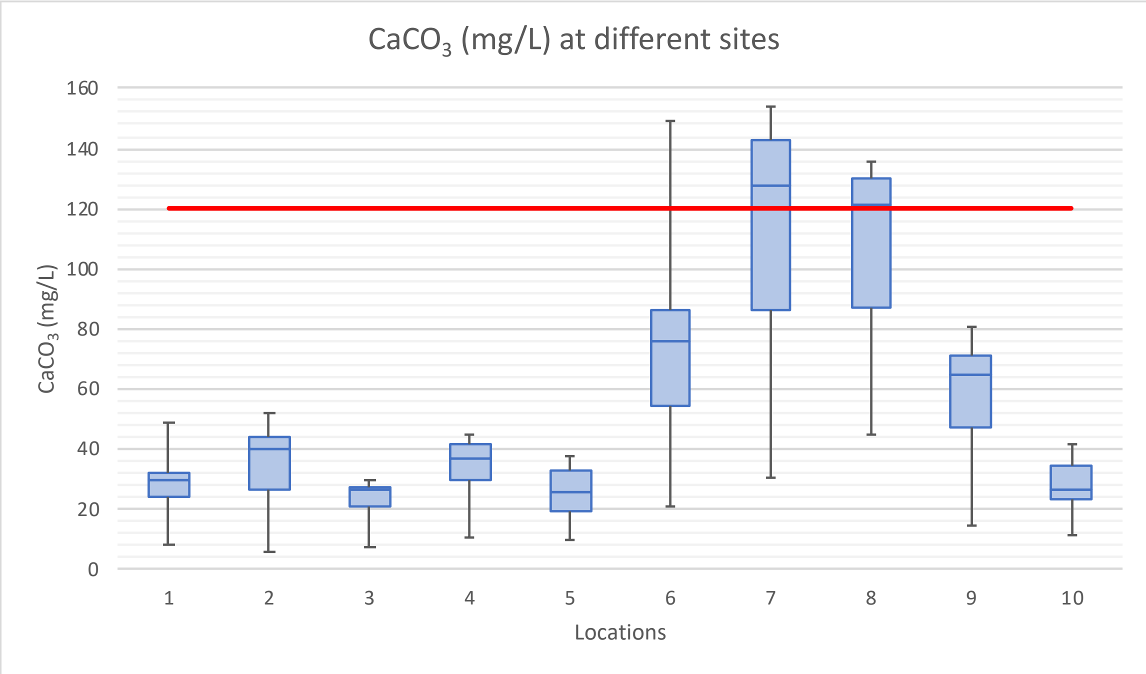

Fig. 15. $CaCO_3$ (mg/L) levels at different sites (Source: data from SEPA, 2016).

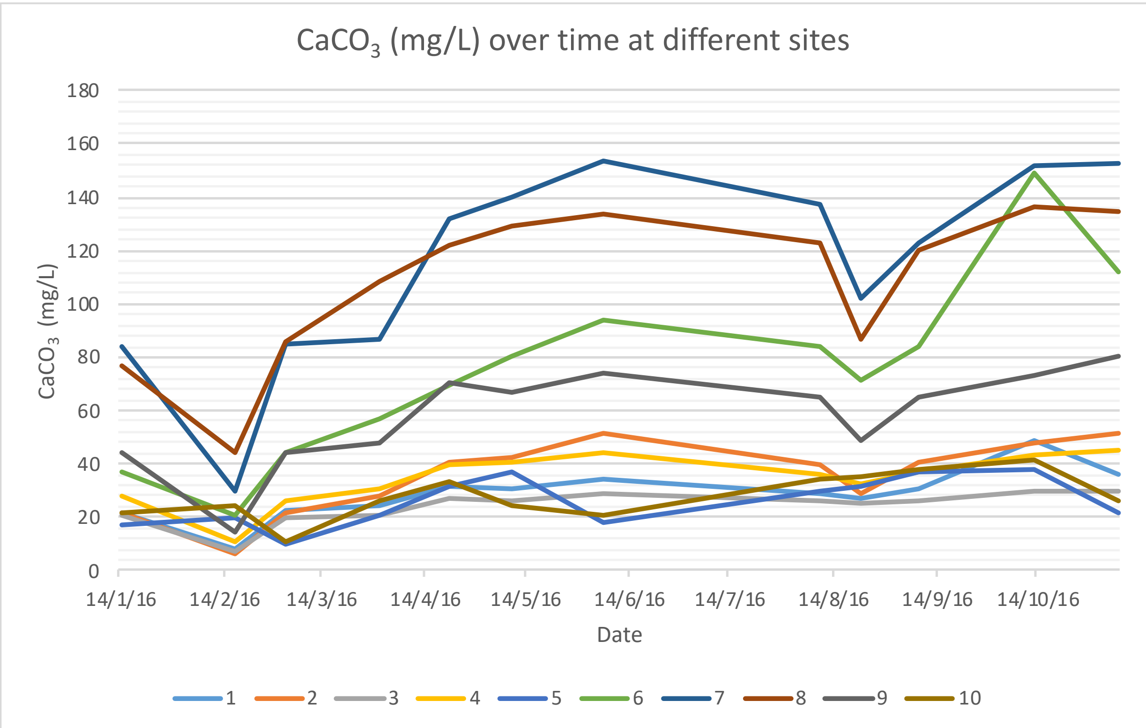

Fig. 16. $CaCO_3$ (mg/L) over time at different sites (Source: data from SEPA, 2016).

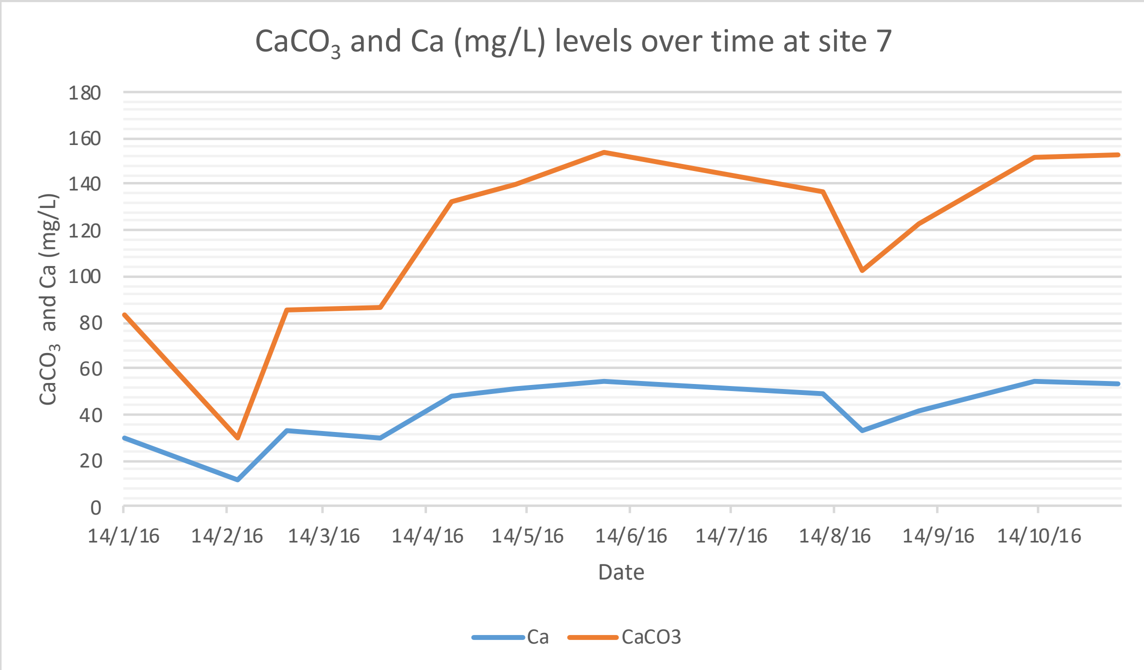

Fig. 17. $CaCO_3$ (mg/L) and Ca (mg/L) levels over time at site 7 (Source: data from SEPA, 2016).

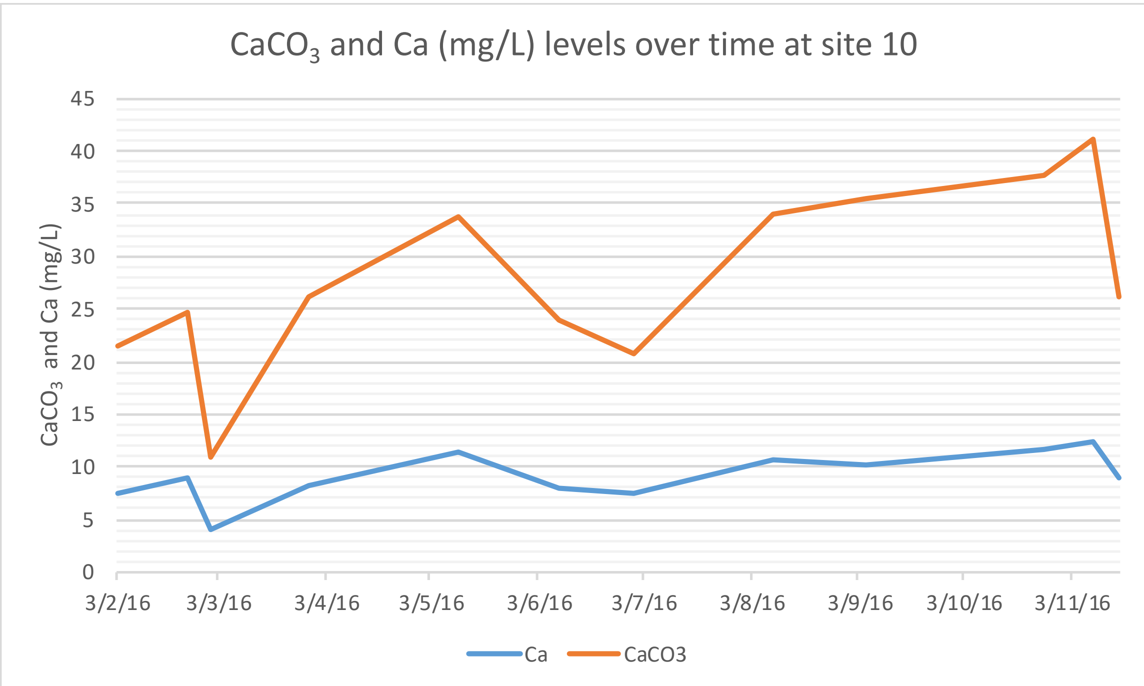

Fig. 18. $CaCO_3$ (mg/L) and Ca (mg/L) levels over time at site 10 (Source: data from SEPA, 2016).

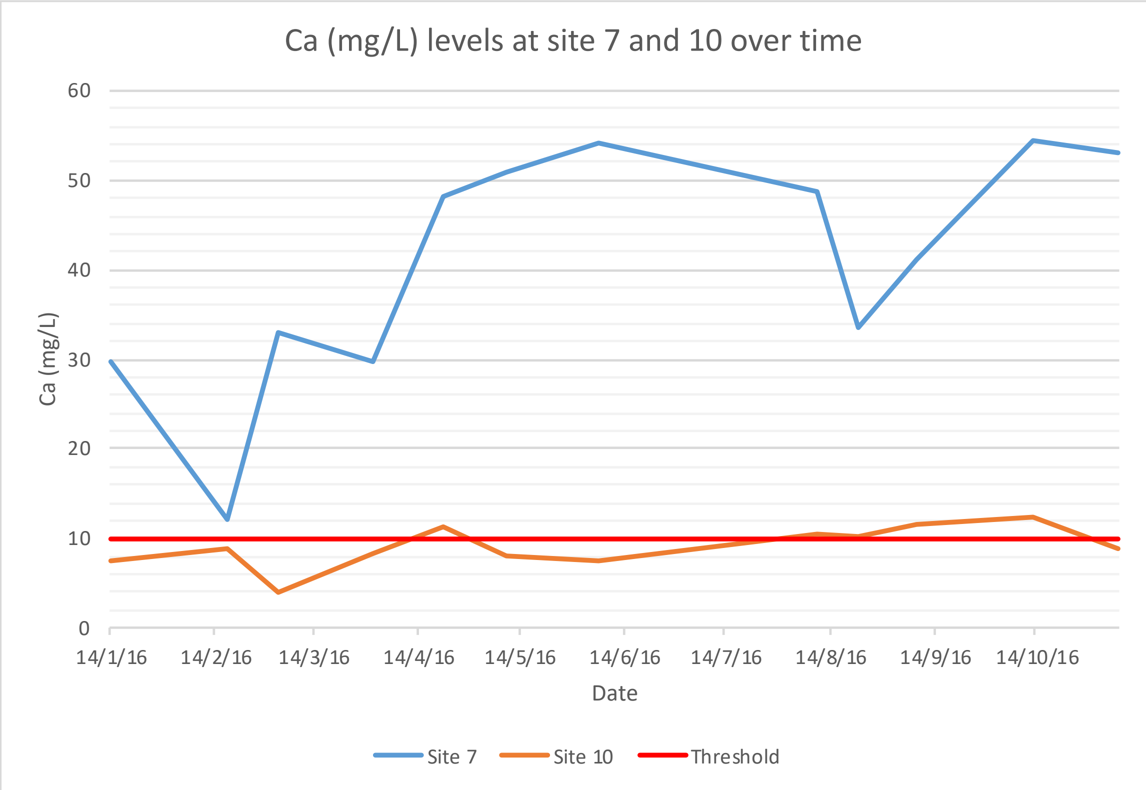

Fig. 19. Ca (mg/L) levels over time at site 7 and 10 (Source: data from SEPA, 2016).

4.3.4. Biochemical oxygen demand

- Figure 20 shows that all average BOD levels are within the safe limit, but none of sites are within the recommended target for the species.
- Some sites show large fluctuations between minimum and maximum BOD levels, especially locations 7 and 8. For example, site 8's average BOD is 2.417 (mg/L), whereas its reaches BOD levels of 7.3 mg/L which is over 2 times higher than the threshold limit.
- Site's 7 peak levels also surpass the threshold for the species.
- Figure 21 shows that there is a negative correlation between BOD and DO levels, where a rise in BOD (mg/L) is followed by a drop in DO (%) levels.
- In Fig. 22 the graph shows that there is generally an abrupt peak in BOD levels from January to February, with the highest BOD levels recorded at most sites in this month, followed by a steep decline until March.
- At locations 10 and 8 there is another sharp peak from August to September, in contrast to other sites, where the lowest BOD levels are recorded in these months.

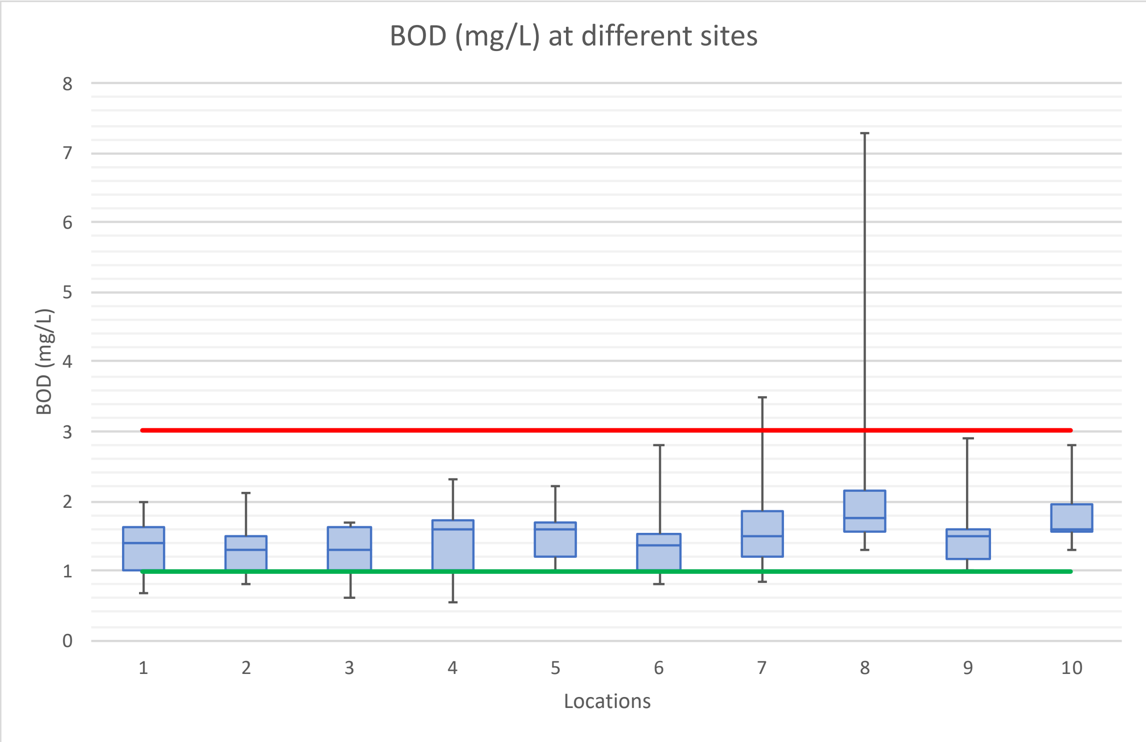

Fig. 20. BOD (mg/L) at different sites (Source: data from SEPA, 2016).

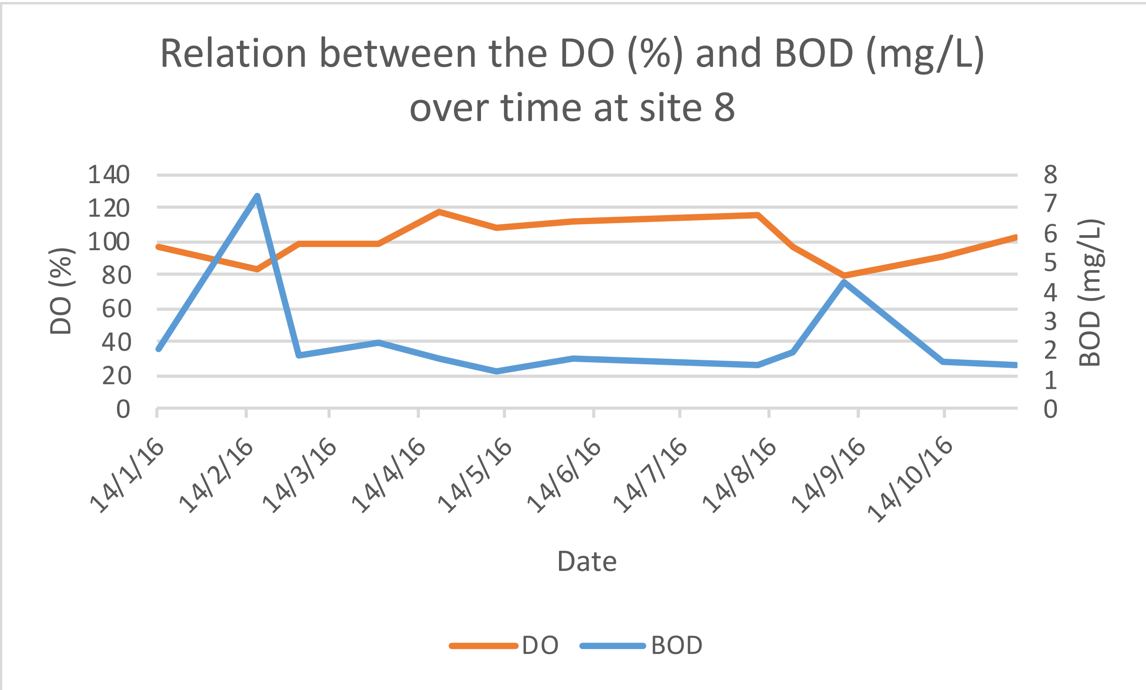

Fig. 21. Relation between DO (%) and BOD (mg/L) over time at site 8 (Source: data from SEPA, 2016).

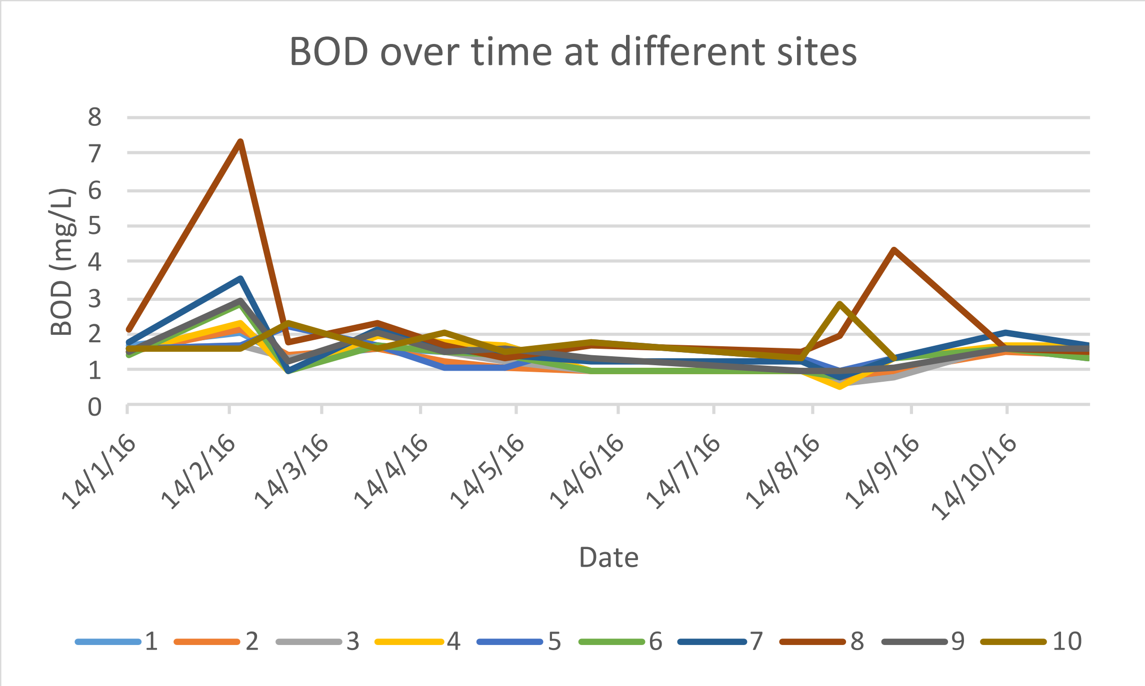

Fig. 22. BOD (mg/L) over time at different sites (Source: data from SEPA, 2016).

4.3.5. Dissolved oxygen

- Figure 23 shows that all average DO (%) levels are within the proposed safe limit for the species, ranging from 94.067 % at site 1 to 103.525 % at location 7 (mean= 98.175 %).
- However, there are large fluctuations between average DO (%) levels and minimum and maximum levels at all sites as seen in Fig. 21 and Fig. 24.
- High peaks of DO of 126% and 125% are recorded at sites 4 and 6, respectively.
- Lowest DO of 77,5 % and 73,2 % seen at locations 2 and 3, respectively.
- Figure 24 shows that DO (%) levels constantly fluctuate from abrupt rises to sharp declines throughout the year at the different sites. However, there is mostly higher DO between early spring and summer months and lower values in the winter months.
- The highest DO (%) levels are documented in May at locations 4 and 6, while the lowest DO is recorded at sites 2 and 3 in February.

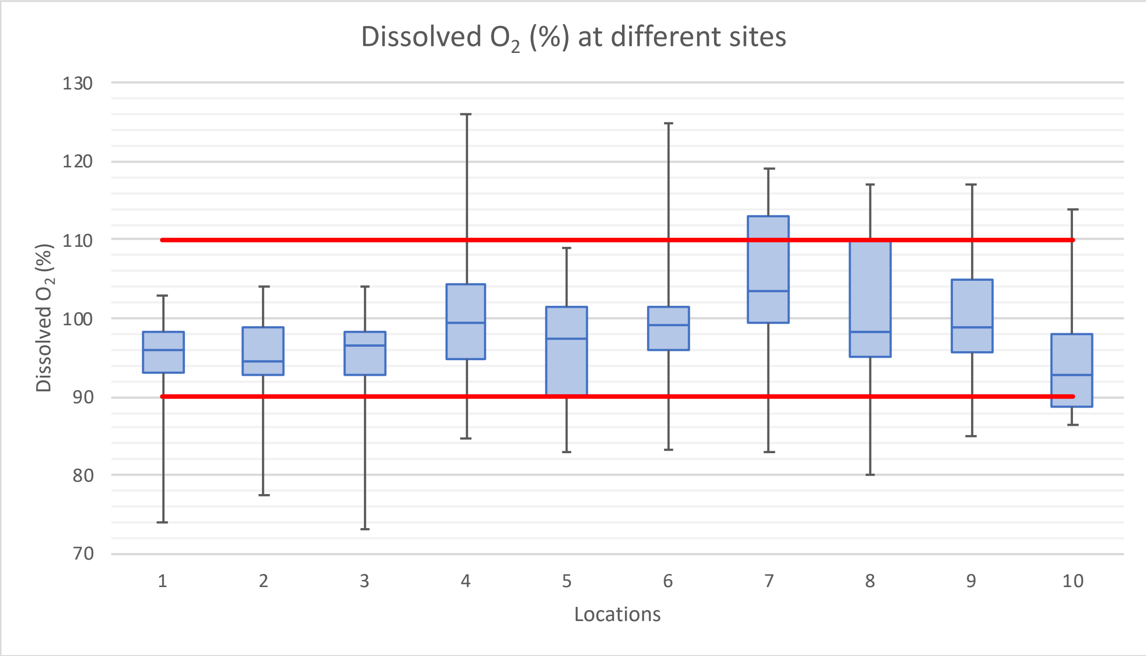

Fig. 23. DO at different sites (Source: data from SEPA, 2016).

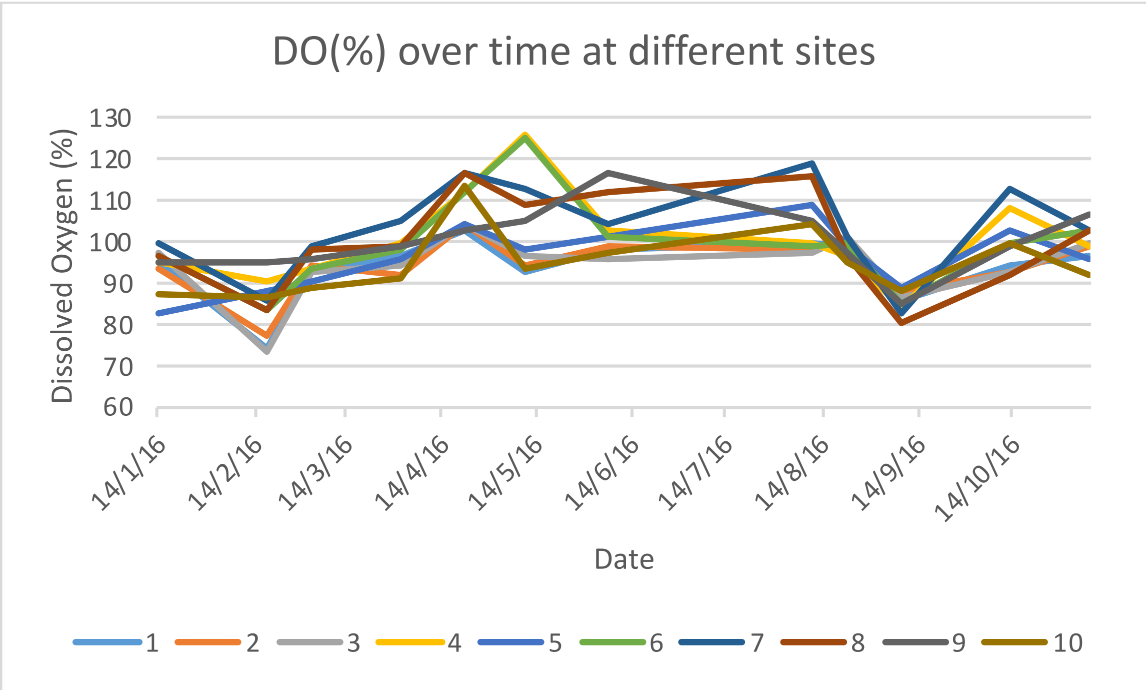

Fig. 24. DO over time at different sites (Source: data from SEPA, 2016).

4.3.6. Nitrate

- Figure 25 shows that excluding locations 6 and 8, all other sites are within the threshold limit for the species. Particularly site 8 which exceeds the safe limit, with an average nitrate level of 2.15 mg/L and reaching up to 2.71 mg/L which is over 2 times higher than the threshold.

- However, only sites 2, 3 and 5 meet the favourable nitrate (mg/L) level objective, with levels on average below 0.05 mg/L.
- There are large fluctuations between average, minimum and maximum nitrate levels at all locations, particularly at sites 6 and 8.
- Figure 26 shows that the only general trend at most sites is a sharp decline in nitrate levels from the month of January to February, apart from site 5 and 10 which increases. The rest of the year, there are multiple peaks and declines with highest values mostly recorded from spring to summer and lowest values in the winter.
- Instead, Fig. 26 shows that at sites 2, 3 and 5, nitrate levels remain quite stable throughout the year, with small increases and decreases.

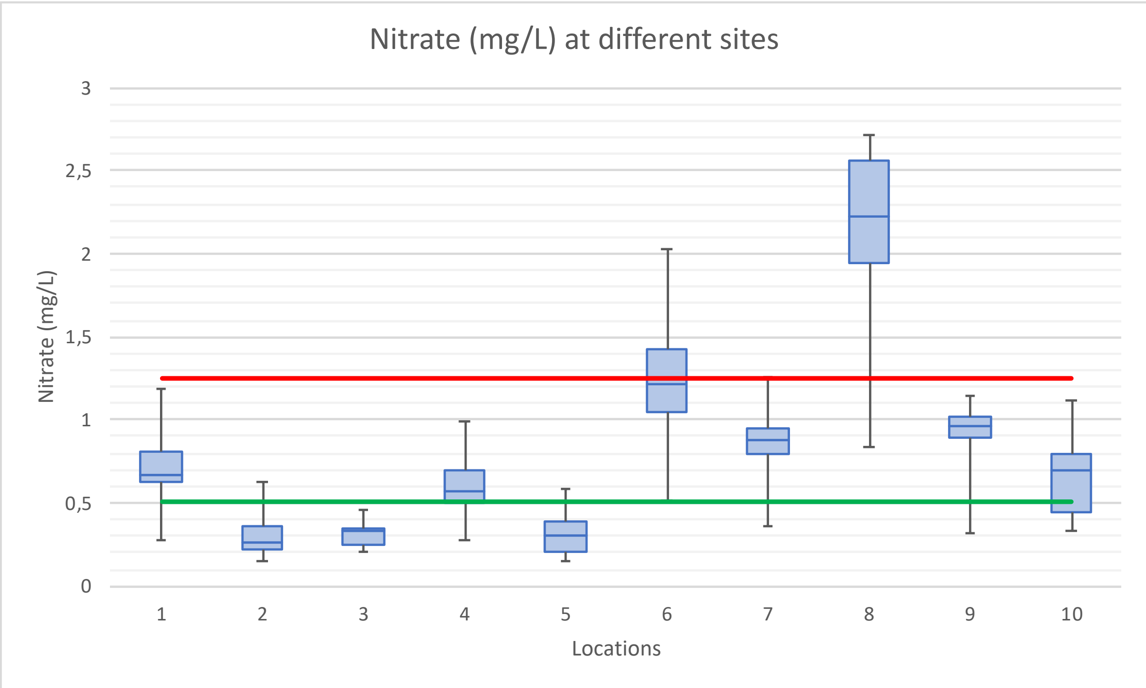

Fig. 25. Nitrate at different sites (Source: data from SEPA, 2016).

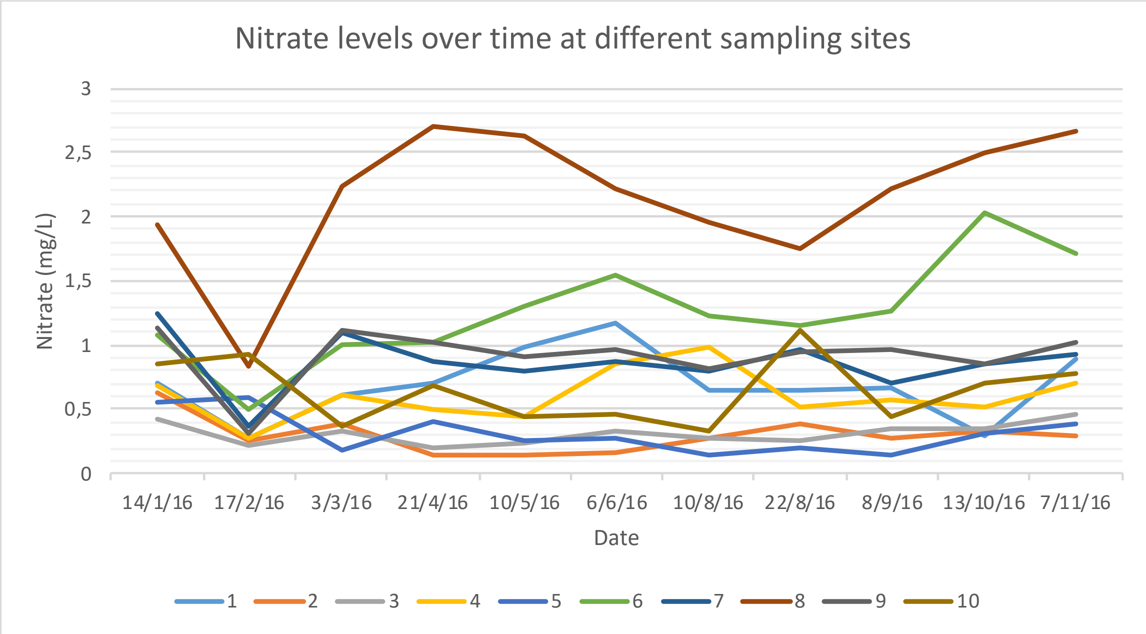

Fig. 26. Nitrate levels over time at different sampling sites (Source: data from SEPA, 2016).

4.3.7. Ortho-phosphate

- Figure 27 shows that average orthophosphate levels range from 0.008 to 0.07 mg/L (mean = 0.02 mg/L) so all sites are within the threshold, except site 8 where the orthophosphate levels rise to 0.234 mg/L which is nearly 4 times greater than the limit. Also, high peaks at location 1 reach 0.111 mg/L which greatly exceed the safe levels.
- However, no site is even remotely close to the recommended target for the species.
- Figure 28 shows that at most locations the orthophosphate levels fluctuate slightly, with major peaks in the month of February and August, but always below 0.05 mg/L.
- Instead, location 1 and 8 have considerably higher average and peak orthophosphate levels throughout the year. In the month of February, the highest peak at location 8 is over 4 times higher than any of the other sites at that time.

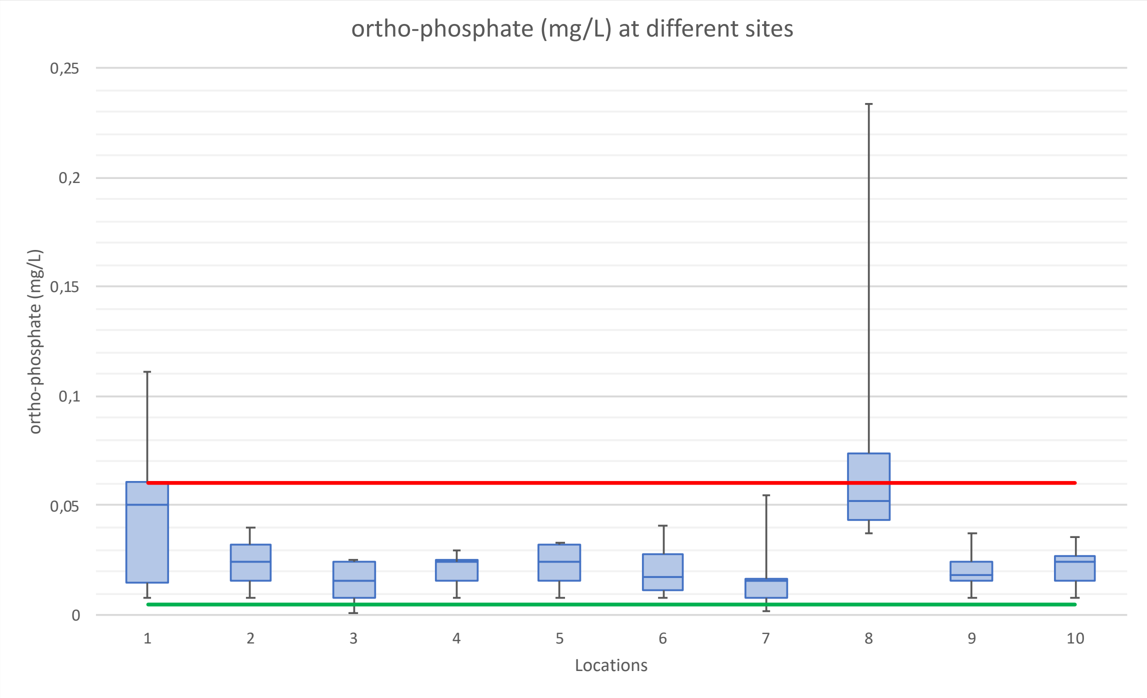

Fig. 27. Phosphate at different sites (Source: data from SEPA, 2016).

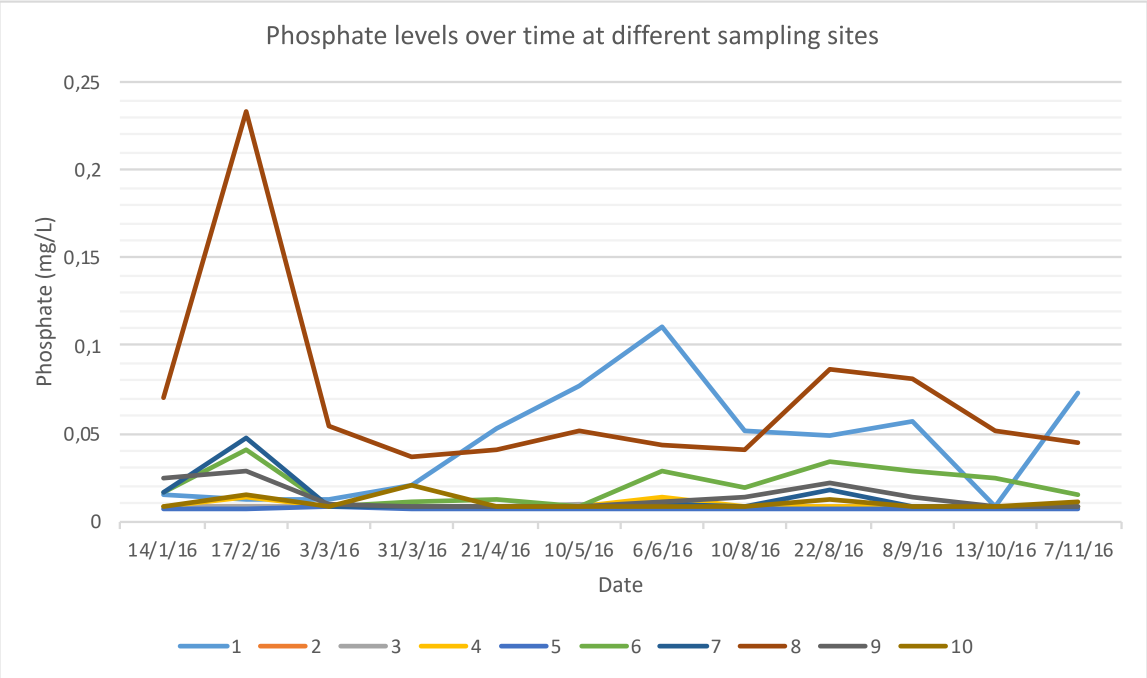

Fig. 28. Phosphate levels over time at different sampling sites (Source: data from SEPA, 2016).

4.3.8. Suspended solids

- Figure 29 shows that the average levels of suspended solids at all locations are below the threshold limit and mostly within the reference target for the species, generally being much lower than 10 mg/L at all sites. However, at location 9 an average level of 20.202 mg/L is at least 2 times greater than the reference level.

- Moreover, there are also notably high variations between average and maximum levels of suspended solids at all sites, with most peaks surpassing the reference level target and at sites 7, 8, 9 and 10 even the threshold limit.
- Levels of suspended solids at site 9 reach up to 109 mg/L which is nearly 4 times greater than the threshold limit and at least 2 times higher than levels at any other site in that month (Fig. 30).
- Figure 30 also shows that the highest levels at all sites are recorded in the month of February – March, with another peak in August - September.
- Sites 1, 2 and 3's levels of suspended solids remain stable the other months, with average levels below 4.0 mg/L. Instead, location 9 shows 3 major peaks.

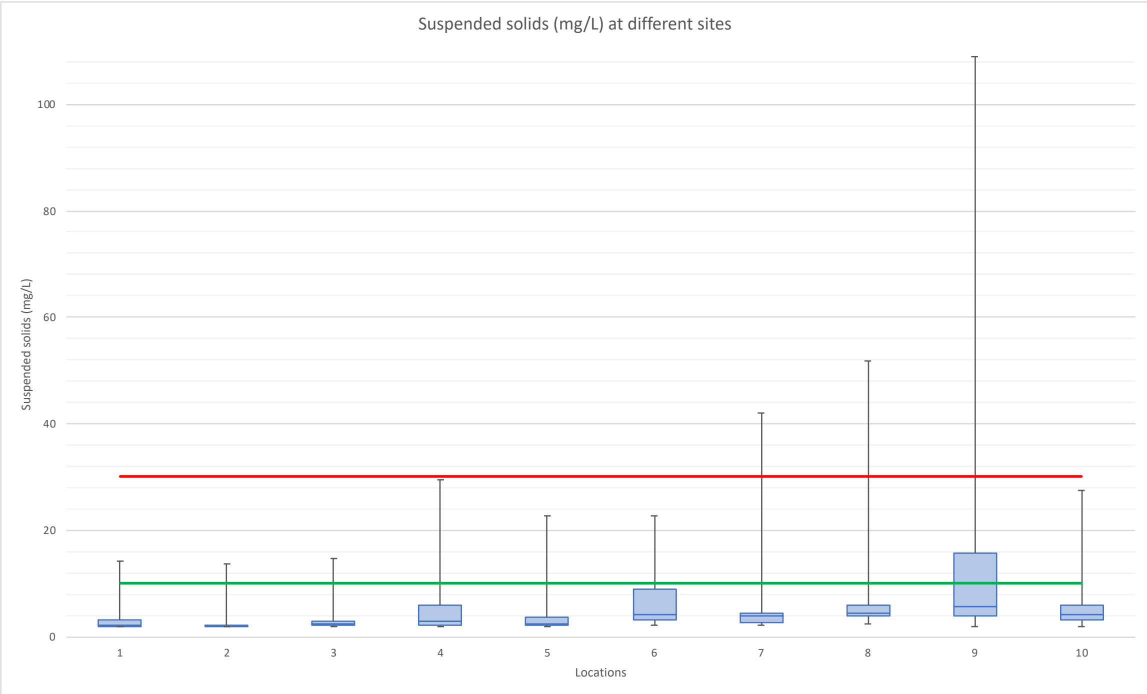

Fig. 29. Suspended solids at different sites (Source: data from SEPA, 2016).

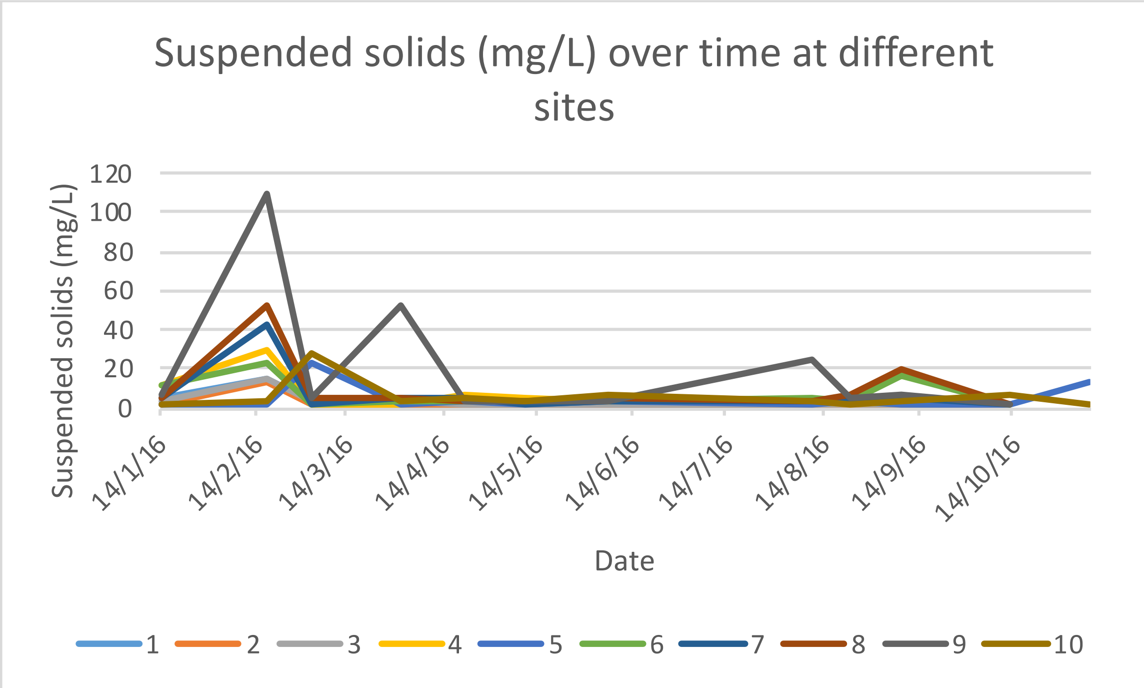

Fig. 30. Suspended solids over time at different sites (Source: data from SEPA, 2016).

4.3.9. Summary by site

Summary statistics are given in Table 5 and Fig. 31 with a traffic light system that displays in green, yellow or red the viability of FPM populations at the different locations.

Fig. 31 shows that the most suitable sites with favourable water chemistry are in the upper catchment or they branch off from the main water body, instead sites 6, 7, 8 and 9 in the lower catchment are completely unfavourable for the species.

Site 1: Meets most of the water chemistry requirements for the species, excluding ortho-phosphate levels, which are considerably higher than at other locations. Also, nitrate levels are slightly high and exceed the reference level target for healthy FPM populations.

Site 2: Meets most of the requirements aside from ortho-phosphate levels, although lower than at most sites. Also, pH and conductivity levels are slightly high.

Site 3: Meets all the requirements for the species, excluding ortho-phosphate levels, which although lower than at other sites, are still below the reference level for the species. Also, DO drops to 73.2% which is below the recommended target for the species.

Site 4: Apart from not meeting safe limit for ortho-phosphate levels, the reference target level for the species is not met for most of the chemical species analysed: pH, conductivity, BOD, nitrate and suspended solids.

Site 5: Aside from ortho-phosphate levels, all minimum requirements for the species are met. However, average BOD is slightly higher than the proposed target, showing wide fluctuations. Does not follow the same annual trend.

Site 6: Does not meet most of the safe limits for the species, with extremely high, unnatural levels of: conductivity, $CaCO_3$, DO, nitrate and ortho-phosphate.

Site 7: Does not meet most of the minimum requirements proposed, with substantially higher pH and $CaCO_3$ than the rest of the sites and unnaturally high conductivity, ortho-phosphate, DO values and high fluctuations in suspended solids that surpass the threshold limit.

Site 8: Does not meet most of the minimum requirements, with the highest recorded conductivity, nitrate and ortho-phosphate levels of all sites that greatly surpass thresholds. Also, extremely high pH, $CaCO_3$, DO levels and high peaks in suspended solids that greatly exceed limit.

Site 9: Does not meet most of the proposed favourable targets for the species, with pH, conductivity, $CaCO_3$, BOD, DO and nitrate levels being too high. However, extremely high levels of suspended solids are the greatest concern as they largely surpass limit.

Site 10: Aside from ortho-phosphate levels, meets all the other safe level targets for the species and most of the proposed favourable requirements, although levels of nitrate, DO, BOD and suspended solids are slightly high. Does not follow the same annual trend.

Location	Average pH	Average Conductivity (µS/cm)	Average $CaCO_3$ (mg/L)	Average BOD (mg/L)	Average Dissolved O_2 (%)	Average Nitrate (mg/L)	Average ortho-phosphate (mg/L)	Average Suspended Solids (mg/L)
1. River Annan: Evan Water	7.295	100.508	28.749	1.362	94.067	0.694	0.045	3.545
2. Evan Water: Beattock	7.55	129.9	35.058	1.301	94.533	0.299	0.008	3.135
3. River Annan: Moffat Water	7.26	75.85	24.058	1.285	94.383	0.314	0.008	3.562
4. River Annan: Johnstonebridge	7.47	113.817	34.425	1.453	100.608	0.606	0.009	6.407
5. Kinnel Water	7.252	81.65	25.925	1.5	96.425	0.316	0.008	5.168
6. River Annan: Shillahill	7.792	232.558	75.342	1.499	99.683	1.26	0.02	7.199
7. Water of Milk: Hoddom Mill	8.163	269.833	114.9	1.636	103.525	0.865	0.012	7.015
8. Mein Water	7.912	321.167	108.45	2.417	100.142	2.15	0.07	9.976
9. River Annan: Brydekirk Gaugin Station	7.718	184.942	57.9	1.525	100.208	0.915	0.014	20.202
10. Water of Ae: Elshieshields	7.228	97.541	28.05	1.79	94.85	0.652	0.01	6.04

Table 5. Traffic light system table showing in green, yellow or red the viability of freshwater pearl mussels at different locations (Source: data from SEPA, 2016).

Fig. 31. Traffic light system map showing in green, yellow, or red the viability of freshwater pearl mussels at different locations in the River Annan in 2016 (Source: modified from Ordnance Survey, 2011).

5. DISCUSSION

5.1. Analysis of water quality results for the River Annan

5.1.1. pH

Average pH at all sites is slightly alkaline, with no values below 6 and most values above pH 7 hence there is no potential threat of a gradual destruction of *M. margaritifera* calcareous shell or acidosis within the animal due to acidity of the river (Vinogradov *et al*., 1987).

Excluding location 8, the average annual pH at all sites meets the minimum requirement proposed. However, peaks at sites 6, 7 and 9 also surpass the threshold. Nonetheless, the bed composition at sites 7, 8 and 9 is mainly limestone (see Appendix C) hence, further investigative monitoring is required to understand if these high pH values can be attributed to a natural variation in alkalinity or if instead there is a nutrient loading problem which would be unsuitable for the species (EPA, 2012). Nonetheless, site 7 is completely unfavourable as it has an average pH of 8.41 and reaches peaks of pH 8.41 and pH values above 8 are moderately high and generally indicate intense photosynthetic activity by periphyton and macrophytes which is completely unsuitable for the species, especially juveniles, as it can block the interchange between surface and interstitial water and cause drops in oxygen levels between bed sediment and the overlying water column (Moorkens *et al.,* 2017). Also, the pH at site 6 cannot be explained by geology, as the bed composition is sandstone (see Appendix C) thus, the high values and wide fluctuations in pH indicate a potential problem in pollution or some other environmental factor (NIWA, 2016). Overall, further investigation is needed to understand the high pH and fluctuations at most sites, as these high values are associated with low numbers or no mussels as seen in Fig. 7. Although, FPM populations have been found in similar pH ranges of 7.5 - 8 in Ireland, Norway, Finland and Germany (Moorkens, 2000; Larsen, 2005; Lucey, 2006; Degerman *et al*., 2009), these sightings do not necessarily specify if specimens were in good health (Moorkens, 2000). Consequently, there is a consensus for pH to be circumneutral, ideally ranging from pH 6.5 - 7.5 for sustainable *M. margaritifera* populations (Cooksley and Blake, 2014) which is only observed at sites 1, 3, 5 and 10.

5.1.2. Conductivity

The reference conductivity target of < 100 µS/cm is only met at site 3 and 5, where slight fluctuations in conductivity levels are most likely due to natural factors such as increased runoff after prolonged or heavy rain or snowmelt (NIWA, 2016). Instead, Moorken's (2000) minimum conductivity requirements for the species of < 200 µS/cm is met at most locations, except at site 6, 7 and 8 which greatly surpass the threshold. Although values above 120 µS/cm may be natural on limestone-influenced areas (Skinner *et al*., 2003) like sites 7 and 8 (Fig. 16), ranges between 250 - 399 µS/cm observed at these sites are generally a sign of moderately enriched waters (NIWA, 2016) which are completely unsuitable for the species. Furthermore, extremely high levels of conductivity and fluctuations at site 6 clearly indicate enrichment of the water body, as bedrock composition does not influence the high conductivity values (see Appendix C) and levels above 400 µS/cm indicate heavily enriched waters with a very poor water quality rating (NIWA, 2016). Therefore, sites 6, 7, 8 are not suitable for *M. margaritifera* as they indicate a nutrient problem and FPM's require very low overall conductivity (Bauer, 1988; CEN working group, 2014). Further research is needed to understand the source of pollution which could be derived from urban and/or rural run-off, containing salt, fertilizers and organic matter (CEN working group, 2014).

5.1.3. Calcium

Measurements for Ca (mg/L) levels at the River Annan are only available for sites 7 and 10. Figure 19 shows that calcium levels are high at site 7 and greatly exceed Oliver's (2000) optimal target for the species of < 10 mg/L by over 5 times, with levels reaching 54.4 mg/L. Therefore, this is an immediate cause for concern as increased levels of calcium are correlated with decreasing survival and establishment of juveniles (Bauer, 1988; Skinner *et al.,* 2003). Instead, measurements at location 10 indicate that overall, the average Ca (mg/L) levels are within the target for the species, although peaks slightly surpass optimal levels. Nevertheless, there is much discrepancy amongst scholars about these Ca (mg/L) thresholds (Cooksley and Blake, 2014) as FPM populations have been recorded at sites with naturally elevated calcium levels in Ireland, Finland and Norway (Chesney and Oliver, 1998; Young *et al*., 2001; Skinner *et al.,* 2003; Moorkens, 2000; Lucey, 2006). In fact, given the variation in calcium levels between different FPM populations, no calcium thresholds have been proposed by CEN working group (2014) which is summarizing expert knowledge on water quality requirements for the species. Rather than calcium levels, most monitoring regimes measure total hardness which can be related to the baseline calcium levels (CEN working group, 2014). Although there are no established standards for calcium carbonate levels (Moorkens, 2000), healthy, reproducing populations of *M. margaritifera* are commonly found in rivers which have 'Soft' water, with levels below 120 mg/L (EPA, 2012; USGS, 2017). Hence, sites 6, 7, 8 and 9 have extremely high calcium carbonate (mg/L) levels which are not favourable for the species. For example, at site 7, peak levels reach to 154 mg/L, which would be classified as 'Hard' (EPA, 2012; USGS, 2017) and calcium levels are also high (Fig. 19). However, high values at sites 7, 8 and 9 may be linked to the natural geology of the catchment (Olson, 2012) as the bed material is carbonate rocks (limestone) (see Appendix C). Therefore, further investigative monitoring is needed to understand if these are the natural levels of calcium content for the river and the elevated alkalinity is as expected for the geology of the catchment (CEN working group, 2014). Nevertheless, at site 6 the high peaks of calcium and conductivity levels are likely to have been caused by liming (Moorkens, 2017) due to the intensively managed farm land use surrounding the area (Fig. 31) and that the high alkalinity values are not related to the bed material as it is sandstone (see Appendix C). Moreover, the Dumfries Soil and Nutrient Network highly recommends rectifying soil acidity in the area with lime to increase the productivity and yield of arable and grassland crops (Dunbar, n.d.). Nonetheless, further monitoring is required to thoroughly assess these high levels and address these concerns as CEN working group (2014) warns about harmful impacts of artificially high calcium levels for the species. More measurements in Ca (mg/L) levels are needed for all sites since an elevated alkalinity in the absence of increased calcium levels can be a direct indicator of pollution (Abril and Frankignoulle, 2001; CEN working group, 2014).

5.1.4. Biochemical oxygen demand

Although all sites average BOD levels are within Moorken's (2000) < 3 mg/L minimum requirement, peak values for site 7 and 8 exceed this threshold, particularly site 8, reaching peaks of up to 7.3 mg/L which surpass the threshold by over 2 times. Thus, these sites are completely unsuitable for the species, as Moorken's (2000) proposed threshold is already disputed by Young (2005) for being too high in comparison with other proposed BOD targets for FPM populations (see Table 2). Also, these sites are not viable because high BOD levels are linked to severe depletion of adult mussels and particularly to the loss of juveniles since elevated BOD levels will accelerate bacterial growth in the river and consume DO levels which are extremely important for their survival and establishment (Bauer, 1988; Young, 2005; CEN working group, 2014). This can be seen in Fig. 21 where a rise in BOD (mg/L) is followed by a drop in DO (%) levels.

Elevated BOD is usually caused by high levels of organic pollution, especially when there are also high conductivity levels (Abril and Frankignoulle, 2001) as is the case for location 8. Furthermore, site 8 also shows an unnatural peak from August to September which does not follow the general trend in rising and dropping BOD levels (Fig. 22) that indicate a source of pollution.

Instead, sites 1, 2 and 3 are within Bauer (1988) and Oliver's (2000) target range (see Table 2). However, CEN working group (2014) has found that reproducing populations of FPM's in the UK, Ireland and Spain have BOD levels consistently below 1.0 mg/L. Therefore, none of the sites at the River Annan meet this lower BOD target therefore, the water quality may not be sufficient to sustain healthy FPM populations.

Further investigative monitoring is needed to understand what may be using up the oxygen in the water and to locate the source of pollution, particularly at sites with high BOD levels. Nonetheless, as the catchment is dominated by farm land use, it can most likely be attributed to agricultural sources (RADSFB, 2014).

5.1.5. Dissolved oxygen

All sites are within the 90-110 % proposed target and thus are classed as 'High' under the Water Framework Directive (2008). However, there are large fluctuations between average DO (%) levels and minimum and maximum levels at all sites and although DO levels fluctuate seasonally and over a 24-hour period (EPA, 2012), excessive deviations are problematic. DO should be

consistently near to 100% for the species (CEN working group 2014), especially for juveniles since they are completely buried for the first few years (Cranbrook, 1976) and oxygen saturation can decrease significantly with depth (Buddensiek *et al*., 1993). Nonetheless, the lowest DO levels recorded of 77.5 % and 80.1% at sites 2 and 8 respectively, are still classed as 'Good' and at site 3 DO levels drop to 73.2% which is 'Moderate' (EPA, 2012). So, there are no sign of dangerous anoxic conditions that could lead to the release of phosphate from sediment and the reduction of nitrate to nitrite, which is a potent neurotoxin (Cooksley and Blake, 2014).
Conversely, site 4 and 6 reach abnormally high DO levels of 126% and 125%, respectively. This is indicative of the production of pure oxygen by photosynthetically-active organisms such as plants or algae in the river (YSI, 2005) which is problematic as even mild enrichment is likely to be a severe problem for *M. margaritifera* communities (Skinner *et al.,* 2003; Young, 2005) as it can lead to eutrophication (EPA, 2012). Sites 7, 8 and 9 also surpass the 110% threshold and indicate mild enrichment.
Further research is needed to understand DO variations across the year as it does not follow a natural seasonal pattern – with high DO levels in the winter and early spring when the water temperature is low and low DO levels in the summer and fall when there is higher water temperature (USGS, 2017). Instead, the reverse is true and trends in DO (%) levels seem to respond to changes in BOD (mg/L) levels as seen in Fig. 21.

5.1.6. Nitrate

Moorken's (2006) nitrate threshold for the species of 0.125 mg/L is met at most locations, except at site 6 and 8, with peaks reaching 2.03 mg/L and 2.71 mg/L, respectively, making them completely unsuitable for the species as Bauer (1988) found increased mortality at sites where nitrate values were >1.5 mg/L. However, the proposed nitrate level target for sustainable *M. margaritifera* populations is only met at sites 2, 3 and 5, with average annual levels below 0.5 mg/L which would indicate natural mortality rates (Bauer, 1988). Also, as seen in Fig. 26, there are only small fluctuations in nitrate levels across the year at these locations, indicating that these may correspond to the natural nitrate levels for the catchment. Instead, most of the sites have high nitrate levels and wide fluctuations throughout the year, particularly at locations 6 and 8, which clearly indicate a problem with pollution.

In fact, nitrate levels do not follow a natural seasonal pattern - with higher nitrate levels in winters due to higher rainfall. Instead, Fig. 26 shows that the highest levels of nitrate are recorded in early spring to late summer, which could be linked to increasing plant growth due to higher temperatures and increased light in the warmer months of the year (EPA, 2012).

Therefore, nitrate levels at the River Annan are alarming, as *M. margaritifera* are sensitive species (EPA, 2012) and excessive nitrogen inputs may enhance productivity and can lead to eutrophication (CEN working group, 2014; Cooksley and Blake, 2014) which has been noted as the main cause for the decline in the species across its range (Young *et al*., 2001). Thus, further research is required to understand the source of the pollution, although it is most likely due to increased use of nitrogen fertilizers and intensification of animal production from predominant agricultural land use surrounding the Annan catchment (Stalnacke *et al*., 2003).

5.1.7. Ortho-phosphate

None of the locations meet the average, annual phosphate level target of < 0.005 mg/L associated with effectively recruiting populations of *M. margaritifera* (Moorkens, 2006; Degerman *et al*., 2009; Cooksley and Blake, 2014; CEN working group, 2014). In particular, location 1 and 8 largely surpass this annual target level and, greatly exceed the maximum peak target of < 0.06 mg/L (Cooksley and Blake, 2014; CEN working group, 2014). Moreover, these locations do not follow the same annual trend seen at the other sites hence, they suggest likely anthropogenic impacts. For instance, Fig. 28 shows that in the month of February, location 8's peak is more than 4 times higher than any of the other sites at that time.

Thus, more detailed investigative monitoring is needed to understand the high phosphate concentrations at all sites, especially at locations 1 and 8, as excessive nutrient inputs have been shown to be the main cause of eutrophication (EPA, 2012) and the leading cause for the international decline of *M. margaritifera* populations (Moorkens, 2011).

Thus, phosphate levels are the greatest immediate concern for potential FPM populations in the Annan catchment given that this species requires nutrient concentrations to be at reference conditions and maintaining low levels at all times is considered essential (CEN working group, 2014).

5.1.8. Suspended Solids

The annual median target of suspended solids < 10 mg/L (Valovirta, 1990; Valovirta and Yrjana; 1997; Skinner *et al*., 2003) is met at most sites, except location 9 which highly exceeds this threshold with an average of 20.20 mg/L and site 8 hardly meets the minimum requirements with an average of 9.98 mg/L. These high levels are alarming, as suspended solids in rivers with sustainable FPM populations should be extremely low (CEN working group, 2014) with levels consistently above 10 mg/L being a cause for concern (Valovirta, 1990; Valovirta and Yrjana, 1997; Skinner *et al*., 2003; JNCC, 2005; Langan et al., 2007). In fact, there should only be short, minor peaks occurring during periods of heavy rainfall or snowmelt and they should be below 25 - 30 mg/L (Valovirta, 1990; Valovirta and Yrjana, 1997; Skinner *et al*., 2003; CEN working group, 2014). Hence, site 7, 8 and 9 are completely unsuitable for *M. margaritifera*, notably site 9 which reaches levels of 109 mg/L, which is almost 4 times higher than the threshold limit. Also, sites 4 and 10 are unfavourable for healthy reproducing populations of FPM's because although adult *M.*

margaritifera appear to be tolerant to silty conditions for a short-term, they are completely unsuitable for the more sensitive juvenile stages (Hastie *et al*., 2000). In fact, excessive amounts of suspended solids are one of the biggest threats to freshwater pearl populations (CEN working group, 2014) as they cause adverse effects on adult and juvenile mussels by inhibiting feeding and preventing oxygen exchange, causing them to clam up and leading to severe stress and death (CEN working group, 2014).

A detailed investigation is required to understand the elevated amounts of suspended sediments at most sites, as they are a visible indicator of a water quality problem (EPA, 2012) due to point sources of pollution such as wastewater and/or diffuse sources, such as soil erosion from agricultural land use and construction sites (EPA, 2012).

5.1.9. Analysis of site suitability

The results indicate that none of the sites meet all the water chemistry criteria required by *M. margaritifera* and that this may be due to nutrient enrichment of the water body caused by point source and/or diffuse pollution. Moreover, since approximately 70% of the Annan catchment is dominated by agricultural land use (RADSFB, 2014), it is likely that this has resulted in diffuse pollution into the catchment from slurry and other waste product runoff into the river. In fact, RADSFB (2014) identified that the main factor limiting fisheries performance is due to agricultural practices and its associated impacts, mainly affecting the catchment from Johnstonebridge to the south (RADSFB, 2014). As seen in Fig. 31 this coincides with sites 6, 7, 8 and 9 having the poorest water quality and failing to reach good ecological status for *M. margaritifera*.

Furthermore, the unfavourable water quality results for these sites can also be linked to the unsuccessful survey results for the species at the Annan catchment, particularly the monitoring in 2013 by RAT where 4 deceased FPM's were found (The River Annan Trust, n.d.-b), since the scope of the survey corresponds to locations 6,7,8 and 9 as seen in Fig. 4 and Fig. 31.

Particularly, sites 7 and 8 have the most unfavourable water quality and substrate conditions for the species, with significantly high levels and fluctuations in: pH, $CaCO_3$, suspended solids, conductivity, BOD, nitrate and phosphate levels and instead, unnaturally low or high DO levels.

Instead, sites 2, 3 and 5 meet most of the criteria for water quality and further investigation is needed to test their suitability. Interestingly, favourable water chemistry results at site 3 and 5 can be associated with the Habitat Improvement Programme undertaken on parts of the Moffat and Kinnel water (RADSFB, 2014) which highlight the potential benefits of fencing projects and erosion control works on water quality.

Location 3 shows the most favourable water chemistry conditions for *M. margaritifera* with low overall pH, BOD, conductivity, phosphate and nitrate levels and high DO levels. However, although ortho-phosphate levels are lower than other sites at the catchment, they are still not favourable for healthy, reproducing FPM populations. Thus, further monitoring is needed. In fact, none of the locations examined at the River Annan meet the annual median minimum requirement for ortho-phosphate levels and this is problematic as Bauer (1988) observed that sustainable FPM populations could only occur when enrichment was not affecting its rivers, as it is directly linked to eutrophication which is the main cause of the international decline in FPM populations (Cosgrove *et al*., 2000) due to:

> "increased organic sedimentation, colmation, oxygen depletion in the substrate, changes in fish communities and increased fluctuations in pH values" (CEN working group, 2014: 25).

Overall, the results point to enrichment and show a general bad status for this critically endangered, sensitive species of mollusc, particularly at the lower part of the Annan catchment which may be due to pollutants being transported downstream. Also, this could potentially explain the similar seasonality trends throughout the year at most sites following the general movement of water through the River Annan. Whereas site 5 and 10, which branch off into the tributaries of the Kinnel water and water or Ae, show completely different seasonality trends for most attributes.

5.2. Limitations and Recommendations

Despite numerous studies on the ecology of FPM's (Hastie *et al*., 2000), there is a lack of research on the key water chemistry and substrate conditions that influence the survival and distribution of *M. margaritifera* (Young, 2005). In summary, there is a consensus that *M. margaritifera* require high water quality to survive and reproduce (Bauer,1983; Buddensiek *et al*., 1993) but few studies quote precise standards which must be met for healthy populations to be maintained (Cooksley and Blake, 2014). The main publications are listed in Appendix B and Table 2 shows a summary of the standards for specific water quality parameters from these key studies. However, the water quality objectives proposed from the different studies are difficult to interpret because many factors must be taken into account when considering safe levels for the species (Young, 2005). For example, at different parts in their life cycle, *M. margaritifera* vary in sensitivity, with juvenile mussels and glochidia being much more vulnerable than adults to poor water conditions (Hastie *et al.,* 2000). Another example is that some pollutants such as metals, may be present in toxic or non-toxic states, dependent on other factors such as pH (Naimo, 1995). Also, FPM's show local adaptation and therefore standards vary at different areas (Purser, 1985). Additionally, proposed targets may be underestimates of water quality requirements for the species, as in most cases FPM populations have declined and there is a lack of data for when the populations were healthy (Moorkens, 2000). Thus, largely there is still a very patchy picture of the requirements of the species and much discrepancy between scholars regarding precise standards (Cooksley and Blake, 2014). Hence, an overall standard for FPM catchments is being developed by a working group of European FPM experts called CEN working group that details what parameters need to be measured and recommends targets that need to be attained to support sustainable *M. margaritifera* populations. However, this study has only been able to draw information from the draft report of CEN working group (2014) so proposed standards for the River Annan need to be revised when the final report is published.

Moreover, summary statistics from this study should be treated as highly preliminary, as a total of 12 samples per site, for a 1-year period, with occasional monthly data gaps and a lack of consistency in the time and date of the sampling is not sufficient. Hence, to fully understand the water quality situation, it will be necessary to analyse longer data sets, at a higher frequency with consistent measurements at certain times and dates to ensure accuracy and precision in the results and to allow for a more robust analysis that will enable the investigation of trends and seasonality.

Furthermore, more parameters need to be monitored to determine the water quality at these sites and its potential suitability for *M. margaritifera* populations. For example, measurements in Ca (mg/L) levels are needed for all sites since an elevated alkalinity in the absence of increased calcium levels can be a direct indicator of pollution (Abril and Frankignoulle, 2001; CEN working group, 2014). Also, it is important to analyse other pollutants, such as metals, which may be toxic for the species. It is also essential to cross-examining other variables such as changes in weather patterns, like heaving rainfall that will affect the amount of runoff and dilution to understand trends across sites.

Moreover, aside from suitable water quality objectives, there are other key criteria that are necessary to determine the viability of a watercourse for *M. margaritifera* populations such as: density of host fish populations, suitability of the physical habitat and flow regime, site-security from illegal pearl fishing and controlling diffuse and point sources of pollution. Adverse changes in any one of these would be sufficient to impact on the size and viability of FPM populations (JNCC, 2007) at the Annan catchment. Therefore, assessing these other parameters is essential to be able to quantify the threats and gain a better understanding of the characteristics and pressures that the River Annan faces. For example, due to historically low salmon and trout stocks at the River Annan (see Fig. 10) management measures should be aimed at enhancing these populations as they are an essential link in the FPM's life cycle and could ultimately threaten the long-term survival of any remaining FPM populations in the catchment.

Furthermore, the results indicate that there is a general problem of nutrient enrichment. Thus, further investigative monitoring is needed to identify the source of pollution and to ensure that it is acted upon, especially targeting the lower end of the catchment near locations 6, 7, 8 and 9 which are failing to reach good ecological status for the species.

Although this project cannot directly attribute the cumulative effects of agricultural practices to the poor water quality results, it is likely to be the case considering the dominant agricultural land use surrounding the catchment, with the worst water quality results being observed in the most intensively managed farm land sites. Furthermore, since various studies link agricultural impacts to the international decline of the species (Young *et al*., 2001) and particularly in Scotland (Henrikson *et al*., 2009), this project recommends conservation measures to control pollution and promote habitat restoration work to obtain favourable conditions for FPM's. Moreover, livestock, agriculture and its associated impacts have been identified by RADSFB (2014) as the main factors limiting fisheries performance at the River Annan, thus it is important to work closely with land managers to control the nutrient and sediment input from diffuse sources through agro-environment schemes, the stabilization of riverbanks where appropriate and the planting of riparian woodlands (Pearls in Peril, 2017). These types of measures will improve the environmental conditions and water quality of the River Annan for *M. margaritifera* populations as already seen at sites 3 and 5 which correspond to the Moffat and Kinnel water, were the Habitat Improvement Programme has already made many bank enhancements to control erosion and prevent livestock encroachment and has had positive results (RADSFB, 2014).

More importantly, for restoration to be effective, there must be a commitment to long-term catchment management plans owing to the age at which breeding commences in *M. margaritifera* and the length of the FPM life-span.

6. CONCLUSION

Overall, there is a strong consensus that sustainable FPM populations require near natural, clean and well-oxygenated water to survive and reproduce. Yet, there is still an important gap in the evidence base for precise water chemistry and substrate conditions for the species. This paper compares the water chemistry within the River Annan to proposed water quality objectives for *M. margaritifera* from key studies to assess the potential viability of the species in the Annan catchment. Although there are many limitations and statistics should be treated as highly preliminary, this study has identified water quality to be an issue at most sites, with none of the locations meeting all of the criteria required for healthy FPM populations. Key findings show that levels of BOD, nitrate and phosphate are generally much higher than the recommended requirements for the species and particularly phosphate levels are the greatest immediate cause for concern, as all sites exceed the threshold limit. Sites 2, 3 and 5 have the most favourable water quality, meeting most of the recommended targets, particularly site 3 with low overall pH, BOD, conductivity, suspended solids, phosphate and nitrate levels and high DO levels. Instead, sites 6, 7, 8 and 9 show completely unfavourable environmental conditions for the species, surpassing most of the thresholds for the chemical species analysed with extremely high, unnatural levels of pH, BOD, conductivity, suspended solids, phosphate and nitrate levels and wide fluctuations in DO.

The water quality results indicate that there is a nutrient enrichment problem, particularly affecting the lower catchment which is most likely caused by point source and/or diffuse pollution from agricultural practices. This is alarming, as enrichment can lead to eutrophication which has been noted as the underlying reason for the global decline in FPM populations. Thus, this study recommends improving the water quality at the River Annan through the close liaison with land managers and through the implementation of more projects like the Habitat Improvement Programme that control erosion, prevent livestock encroachment and reduce nutrient loading into the river. Furthermore, it is also be necessary to consider other key factors that are detrimental to the species like low numbers of host fish populations, degraded riparian habitat and illegal pearl fishing; as adverse changes in any one of these would be sufficient to prevent improvements in the size and viability of FPM populations in the catchment.

In conclusion, this study shows that if environmental conditions do not change soon, there are very bad prospects for any remaining *Margaritifera margaritifera* in the River Annan, particularly juveniles as they are far less tolerant than the adults. However, with adequate and effective catchment management plans over a long-term period it may be possible to reverse this trend, as historical evidence suggests that mussel populations can recover naturally from low levels. Moreover, a more robust analysis of water quality is required, particularly when CEN working group summarizes water quality standards for the species. This will enable the investigation of trends and seasonality and could ultimately lead to the protection of an internationally protected, keystone species which will enhance the overall river ecosystem.

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8. APPENDICES

8.1. Appendix A. Raw water chemistry data from SEPA.

	A	B	C	D	E	F	G	H	I	J	K
1	Location code	Name	NGR	Sample date	Sample time	Sample no.	Media	Determinand	Det. No.	LoD sign	Sample result
3	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	8/9/16	1000	3054281	RIV_WATER	Alk as CaCO3 (mg/L)	200200		30,4
5	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	10/8/16	1000	3034260	RIV_WATER	Alk as CaCO3 (mg/L)	200200		29,1
7	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	13/10/16	1610	3075570	RIV_WATER	Alk as CaCO3 (mg/L)	200200		48,4
8	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	22/8/16	1030	3042538	RIV_WATER	Alk as CaCO3 (mg/L)	200200		27,2
14	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	14/1/16	1100	2900554	RIV_WATER	SuspSolids (mg/L)	140100		4,25
15	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	17/2/16	1030	2921916	RIV_WATER	SuspSolids (mg/L)	140100		14,2
16	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	6/6/16	1045	2990365	RIV_WATER	SuspSolids (mg/L)	140100		2,12
25	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	8/9/16	1000	3054281	RIV_WATER	SuspSolids (mg/L)	140100	<	2
26	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	13/10/16	1610	3075570	RIV_WATER	SuspSolids (mg/L)	140100	<	2
27	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	10/5/16	1000	2973131	RIV_WATER	SuspSolids (mg/L)	140100	<	2
28	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	31/3/16	1120	2947510	RIV_WATER	SuspSolids (mg/L)	140100	<	2
29	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	21/4/16	1020	2961020	RIV_WATER	SuspSolids (mg/L)	140100	<	2
30	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	3/3/16	1030	2932552	RIV_WATER	SuspSolids (mg/L)	140100		2,98
31	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	10/8/16	1000	3034260	RIV_WATER	SuspSolids (mg/L)	140100		2,22
32	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	22/8/16	1030	3042538	RIV_WATER	SuspSolids (mg/L)	140100		3,22
45	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	13/10/16	1610	3075570	RIV_WATER	ElecCond-25 (µS/cm)	200160		149
46	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	8/9/16	1000	3054281	RIV_WATER	ElecCond-25 (µS/cm)	200160		98
47	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	10/8/16	1000	3034260	RIV_WATER	ElecCond-25 (µS/cm)	200160		92
48	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	22/8/16	1030	3042538	RIV_WATER	ElecCond-25 (µS/cm)	200160		87,7
49	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	14/1/16	1100	2900554	RIV_WATER	ElecCond-25 (µS/cm)	200160		84,9
50	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	13/10/16	1610	3075570	RIV_WATER	O2 -%sat (%)	210200		94,2
51	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	22/8/16	1030	3042538	RIV_WATER	O2 -%sat (%)	210200		99,3
52	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	10/8/16	1000	3034260	RIV_WATER	O2 -%sat (%)	210200		100
53	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	8/9/16	1000	3054281	RIV_WATER	O2 -%sat (%)	210200		85,5
54	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	3/3/16	1030	2932552	RIV_WATER	O2 -%sat (%)	210200		93,1
55	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	7/11/16	1035	3091772	RIV_WATER	O2 -%sat (%)	210200		96,9
56	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	31/3/16	1120	2947510	RIV_WATER	O2 -%sat (%)	210200		96,9
57	121093	River Annan, 500m u/s Evan Water	NT 09218 02891	21/4/16	1020	2961020	RIV_WATER	O2 -%sat (%)	210200		103

Fig. 32. Extract of raw water chemistry data from SEPA for the River Annan (Source: data from SEPA, 2016).

Full data set available at:
https://1drv.ms/x/s!AhQPYeGhKmLKozobSNWbNF-u3Yfb

8.2. Appendix B. Overview of key literature.

Citation	Key findings
Purser (1985)	Using data from River Purification Boards, Purser investigated the favourable water chemistry values for freshwater pearl mussels in the UK, particularly focusing on the North West of Scotland. Summarized the normal range of values in mussel rivers for commonly measured parameters and demonstrated that the standards varied throughout Britain implying mussel populations show local adaptation.
Bauer (1988)	Favourable water quality conditions for freshwater pearl mussels in central European rivers. Results are very similar to Purser (1985).
Oliver (2000)	Produced water chemistry recommendations for freshwater pearl mussels consulting Purser (1985) and Bauer (1988). Recommended water quality objectives for freshwater pearl mussels.
Young *et al.* (2001)	Summarized the main causes of decline in freshwater pearl mussel populations throughout European countries. Eutrophication found as the main cause of the decline in freshwater pearl mussel populations.
Young (2005)	Literature review of results from Purser (1985), Bauer (1988) and Oliver (2000) summarizing the water quality requirements of the species, including toxicology.
Moorkens (2000)	Surveyed 526 sites in 149 Irish rivers and compared water chemistry between the sites with and without freshwater pearl mussels to establish favourable water quality requirements for the species.
Degerman (2009)	A project in Sweden that implemented different restoration methods in 21 rivers to restore freshwater pearl mussels and its host fish. Proposed guidelines for water quality thresholds for a range of attributes.
Cooksley and Blake (2014)	A review of current understanding of water quality requirements of freshwater pearl mussels to propose reference conditions for the River Spey and analyse trends over time and seasonality.

CEN working group (2014)	Draft document awaiting approval that summarizes expert knowledge on water quality requirements for freshwater pearl mussels.

Table 6. Summary of the key literature on freshwater pearl mussel water chemistry requirements (Source: modified from Cooksley and Blake, 2014).

8.3. Appendix C. Bed composition.

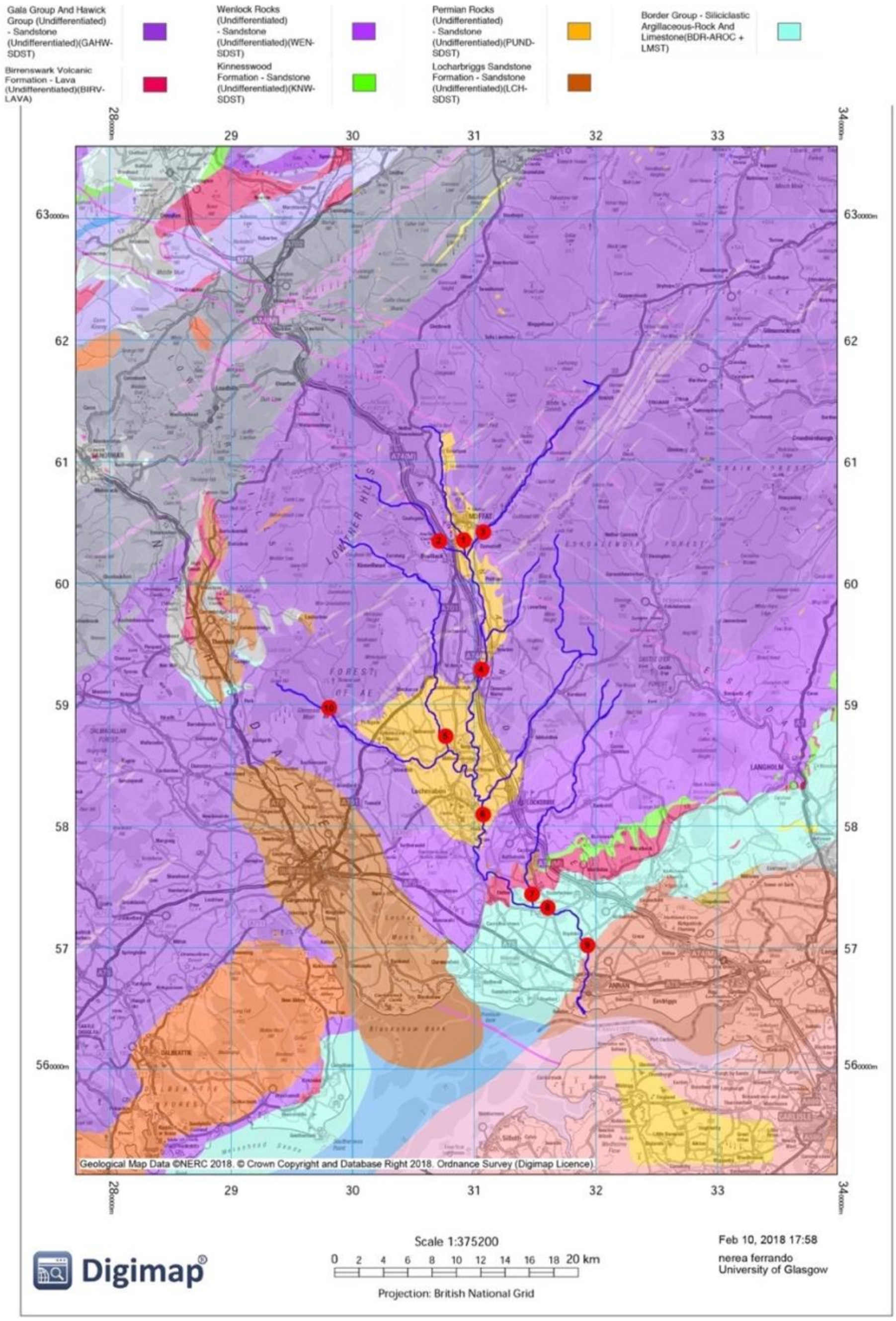

Fig. 33. Bed composition of the River Annan catchment (Source: modified from Digimap, 2018).

Figure 33 shows that location 7, 8 and 9 are found in an area predominantly made of limestone, whereas the rest of the site's main bedrock composition is sandstone.