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Review: approaches to monitoring and surveillance of antimicrobial resistance in bathing waters

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Dr Robert Bradburne
Chief Scientist

Contents

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Executive summary

Antimicrobial resistance (AMR) represents a major concern for human, animal and plant populations, and the wider environment. The Government's 20-year Vision for AMR and the 5-year National Action Plan outline how the UK will address the AMR challenge. This specifically communicates the importance of better understanding the dissemination, selection, and transmission of AMR to and from the environment.

The microbiome of a bathing water (BW) reflects the microbiome of the wider catchment and thus surveillance of a BW can provide valuable insights into the prevalence and dissemination of AMR in the aquatic environment. Additionally, studies have indicated the potential of AMR exposure through the recreational use of coastal BWs. The main objectives of this work were to improve understanding of the approaches that have or could be applied to surveillance of AMR in BWs, and consolidate knowledge in relation to antifungal resistance (AFR) in coastal BWs and sands. The research project was divided into tasks:

Task 1 set out to review how AMR is assessed in designated BWs, focusing on jurisdictions in the UK (other than England) and Europe. Dedicated searches of the literature were undertaken, whilst information for review was also obtained following consultation with AMR specialists. Specific details on the sampling undertaken, the rationale for site selection, and the methods employed to measure AMR were recorded. The only example of AMR surveillance being included in national scale monitoring at designated BWs was found to take place in Scotland.

Task 2 set out to collate and assess the current state of knowledge in relation to AFR in coastal BWs and sands. A literature review was undertaken to assess what (if any) AFR fungal species have been identified in coastal environments, as well as typical fungal pathogens that inhabit coastal environments, and any known (or suspected) clinically significant AFR potential within these species (e.g. identified in clinical settings or other environments).

Task 3 developed an approach to select and prioritise designated BWs in England for AMR surveillance. Principally this involved the development of criteria (which reflect AMR risk) that can be used to prioritise BWs over one another. An excel workbook which facilitated the calculation of risk scores and can be used to select BWs based on chosen criteria was developed. In this study, BWs were selected based on criteria agreed with the Environment Agency. A subsequent step was included in the approach, involving more detailed review of these BWs to recommend priority for inclusion in future AMR surveillance.

Studies have indicated the potential for AMR exposure at coastal bathing waters, and this research work explored how we could pilot surveillance in this area. Whilst currently there is no statutory driver for AMR monitoring at bathing water sites, this research project is to highlight potential areas of future concern. This work has found that there are few environmental regulators assessing AMR in the environment and has consolidated knowledge in relation to potential antifungal resistance in coastal bathing waters and associated sand. This research has also developed an approach to prioritise costal bathing waters for surveillance so that any future surveillance programme could monitor AMR in coastal bathing waters in a cost-effective manner.

Introduction

Antimicrobial Resistance (AMR) arises when microorganisms evolve or aquire mechanisms to become resistant to antimicrobial substances. The term antimicrobial includes antibiotic (antibacterial), antiprotozoal, antiviral and antifungal substances.

The World Health Organisation (WHO) identify AMR as ‘one of the biggest threats to global health, food security, and development today’. Whilst the development of AMR in clinical and agricultural settings has received significant attention, increasing evidence suggests that environmental drivers play a significant role in the development, proliferation and transmission of clinically relevant AMR (Singer et al., 2016; United Nations Environment Programme (UNEP, 2023). This is reflected in the One Health approach to tackling AMR, which includes the coordination of human, animal, plant and environmental policies.

The Governments 20-year Vision for AMR (HM Government, 2019a) and the 5-year National Action Plan (HM Government, 2019b) outline how the UK will address the AMR challenge. This makes particular reference to the importance of better understanding the potential spread, transmission, and risk of AMR in the environment.

Bathing waters (BWs) are defined (2013) as ‘*surface waters that have been identified in England, other than excluded pools and waters, at which the Secretary of State expects a large number of people to bathe, having regard in particular to past trends and any infrastructure or facilities provided, or other measures taken, to promote bathing at those waters*’ (Bathing Water Regulations, 2013). The microbiome of a BW reflects the microbiome of the wider catchment; therefore, surveillance of a BW can provide valuable insights into the prevalence and dissemination of AMR in the aquatic environment. Additionally, studies have indicated the potential from AMR exposure through recreational use of coastal BWs (Leonard et al., 2015).

The main objectives of this work were to improve understanding of the approaches that have or could be applied to surveillance of AMR in BWs, and consolidate knowledge in relation to antifungal resistance (AFR) in coastal BWs and sands. The study encompasses three key tasks, which will provide information for a potential BW surveillance pilot in England:

- Task 1: Review of AMR surveillance at designated BWs
- Task 2: Literature review of AMR fungi at coastal BWs
- Task 3: Development of an approach for the selection of designated BWs for AMR surveillance.

The report sections below provide more detail on each of these tasks in turn.

Task 1: Literature review of AMR surveillance at designated bathing waters

Introduction

Current research of AMR in designated BWs is dominated by studies using *Escherichia coli* (*E. coli*) and *Enterococcus* species (spp.)*,* as these are the usual bacterial faecal indicator organisms (FIOs) for BW quality. Most of the designated BWs within the UK (England, Wales, Scotland and Northern Ireland) are coastal waters and thus most studies focus on coastal waters as opposed to inland waters. There is, however, a need to understand if AMR surveillance in designated BWs in the UK and Europe exists, and if so, what it entails, as this will help inform decisions relating to the design of AMR surveillance to be undertaken in the future.

Methodology

Dedicated searches of the literature for available datasets and information associated with existing or proposed AMR surveillance in European BWs were undertaken, principally using the term 'AMR surveillance bathing water' in the Google search engine. This was used so that grey literature would also be captured. Information for review was also obtained following consultation with AMR specialists at Scottish Environment Protection Agency (SEPA) and the University of Galway, Ireland.

Information to be recorded from the search was agreed with the Environment Agency. As well as general information for each study (e.g. aim/objective), specific details on the sampling undertaken (e.g. time-period, frequency, depth/position), the rationale for site selection and the method(s) employed to measure AMR were recorded.

Types of studies found in the search were separated into categories (or 'Tiers') ordered by relevance:

- Tier 1 – AMR surveillance included in national monitoring at designated BWs
- Tier 2 – AMR surveillance at designated BWs (coastal & inland)
- Tier 3 – AMR surveillance at non-designated bathing sites
- Tier 4 – Selected studies, which have investigated AMR at locations not defined as BWs but that have a potential for bathing activity (i.e. river, lake, estuarine, seawater sites).

Findings

Findings from the review are summarised in Table 1. Key findings noted at this stage, included:

- The only example found of AMR surveillance being included in national monitoring at designated BWs was for Scotland. There, AMR surveillance is currently undertaken as an extension of existing monitoring as part of the Bathing Water Regulations (2013) at Scotland’s designated BWs (n = 87). This includes testing for cefotaxime resistance in *E. coli* and testing for vancomycin resistance in *Enterococcus* spp.

- Across the studies found in this review sampling was typically reported to occur during the BW season only (which extends from May to September), whilst the frequency of sample collection varied substantially.
- Only one study investigated sand as well as water. In this study, composites of water and sand were collected, as opposed to single grab samples, which can improve representation of the site investigated.
- Methods used to measure AMR included culture-based analysis (such as enumeration of resistant bacteria, Antibiotic Susceptibility Testing (AST of isolates) and Whole Genome Sequencing (WGS)) or molecular-based analysis (typically quantitative Polymerase Chain Reaction (qPCR)) or both. As shown in Table 1, the types of analysis employed varied between studies, whilst methodologies also varied in terms of selected bacteria species, selection of tested antibiotics and genes targeted.

Table 1 – AMR surveillance in BWs: summary of findings (Task 1).

Tier *	**Ref**	**Location**	**Site (n)**	**Site Type** (designated BW or non-designated bathing site)	**Media Sampled**	**Sampling Period** [Frequency of sample collection]	**Sample Depth/ Position**	**Rationale for Site Selection*****	**Method(s) used to measure AMR**
Tier 1	[1]	Scotland	87	Designated BW	Coastal / lake water	BW season (1 June to 15 September) [18 samples per site, 10 for remote sites, 5 for sites which have consistently demonstrated excellent water quality]	Depth of ~30cm, ideally beyond the wave breaking zone**	Holistic coverage of Scotland's designated BWs - samples collected in accordance with Bathing Water Directive are used.	Enumeration of resistant bacteria[a]

Tier *	**Ref**	**Location**	**Site (n)**	**Site Type** (designated BW or non-designated bathing site)	**Media Sampled**	**Sampling Period** [Frequency of sample collection]	**Sample Depth/ Position**	**Rationale for Site Selection*****	**Method(s) used to measure AMR**
Tier 2	[2]	Dublin Bay, Ireland	3	Designated BW	Coastal water	August 2017 – August 2018 [bi-monthly]	20 cm where possible, 2 m from bank	> As the River Liffey discharges into Dublin Bay it receives treated effluent from Wastewater Treatment Works (WwTWs) at Ringsend. > Dublin Bay is a UNESCO biosphere and is home to thousands of protected native and migratory birds that roost on or near the coast. > Several small streams that are completely urban along their courses discharge into Dublin Bay. > Streams flow through urban areas before discharging into designated bathing areas (in 2 out of 3 cases)	qPCR

Tier *	**Ref**	**Location**	**Site (n)**	**Site Type** (designated BW or non-designated bathing site)	**Media Sampled**	**Sampling Period** [Frequency of sample collection]	**Sample Depth/ Position**	**Rationale for Site Selection*** **	**Method(s) used to measure AMR**
	[3]	Austria	27	Designated BW	River / lake water	July – August 2017 [1 per site]	30 cm below the river/lake surface, 2 m from the bank	Three sites were arbitrarily chosen per Austrian state.	AST of isolates^, WGS
	[4]	North Rhine-Westphalia, Germany	20	Designated BW	Water	BW season (2018, May to September) [4 per site]	Not stated	Risk based strategy, including the following factors: (i) Risk of entry of antibiotic resistant bacteria and antimicrobial substances (ii) Known burdens (iii) Region and usage (number of bathing guests) (iv) Type of water	Enumeration of resistant bacteria[a] and AST of isolates^

Tier *	**Ref**	**Location**	**Site (n)**	**Site Type** (designated BW or non-designated bathing site)	**Media Sampled**	**Sampling Period** [Frequency of sample collection]	**Sample Depth/ Position**	**Rationale for Site Selection*****	**Method(s) used to measure AMR**
Tier 3	[5]	Ireland	2	Non-designated bathing sites	Coastal water	May – September 2016 [1 per site]	Not stated	> Beach A - used for bathing and recreation, crossed by 2 freshwater streams (in which detection of NDM† -producing *E. coli* previously occurred), human sewage was being discharged into the sea in the vicinity of the beach, and the freshwater streams can become immersed in seawater at high tide. > Beach B - 950 m in a direct line from Beach A	Enumeration of resistant bacteria[a], AST of isolates^, qPCR, PFGE[Ø]
	[6]	West Ireland	1	Non-designated bathing site	Coastal water	May – September 2017 [6 in total]	~30 cm below surface, in water at least 1 m in depth.	Site is located within close proximity to a secondary wastewater treatment plant.	enumeration of resistant bacteria[a], qPCR, WGS

Tier*	**Ref**	**Location**	**Site (n)**	**Site Type** (designated BW or non-designated bathing site)	**Media Sampled**	**Sampling Period** [Frequency of sample collection]	**Sample Depth/ Position**	**Rationale for Site Selection*****	**Method(s) used to measure AMR**
	[7]	Italy	2	Non-designated bathing sites	Coastal water	July – late August 2016 [3 per site]	Not stated	Sites both located approximately 3 km away from river mouths - both rivers receive discharge from WwTWs close to river mouth.	AST of isolates^, End-point PCR

Tier *	**Ref**	**Location**	**Site (n)**	**Site Type** (designated BW or non-designated bathing site)	**Media Sampled**	**Sampling Period** [Frequency of sample collection]	**Sample Depth/ Position**	**Rationale for Site Selection*****	**Method(s) used to measure AMR**
	[8]	Prophète beach, France	1	Non-designated bathing site	Coastal water, Sand	July 2018 [Water collected at ten sampling points spaced evenly across the bathing area were combined in a sterile bottle, samples collected hourly 8am-8pm; *Sand* was collected at ten points located at the water edge in the area of the surf spaced evenly across the beach, samples collected 3 times across day]	Sample depth not stated	Beach is open to the sea on either side of a dike running parallel to the coast and offers two bathing areas.	qPCR

Tier *	Ref	Location	Site (n)	Site Type (designated BW or non-designated bathing site)	Media Sampled	Sampling Period [Frequency of sample collection]	Sample Depth/ Position	Rationale for Site Selection***	Method(s) used to measure AMR
Tier 4	[9]	Ireland	5, 27	-	Coastal / River / Lake Water	May – September 2016 and May - September 2017 [22 in total], December 2018 – December 2019 [89 in total]	Not stated	Covering 3 local authority areas in Ireland.	qPCR
	[10]	Ireland	45	-	Coastal / Estuarine / River / Lake Water	November 2018 – July 2019 [Sampling frequency varied depending on location and site type]	Not stated	Covering 4 local authority areas in Ireland. Water bodies chosen for sample collection included ‘hot spot’ areas receiving discharges (storm water overflows, raw sewage discharges, primary and/or secondary wastewater treatment discharges). Where possible, ‘cold spots’ were also chosen which included waters receiving little or no contaminating discharges for comparison.	AST of isolates^, qPCR, WGS

Tier *	**Ref**	**Location**	**Site (n)**	**Site Type** (designated BW or non-designated bathing site)	**Media Sampled**	**Sampling Period** [Frequency of sample collection]	**Sample Depth/ Position**	**Rationale for Site Selection*****	**Method(s) used to measure AMR**
	[11]	Ireland	60	-	Coastal / Estuarine / River / Lake Water	August 2019 – January 2020 and February – November 2020 [118 in total]	Not stated	> Sample collection points were chosen based on the findings of point prevalence survey [10]. > Additional sampling points were added in areas of interest in which carbapenem resistant, extended spectrum beta-lactamase-producing (ESBL) or carbapenemase-producing Enterobacterales were previously detected in water bodies.	AST of isolates^, qPCR, WGS

Tier *	**Ref**	**Location**	**Site (n)**	**Site Type** (designated BW or non-designated bathing site)	**Media Sampled**	**Sampling Period** [Frequency of sample collection]	**Sample Depth/ Position**	**Rationale for Site Selection*** **	**Method(s) used to measure AMR**
	[12]	Lahn River, Germany	2	-	River water	October 2011 – December 2012 [weekly]	~30 cm below surface, 1 m offshore	> Study area: proportion of municipal wastewater effluent at the studied river stretch is in the range of 10–20% during average flow conditions and greatly exceeds 50% under low flow conditions [13]. > Sampling site 1 located 1km downstream of WwTW outfall, sample site 2 located 18 km upstream (no outfalls from tributaries or WwTWs over a stretch of 9 km). > Industrial discharge from clinic approximately 10 km upstream of site 1.	qPCR

Tier *	**Ref**	**Location**	**Site (n)**	**Site Type** (designated BW or non-designated bathing site)	**Media Sampled**	**Sampling Period** [Frequency of sample collection]	**Sample Depth/ Position**	**Rationale for Site Selection*****	**Method(s) used to measure AMR**

* as described in the methodology Section for Task 1; ** providing that it is safe to do so. No minimum depth requirement as some locations are on tidal estuaries with thixotropic sands where sampling at this sort of depth is not practical or safe; *** Where directly stated, else deduced from site-specific information provided for study; [a]Enumeration of resistant bacteria by membrane filtration ^Antibiotic susceptibility where a specific species of bacterium isolated from the BW sample is interrogated with a singular antibiotic, or suite of antibiotics, to determine its susceptibility (AST of isolates), † New Delhi metallo-beta-lactamase; ø Pulsed-field gel electrophoresis.

References [1] SEPA (2022), [2] Reynolds et al. (2020), [3] Lepuschitz et al. (2019), [4] Döhla et al. (2020), [5] Mahon et al. (2017), [6] Mahon et al. (2019), [7] O'Flaherty et al. (2019), [8] Toubiana et al. (2021), [9] O'Connor et al. (2021), [10] Hooban et al. (2021), [11] Hooban et al. (2022), [12] Herrig et al. (2020), [13] Drewes et al. (2018) in Herrig et al. (2020).

Task 2: Literature review of AMR fungi at coastal bathing waters

Introduction

The microbiological components of beach sand and coastal water and the organisms they may contain have implications for human health and have been the subject of research for decades. Whilst the majority of research focusses on AMR in bacteria, AMR is also present in pathogenic fungi (antifungal resistance, AFR), although this area is typically less well understood. Fungi can persist for long periods of time, with evidence of fungi remaining viable for six months in beach sand (under laboratory conditions) (Carillo-Munoz et al., 1990) and for a year in seawater (Anderson, 1979). Some species are also capable of surviving at temperatures and salinities that exceed their natural habitats (Sabino et al., 2014).

Exposure to environmental fungi can result in opportunistic infections, with immunocompromised people most at risk (de Hoog et al., 2000). Studies suggest that in locations with warmer climates, fungi in beach sand and BW act as a potential source of disease (Sabino et al., 2011). At present, there are only four classes of antifungal drugs available (azoles, echinocandins, pyrimidines and polyenes) (WHO, 2022), meaning the prevalence of AFR can significantly impact treatment (CDC, 2021).

Azoles are a high-use class of antifungal substancesand are commonly used in human/veterinary medicine and agriculture (Chowdhary and Meis, 2018; Fisher et al., 2018). They are widely detected in surface water and sediment due to incomplete removal in wastewater treatment works (WwTWs) where they are resistant to microbial degradation (Li et al., 2020; Spurgeon, 2021). Prolonged exposure to trace levels of antifungals can cause potentially toxic effects to aquatic organisms and may drive the development of AFR.

Fungi at coastal bathing sites are not currently monitored or regulated in England nor within the wider UK. Little is known about which fungal pathogens inhabit English coastal environments, or the potential risk they pose to human health, either directly or through the development and proliferation of AFR. This tasked aimed to address these knowledge gaps by identifying:

i. What is known about AMR fungi at coastal bathing sites globally.
ii. What types of fungi are present at coastal bathing sites and whether there are any common resistances found in these species.
iii. Anthropogenic drivers of AFR at coastal sites.
iv. Considerations and implications for monitoring AFR at coastal sites.
v. Areas for future research.

Methodology

A literature review was undertaken to assess the current state of knowledge in relation to AFR in coastal BWs and sands. The main sources of relevant literature were the Web of Science and Google Scholar, using various combinations of the key search terms (detailed inTable 2). Searches were conducted in November 2022.

Additional 'grey literature' searches were also conducted which identified several key reports from the WHO:

- WHO fungal priority pathogens list to guide research, development and public health actions (2022)
- Guidelines on recreational water quality: Volume 1 coastal and fresh water (2021)
- Guidelines for safe recreational water environments. Volume 1: Coastal and fresh waters (2003)

Limited studies were identified that specifically assessed AFR in fungal pathogens in coastal environments. Therefore, the literature review largely focussed on assessing pathogenic fungal communities identified in coastal environments (sand and seawater), and documented AFR potential within these communities.

Table 2 – Search terms used for the literature review (Task 2).

Primary search term	**Secondary search term**		**Third search term**
	Water	**Sand**	
Antifungal resistance	Estuar(y/ine)	Coast(al)	Human health
AMR fungi	Bath(ing)	Sediment(s/ary)	Human
Fungi	Water quality	Beach(es)	Pathogen
Fungal	Ocean	Sand quality	
AFR	Sea	Sand(s)	
	Recreational water(s)		
	Water		

Findings

The academic studies found which investigated fungi in coastal environments are summarised in Table 3.

None of the studies focused on UK beaches investigated pathogenic fungi, however, there were some European studies that had such a focus. Some of these formed part of the pan-European “Mycosands” initiative (covering 28 sites across 13 countries between 2018 and 2020) aiming to investigate the fungal diversity and abundance in beach sand and seawater under different environmental conditions and to assess the potential health risk fungi pose to humans (Brandão et al., 2021).

A list of 42 pathogenic fungi were identified in coastal environments in the literature and are summarised in Table 4. These species were then cross-referenced with the WHO fungal priority pathogen list (WHO, 2022) and any documented AFR from other sources was recorded. As shown in Table 4, 14 species were found to have documented AFR potential, eleven of which were on the WHO fungal priority pathogen list (WHO, 2022), and five of which were found to have AFR at a coastal location. Further information obtained from the literature on the typical habitat of fungi species, the source of the species to the coastal environment, the type of disease, condi are known faecal contaminants tion or infection typically caused by the pathogen and details of how the pathogen is transmitted to humans has been complied in the *‘Pathogenic and AFR Fungi’* table included in the *‘Task 2_AFR Fungi_Workbook’* which accompanies this report.

The following sections provide a brief description of the AFR fungi species identified in coastal environments as well as the common fungal pathogens with documented clinically significant AFR potential. Other potentially pathogenic fungi (i.e. those not identified in the literature review as having AFR potential), will not be discussed here; however it should be noted that AFR may still occur in some of these species.

Following this, the chapter describes some anthropogenic drivers of AFR at coastal sites, summarises insights from the literature relevant to sampling for AFR in coastal environments, and identifies key knowledge gaps in the field.

Table 3 – Summary of academic studies found which investigated Fungi in coastal environments.

Author and date	Country of analysis	Sea water	Sand	Sand type (if specified)		Other
				Dry sand	Wet sand	
Arora et al., 2021	India	X	X			
Arvanitidou et al., 2002	Greece	X				
Bernard et al., 1988	France		X			
Boiron et al., 1983	Guadeloupe Island	X	X		X	
Frenkel et al., 2020	Israel		X	X		
Ghinsberg et al., 1994	Israel		X			
Gomes et al., 2008	Brazil	X	X			
Izquierdo et al., 1986	Spain		X		X	
Larrondo and Calvo, 1989	Spain		X		X	
Novak Babič et al., 2022	Slovenia	X	X	X		
Maciel et al., 2019	Brazil	X	X			
Oliveira et al., 2020	Brazil	X	X			
Papadakis et al., 1997	Greece	X	X		X	

Author and date	Country of analysis	Sea water	Sand	Sand type (if specified)		Other
				Dry sand	Wet sand	
Periera et al., 2013	Portugal		X	X		
Roses Codinachs et al., 1988	Spain		X			
Sabino et al., 2011	Portugal		X	X		
Shah et al. 2011	Florida, USA	X	X	X	X	
Sousa, 1990	Portugal		X	X		
Stevens et al., 2012	South Carolina, USA		X	X		
Vezzulli et al., 2009	Italy		X	X	X	Pore water
Vogel et al., 2007	Florida, USA		X	X	X	

Table 4 – Summary of pathogenic fungi identified in beach sand and seawater from the literature review, and any clinically significant AFR potential within these species (adapted from the full table presented in the *'Task 2_AFR Fungi_Workbook'* provided with this report).

Species	WHO Priority Classification*	Descriptions of AFR potential from WHO (2022) (unless otherwise specified)*
Acremonium spp.	Unclassified	
Alternaria (genus)	Unclassified	
Aspergillus candidus	Unclassified	
Aspergillus fumigatus	CRITICAL	AFR is on the rise
Aspergillus niger	Unclassified	Found to have resistance to antifungal agent Amphotericin B in beach sand (Novak Babič et al., 2022)
Aspergillus ochraceus	Unclassified	
Aspergillus sp. (other species, or non-specified)	Unclassified	
Blastomyces dermatidis	Unclassified	
Candida albicans	CRITICAL	AFR is uncommon (low), but it is capable of developing resistance follow prolonged exposure to antifungals (Costa-de-Olivers and Rodrigues, 2020)
Candida auris	CRITICAL	High rates of AFR. It is intrinsically resistant to most available antifungal medicines and some strains are pan-resistant

Species	**WHO Priority Classification***	**Descriptions of AFR potential from WHO (2022) (unless otherwise specified)***
Candida catenulate	Unclassified	
Candida haemulonii	Unclassified	Wide, yet variable resistance to many common antifungals (Coles et al., 2020; Maciel et al., 2019).
Candida glabrata (*Nakaseomyces glabrata*)	HIGH	High resistance to azoles, increasing resistance to echinocandin
Candida guillermondii (*Meyerozyma guilliermondii*)	Unclassified	Reduced susceptibility to antifungals: fluconazole, polyenes and echinocandins (Marcos-Zambrano et al., 2017)
Candida parapsilosis	HIGH	Moderate rates of AFR
Candida sp. (other species, or non-specified)	Unclassified	
Candida tropicalis	HIGH	Some AFR identifed. AFR to azoles generally ranged from 0% – 20%, with some studies reporting higher resistance rates of 40 – 80% (WHO, 2022). Isolate from beach sand (Brazil) was found to be resistant to all three of the tested antifungals (Maciel et al., 2019).
Cephalosporium	Unclassified	
Chrysosporium (genus)	Unclassified	
Chrysosporium spp.	Unclassified	
Cladosporium spp.	Unclassified	
Cryptococcus albidus	Unclassified	

Species	**WHO Priority Classification***	**Descriptions of AFR potential from WHO (2022) (unless otherwise specified)***
Cryptococcus neoformans	CRITICAL	AFR poorly understood
Cryptococcus spp.	Unclassified	
Epidermophyton	Unclassified	
Fusarium spp.	HIGH	High rates of AFR: resistance to many currently available antifungal agents
Geotrichum spp.	Unclassified	
Histoplasma capsulatum	HIGH	Moderate rates of AFR
Microsporum nanum	Unclassified	
Microsporum spp.	Unclassified	
Mucor spp.	HIGH	Some AFR identified, however AFR is difficult to determine, as clinical breakpoints have not been established.
Penicillium spp.	Unclassified	
Rhodosporidium paludigenum	Unclassified	
Rhodotorula mucilaginosa	Unclassified	
Rhodotorula spp.	Unclassified	

Species	**WHO Priority Classification***	**Descriptions of AFR potential from WHO (2022) (unless otherwise specified)***
Scedosporium spp.	MEDIUM	Treatment is threatened by high rates of AFR
Scopulariopsis spp.	Unclassified	
Scytalidium spp.	Unclassified	
Trichophyton mentagrophytes	Unclassified	
Trichophyton ruben	Unclassified	
Trichophyton spp.	Unclassified	
Trichosporon asahii	Unclassified	

* This column only contains information gained from the literature review and is not based on an exhaustive survey of AFR potential within all the species listed. AFR potential may also be present in other species.

AFR fungi identified in coastal environments

Only a few academic studies were identified that specifically assessed AFR in fungal pathogens in coastal environments. These studies found AFR in *Candida auris* in India (Arora et al., 2021), in *Candida albicans, Candida tropicalis and Candida haemulonii* in Brazil (Maciel et al., 2019) and in *Aspergillus niger* in Slovenia (Novak Babič, 2022).

Candida auris (C. auris)

C. auris is a globally distributed pathogenic yeast species first isolated in 2009, which has since caused prolonged outbreaks across the world, including Columbia, India, Israel, Pakistan, South Africa, Spain, Venezuela and the USA (Public Health England, 2016).

C. auris is a species with some intrinsic resistance that can develop resistance to all three key antifungal drugs (Ostrowsky et al., 2019). It can result in severe infections in hospitalised patients (Arora et al., 2021) by causing invasive candidiasis, which is a life-threatening disease with a high mortality rate (WHO, 2022). *C. auris* was included in the top priority 'critical group' on the WHO fungal priority pathogen list (Figure 1) (WHO, 2022).

A recent study found *C. auris* present in a salt marsh (with no human activity) and a tourist sandy beach in India, suggesting this species existed as an environmental fungus prior to recognition as a human pathogen, and can survive in harsh coastal environments (Arora et al., 2021).

***Candida albicans* (*C. albicans*)**

C. albicans was one of the most common fungi identified in the literature. Its presence in the environment is almost exclusively linked to human faecal contamination. It can cause life-threatening candidiasis and AFR in this species is considered low, but may be increasing (WHO, 2022). It was included within the 'critical group' on the WHO fungal priority pathogen list (Figure 1) (WHO, 2022).

A study in Brazil identified *C. albicans* in five beach sand samples. Out of the five isolates, two were resistant to itraconazole, one of which was also resistant to fluconazole, whereas all were susceptible to amphotericin B (Maciel et al., 2019).

***Candida tropicalis* (*C. tropicalis*)**

C. tropicalis was commonly identified in the literature from wet and dry beach sand. It can be found as part of the healthy human biome, but is also capable of causing invasive infections that can have a high mortality rate (up to 60%). The WHO (2022) report highly variable resistance in C. *tropicalis* (0-80%) and class it as a high priority fungal pathogen (Figure 1).

Studies in Brazil isolated *C. tropicalis* from beach sand and seawater and found resistance to fluconazole, voriconazole, itraconazole and amphotericin B (Zuza-Alves et al., 2016; Maciel et al., 2019).

***Candida haemulonii* (*C. haemulonii*)**

C. haemulonii (closely related to *C. auris*) is an emerging pathogen whose resistance to multiple antifungal medications represents a challenge to treatment (Coles et al., 2020; Maciel et al., 2019); however it is not identified on the WHO priority fungal pathogen list (WHO, 2022, Figure 1). An isolate of *C. haemulonii* from seawater in Brazil displayed resistance to all three antifungals tested (fluconazole, itraconazole and amphotericin B.) (Maciel et al., 2019).

Aspergillus niger

Aspergillus niger (black mould) is an opportunistic pathogen which may cause severe lung problems in humans if inhaled in sufficient quantity and is associated with various plant diseases resulting in huge economic loss (Guatam et al., 2010). However, it is generally recognized as safe by the US Food and Drug Administration (Powell et al., 1994) and was not included in the WHO fungal priority pathogen list (WHO, 2022).

A recent study on one of the most popular (artificially created) sandy beaches in Slovenia *(*Novak Babič, 2022) found the most numerous fungi in beach sand to belong to the genus *Aspergillus,* members of which can cause allergies, sinusitis, otitis, keratitis, but also life-threatening infections (de Hoog et al., 2020). *Aspergillus niger* and its close relative *Aspergillus welwitschiae* (both isolated from beach sand) were resistant to the antifungal amphotericin B (Novak Babič, 2022).

Pathogenic fungi in coastal environments with documented antifungal resistance potential

Many of the common fungal pathogens identified in both beach sand and BWs have AFR documented in other environments. These include several members of the *Aspergillus* and *Candida* genus, *Cryptococcus neoformans*, *Fusarium* spp., *Histoplasma capsulatum* and *Mucor* spp., which were all included on the WHO fungal priority pathogen list (Figure 1).

Aspergillus fumigatus was one of the most common fungal pathogens identified in the literature review. It is the leading cause of invasive fungal infections in people. It is common in the natural environment, typically associated with decaying vegetation, and can enter the human body when spores are released into the air and inhaled. Patients contracting the azole-resistant strain have a high mortality rate (47-88% up to 100% in some studies) (WHO, 2022). AFR is on the rise, with the widespread use of azole fungicides in agriculture contributing to the rising rates of resistant aspergillosis in humans (WHO, 2022).

Candida species are commonly detected yeasts in the beach environment, such as *C. albincans*, *C. tropicalis*, *C. glabrata* (*Nakaseomyces glabrata*), *C. guillermondii* (*Meyerozyma guilliermondii*) and *C. parapsilosis.* Many Candida species identified in the literature review are associated with faecal contaminantion, opportunistic pathogens and featured on the WHO fungal priority pathogen list (WHO, 2022) (Figure 1). Several studies suggest that the supralittoral zone (dry sand) harbours the highest yeast concentrations compared to the wet sand (Vogel et al., 2007; Maciel et al., 2019).

C. glabrata has high resistance to azoles and increasing resistance to echinocandin which is posing a challenge to treatment. Resistance is considered moderate in *C. parapsilosis* (azole resistance rates of ~10% are frequently observed) and *C. guillermondii* is described as having high AFR and low mortality rates (Zaragoza et al., 2017) with reduced susceptibility to fluconazole, polyenes and echinocandins (Marcos-Zambrano et al., 2017).

Cryptococcus neoformans is an opportunistic fungal pathogen that enters the body through inhalation from the environment. It can result in cerebral cryptococcosis which is a life-threatening disease with high mortality despite antifungal therapy. Whilst reduced susceptibility to fluconazole has been described, the mechanisms and prevalence of AFR is poorly understood. Clinical breakpoints are only available for amphotericin B (WHO, 2022).

Fusarium spp. can cause invasive fusariosis which is a life-threatening disease with mortality ranging from 43% to 67%. Treatment is challenging due to an innate resistance to many of the currently available antifungal agents (WHO, 2022).

Histoplasma spp. are globally distributed pathogens that cause histoplasmosis (a life-threatening disease) and has the potential to cause outbreaks. AFR is classed as moderate, however studies are limited, and AFR is rarely measured (WHO, 2022).

Mucor spp*.* belongs to the Mucorales Order which commonly affect the lungs and sinuses, and can spread to the eye, central nervous system and gastrointestinal tract. They are inherently resistant to fluconazole, voriconazole and echinocandins (WHO, 2022).

Scedosporium are a globally distributed fungal pathogens found in the natural environment that can infect humans and produce scedosporiosis (mortality rate 42 - 46%) (WHO, 2022). They are increasingly recognised as an emerging pathogen for immunocompromised individuals (Sabino et al., 2014), though treatment is threatened by high rates of AMR (WHO, 2022).

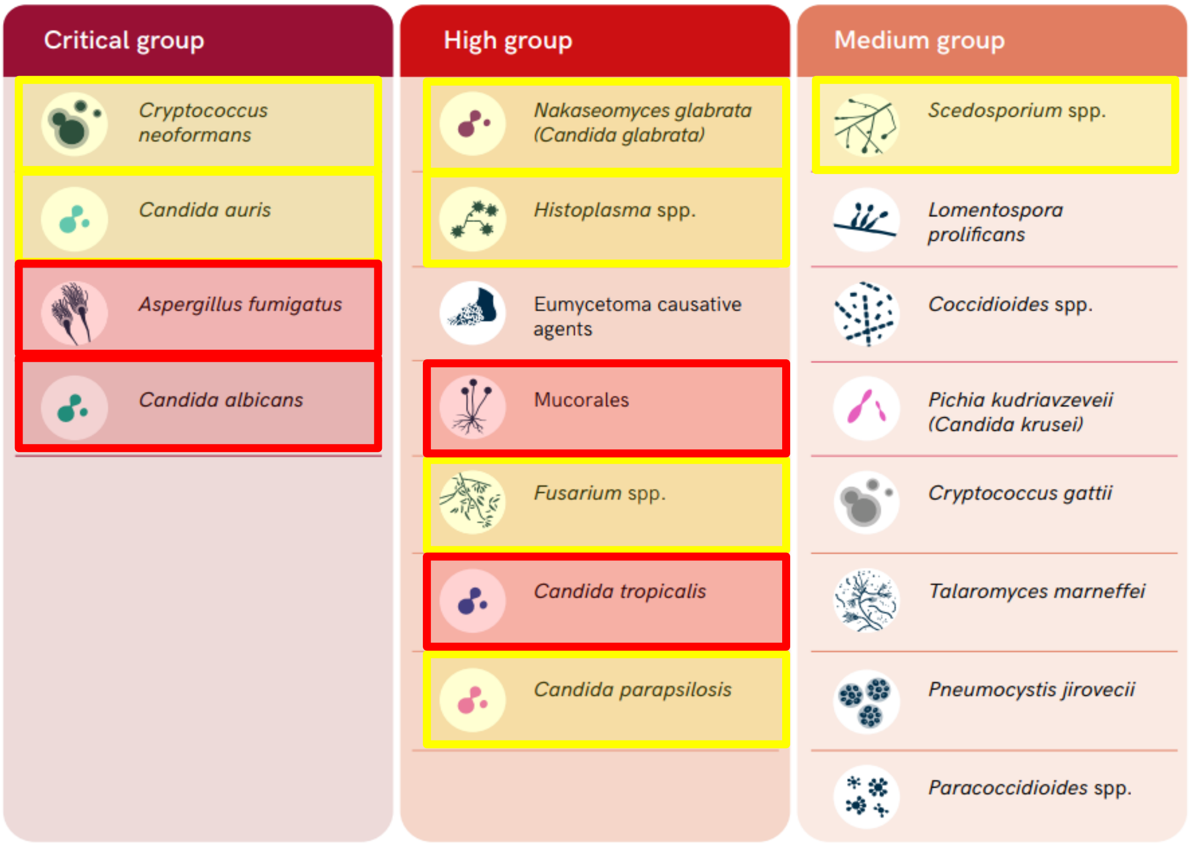

Figure 1 – The WHO fungal priority pathogens list. Boxes highlight species identified in coastal environments (sand and water) in the literature review (image adapted from WHO, 2022). *Coloured boxes represent the number of times each species was detected in the literature review. Yellow: identified 1-3 times, red: 4-7 times.

Anthropogenic drivers promoting AFR in coastal environments

Coastal environments can act as a natural reservoir for some pathogenic (potentially AFR) fungi, and their prevalence may be exacerbated by human-induced climate change via changes to water temperature, salinity, sea level, precipitation and wave characteristics (Brandão et al., 2022).

Furthermore, increased population and/ or changing habits can result in more human activity at coastal sites. Humans travel and relocate, often carrying endemic allochthonous microbiota (Brandão et al., 2022). Increased temperatures in the UK may result in increased recreational use of coastal bathing sites. Additionally, pressures associated with increased population may drive increased nutrient loading to coastal sites and may result in the delivery of more AMR microorganisms and antimicrobial agents (e.g. detergents, disinfectants, pesticides, fungicides, etc.) from a range of sources (e.g. wastewater/sludges, combined sewer overflows, agricultural runoff, hospitals, and pharmaceutical manufacturing plants), potentially allowing evolution and spread of AMR within coastal environments.

Human pathogenic fungi constitute a very small proportion of fungal species. Typically, mammalian body temperature is too high to support many fungi which are better adapted to environmental temperatures. It has been suggested that increased environmental temperatures will allow the selection of more thermally tolerant fungal lineages, which may be better adapt to infect humans (Garcia-Solache & Casadevall, 2010; Robert et al., 2015; Casadevall et al., 2019). *Candida auris* had been suggested as the first example of a new pathogenic fungi emerging from human-induced global warming, with the proposed mechanism described in Figure 2 (Casadevall et al., 2019).

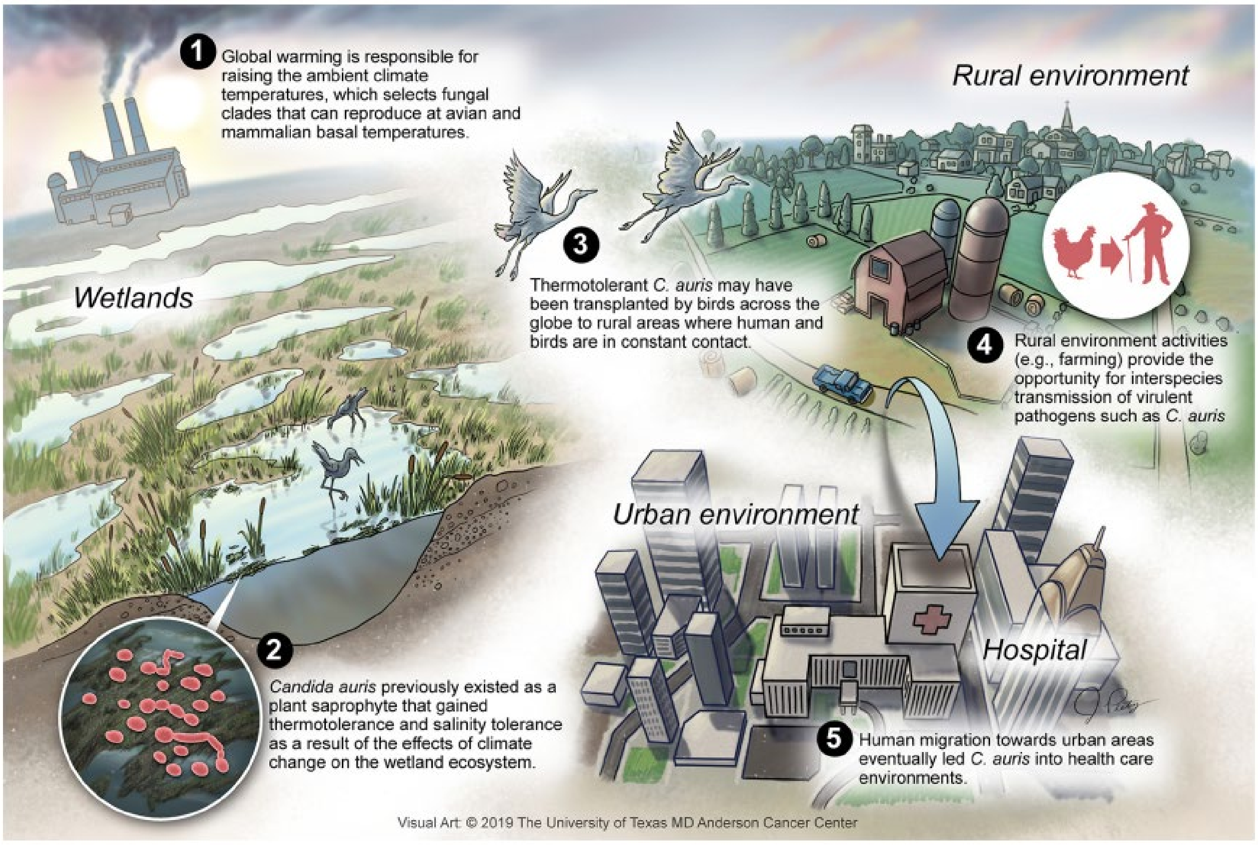

Figure 2 – Proposed scheme for the emergence of *Candida auris* (Casadevall et al. 2019).

Consideration for sampling/ monitoring fungal pathogens and AFR in coastal environments

The WHO identify AMR as ‘one of the biggest threats to global health, food security, and development today’. The recent ‘Guidelines on Recreational Water Quality’ (WHO, 2021) identify that the potential health impacts associated with AMR organisms in recreational waters and beach sands are currently not well understood and suggest that more research is needed to provide a better understanding of these hazards (Sanseverino et al., 2018).

The WHO Guidelines (2021) also suggest that it is desirable to limit peoples’ exposure to fungi through the environment. However, fungi (either at the organism or genetic level) are not included within recreational water and sand regulatory programmes designed to protect human health (Brandão, et al. 2021). Several considerations for sampling/ monitoring fungi (and potentially AFR) arose from the literature review, relating to sampling location and methodology, acceptable concentration standards and indicator organisms.

Sampling sand

Recently, the WHO recommended that recreational beach sands be added to the matrices monitored for Enterococci and fungi (WHO, 2021). Whilst sand is not typically screened as an indicator of pollution, evidence suggests it may act as a reservoir of pathogens and AMR and may be a particularly important reservoir for (pathogenic) fungi which typically have a longer survival rate in sand than other microorganisms (WHO, 2003).

Growing evidence suggests sand can harbour pathogenic microbes in higher concentrations than the adjacent seawater (Sabino et al., 2014; Maciel et al., 2019; Brandão, et al., 2021; Novak Babič, 2022). A five-year study in Portugal (across 33 beaches) found potentially harmful fungi and bacteria in 66.5% of all beach sand sampled (Sabino et al., 2011).

The presence and abundance of fungi in beach sand has been linked to contamination (direct or indirect) from residues and detritus, delivered by beach users or tidal influence (Mendes, 1998), suggesting that the degree of use and tidal regime may be useful considerations when prioritising sampling locations. Wave, tide and beach characteristics may impact the fungal communities in the sand in several ways:

- Higher abundances of sand microorganisms are typically present on beaches with low-energy conditions (Feng et al. 2016). Therefore, enclosed beaches may have a higher density of sand microorganisms than beaches with high energy conditions (e.g. direct ocean-facing beaches).
- Extreme events (e.g. storms, hurricanes) remove sand and reduce microorganism numbers (Roca et al. 2019).
- Tidal fluctuations mean the area with the highest levels of FIOs is typically the dry sand just above the high tide mark (Whiley et al. 2018).
- In many locations, the majority of beach contamination is delivered by the sea; in which case the preferable sampling location would be near the top of the tidal line, where there is impact from the tide, but minimal energy would have been available to disturb the sand.
- However, this may not be the case for beaches with discharges from heavily polluted creek water and/or run-off from nearby urban/agricultural land (Sato et al., 2005). In this case, the most polluted part of the beach (and thus the target for sampling) would be sand above the high-tide line, proximal to the sources of pollution (e.g. creek).

Sampling methodology

Fungi are an under-investigated biological group in the field of BW quality research and are not included in the Bathing Water Directive (Brandão, et al., 2021). No sampling strategies were identified specifically for detecting AFR in beach sands and seawater. However, some researchers involved in the Mycosands initiative (Brandão, et al., 2021) did investigate AFR within selected species (e.g. Novak Babič, 2022); they followed the same methodology as for mycological sample analysis (Sabino et al. 2011), which was adopted by the Mycosands initiative. This methodology is summarised as follows:

Methodology

- Sand: samples were collected at 10 cm depth, with sterile gloves, sterile plastic container, from the middle of the dry sand section of the shore. Three equidistant samples were combined to form one beach composite. 40 g of sand (not oven-dried prior to processing) diluted with 40 ml of sterilised distilled water, agitated for 30 min at 100 rpm (unlike bacteria samples, fungi samples must not be vigorously agitated as this would break the hypha units resulting in extra colony forming units (CFU)).
- Water: 400 ml of water was collected underwater (~20 cm deep, in a 1 m deep water column) into a sterile vessel and transported to the laboratory cooled (< 20 °C), for direct transfer to plates.
- 0.2 ml of the water/suspension was spread (in triplicate) onto Petri dishes containing:
    - Mycobiotic agar for dermatophytes (up to three-week incubation at 27.5 °C)
    - Malt extract agar (2%) with chloramphenicol (0.05 g/L) for non-dermatophytes fungi (5-7 days incubation at 27.5 °C).
- Fungal identification was carried out by macroscopic and microscopic (using lactophenol blue staining) observation of colonies for filamentous fungi, using identification atlases and using the biochemical identification galleries ID32C (bioMérieux SA, Marcy-l'Etoile, France) for yeasts.

Standards for acceptable fungal concentrations in beach sand

Several epidemiological studies have demonstrated positive correlations between swimming at beaches affected by human activities and adverse human health effects; with symptoms such as gastrointestinal and dermatological diseases, and respiratory, eye, nose, and throat infections reported (Maciel et al., 2019). However, the relationship between human health and exposure to pathogenic fungi from beach sand and recreational surface water has not been established (Sabino et al., 2014; WHO, 2021).

Nevertheless, some beach users (especially immunocompromised people, or children with less developed immune systems) may be at a higher risk of exposure to some fungal pathogens through direct skin contact or by inhalation of fungal spores from the beach environment (de Hoog et al., 2000; Maciel et al., 2019). Heaney et al. (2012) reported a positive correlation between incidence of gastroenteritis and sand activities, such as digging and burying, suggesting children may be at a greater risk of illness following such exposures (Maciel et al. 2019).

The WHO Guidelines (2021) identify that more studies are needed to establish guideline values for acceptable levels of microorganism in beach sands. This is particularly necessary for addressing concerns around opportunistic fungi, which are not currently addressed in water quality recommendations. The Guidelines recommend that it is desirable to limit peoples' exposure to fungi through the environment, and that the public should be informed about the presence of allergenic fungi in beach sand (e.g. *Aspergillus fumigatas*), which may trigger an immune response if spores are inhaled by susceptible individuals (WHO, 2021).

Quantitative microbial risk assessments (QMRA) can be a powerful tool for informing public health policy in recreational areas, however, characterisation of the virulence of pathogenic fungi in beaches has rarely been done (Whitman et al. 2014), and median infectious dose information required for QMRA is lacking.

Whilst no information was found regarding acceptable limits of AFR in beach sands or seawater, there have been recent developments around acceptable limits for total fungi in beach sands.

- Sabino et al. (2011) suggests sand threshold values for fungi of 15 CFU/g for yeasts, 17 CFU/g for potential pathogenic fungi and 8 CFU/g for dermatophytes.
- During the 2021 bathing season in Portugal, the Blue Flag organisation included sand quality into the list of awarding criteria which was based on total fungi, Enterococci and *E. coli* per gram of sand. They propose a limit for total fungi in sand of 89 CFU/g (determined by Brandão et al., 2021), with a rejection limit at the 80th percentile of 490 CFU/g. For example, in five sampling events, only one is allowed to exceed the value for total fungal count of 490 CFU/g.
- The WHO (2021) support the finding based on a pan-European average (Brandão et al., 2021) with an indicative reference value of 90 CFU/g of wet weight for fungi.

Indicator organisms

Unlike sampling for bacteria, there are no well-established indicator organisms for assessing fungal contamination. A recent study in Slovenia suggested the species *Meyerozyma* could be used as an indicator species during the development of beach microbial regulation (Novak Babič et al., 2022);however, this may be specific to that location. Research is needed to establish appropriate indicator organisms for the UK.

Maciel et al. (2019) suggests that in addition to the traditionally used bacterial FIOs, other microbiological parameters could be adopted to improve water and sand quality evaluation. For example, yeasts provide a good alternative to traditional indicator organisms, as they represent a widely distributed group that have a well-developed taxonomy and are easy to cultivate. Some studies identified positive correlations between faecal indicator microorganisms and some fungal pathogens. Shah et al. (2019) found yeasts in the *Candida* species correlated significantly with faecal coliforms and Sabino et al. (2011) demonstrated a significant correlation with *C. albicans* and *E. coli* in Portugal (Sabino et al. 2011).

It is worth noting that indicators are only relevant if you have a suitable indicator for all sources of contamination. There are two separate sources of fungal contamination in coastal environments, hence at least two indicators/proxy measures are required to capture both sources:

1. A sewage indicator: as human pathogens are found in sewage, which can be discharged in coastal waters.
2. Bird and wild animal waste indicator: this will always be present on a beach and will not co-correlate with sewage indicators.

Knowledge gaps and areas for future research

Several knowledge gaps were identified relating to AFR in beach sands and seawater, including:

- Unlike many European countries, there has been no assessment of fungal pathogens in UK beach sands and BWs, despite the WHO recommendations that recreational beach sands be added to the matrices monitored for Enterococci and fungi (WHO, 2021).
- It is not clear whether the environmental pathogenic fungi found in beach sands are more common in beach sand compared to other ecosystems (Segal and Elad, 2012).
- The coastal environment likely contains numerous fungal species that have pathogenic potential, and which are presently not able to grow at mammalian temperatures. The direct and indirect effects of human-induced climate and environmental changes on fungal evolution should be an area of future research in the coming decades (Casadevall et al., 2019).
- There are no established indicators of fungal pollution of beaches and the underlying sand. Identification of such indicator fungi could help to establish a suitable monitoring method.
- The application of quantitative microbial risk assessments for informing public health policy in recreational areas is limited, as the median infectious dose information for many fungi is unavailable.
- It is largely unknown to what degree AFR is developing in coastal environments within the UK (e.g., driven by antimicrobial agents' pollution such as azole fungicides).

Task 3: Development of an approach for the selection of designated bathing waters for AMR surveillance

Introduction

In England there are currently 424 designated BWs which are monitored for bacterial FIOs as part of the Bathing Water Regulations (2013), but currently there is no statutory driver for AMR microorganisms and their associated genes. Undertaking AMR monitoring at all sites in the future may be an option, but this would be costly and would not account for potential significant temporal and spatial variation of AMR within a BW. A smaller scale surveillance programme would be more cost-effective and flexible (as a pilot). For example, a pilot is likely to be better suited to methodological developments such as sampling dry sand and wet sand for an experimental period.

It is in this context that an approach to select and prioritise designated BWs in England for AMR surveillance has been developed. Principally this involved the development of criteria (which reflect potential AMR risk) that can be used to prioritise BWs over one another. An excel workbook which facilitated the calculation of 'risk' scores and can be used to select BWs based on chosen criteria accompanies this report. In this study, BWs were selected based on criteria agreed with the Environment Agency and a subsequent step, which involved a more detailed review of these BWs to recommend priority for inclusion in future AMR surveillance, was included in the approach.

Methodology

1. Identification of variables for consideration

The first stage in the process was the selection of variables for consideration when selecting BWs for AMR surveillance. The variables included in the 'preliminary list' were those which (i) are likely or known to influence AMR in BWs, (ii) have a quantifiable attribute and (iii) have a dataset freely available from which attributes can be obtained.

The three main sources of information from which the variables were derived, were:

- Datasets and information collated from literature reviewed as part of previous tasks in this study;
- AMR Geodatabases, an outcome of previous Environment Agency projects that have collated data to investigate the potential sources of AMR in the environment (Environment Agency, 2022); and
- Dedicated searches for datasets of attributes likely/ known to influence AMR in BWs.

The variables identified were separated into the following categories and were assigned weightings (which reflect relative importance in the selection of BWs for AMR surveillance) when presented in the first version of the variables and attributes (V&A) table[1]:

- *Source* – source of AMR microbes and compounds to BWs.
- *Pathway* – pathway for delivering AMR microbes and compounds to BWs.
- *BW Environment* – characteristics of the BW environment that may indicate potential risk of AMR (i.e. BW 2022 classification, pollution incidents and risk warnings).
- *Receptor* – risk of transmission to humans from the environment.

2. Discussion of variables with the Environment Agency

Discussion with the Environment Agency scoped out a long-list of variables and attributes that would drive a bespoke systematic checklist for the selection of BWs for surveillance of AMR in the environment. Weightings which reflect relative importance of the variables were also agreed. The refined list was saved in a revised version (v2) of the V&A table.

3. Consultation by consulting the wider AMR community

A short list of high-priority variables and their weightings was consulted with three AMR specialists . Interviews were conducted by MS Teams with three AMR specialists;

- Dr Johanna Rhodes (Department of Medical Microbiology at Nijmegen, the Netherlands);
- Prof Dearbháile Morris (Head of the Discipline of Bacteriology at the School of Medicine, National University of Ireland Galway);
- Dr Anne Leonard (University of Exeter Medical School).

The primary focus of the interviews was to ascertain feedback on whether (i) variables included in the V&A table[2] were considered to be appropriate when selecting BWs for AMR surveillance, and (ii) whether the weightings included in the table appropriately reflect the relative importance of each variable.

The project team also took the opportunity to discuss further aspects with the AMR specialists to help inform subsequent steps in the approach, which included:

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[1] Document titled: Task 3_VA_AMR in Bathing waters_v1.0_13122022 (submitted to the Environment Agency on 13/12/2022)

[2] The revised version (v2) was submitted to AMR specilaists in advance of the interviews (in the document titled: Task 3_VA_AMR in Bathing waters_v2.0_05-01-2023)

- *Quantifying point sources impacting on a BW by distance*[3] – Discussion to understand what AMR specialists would consider to be a suitable threshold value above which point sources of AMR can be discounted (as having an impact); and suitable distances from BWs that could be used to categorise the likely importance of point sources.
- *Availability of datasets on the number of BW users* – Discussion to understand if AMR specialists were aware of any datasets available on the number of people using BWs. To date the only such data found in the literature was at regional resolution.
- *Relevant studies* – Discussion to understand awareness of any current or upcoming studies relevant to the ongoing work.
- *Approach for selection of BWs for AMR surveillance* – Discussion to ascertain how AMR specialists might prioritise BWs to be monitored in the future.

Key findings from the discussions and (where applicable) how they have been incorporated into the approach are shown in Table 5.

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[3] Though the project team communicated that there are significant limitations with using distance as a metric, the Environment Agency were keen to explore the potential to use this in the approach (email received from Jono Warren [Environment Agency] on 21/12/2023).

Table 5 – Key findings from the discussions with AMR specialists and (where applicable) how they have been incorporated into the approach.

Key findings from discussions with AMR specialists	**How findings have been incorporated into the approach?**
Discussion of V&A Table (v2)	
Good agreement that this is comprehensive and that weightings assigned are reflective of relative importance of variables.	N/A
Weightings applied in calculation of 'risk score' should account for 'strength' of dataset and attribute used.	New column included in the final V&A table (sub-section 4) which details the limitations/ assumptions when using the attribute and dataset associated with each variable. The assigned weighting score accounts for this.
Further variables that would 'ideally' be accounted for, including: Untreated sewage discharges (such as storm-water overflows), Long-term care facilities (LTCFs).	Availability of spatial datasets for these variables was briefly investigated. It is likely that data on raw sewage discharges may need to be requested directly from water companies, whilst, though not readily available, high-resolution data on the location of LTCFs may be requested from the NHS or Care Quality Commission (CQC)*. It is recommended that this is explored further in the future and to capture this, as well as any further considerations associated with each of the variables, a new column was included in the final V&A table (sub-section 4).
Alternative sources of data that would strengthen approach include: Bird populations, Agricensus data.	Possible use of alternative datasets suggested was briefly explored. Bird populations could be acquired from the British Trust for Ornithology (BTO) though expertise would be needed to quantify birds which frequent beaches to establish an appropriate attribute

Key findings from discussions with AMR specialists	How findings have been incorporated into the approach?
	that could be used. Agricensus data which provides data on animal characteristics is not freely available. As above, it is recommended that these areas are explored in the future and thus have been captured in the final V&A table (sub-section 4).
Quantifying point sources impacting on a BW by distance	
Use of distance has many limitations, would be preferable if distance was not used when assessing potential risk.	To take on the feedback provided and also alleviate the concerns that the Environment Agency wished to address by including distance – that is that larger river catchments would be disproportionately targeted for AMR surveillance – the following solution was proposed, and was implemented upon agreement with the Environment Agency: • Use of a metric which accounts for the size of the catchment associated with a BW (e.g. Total WwTW capacity per km2 (of river length in a BW catchment) in the calculation of risk scores for a BW (sub-section 5). • Inclusion of a subsequent step in the approach, which involves a more detailed review of BWs selected using the workbook developed in this study (see sub-section 6) in order to prioritise their importance for inclusion in AMR surveillance. This would allow appraisal of point source locations and length of watercourse to BWs, as well as providing further advantages (sub-section 7).
Datasets on the number of BW users	
No awareness of a national dataset that currently exists, data on number of surfers may be available and used as proxy (noting	Surfing England were contacted and indicated that they themselves use data from Sports England (active lives surveys) to quantify number of surfers**. Though data on participation is available at local authority resolution (Sport England, 2023), this does not

Key findings from discussions with AMR specialists	**How findings have been incorporated into the approach?**
this approach has limitations, and may not capture other vulnerable BW users, such as children playing in the sand).	appear to be available for export. It is plausible that this data could be requested for use in the future and has been captured as a future consideration in the final V&A table (sub-section 4).
Surfers Against Sewage app is being developed as part of Blue Adapt – to collect data on people entering the water.	It is recommended that once available this data is considered to support the inclusion of receptors as a variable in the approach. This has been communicated in the final V&A table (see sub-section 4).
Limitation of the dataset (MENE) included in the V&A table (v2) is that it accounts for all beach users, and not specifically those using the water.	This is also a low-resolution dataset (i.e. data only available at regional resolution) and therefore was not applied in this study.
Approach for selection of BW for surveillance	
Good agreement that BWs for AMR surveillance should include those with high (hotspots) and low (coldspots) perceived risk.	BW with the highest calculated AMR risk score (hotspots) and the lowest AMR risk score (coldspots) were selected using the excel workbook developed (see sub-section 6) and have been reviewed in order to prioritise their importance for inclusion in AMR surveillance (see sub-section 7).

* Data at local authority resolution is available to be downloaded in CSV format (CQC, 2023) though at this resolution data is of limited use for the selection of BWs and has not been processed or applied in this study. The underlying (high resolution) dataset which the online mapping service uses (https://www.cqc.org.uk/help-advice/help-choosing-care-services/services-in-your-area) may however be available on request.

** Email received from Hannah Brand (Surfing England) on 17/01/2023.

*** Monitor of Engagement with the Natural Environment

4. Final V&A table

The short list of high-priority variables and their weightings was included in the updated (and final) version of the V&A table that is saved within the Excel workbook titled 'Task 3_VA_Workbook' (hereafter referred to as 'V&A workbook'). As indicated in Table 5, in response to findings from interviews with AMR specialists additional columns were added to the table recording (i) limitations of the attributes and datasets associated with each variable, (ii) weighting scores which account for these and (iii) future considerations (i.e. further variables that would ideally be accounted for, alternative sources of data or further processing of datasets that could strengthen the approach).

Further columns were also added to the table which provide additional information associated with the chosen datasets (e.g. date, spatial extent, data format) and the processing that has been undertaken for data to be used in the calculation of AMR risk scores in this study (see sub-section 5 below); both of which were important to consider when identifying limitations and future considerations.

5. Calculation of AMR 'risk scores'

To calculate AMR 'risk scores' for each designated BW the datasets detailed in the final V&A table (see sub-section 4) were typically processed via a Geographical Information System (GIS) software. A description of the processing carried out for each dataset, and where applicable any proxies used or assumptions made, can be found in column O of the final V&A Table provided with this report. Once processed, data was exported from GIS to Excel as required for the calculation of the scores.

In Excel, the 0, 20, 40, 60, 80 and 100th percentiles were calculated[4] for each variable (with the exception of BW quality). From this, a new column was added adjacent to the variable where each variable was scored 1 to 5 based upon which percentile group the value each BW fell within (see Table 6); where the value of an attribute was 0, a score of 0 was assigned. Bespoke scoring criteria was developed for BW quality, also shown in Table 6[5].

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[4] Where the value of an attribute was 0, this value (0) was not included in the calculation of percentiles.

[5] If BW classification did not match any of those included in the table, then a score of 0 was assigned.

Table 6 – Scoring based on percentile ranges and bespoke scoring for BW quality

Scoring	Percentile
1 (Very Low)	>0 – 20
2 (Low)	20 – 40
3 (Medium)	40 – 60
4 (High)	60 – 80
5 (Very High)	80 – 100

Scoring	BW Classification
2	Excellent
3	Good
4	Sufficient
5	Poor

To calculate a ‘risk score’ for each variable, the score based on percentile ranges was multiplied by the weighting score for the variable (included in the final attached V&A Table, column P). An overall ‘AMR risk score’ for a BW was calculated as the sum of the risk scores for each variable. These are provided in the ‘BW AMR Risk’ tab of the V&A workbook.

Attributes used to calculate ‘risk scores’ predominantly relate to BW catchments i.e. proportion of a certain type of landcover (e.g. grassland, artificial surfaces) or count or properties of features (e.g. hospitals, WwTWs) within a catchment. To represent BW catchments, the Environment Agency’s dataset was used (Environment Agency, 2021a; and is also included in the final V&A Table).

It is important to note that not all BWs have catchments defined in this dataset; very small catchments or run off areas have not been defined. Only BWs that have a catchment defined in this dataset (n = 401) have been included in the V&A workbook, and thus have been considered for AMR surveillance as part of this study.

6. Selection of bathing waters for review

Ultimately the BWs selected using the V&A workbook are dependent on the criteria investigated. The data has been complied in the workbook so that BWs may be selected in several different ways. Scores derived for individual variables may be used in isolation or combination (e.g. total score for non-point sources), whilst data used to derive ‘risk scores’ for each variable (for example, arable landcover in BW catchments) are also available in the ‘BW AMR Risk’ tab and may be used directly to select BWs.

Types of BWs that could be monitored as a priority in a future pilot study were selected based on findings from interviews with AMR specialists (see sub-section 2) and following discussion with the Environment Agency. These are included, along with the criteria applied for selection when using the V&A workbook, in Table 7. The Agri-Arable type was included with the intended focus of including BWs where antifungal resistance is likely in AMR surveillance.

As several of the 'Hotspot' and 'Top Non-Point' BWs selected had the same (or almost exactly the same) BW catchment in the Environment Agency's dataset, five unique catchments containing BWs with the criteria shown in Table 7 were taken forward for review (see sub-section 7 below), thus allowing a more diverse range of catchments to be explored. For 'Hotspots' and 'Coldspots' the 5th unique catchment identified had equally as high/low scoring BW(s) included as another catchment, which was also included for review. Selected BWs are shown in the 'FC_BWs' tab in the V&A workbook.

Note that only BWs typified as 'Coastal' were considered, as requested by the Environment Agency. BWs typified as 'Estuarine', 'River' or 'Lake' were therefore not considered, but for those with a BW Catchment (in the Environment Agency's dataset; Environment Agency, 2021a), data have been collected and risk scores calculated in the V&A workbook.

Table 7 – BW 'types' and associated criteria

AMR Surveillance Type	Criteria applied for selection of BWs (using V&A workbook)
Hotspots	5 Highest Scoring BWs
Coldspots	5 Lowest Scoring BWs
Agri-Arable	5 BWs with highest proportion of arable landcover.
Top Non-Point	5 BWs with Highest Score for non-point sources

7. Review of bathing waters for AMR surveillance

This step involved taking a more detailed look at the BWs selected using the V&A workbook (as described in sub-section 6) and providing a recommendation as to priority (high, medium, low) for inclusion in AMR surveillance. The key reasons as to why this stage was included in the approach (as opposed to solely basing decisions on selections made using the 'BW AMR RISK' tab of the V&A workbook), were:

- To allow point source discharges in proximity to BWs (rather than just density within BW catchments) to be considered.
- To allow extent of landcover in proximity to BWs (rather than just the amount within BW catchments) to be considered.
- To allow coastal discharges which sometimes sit outside of the 'direct' BW catchments (for example may be a little way up/ down the coast), but which are likely

to have an impact at the BW site due to tidal currents to also be considered – in this way removing one of the main limitations of using BW catchments.
- To allow information on BWs not found within spatial datasets, but which provides important details on factors influencing potential AMR risk, to be accounted for in decisions made.

The review was carried out using:

- *ArcGIS maps* – the majority of the datasets applied in the calculation of 'AMR risk scores' (sub-section 5) were plotted in GIS to show the location of BWs (and associated catchments) in relation to variables (where applicable)[6]. In certain instances underlying datasets were explored, for example to understand the quantity/ duration of combined sewer overflow (CSO) spills.
- *BW Profile* – select information was extracted from the Environment Agency's 'Swimfo' resource (Environment Agency, 2021b) from which profiles for BWs are available. Information was extracted from headed sections of the profile deemed of greatest relevance from a brief review of the information included. It is recommended that in the future the sources of the information included are explored in further detail to understand origin and relevance for AMR. This could support the development of a revised performa which would allow full extent of the most suited information to be utilised at this stage in the approach in the future.

The review aimed to prioritise the BWs based on how representative they are likely to be of the AMR surveillance type (e.g. 'Hotspot'; Table 7) for which they were selected. To inform decisions, definitions were derived for each AMR surveillance type (based on professional opinion; see Table 8).

It is acknowledged that recommendations are likely to be subjective, and may be subject to change should additional/alternative information be reviewed or new information become available. The information extracted and the rationale for the deduced recommendations in this study have been provided in the 'Review_BWs' tab of the V&A workbook.

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[6] When presenting the 'Treatment plants reported under UWWTD Works' dataset on the maps, works which employ Ultraviolet (UV) treatment were separated from those which do not, as the presence/absence of this treatment type has a major effect on concentration of microbes discharged.

Table 8 – AMR surveillance type definitions

AMR Surveillance Type	Definition of Term
Hotspots	Multiple point and non-point sources of AMR microbes and compounds in the BW catchment and/or near to the BW. Multiple pathways which can deliver AMR microbes and compounds from point and non-point sources to the BW and its users.
Coldspots	None or few point and non-point sources of AMR microbes and compounds in the BW catchment and/or near to the BW. None or limited pathways which can deliver AMR microbes and compounds to the BW.
Agri-Arable	A large amount of arable land-cover within the catchment, in particular in the area local to a BW, with pathways for run-off from land into watercourses which flow to a BW. None or limited influence from other non-point and point sources of AMR microbes and compounds in the BW catchment and/or near to the BW.
Top Non-Point	Multiple non-point sources of AMR microbes and compounds in the BW catchment and/or near to the BW. Multiple pathways which can deliver AMR microbes and compounds from non-point sources to the BW. None or limited influence from point sources.

Findings

The designated BWs selected using the V&A workbook (developed in this study) for the criteria agreed with the Environment Agency (see Methodology, sub-section 6) are shown in Table 9 – Table 12. As per the approach described (in Methodology, sub-section 7), maps were produced to inform the review of selected BWs and these are shown in Figure 3 to Figure 28. Information from the BW profiles provided by the Environment Agency was used in conjunction with the maps produced to recommend priority for inclusion of BWs in future AMR surveillance; this along with the rationale (for priority selected) is also included for each BW in Table 9 – Table 12.

Recommendations

It is recommended that for each of the BW types that were identified as a priority (following discussion with the Environment Agency; Table 7), a minimum of three unique catchments in which BWs of this type are found are selected for future surveillance. It is, nevertheless, also advised that multiple BWs located in the same catchment (for example, *St Annes*, *St*

Annes North and *Southport*; Figure 22) are additionally monitored so that differences within catchments can also be explored.

Studies have indicated the potential for AMR exposure at coastal bathing waters and whilst there is currently no statutory driver for the monitoring of AMR in bathing waters, this research project explored new areas of potential concern, which may warrant potential future surveillance. Once the scope of the Environment Agency's intended AMR surveillance is fully defined, including the number of BWs to be monitored, the two 'key' stages of the developed approach, it is reccomended that selection of BWs using the developed workbook (see Methodology, sub-section 6) and subsequent more detailed review (see Methodology, sub-section 7) – are revisited as required. For example, additional 'types' of BWs may be considered for monitoring.

In the longer term it is also recommended that future considerations captured in this study (and included in the final V&A table) are explored; further processing of datasets or inclusion of additional variables or more detailed data in the calculation of risk scores could improve the results. Particularly if an improved understanding of the relationship between specific features, such as crop type or wastewater treatment type, and AMR is developed.

It is to note, that this project has not allowed for sensitivity testing of the weightings applied in the calculation of 'risk scores'. It is suggested this is undertaken in future phases of work.

Development of the V&A workbook into a more refined spreadsheet-based tool is also recommended. Refinements could be made following sensitivity testing and exploration of future considerations (described above), whilst could also look to include additional functionality to support the needs of the Environment Agency.

Table 9 – Review of coldspots (ordered by recommended priority, High – Low)

BW Name	**Total AMR Risk Score***	**Figure (s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency's 'Swimfo' resource and observations from maps (and underlying datasets)	**Comments**
Beachlands Central	11	Figure 3	High	There are no streams within the beach area. Most streams and drainage enter into the adjacent harbours. There is a storm overflow (Green lane storm overflow) <1 km to the east of the BW - *no spills in 2021 dataset*. Another storm overflow (Fort Cumberland storm overflow) is located in the mouth of Langstone Harbour but this is over 3 km to the west.	-
Beachlands West	13	Figure 3	High	There are no streams within the beach area. Most streams and drainage enters into the adjacent harbours. There is a storm overflow (Fort Cumberland storm overflow) in the mouth of Langstone Harbour 2 km to the west of the BW and another storm overflow (Green lane storm overflow) 1.5 km to the east - *though no spills recorded in 2021 dataset for the latter.*	-
Eastoke	10	Figure 4	High	There are no streams within the beach area. Most streams and drainage enters into the adjacent harbours. The nearest outfalls are within Chichester Harbour (>2 km from BW). Nearest storm overflow is less than 1 km to the west of the BW - *but no spills in 2021 dataset*. Another storm overflow (Fort Cumberland storm overflow) is located in the mouth of Langstone Harbour but this is over 4 km to the west. *Defined catchment does not extend from beach.*	-
Babbacombe	14	Figure 5	Medium	There are no streams within the beach area. There is an outfall beside the pier which can contain surface water from the surrounding area. There is a storm overflow from the Beach Road pumping station, that discharges to the sea 200m to the west of the beach at Withy Point *(there is also a point*	-

BW Name	**Total AMR Risk Score***	**Figure (s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency's 'Swimfo' resource and observations from maps (and underlying datasets)	**Comments**
				further east too, though no spills recorded for this location in 2021 dataset). Approximatly 50% of landcover in the defined catchment is artificial surfaces.	
Porthgwidden	14	Figure 6	Medium	There are no streams discharging directly to the beach. Sewage from the St Ives area is pumped to Hayle STW for treatment, and discharges to the sea four and a half kilometres northeast of the BW. There is an emergency/storm overflow from Porthgwidden pumping station, that discharges to the sea approximately 120m east of the BW. Land use in the catchment is evenly split between coastal grassland and urban.	-
Tunstall	8	-	N/A	BW is closed as there is no safe access to bathers due to coastal erosion (*thus not considered feasible for this BW to be included for AMR surveillance*).	-

* Calculated in the 'BW AMR Risk' tab of the V&A workbook.

Table 10 – Review of Agri-Arable BWs (ordered by recommended priority, High – Low)

BW Name	Arable landcover / Total area*	Figure(s)	Recommended priority	**Rationale** – Information from the Environment Agency's 'Swimfo' resource and *observations from maps (and underlying datasets)*	Comments
Praa Sands East	0.96	Figure 7	High	A small stream approximately 300m long and channelled underground, enters the sea at the beach. Most of the freshwater reaching Praa Sands East is from direct runoff along the steeply sloping coast. Land use is agricultural with just one or two farms, and there is a large amount of arable land. There are caravan parks, homes, and hotels that have private sewage treatment arrangements (as no mains sewerage in the local village) though the Environment Agency do not believe these are a source of pollution to the BW.	-
Ladram Bay	1.0	Figure 8	Medium	There is a small stream 100m west of the BW monitoring point which can be affected by inputs from the catchment. The steep catchment means rain runs off rapidly into the small stream which flows across the beach. The Otterton STW outfall discharges to the sea one km south of Ladram Bay. There is a caravan park at the top of the path leading to the beach (misconnections have been checked), and land use in the remainder of the catchment is rural. There is 1 farm in the catchment. Sand and shingle beach characterised by two high red rock sea stacks, popular with sea birds.	-
Seaham Hall Beach**	1.0	Figure 9	Medium	The natural drainage catchment surrounding the BW is a mixture of arable and grassland in the upper catchment and urban in the lower. There are no emergency or storm overflow outfalls discharging directly onto the bathing	-

				beach, but a number of outfalls discharge to local streams and can temporarily affect BW quality after heavy rainfall.	
Perranuthnoe	1.0	Figure 10	Low	*There are no watercourses in catchment (according to OS map used in this study).* Surface water drains to the beach from the catchment. *Perranuthoe Pumping Station discharge noted to be 500m West of the BW monitoring point.*	-
West Wittering	0.96	Figure 11	Low	There are no streams within the beach area, but the mouth of Chichester Harbour is situated immediately to the west. There are three sewage treatment work outfalls in Chichester Harbour. Most streams and drainage in the surrounding area enters into the harbour or flows away to the east.	-

* Calculated in the 'BW AMR Risk' tab of the V&A workbook; ** Incorrectly labelled as Seaham Beach in BW ZOI catchment dataset and should be Seaham Hall Beach.

Table 11 – Review of ‘Top Non-Point’ BWs (ordered by recommended priority, High – Low)

BW Name	**NP Sources Risk Score***	**Figure(s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency’s ‘Swimfo’ resource and *observations from maps (and underlying datasets)*	**Comments**
Hillhead	44	Figure 12	High	*Hillhead BW is in IBA.* The River Meon inputs into the north-western end of the BW at the Titchfield Haven Nature Reserve. The Haven has an abundance of birds which may increase contamination in the river. There are also two small culverted streams or ditches which drain through pipes onto the beach. These drain surface water from the surrounding area, this can result in a lower standard of water quality after heavy rainfall. There are two storm overflows within the beach area (*no spills recorded in 2021 dataset*). There is a treated sewage treatment works discharge 5km from the shore of the beach	-
Wherry Town	49	Figure 13	High	*Nearest CSO outfall is located approx. 2km North East (Albert Pier pumping station), but no spills recorded in 2021 dataset*. Sewage from the Penzance area is pumped to Hayle STW *(7 miles east of Penzance; https://waterprojectsonline.com/custom_case_study/hayle-stw-inlet-refurbishment-works/)* for treatment, and discharges to the sea off the North Cornwall coast. The Lariggan and the Newlyn rivers flow into the sea on either side of this beach. The Environment Agency have monitored these rivers between 1986 and 2010, and found that water quality is temporarily worse during and after heavy rainfall. Land use is a mixture of rural and urban. The rural areas are mostly improved and unimproved grasslands and heath, the urban areas are concentrated at the coast. *National Lobster Hatchery discharge is located <800m from the Wherry Town BW monitoring point.*	Penzance and Wherry Town have same BW catchment.

BW Name	NP Sources Risk Score*	Figure(s)	Recommended priority	Rationale – Information from the Environment Agency's 'Swimfo' resource and *observations from maps (and underlying datasets)*	Comments
Christchurch Highcliffe Castle	45	Figure 14, Figure 15	Medium	This BW is close to the mouth of the Rivers Stour and Avon which flow into Christchurch Harbour. The natural effects of wind and tide outside the harbour can mean that water quality at the beach is impacted by the quality of the rivers and long-term monitoring has shown that the rivers may affect water quality after periods of heavy rainfall. The largely agricultural catchments of the rivers Stour and Hampshire Avon have significant areas of land used for livestock. During and after periods of heavy rainfall, runoff from agricultural land is greatly increased, and the quality of the BW can be reduced. All the significant sewage treatment works in the lower catchment receive UV disinfection, and further up the catchment there are smaller, treated, continuous discharges. Within the catchment there are storm, emergency and surface water outfalls that discharge to the Stour and Avon rivers. The operation of the overflows can lead to a drop in BW quality.	-
Dawlish Coryton Cove	47	Figure 16	Medium	*Dawlish Water flows to the near-by Dawlish Town BW.* Land in the catchment is mainly used for agriculture, with more than 20 farms. *Langley Trout Farm outlet located <1.1km from BW monitoring point.* The Dawlish STW outfall discharges to the sea 1.4 kilometres northeast of Coryton Cove BW. There is an emergency overflow from the Oaklands (Holcombe) pumping station that discharges to the sea 200m south of the beach. The operation of the overflow can lead to a temporary drop in BW quality. *Several CSO discharge points north of this BW also.*	-

BW Name	**NP Sources Risk Score***	**Figure(s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency's 'Swimfo' resource and *observations from maps (and underlying datasets)*	**Comments**
Penzance	49	Figure 13	Medium	There are no streams directly affecting this BW, however, there are several flowing into Mounts Bay nearby which can affect water quality during and after heavy rainfall. There are no storm or emergency overflows affecting this BW, however, there are several that discharge directly and indirectly into the Mounts Bay area. Sewage from the Penzance area is pumped to Hayle STW for treatment (*7 miles east of Penzance; https://waterprojectsonline.com/custom_case_study/hayle-stw-inlet-refurbishment-works/),* and discharges to the sea off the North Cornwall coast. Land use is a mixture of rural and urban. The rural areas are mostly improved and unimproved grasslands and heath, the urban areas are concentrated at the coast. *2 hospitals located in the catchment as well as non-water company sewage discharges within 400m of the BW sampling point.*	Penzance and Wherry Town have same BW catchment.

BW Name	**NP Sources Risk Score***	**Figure(s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency’s ‘Swimfo’ resource and *observations from maps (and underlying datasets)*	**Comments**
Weymouth Lodmoor	48	Figure 17	Low	There are two streams piped to the sea under the beach. The stream to the south of the Environment Agency monitoring point drains water from a network of man-made drainage channels to the east of Weymouth. The stream to the north of the monitoring point drains water from the marshes of Lodmoor Nature Reserve. The River Jordan and the River Wey enter the sea two kilometres either side of the beach. *The majority of the land cover in the catchment is artifical surfaces. The 3 outlets closest to the monitoring point are for the Sealife Centre (this is not an aquaculture source as such).* Sewage from the Weymouth and Portland area is treated at Weymouth STW and discharges to the sea one kilometre offshore, west of Portland Harbour. Storm overflows from the Melcombe Avenue and Cranford Avenue CSO's share an outfall, that discharges to the sea 230 metres from the monitoring point.	-
Dawlish Town	45	Figure 18	N/A	*This BW was also included in the Hotspots selected and has been reviewed as such (see Table 12).*	-

*Sum of risk score for all non-point (NP) sources. Calculated in the ‘BW AMR Risk’ tab of the V&A workbook.

Table 12 – Review of Hotspots (ordered by recommended priority, High – Low)

BW Name	**Total AMR Risk Score***	**Figure(s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency's 'Swimfo' resource and *observations from maps (and underlying datasets)*	**Comments**
Dawlish Town	126	Figure 18	High	Dawlish Water flows to the BW. The Environment Agency have monitored the Dawlish Water since 1993, and found that inputs higher in the catchment can affect the BW. The steep catchment means rain runs off rapidly into the stream. The Dawlish STW outfall discharges to the sea 930m offshore from Dawlish Town BW. CSO discharges into multiple watercourses (including Dawlish Water and Lyme Bay) which flow to Dawlish Town BW. *Several CSO discharges located within 1km.* Land in the catchment is mainly used for agriculture, with more than 20 farms used for livestock. There are farms in the catchment, most of which are beef and sheep. *Langley Trout Farm is in catchment - discharges to a watercourse that discharges to sea 1.5km from the BW.*	-
Fleetwood	127	Figure 19, Figure 20	High	Surveys of the River Wyre located to the east of Fleetwood have shown the river can impact on BW quality. The BW is flanked by urban areas all along the coastline with agricultural land dominating further inland. There are large areas of agricultural land within the Wyre catchment which are used for livestock. Garstang Sewage Treatment Works (located far East of catchment) on the River Wyre provides disinfection. *Fleetwood BW is located in IBA.*	Cleveleys and Fleetwood have the same BW catchment.

BW Name	**Total AMR Risk Score***	**Figure(s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency's 'Swimfo' resource and *observations from maps (and underlying datasets)*	**Comments**
Southport	139	Figure 22, Figure 23	High	Immediately to the north of Southport the River Ribble flows out to the Irish Sea through the Ribble Estuary. The River Ribble catchment contains significant areas of farmland both around the estuary and further inland. Grazing on the Ribble Estuary salt marshes can impact on the BW. High spring tides can cover the salt marsh and cause wash off from the land.	St Annes, St Annes North and Southport have the same BW catchment.
St Annes	143	Figure 22, Figure 23	High	The River Ribble discharges to the sea immediately south of the BW. There are numerous storm, emergency and surface water outfalls that discharge to the River Ribble and its estuary. The River Ribble catchment contains significant areas of farmland both around the estuary and further inland. Grazing on the Ribble Estuary salt marshes can impact on the BW. High spring tides can cover the salt marsh and cause wash off from the land. Southport and Preston STWs can impact BW quality. *BW is located in IBA.*	St Annes, St Annes North and Southport have the same BW catchment.

BW Name	**Total AMR Risk Score***	**Figure(s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency's 'Swimfo' resource and *observations from maps (and underlying datasets)*	**Comments**
St Annes North	143	Figure 22, Figure 23	High	*The River Ribble discharges to the sea approximately 5km south of the BW.* River Ribble can cause reduced water quality particularly after rainfall. There are numerous storm, emergency and surface water outfalls that discharge to the River Ribble and its estuary. The River Ribble catchment contains significant areas of farmland both around the estuary and further inland. Grazing on the Ribble Estuary salt marshes can impact on the BW. High spring tides can cover the salt marsh and cause wash off from the land. Southport and Preston STWs can impact BW quality. *BW is located in IBA.*	St Annes, St Annes North and Southport have the same BW catchment.
Blackpool South	144	Figure 23, Figure 24	Medium	Though most surface water in the catchment is diverted away from the BW, Blackpool promenade has highway and surface water drains which flow onto the beach. River Ribble can cause reduced water quality at BW particularly after rainfall - there are numerous storm, emergency and surface water outfalls that discharge to the River Ribble and its estuary. The River Ribble catchment to the south of the BW contains large areas of farmland both around the estuary and further inland. Southport and Preston STWs can impact Blackpool South BW quality.	-

BW Name	**Total AMR Risk Score***	**Figure(s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency’s ‘Swimfo’ resource and *observations from maps (and underlying datasets)*	**Comments**
Meols	126	Figure 25, Figure 26	Medium	To the south west of the peninsula is the Dee Estuary. To the north east is the Mersey Estuary which is understood to have limited impact as the estuary generally flows directly out into the Irish Sea and does not reach the BWs. There are several local surface water outfalls draining through the sea wall. These drain the urban area surrounding the BW. Meols STW (*closest STW to the BW*) discharges through a long sea outfall which runs five kilometres out to sea. *CSO discharges into multiple watercourses which flow to Meols BW or near-by Moreton BW.* Although the wider surrounding area is predominantly urban, there is some agricultural land use in the catchment. *Meols is located in IBA.*	Meols and Moreton have the same BW catchment.
Moreton	126	Figure 25, Figure 26	Medium	To the south west of the peninsula is the Dee Estuary. To the north east is the Mersey Estuary which is understood to have limited impact as the estuary generally flows directly out into the Irish Sea and does not reach the BWs. Meols STW (*closest STW to the BW*) discharges through a long sea outfall which runs five kilometres out to sea. *CSO discharges into multiple watercourses which flow to Meols BW or near-by Moreton BW.* The land between the beach and Moreton (town) has mixed agricultural areas, industrial areas and a nature reserve. *Moreton is located in IBA.*	Meols and Moreton have the same BW catchment.

BW Name	**Total AMR Risk Score***	**Figure(s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency's 'Swimfo' resource and *observations from maps (and underlying datasets)*	**Comments**
Bispham	133	Figure 27, Figure 28	Low	*No 'direct' flow of watercourses to this BW is shown on OS mapping.* Fleetwood Sewage Treatment Works discharges via a long sea outfall. The surrounding area is mainly the urban area of Blackpool but further inland is largely rural. Most surface water in the catchment is diverted away from the BW.	Blackpool North, Blackpool Central and Bispham have same BW catchment.
Blackpool Central	141	Figure 27, Figure 28	Low	*No 'direct' flow of watercourses to this BW is shown on OS mapping.* Fleetwood Sewage Treatment Works discharges via a long sea outfall Flanked by urban areas with agricultural land dominating further inland. Most surface water in the catchment is diverted away from the BW.	Blackpool North, Blackpool Central and Bispham have same BW catchment.

BW Name	**Total AMR Risk Score***	**Figure(s)**	**Recommended priority**	**Rationale** – Information from the Environment Agency's 'Swimfo' resource and *observations from maps (and underlying datasets)*	**Comments**
Blackpool North	145	Figure 27, Figure 28	Low	*No 'direct' flow of watercourses to this BW is shown on OS mapping.* Fleetwood Sewage Treatment Works discharges via a long sea outfall. Flanked by urban areas with agricultural land dominating further inland. Most surface water in the catchment is diverted away from the BW.	Blackpool North, Blackpool Central and Bispham have same BW catchment.
Cleveleys	139	Figure 19, Figure 20	Low	*No 'direct' flow of watercourses to this BW is shown on OS mapping.* Fleetwood Sewage Treatment Works discharges via a long sea outfall Flanked by urban areas with agricultural land dominating further inland. Most surface water in the catchment is diverted away from the BW.	Cleveleys and Fleetwood have the same BW catchment.

* Calculated in the 'BW AMR Risk' tab of the V&A workbook.

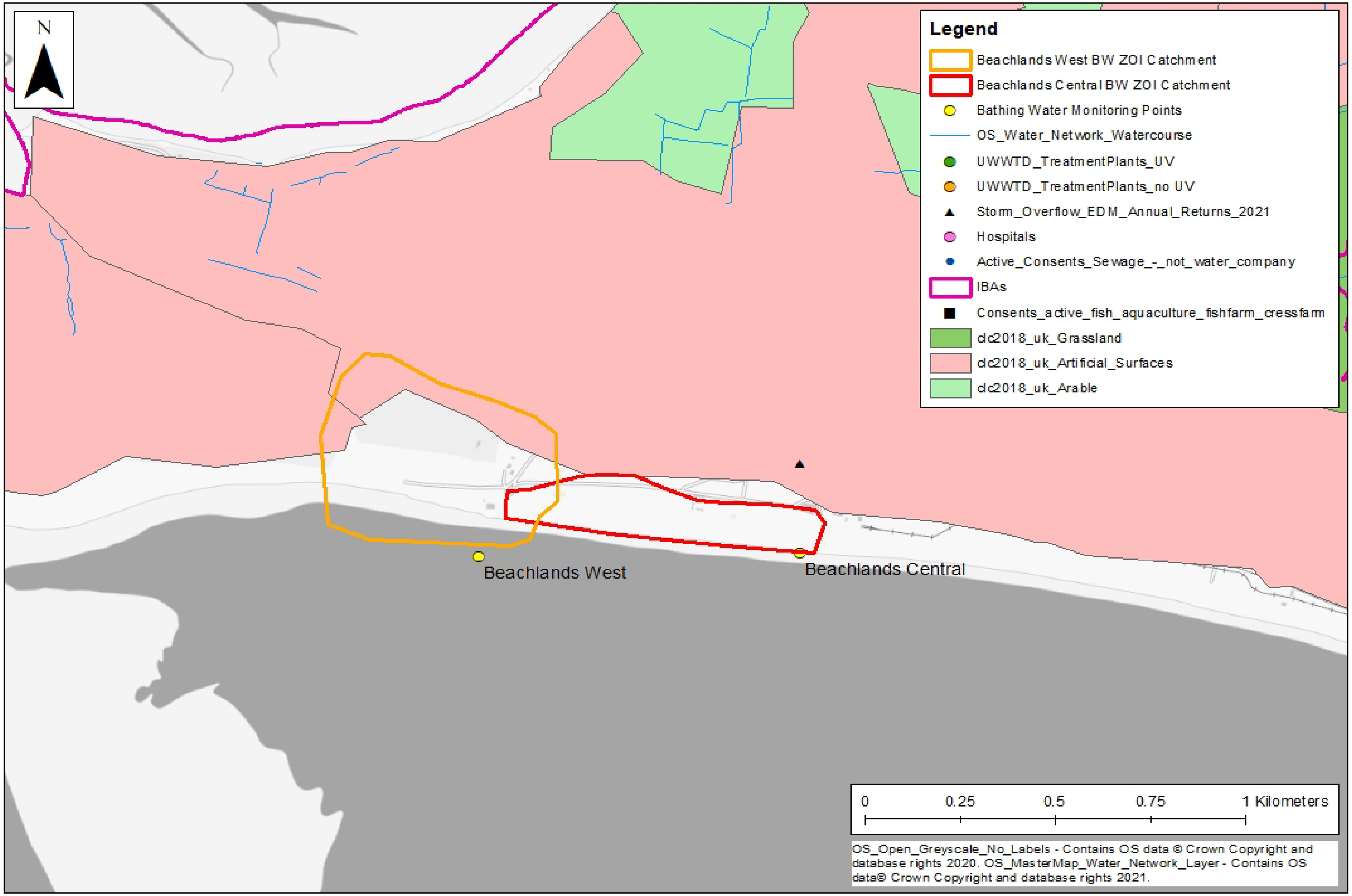

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Figure 3 – *Beachlands West* and *Beachlands Central* (Coldspots)

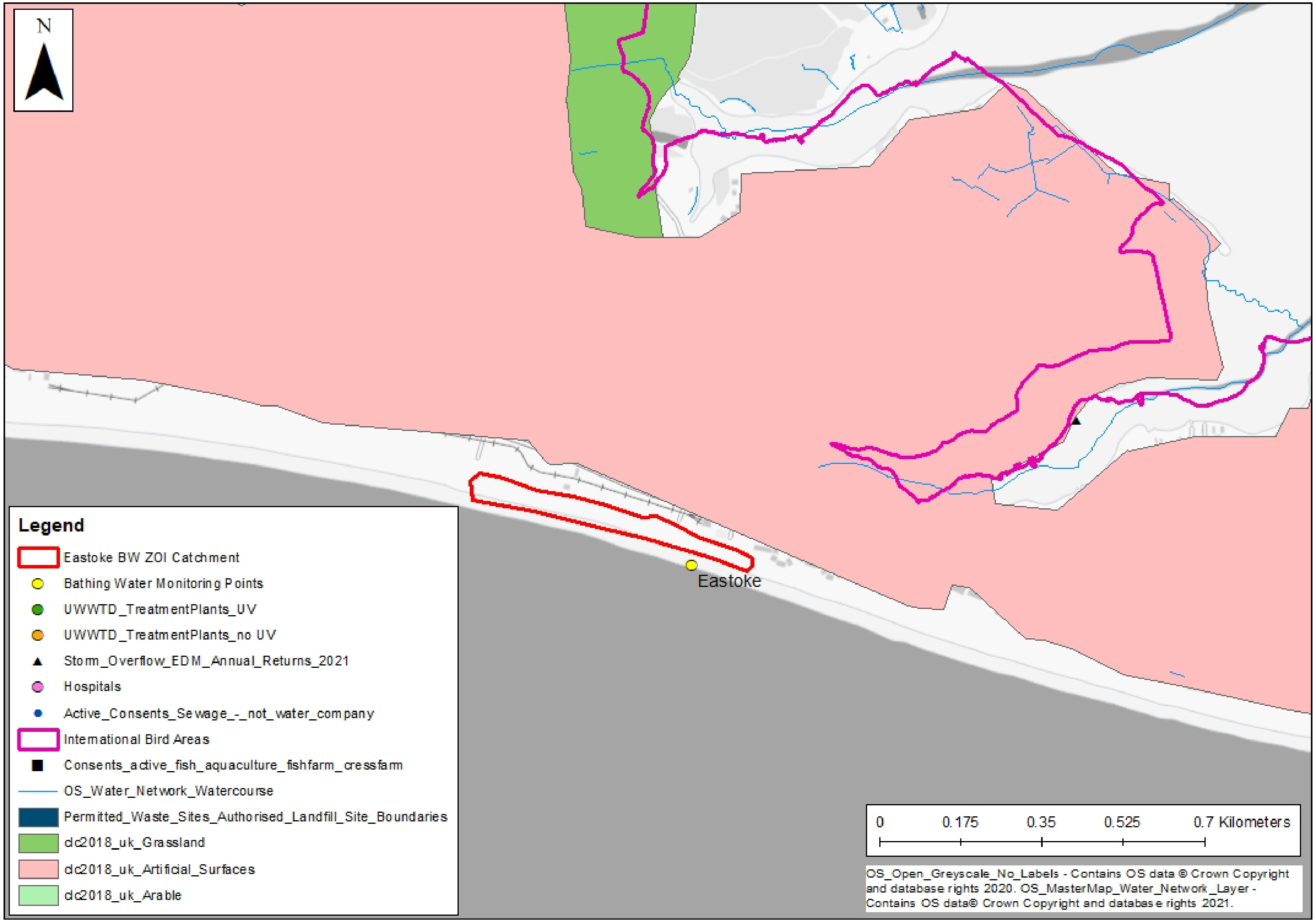

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Figure 4 – *Eastoke* (Coldspot)

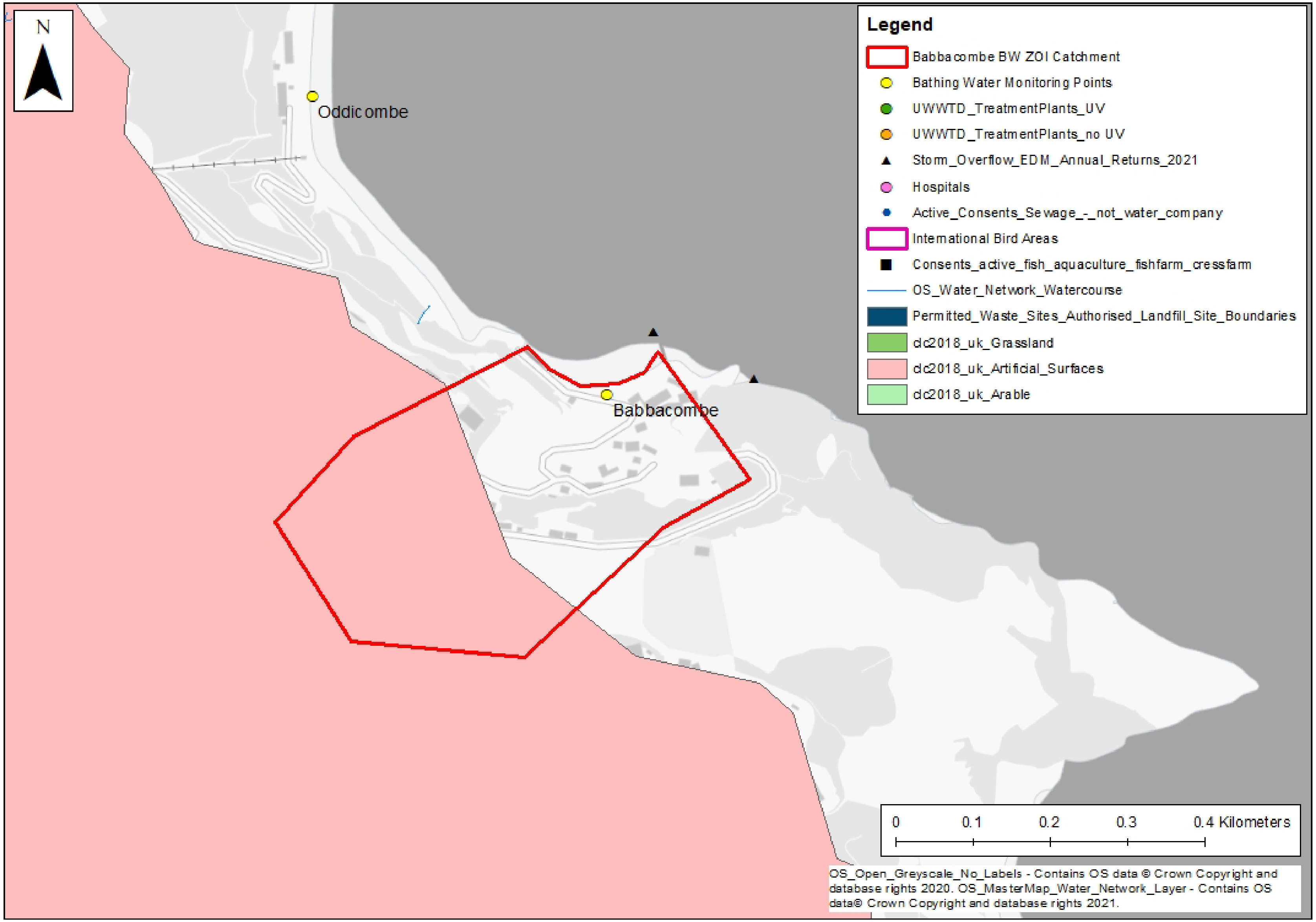

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Figure 5 – *Babbacombe* (Coldspot)

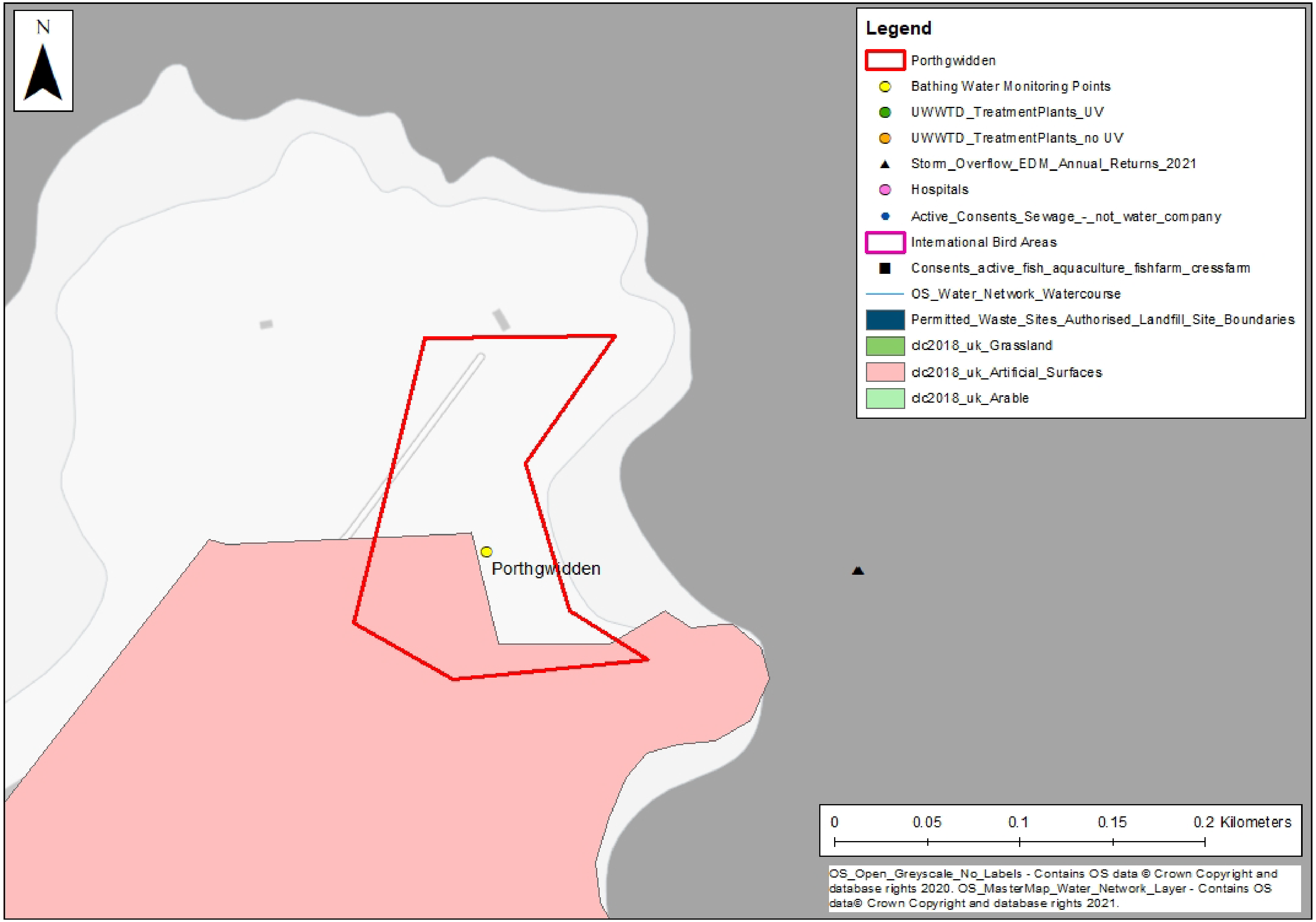

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Figure 6 – *Porthgwidden* (Coldspot)

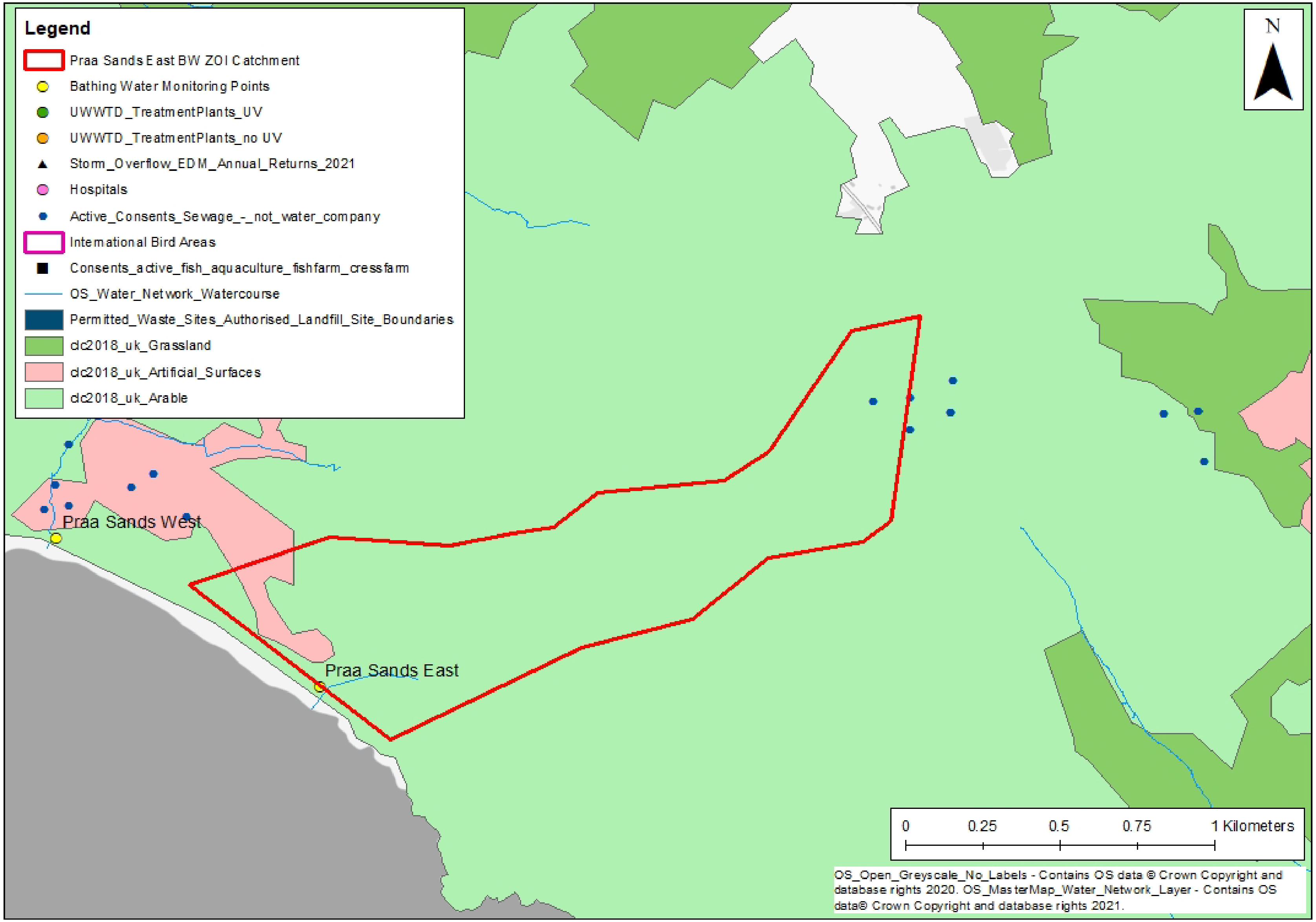

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Figure 7 – *Praa Sands East* (Agri-Arable)

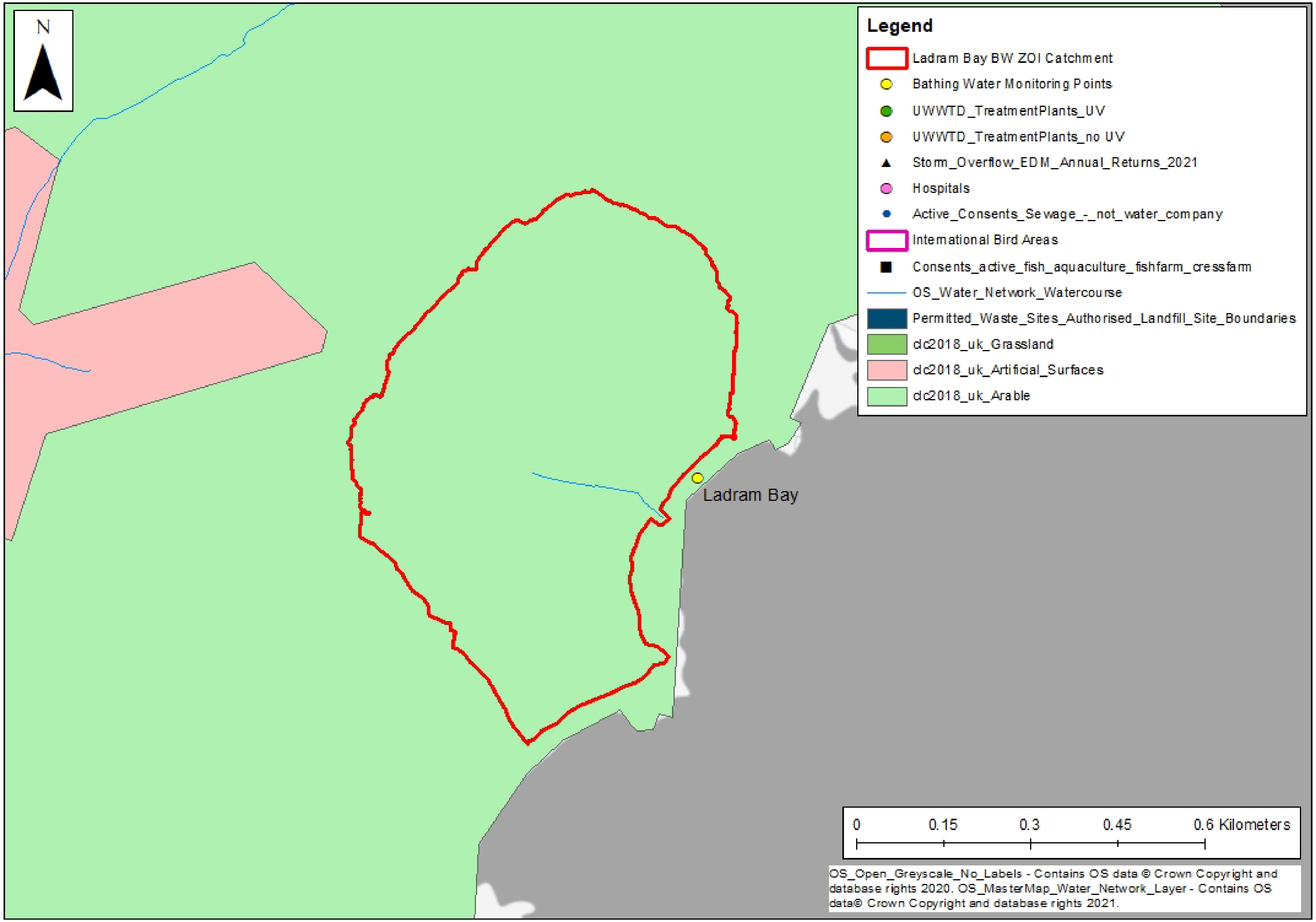

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Figure 8 – *Ladram Bay* (Agri-Arable)

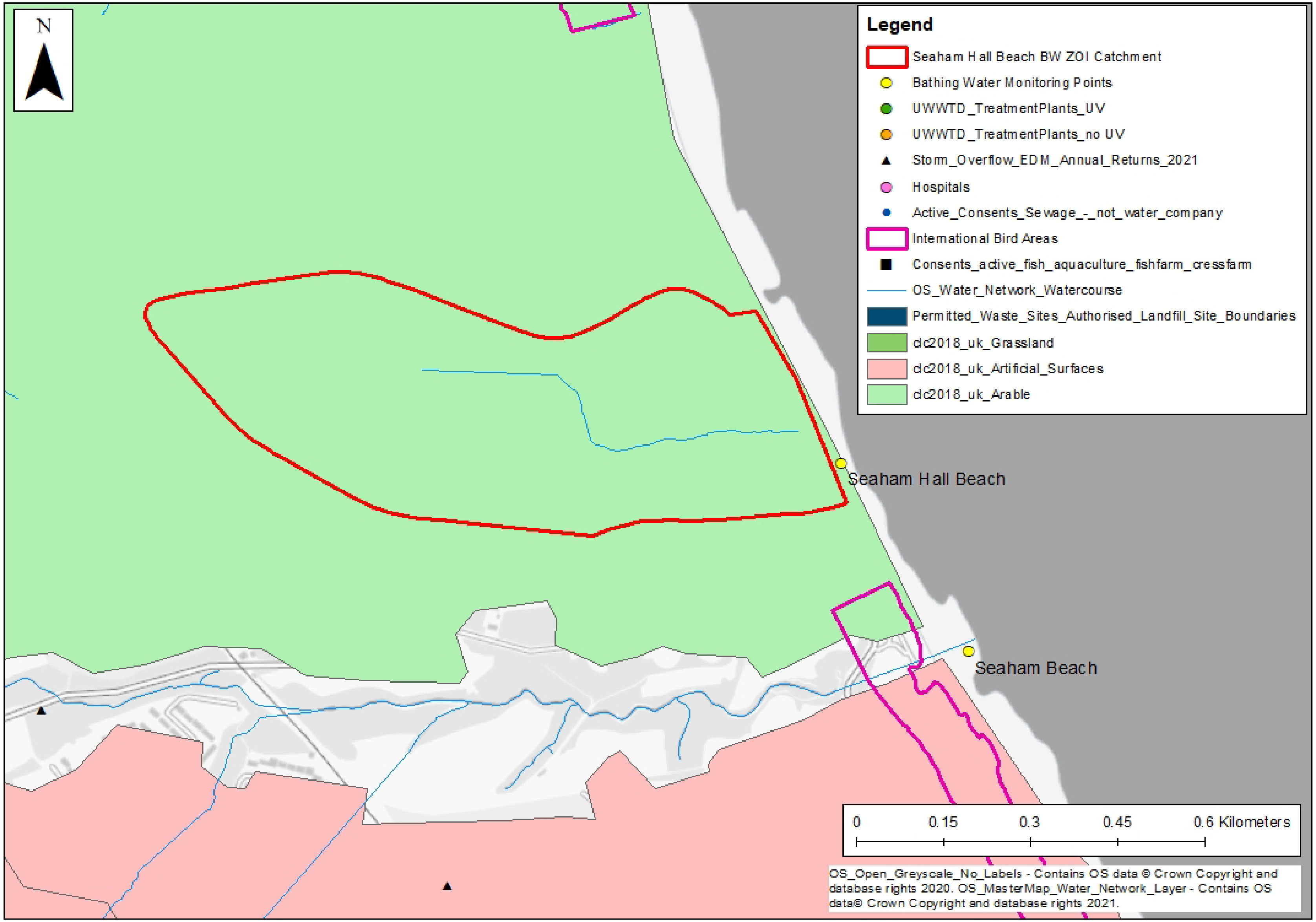

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Figure 9 – *Seaham Hall Beach* (Agri-Arable)

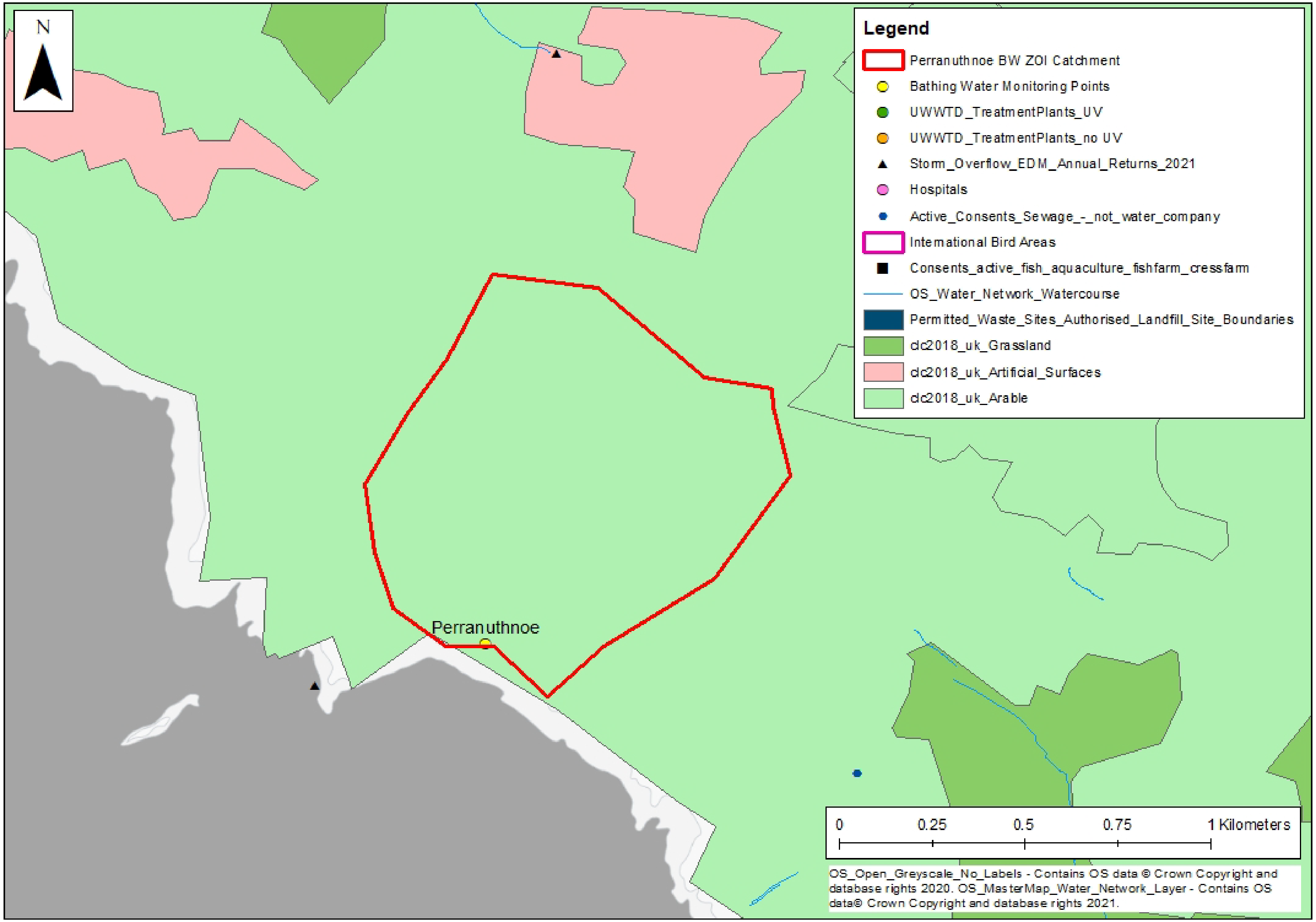

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Figure 10 – *Perranuthnoe* (Agri-Arable)

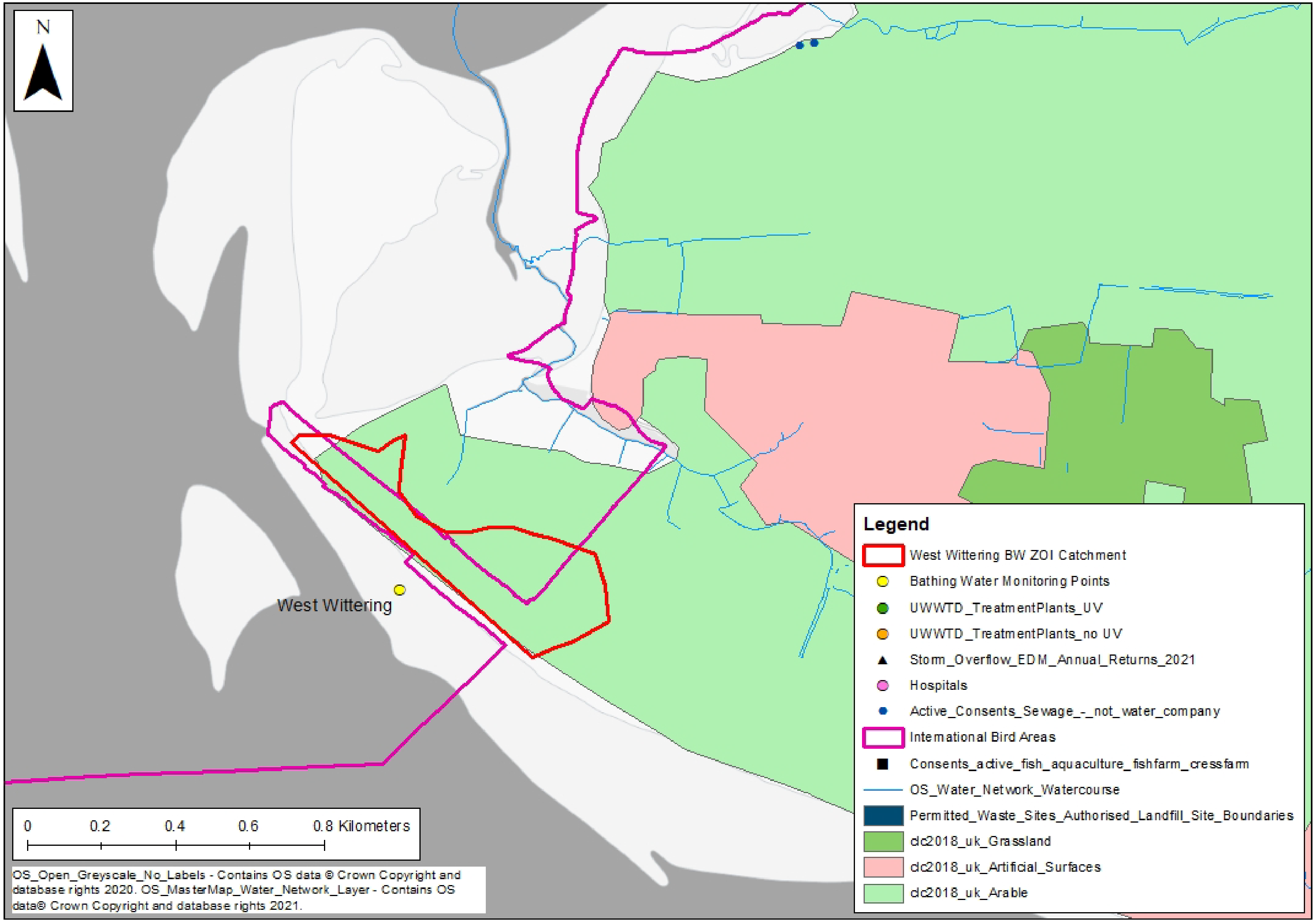

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Figure 11 – *West Wittering* (Agri-Arable)

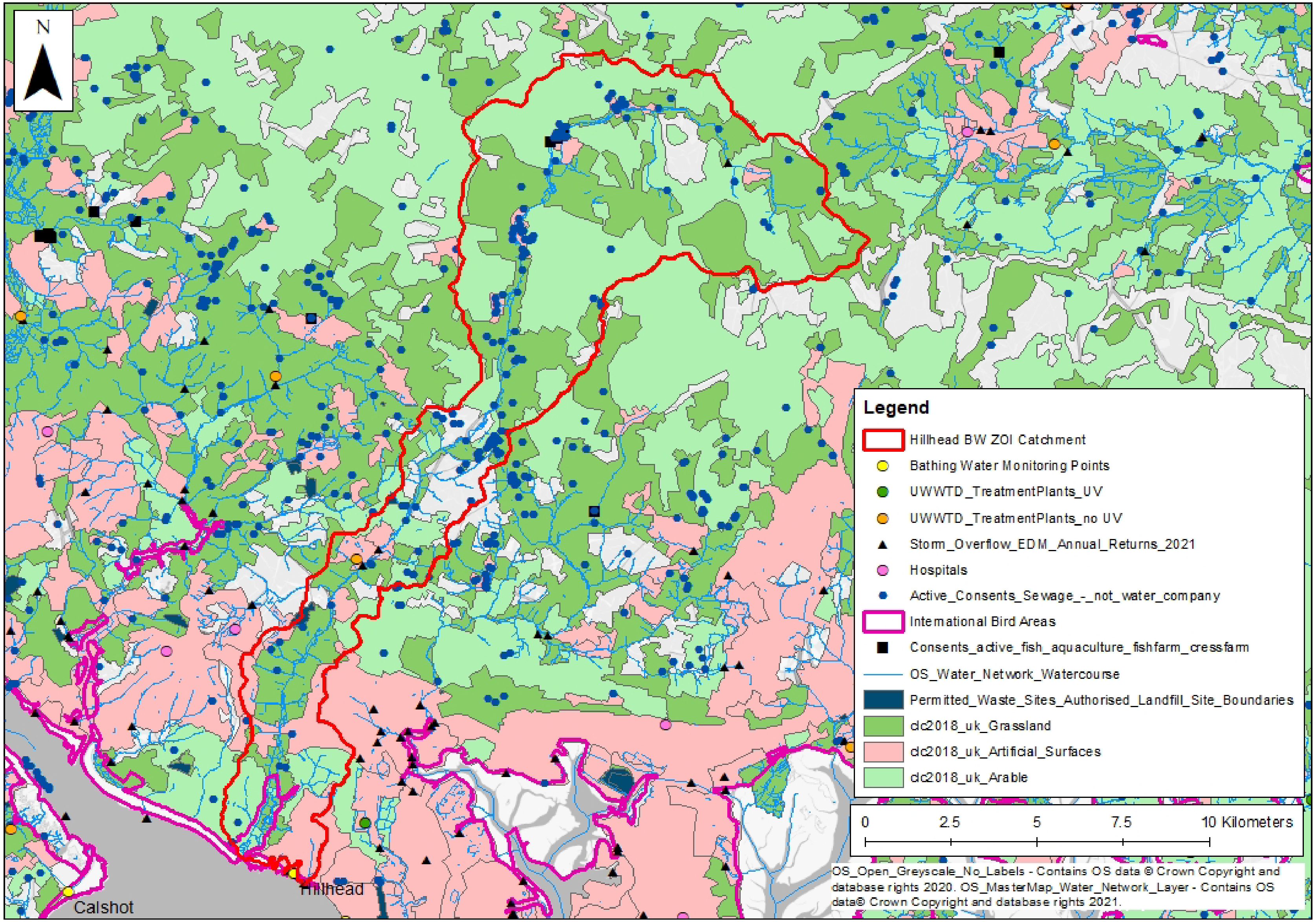

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Figure 12 – *Hillhead* (Top Non-Point)

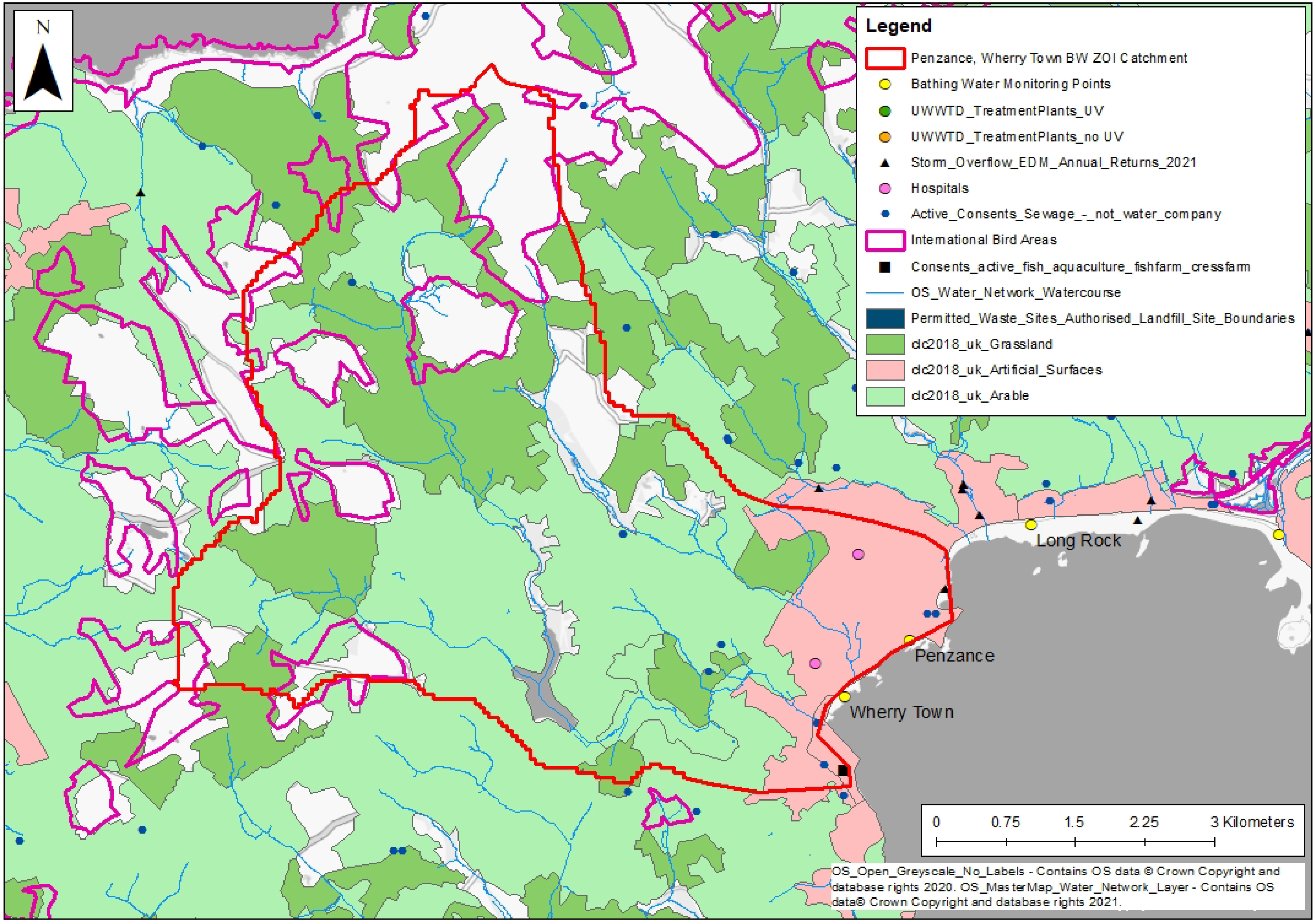

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Figure 13 – *Penzance* and *Wherry Town* (Top Non-Point)

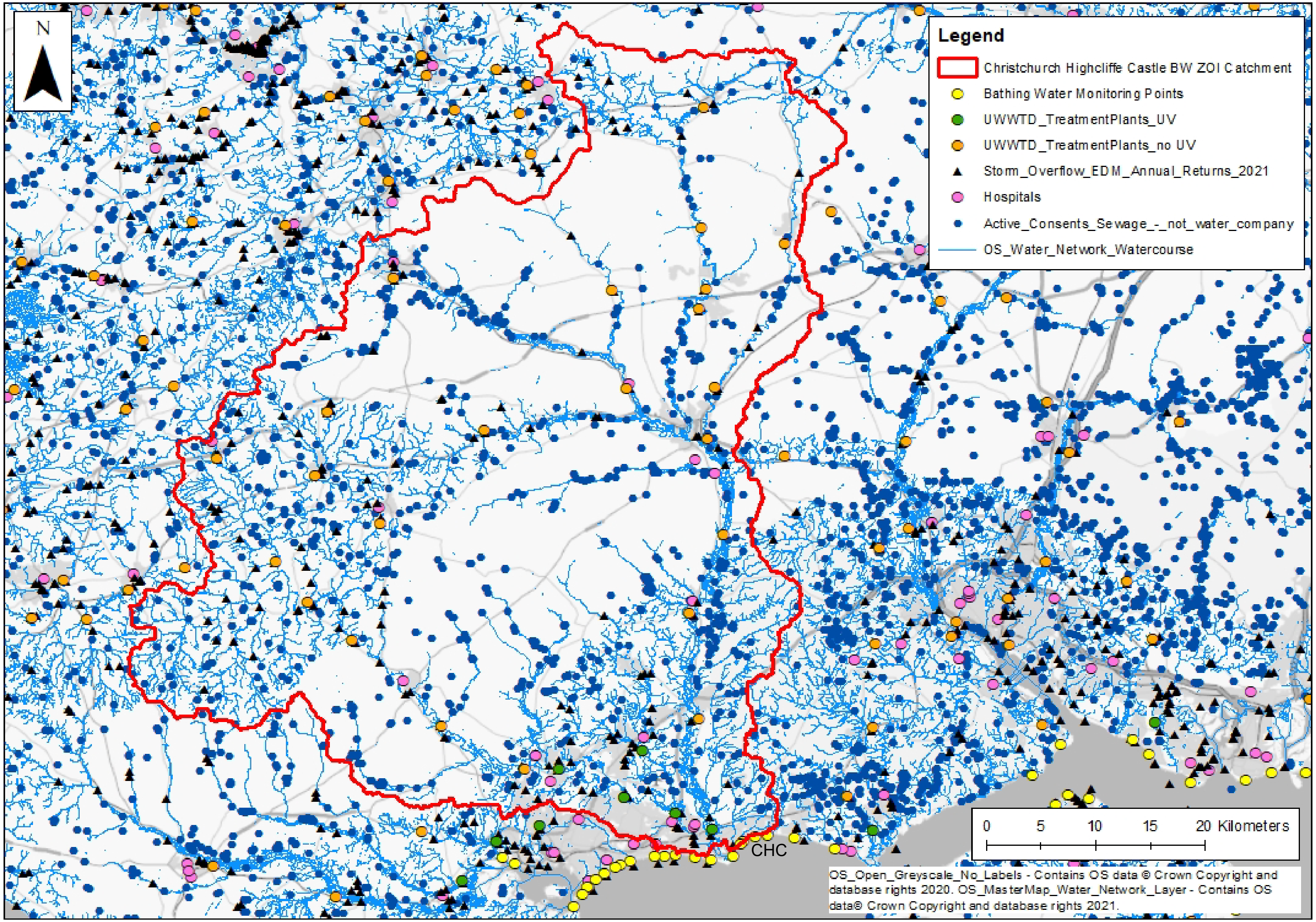

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Figure 14 – *Christchurch Highcliffe Castle* (*CHC*; Top Non-Point) point sources

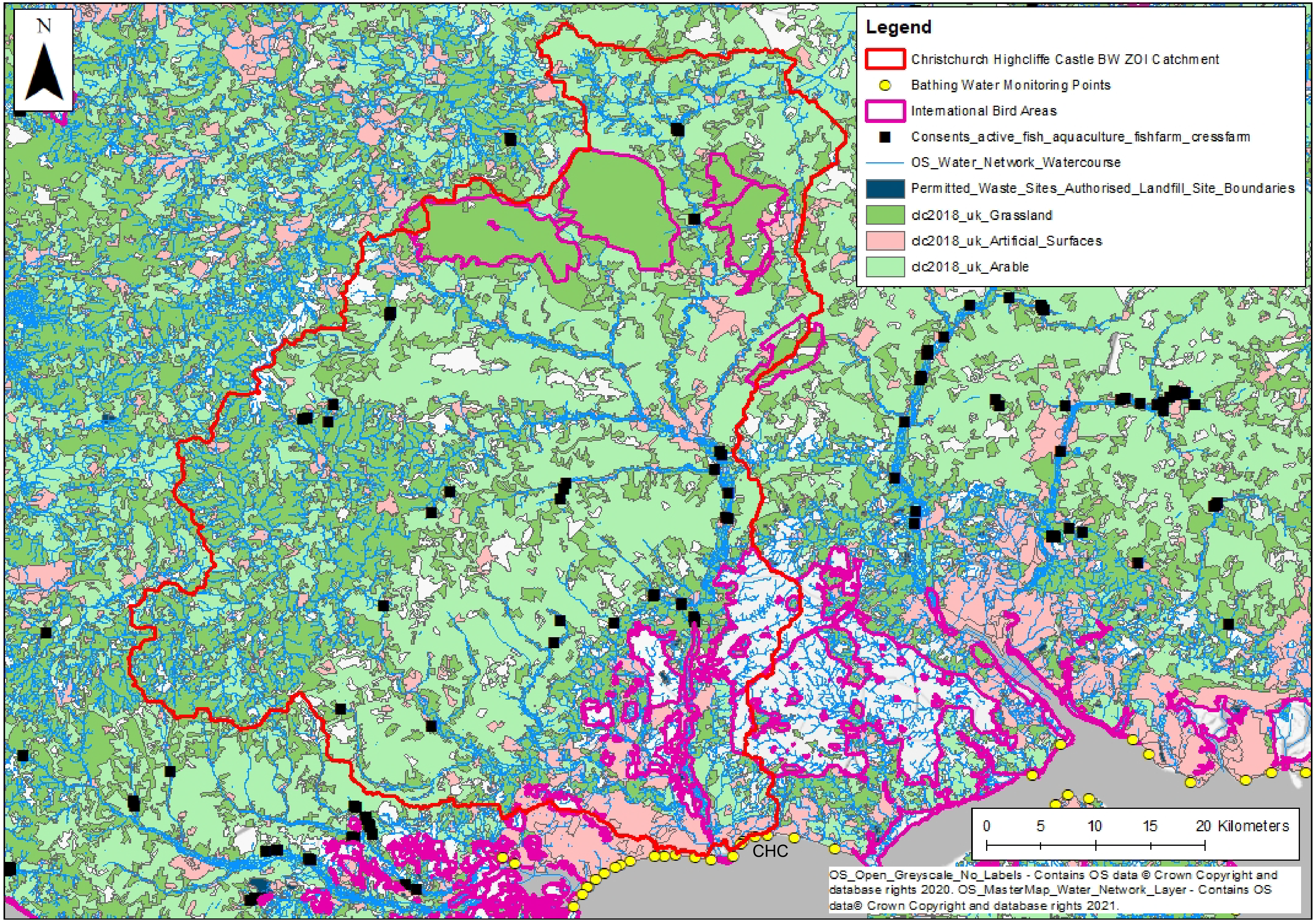

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Figure 15 – *Christchurch Highcliffe Castle* (*CHC;* Top Non-Point) non-point sources

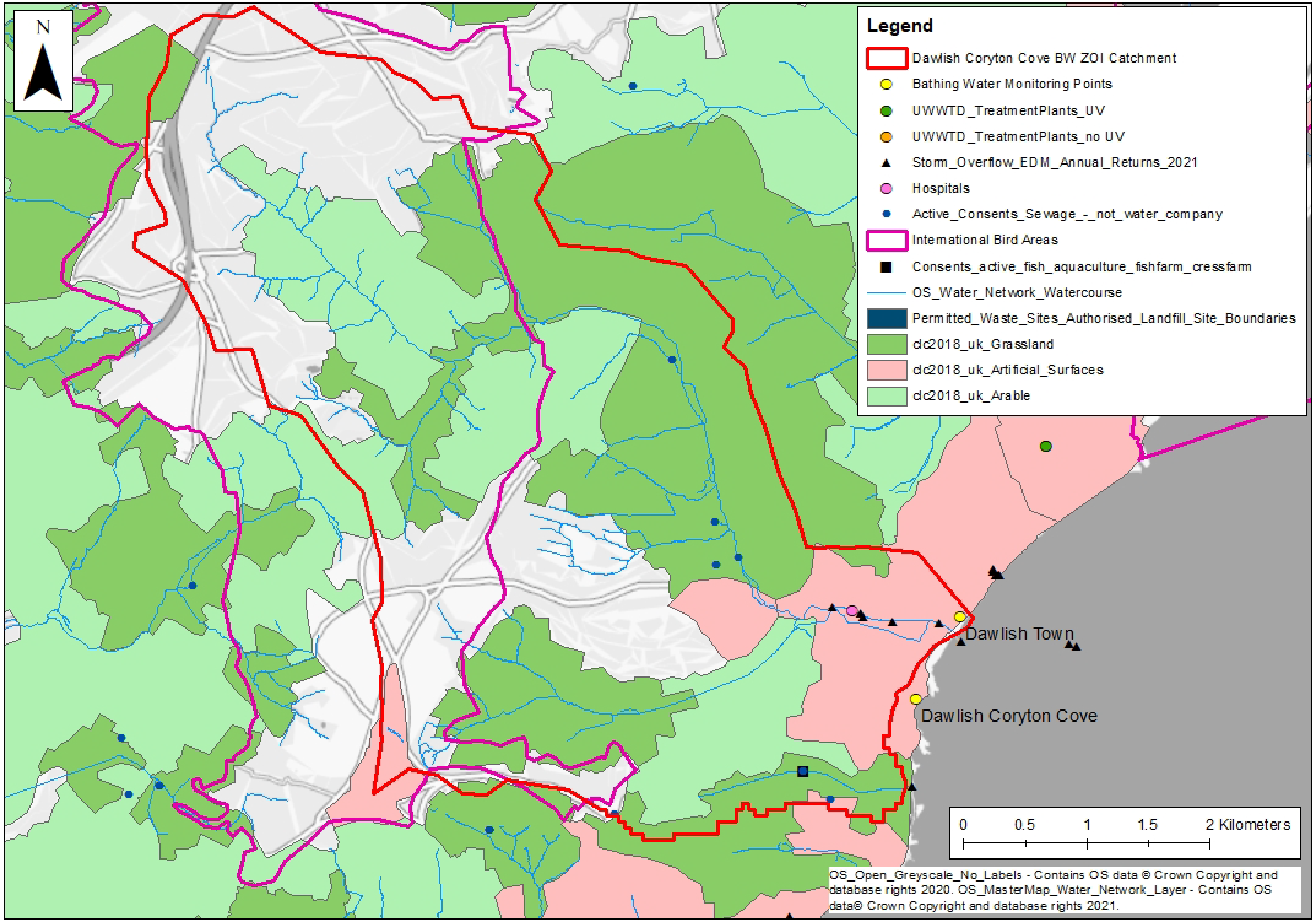

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Figure 16 – *Dawlish Croyton Cove* (Top Non-Point)

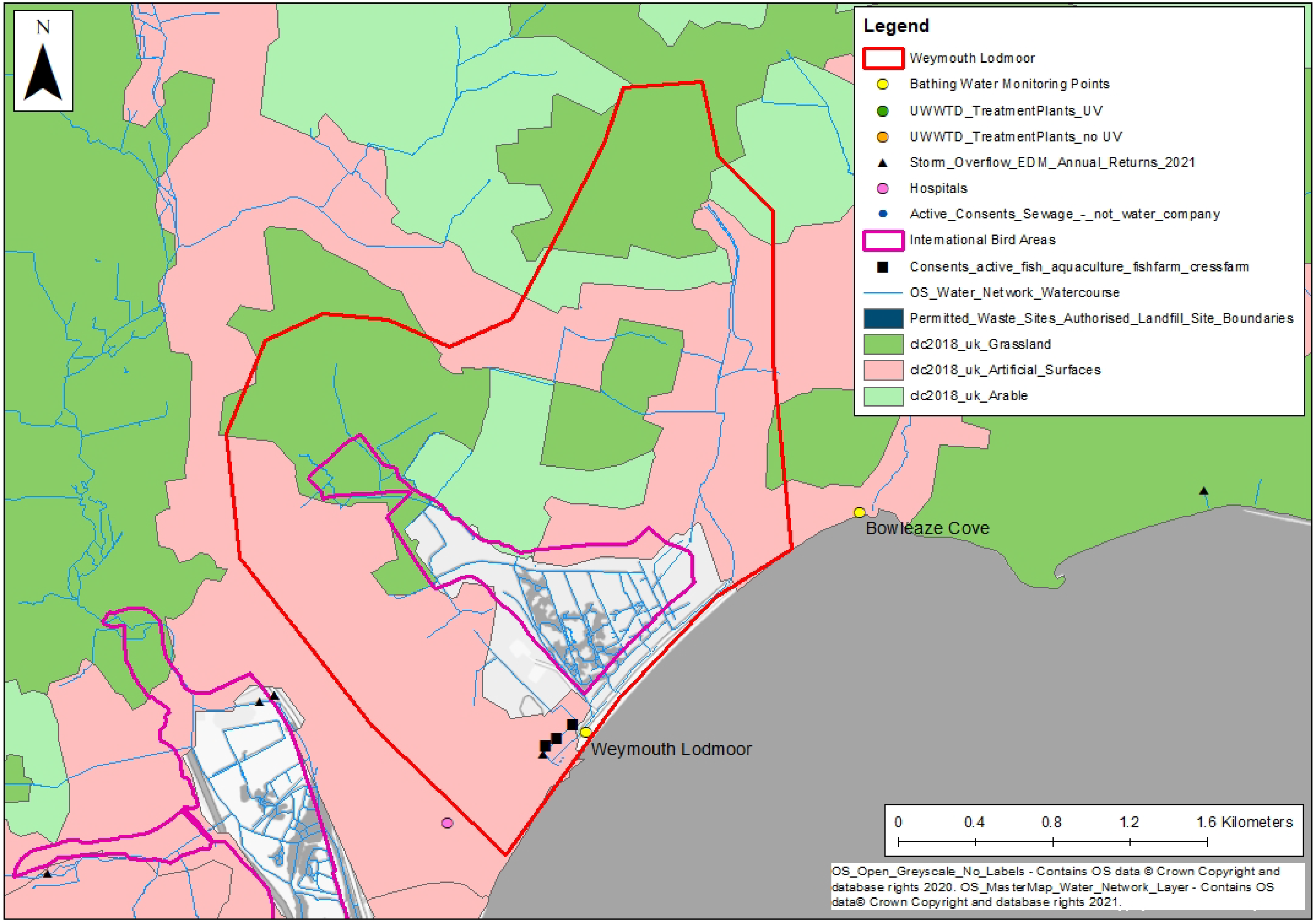

Figure 17 – *Weymouth Lodmoor* (Top Non-Point)

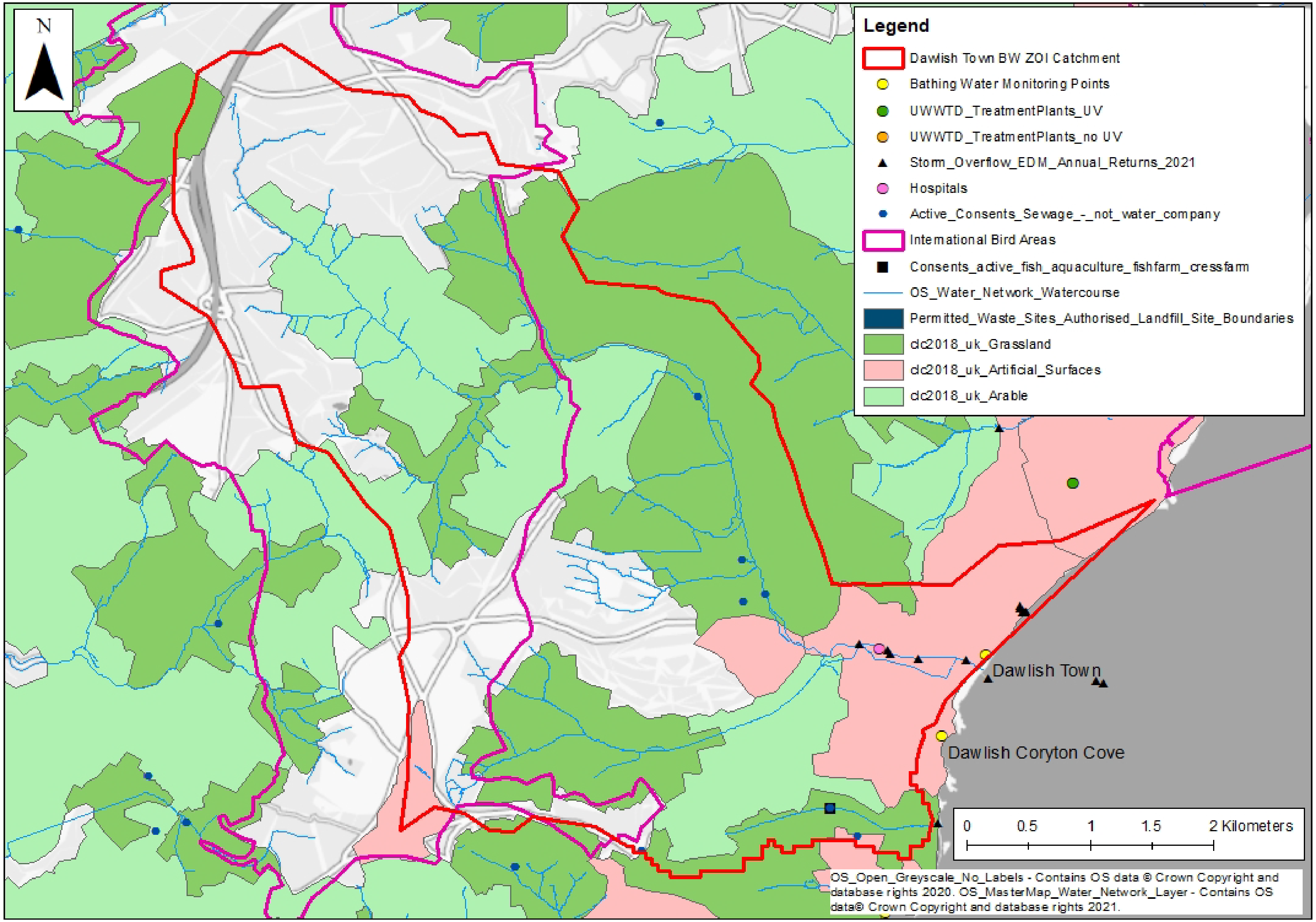

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Figure 18 – *Dawlish Town* (Hotspot)

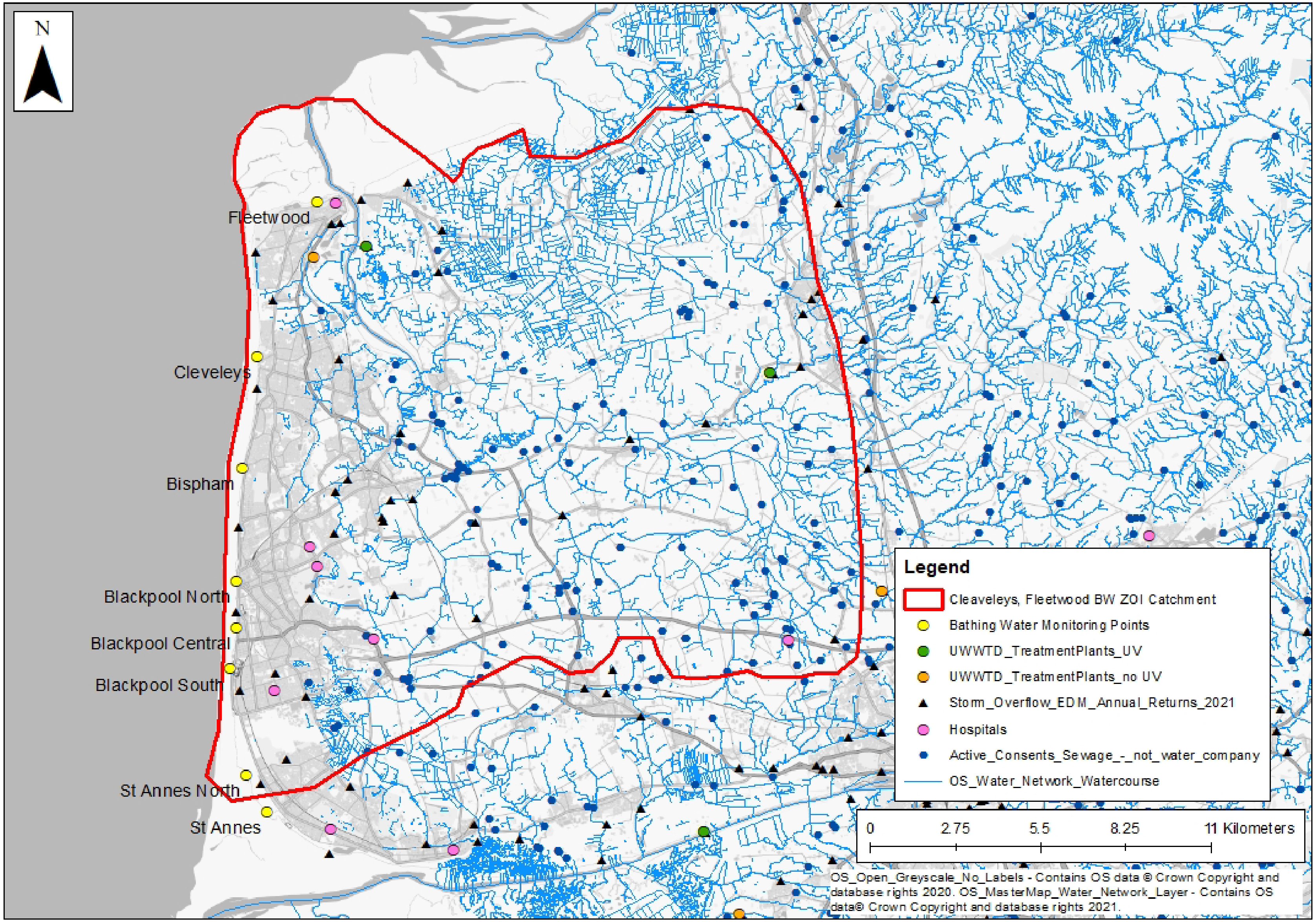

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Figure 19 – *Cleaveleys* and *Fleetwood* (Hotspots) point sources

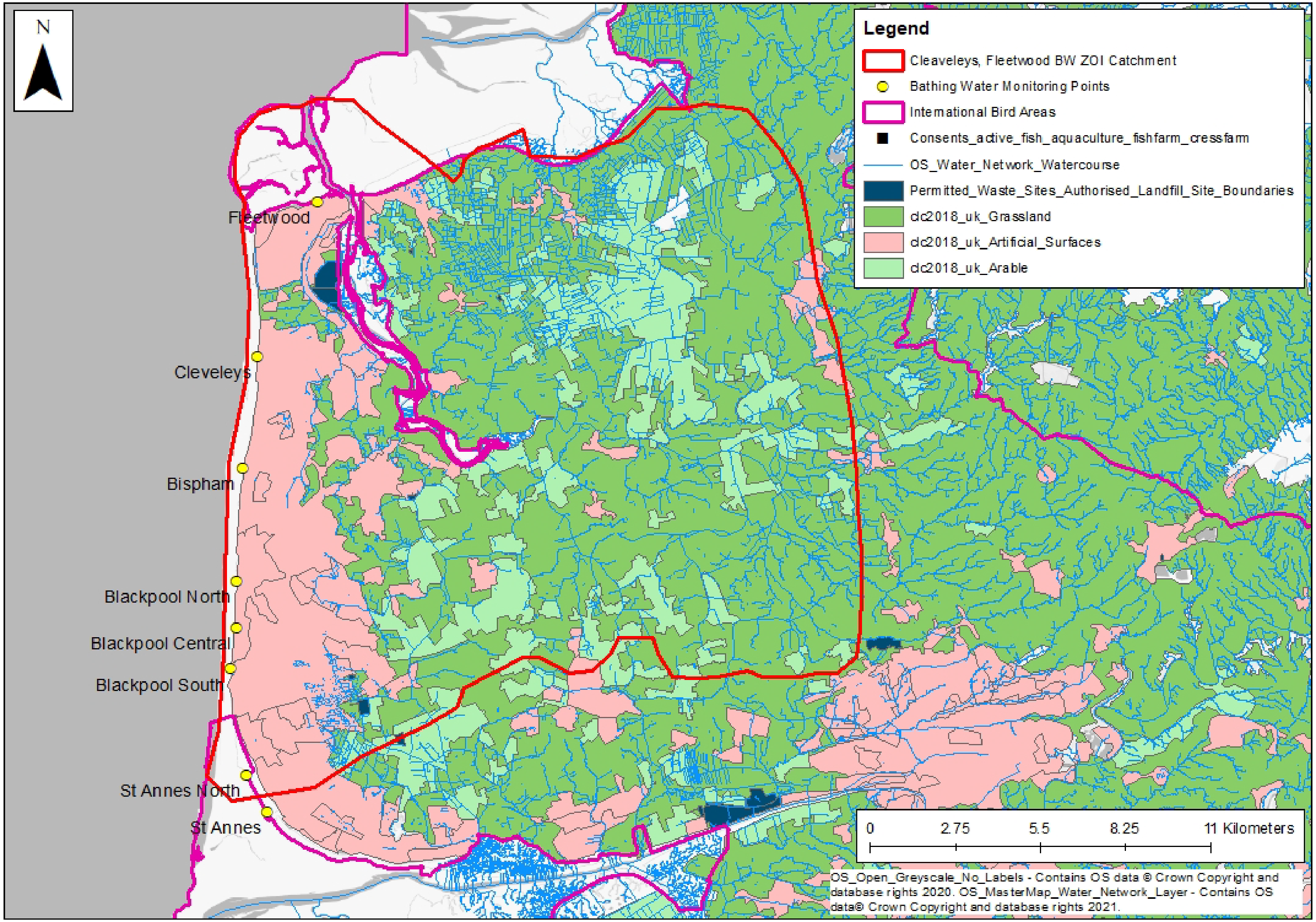

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Figure 20 – *Cleaveleys* and *Fleetwood* (Hotspots) non-point sources

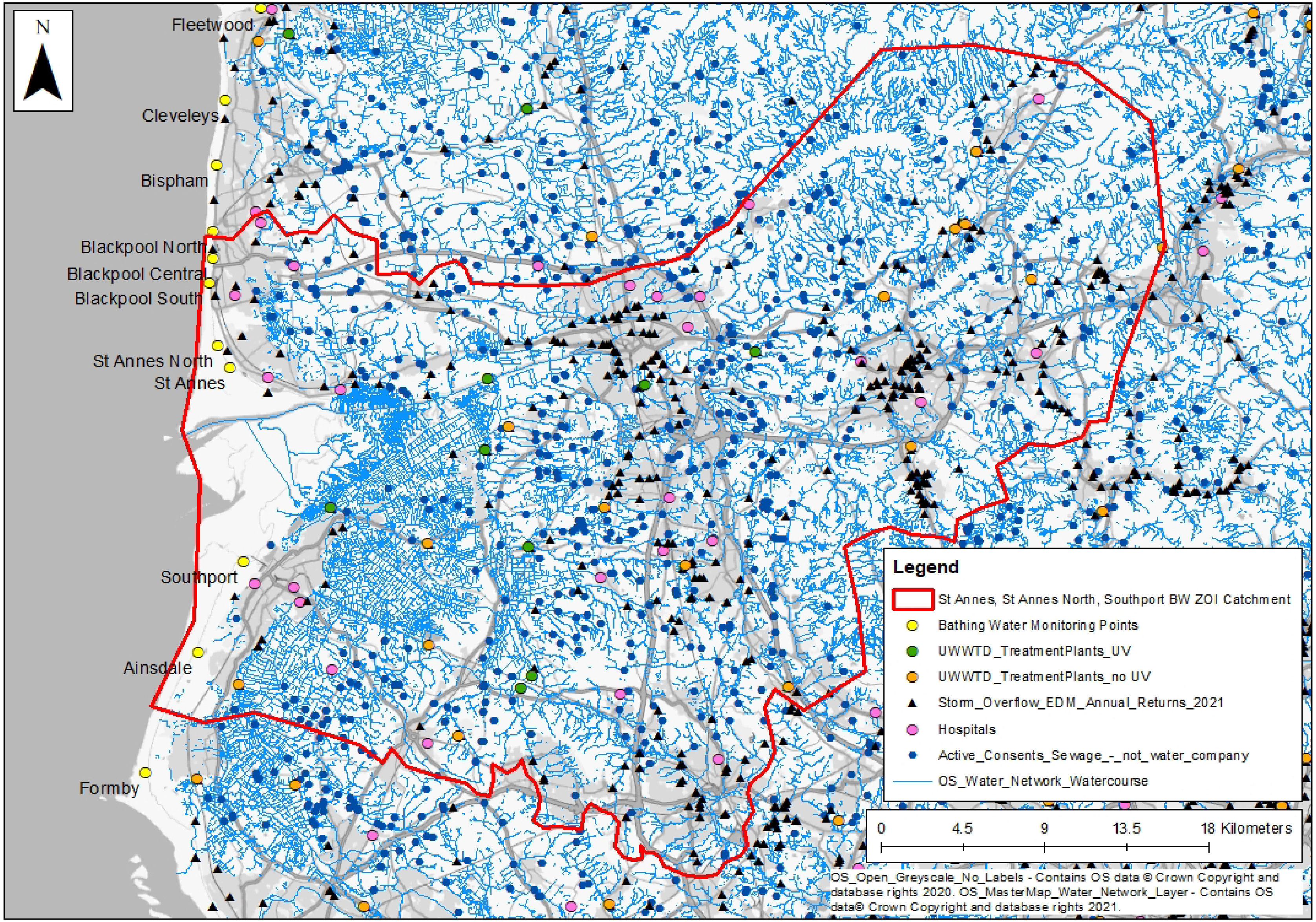

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Figure 21 – *St Annes*, *St Annes North* and *Southport* (Hotspots) point sources

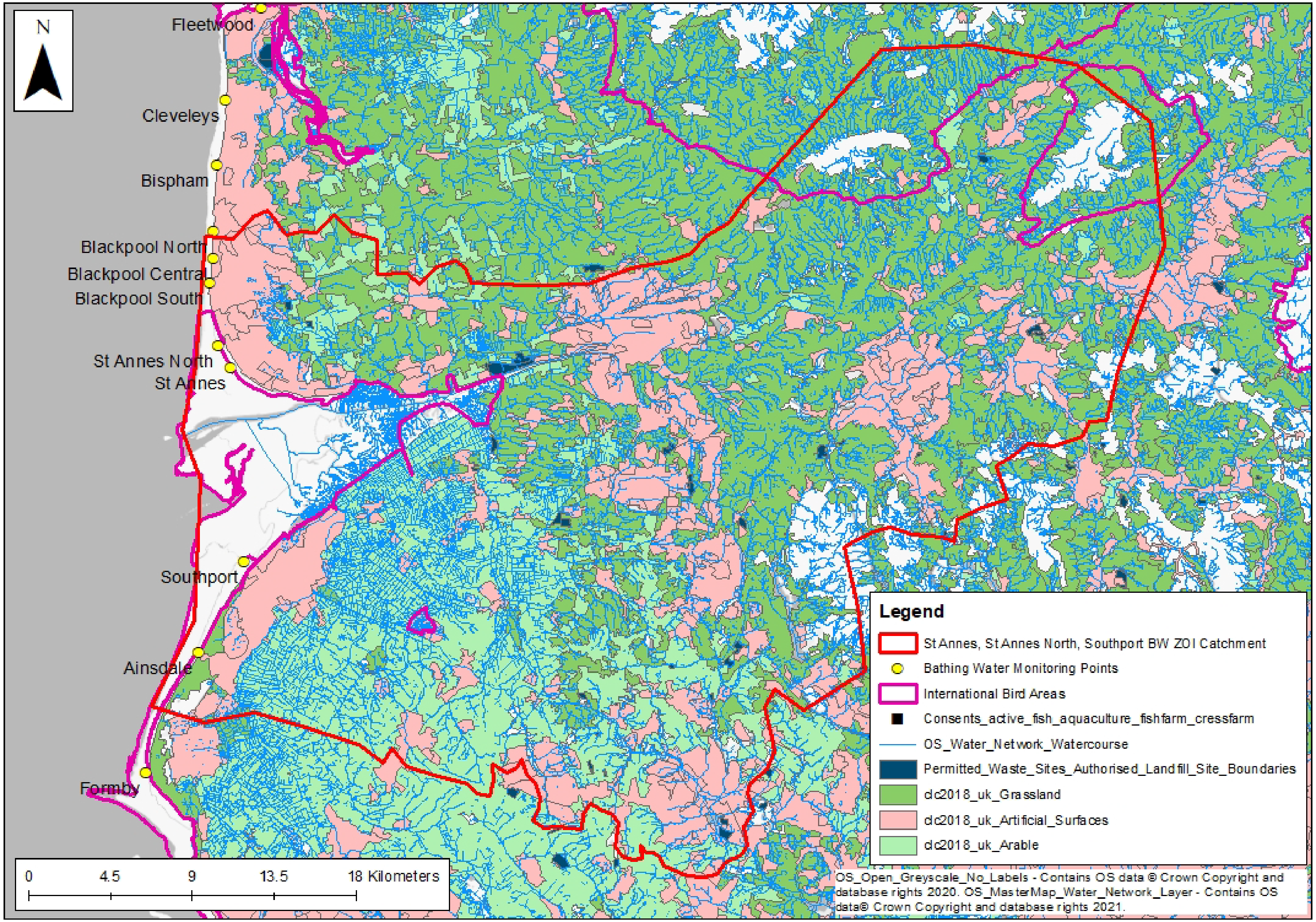

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Figure 22 – *St Annes*, *St Annes North* and *Southport* (Hotspots) non-point sources

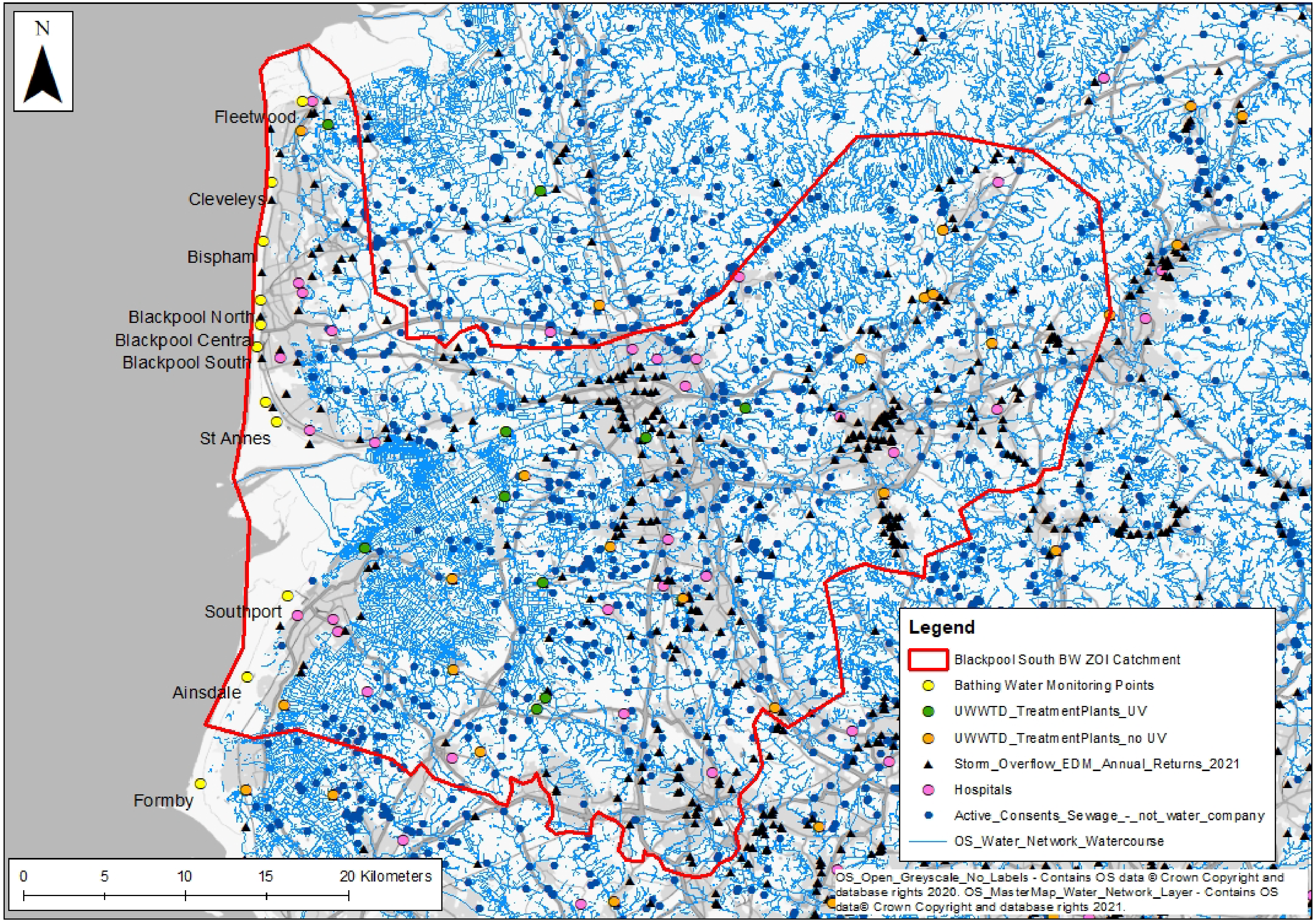

Figure 23 – *Blackpool South* (Hotspot) point sources

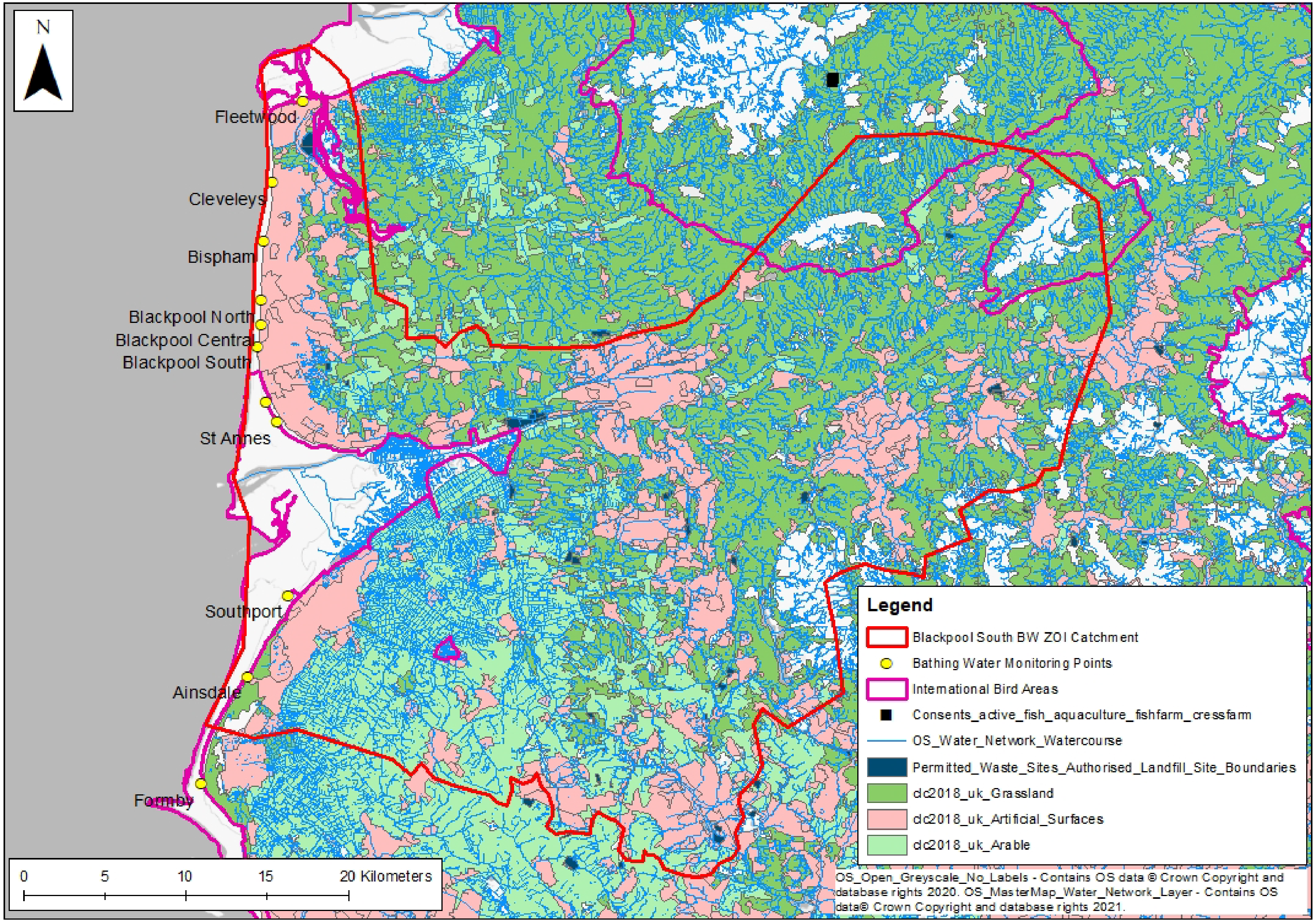

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Figure 24 – *Blackpool South* (Hotspot) non-point sources

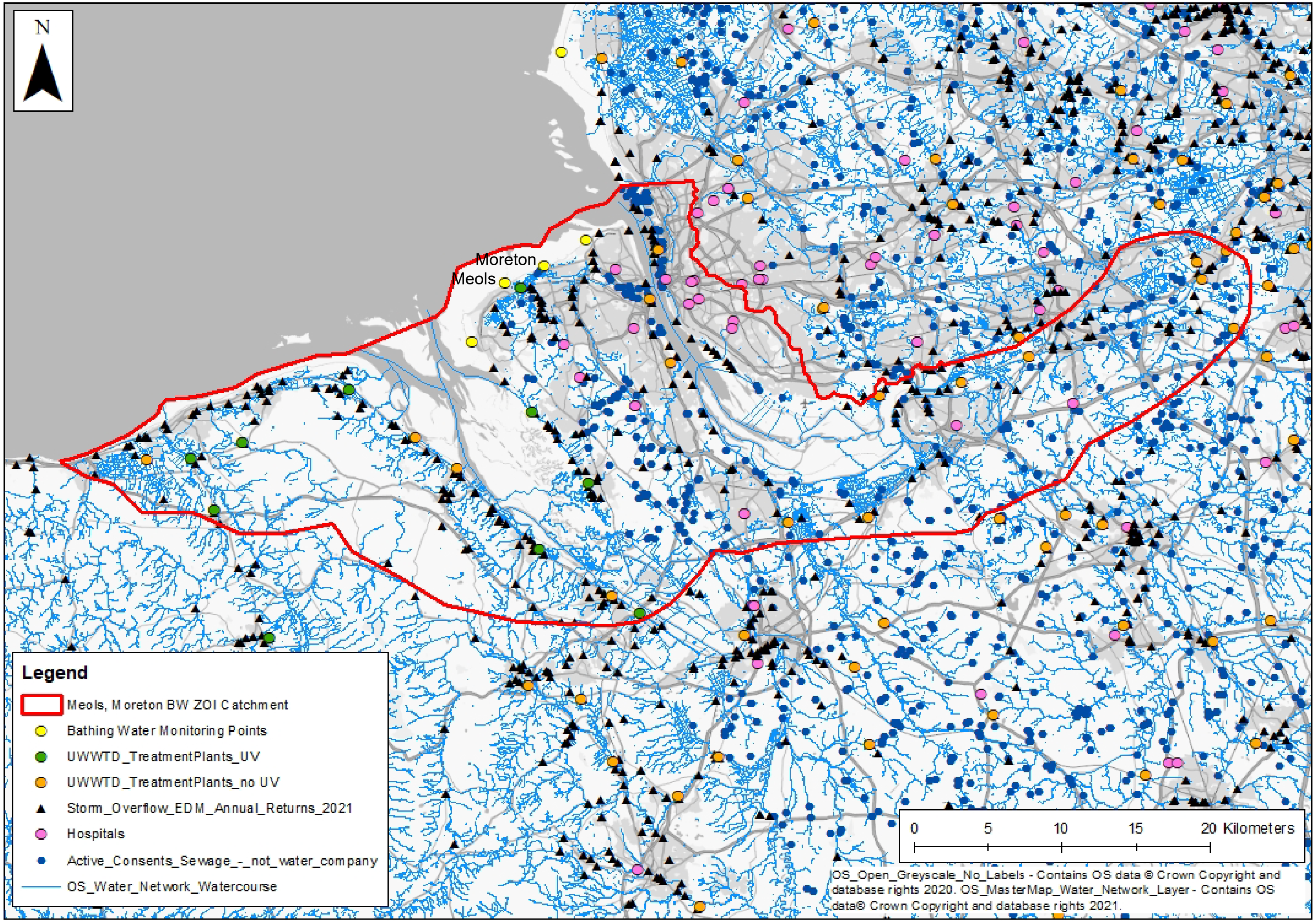

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Figure 25 – *Meols* and *Moreton* (Hotspots) point sources

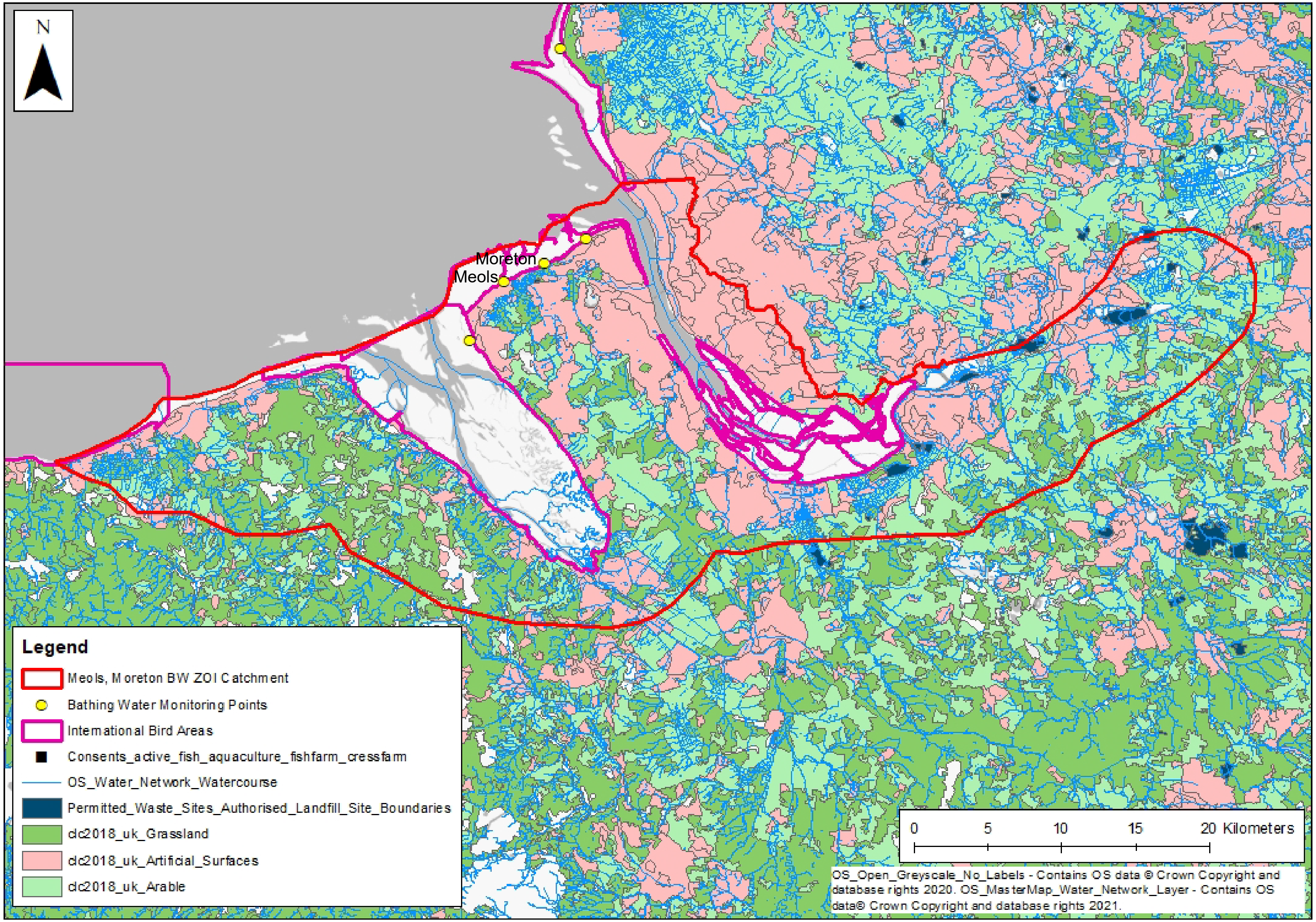

Figure 26 – *Meols* and *Moreton* (Hotspots) non-point sources

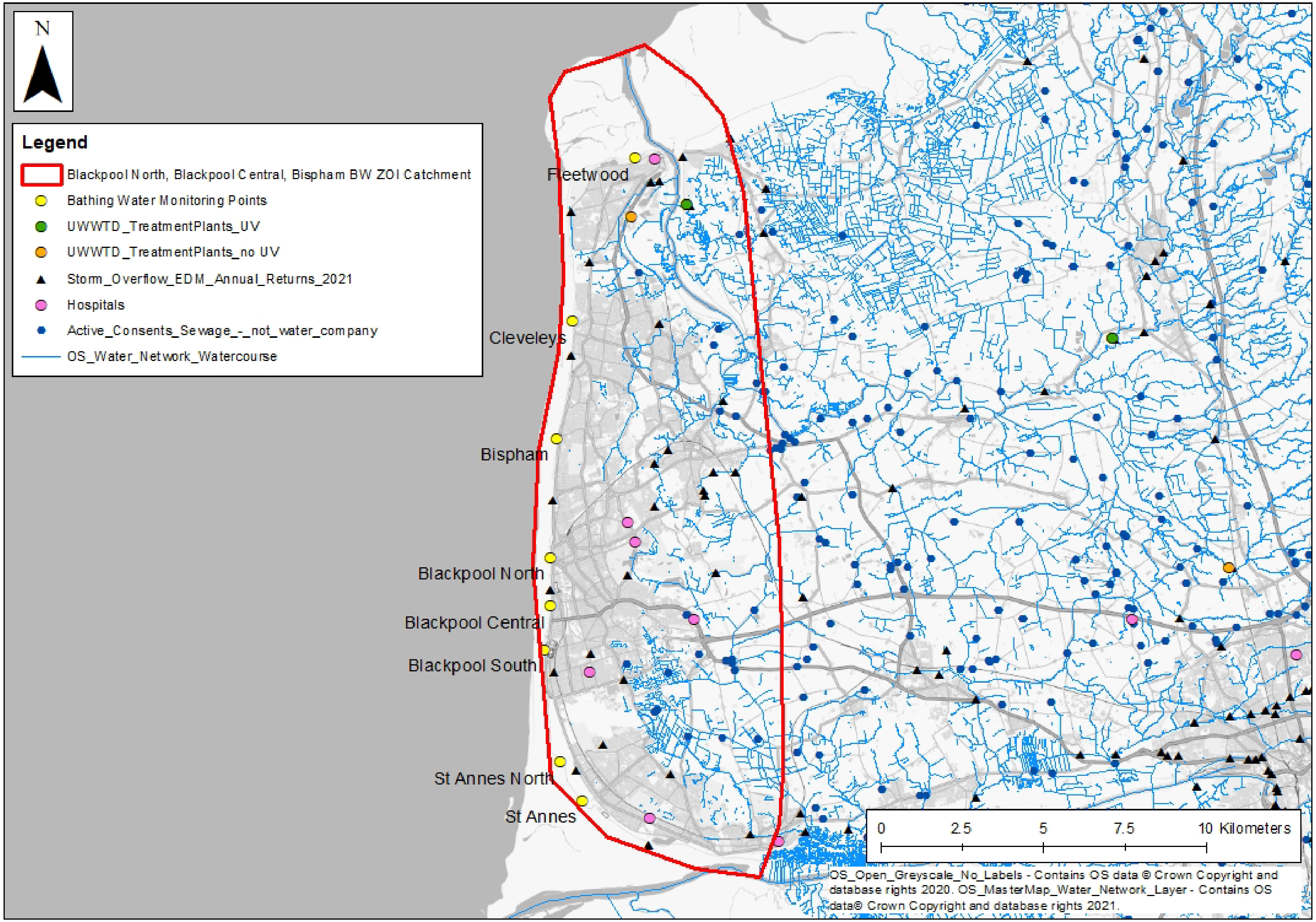

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Figure 27 – ***Blackpool North***, ***Blackpool Central*** **and** ***Bispham*** **(Hotspots) point sources**

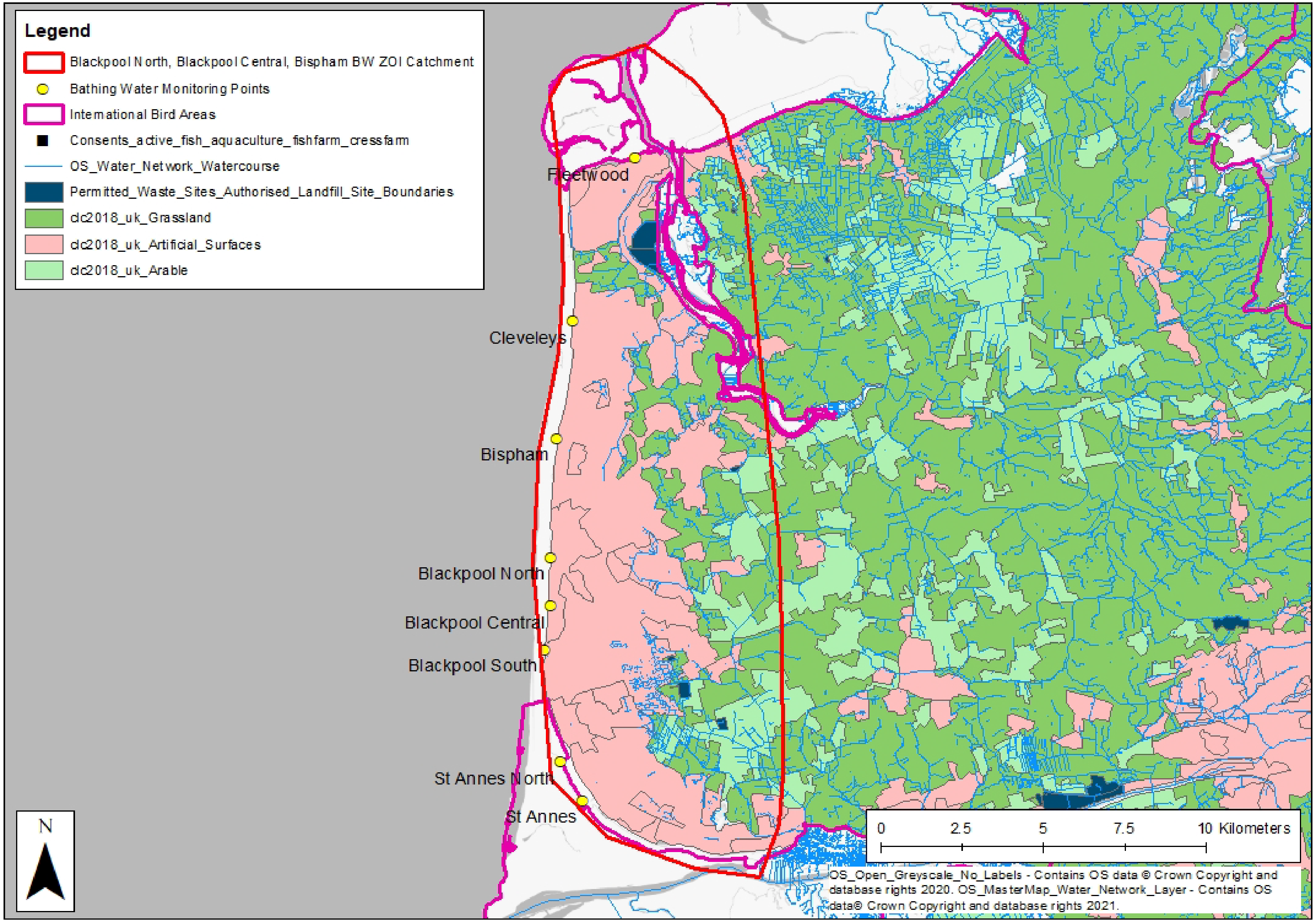

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Figure 28 – ***Blackpool North***, ***Blackpool Central*** **and** ***Bispham*** **(Hotspots) non-point sources**

Summary & conclusion

Following increased interest by the scientific community and the public with regards to AMR, this project has provided information for a potential pilot AMR monitoring programme in English BWs. Key outcomes from each of the project tasks are outlined below. A number of recommendations arising from each task are outlined at the end of each task section in the report.

- Task 1: A search of relevant information was completed to develop an understanding of how AMR is assessed in designated BWs focusing on jurisdictions in the UK (other than England) and Europe.
- Task 2: A review of relevant literature was undertaken to develop an understanding of the current state of knowledge in relation to AFR in coastal BWs and sands. No studies were found identifying pathogenic/ AFR fungi in coastal environments in the UK, and limited studies were available detailing AFR fungi in coastal environments globally. Therefore, this task focused largely on compiling a list of common fungal pathogens in coastal environments and clinically significant AFR potential within those species.
- Task 3: In this task, we developed an approach to select and prioritise designated BWs in England for a future AMR surveillance. Criteria (which reflect potential AMR risk) that can be used to prioritise BWs over one another were developed. Also an Excel workbook, which facilitated the calculation of ‘risk scores’ and can be used to select BWs based on chosen criteria, accompanies this report. In this study, BWs were selected based on criteria agreed with the Environment Agency. A subsequent step, which involved a more detailed review of these BWs to recommend priority for inclusion in future AMR surveillance, was included in the approach. Recommended priorities for inclusion of ‘selected’ BWs in future AMR surveillance are provided within this report.

This study has provided a foundation for future work to improve our understanding of AMR in BWs across England.

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Appendix:

Appendix 1 - Discussion of options to sample for AMR at bathing waters

AMR surveillance

The AMR surveillance table (developed in Task 1) was re-visited and additional information was extracted from the studies identified in the Task 1 search. This included further details on the sampling approach (including the sample volume collected) and details of how samples were processed for methods used to measure bacterial AMR. The updated *'AMR Surveillance Table'* was included in the *'Task 1_AMR Surveillance_Workbook'* which accompanies this report. Information from the study by Novak Babič et al. (2022) (identified in Task 2), who investigated AFR in seawater and sand taken from a designated BW in Slovenia, was also included in the updated table.

Considerations for AMR sampling

From the information included in the updated *'AMR Surveillance Table'*, a list of considerations for AMR sampling at BWs was developed. The different types of approaches that might be considered and the advantages and limitations of adopting an approach for national scale AMR monitoring were also explored.

Findings for each ***consideration*** are noted below, whilst a full list of findings can be found in the *'Considerations'* table in the *'Task 1_AMR Surveillance_Workbook'*:

- ***Number and location of sites***
    - It is plausible that AMR surveillance could be undertaken as an extension of monitoring as part of the Bathing Water Regulations (2013) at England's designated BWs. However, a smaller scale surveillance programme of 'hotspots' (with multiple point and non-point sources of AMR microbes and compounds, and multiple pathways which can deliver these to the BW) and 'cold-spots' (with no point or non-point sources of AMR microbes and compounds), for example, would be more cost-effective than sampling all 421 designated sites. This stepwise approach would lend itself to methodological development (for example, sampling dry sand and wet sand for an experimental period, or methodological development for sampling fungi), which is well-suited to a pilot scheme (see Introduction).
    - Following the assessment of the pilot scheme outcomes, the next step may be to roll out AMR surveillance across all bathing sites.
    - Additionally, another pilot scheme could be developed to trial automated sampling and analysis technologies, assessing the potential benefits provided by near-real time surveillance, which could be incorporated into future monitoring schemes. If a relationship can be drawn between water-derived AMR and the sand AMR across the different beaches, it could potentially

alleviate the need to sample both water and sand, thereby enabling future monitoring schemes to rely solely on automated sampling technologies and site-specific models to predict pathogen risk in the underlying sand.

- ***Timing of sampling***
    - Sampling only during the BW season would provide the most cost-effective approach and would benefit a significant number of beachgoers. However, it does not offer protection to people (and companion animals), who use BWs and the beach year-round.
- ***Frequency of sampling***
    - The frequency of sampling should be justified through an initial pilot that includes high sampling frequency (i.e. multiple times per day), over an extended period of time, e.g. weeks, inclusive of a range of weather conditions. Based on such data, for each site (e.g. enclosed vs open beaches), a sampling frequency can be derived that captures the majority of conditions present at a particular site. A consistent frequency of sampling across all beaches with similar characteristics (e.g. open vs enclosed beaches), would be justified; however, some compromises on sampling frequency might be needed to simplify logistics and produce a robust and comparable data set for all sites, in the case where sampling is not automated.
    - A fixed sampling frequency might be more costly than a variable sampling frequency (different sampling frequency at different sites) that could support flexibility and more reactive monitoring. For example, targeted sampling during a pollution event could offer enhanced awareness and protection to beach users.
    - In future, technological developments in automated sampling or the application of models could provide near real-time information that may be beneficial to protecting human health.
- ***Method for sampling (automated verses manual)***
    - Whilst automated sampling would provide most protection at BWs, as it could generate reliable near real-time data that could influence bather behaviour and exposures, the technology is not currently available.
    - Whilst the development of automated sampling methodologies could be applied to water analysis in the future, analysis of sand would require manual sampling. However, the relationship between microbial contamination of water and sand can be derived and tested. If such a relationship can be modelled, it would support a future automated water sampling approach that could predict the extent and length of sand contamination.
- ***Media to be sampled***
    - Water is already sampled at BWs to assess water quality, requiring minimal additional sampling resources. However, growing evidence suggests that sand can harbour pathogenic microbes in higher concentrations than the adjacent seawater (Sabino et al., 2014), and may pose a more significant risk to younger children, who are more vulnerable to infection (Brandão et al., 2021) and may typically spend more time playing/digging in the sand.
- ***Sampling point selection***

  - Whilst random sampling removes selection bias, it may fail to capture the worst-case scenario (pollution) or the most frequently used part of the beach, which are the most relevant areas to sample in terms of protecting public health.
  - A targeted sampling strategy has the flexibility to change seasonally and can target likely ‘hot-spots’, although may require additional studies to support site selection.
  - The part of the beach containing the highest concentrations of fungal contamination (e.g., dry verses wet sand) is likely to vary across different locations and for different species. Additional research is required to determine the optimal sampling location for protecting human health from potential pathogens within beach sand in England (and the wider UK). It would therefore be preferential to sample both wet and dry sand for an experimental period until there is a better understanding of which fungal species are common in English beaches, and how these communities respond to pollution events.
  - If automated sampling stations were developed, these will be stationary (at least at the outset), but would be positioned in the locations that would be most protective of the beach (i.e. highest risk of being contaminated first if there was a contamination event).
- ***Type of sample***
  - An initial assessment could be employed to sample several spatially separated (e.g. 50-100 m) locations along a beach/coastal BW site, potentially at different days/times to determine the degree of spatial and temporal variation at a given site. This is the suggested methodology for an 'initial microbiological water quality assessment' in the WHO (2021) Guidelines. If there is no significant variability, then subsequent sampling campaigns could utilise a composite sampling approach.
  - For beach scale analysis, one solution for high heterogeneity is to collect samples from many different locations and analyse a composite (Phillips et al., 2011). For fungal sampling, the consensus of participants from the “Microareias 2012” workshop (Sabino et al., 2014) was that sand (one set wet, one set dry) should be collected in three equidistant points along the beach, attempting to represent the beach as a whole. This was agreed to be sufficient, considering the cost of performing multiple analysis for a single beach.
- ***Depth/ position of sample***
  - Surface water may be easier (and safer) to collect manually at some bathing sites than sub-surface water, and is likely to be the most representative part of the sea that most beach users are exposed to.
  - On many UK beaches, the sand surface is the most frequently used part of the beach; however fungi may be more concentrated in the sub-surface. In the Mycosands initiative (Brandão, et al., 2021), samples for fungal analysis are obtained from 5 – 10 cm depth.
- ***Methods used for measuring AMR in bacteria and fungi***
  - Two separate approaches to AST were identified:

        - (1) AST of isolates - where a specific species of bacterium isolated from the BW sample is interrogated with a suite of antibiotics to determine its susceptibility. This approach offers limited insight into the AMR present in a BW sample; for example if the desired target is not present, then no data is obtained.
        - (2) Enumeration of resistant bacteria - where a chosen sub-set of all microorganisms is selectively cultured from the BW sample on a chosen antibiotic selective media to determine the prevalence of particular antibiotic resistance phenotypes within the sample. This technique provides insight into the phenotypic resistance carried by a suite of microorganisms which is better suited to a national surveillance programme than assessing AST of individual isolates that might not be abundant or present.
    - Amplification-based methods (e.g. qPCR,) provide opportunities to rapidly analyse multiple targets simultaneously, for example they could detect antibiotic resistance genes (ARGs) in non-culturable bacteria and fungi and can be applied to complex samples with many different species. Whilst amplification-based methods are restricted to a predetermined set of target ARGs (or other types of genes), high-risk genes are fairly well understood, enabling adequate identification of hotspots. If required, the limiting factor of predefined targets can be partially overcome by using qPCR assays with hundreds of genes (high throughput qPCR), but this also comes with increased costs and may not be feasible for large-scale routine monitoring of environmental AMR.
    - Additional work is needed to establish suitable proxy/indicator fungi for monitoring, and to identify AFR in the indicator fungi. Once a few proxy/indicator fungi are agreed upon, then standardised practices can be developed for applying antifungal susceptibility testing to field samples, or developing molecular assays. Additional work will need to be done to simultaneously identify AFR in the indicator fungi.

Options

Several options for future work were identified in this review:

- The relationship between contaminated water and contaminated sand is poorly understood. If a good understanding of this relationship was developed, water monitoring would enable the predictions of the hysteresis of the pathogens in the sand if such a relationship exists.
- Separate work is also recommended to identify suitable proxy/indicator fungi for monitoring purposes that represent antifungal resistance.
- Future research is needed to better understand the main sources of AMR in coastal environments (e.g. are AMR microbes being delivered to the coastal environment, or is AMR developing in these environments). A subset of the water/sand samples analysed for AMR could be retained to test for concentration of antimicrobial agents, if required.

- The development of an automated in-field sampler could allow a high-frequency, near real-time assessment of *E. coli*/ AMR in BWs, although we note such technology is still under development).

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