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1 **Atypical *Aeromonas salmonicida vapA* type V and *Vibrio* spp.**
2 **are predominant bacteria recovered from ballan wrasse (*Labrus***
3 ***bergylta* A.) in Scotland**

4
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14 **Running page head:** Atypical *Aeromonas salmonicida* in Scottish ballan wrasse (*Labrus*
15 *bergylta* A.)

16 **Abstract**

17 Healthy and / or moribund farmed and wild ballan wrasse, *Labrus bergylta* (>0.5 to 900 g)
18 were sampled from hatcheries (n= 2) and Atlantic salmon cage sites (n= 8) in Scotland
19 between February 2016 and October 2018. Less than half of the sampled individuals (n= 43,
20 32.3 %) had been vaccinated (autogenous polyvalent vaccine; dip and / or injection) against
21 atypical furunculosis (type V and VI) while 20 (15.0 %) fish were not vaccinated and the
22 rest (70 individuals, 52.7 %) were of unknown vaccination status. Swab samples from skin
23 lesions, gill, liver, spleen and kidney were inoculated onto a variety of bacteriological agar

1 plates and bacteriology identification and sequencing analysis was performed on significant
2 bacterial colonies. Atypical *Aeromonas salmonicida* (aAs) *vapA* type V was the predominant
3 bacterial species (70/215 bacteria isolates; 32.5 % of bacteria samples – 43/117 positive
4 individual fish; 36.8 %) isolated in this survey followed by *Vibrio* species which were the
5 most geographically prevalent bacteria. *Photobacterium indicum/profundum* was also
6 isolated from *L. bergylta* for the first time during this study. The collection of these bacterial
7 isolates provides useful information for disease management. Identifying the aAs isolates
8 involved in disease in ballan wrasse could provide vital information for improving / updating
9 existing autogenous vaccines.

10 **Key words:** atypical *Aeromonas salmonicida*, ballan wrasse, health survey, cleaner fish

11

12 **1. INTRODUCTION**

13 Ballan wrasse (*Labrus bergylta* Ascanius, 1767) and lumpsucker (*Cyclopterus lumpus*
14 Linnaeus, 1758) are two cleaner fish species that have been intensively used by the Atlantic
15 salmon (*Salmo salar* L.) farming industry as an alternative means to control sea lice
16 (*Lepeophtheirus salmonis* Krøyer, 1837). The latter is an ectoparasite of the Northern
17 hemisphere that causes major economic and welfare implications on this aquaculture
18 industry (Treasurer 2012, Skiftesvik et al. 2013). Initially wild wrasse species (cuckoo;
19 *Labrus mixtus* L., corkwing; *Symphodus melops* L., goldsinny; *Ctenolabrus rupestris* L. and
20 rockcook; *Centrolabrus exoletus* L.) were used in salmon cages. However, the demand for
21 fish and biosecurity concerns regarding the health status of wild deployed cleaner fish along
22 with sustainable supply of wild wrasse on cage sites has led to rearing of ballan wrasse in
23 Scotland since 2010.

24 Ballan wrasse are known to be susceptible to bacterial (*e.g.* atypical strains of *Aeromonas*
25 *salmonicida* (aAs) and *Vibrio* spp.) (Biering et al. 2016, Gulla et al. 2016, Brooker et al.

1 2018), parasitic (*e.g.* amoebic gill disease (AGD) (Karlsbakk et al. 2013) and viral (*e.g.*
2 piscine myocarditis virus; PMCV) (Scholz et al. 2018) diseases. Various *Vibrio* species
3 (*Vibrio anguillarum*, *V. ordalii* and *V. splendidus*) have also been isolated from diseased
4 (symptomatic to vibriosis) ballan wrasse but only *V. anguillarum* originally isolated from
5 Atlantic salmon caused high mortalities (up to 60%) in ballan wrasse under experimental
6 conditions (Biering et al. 2016). Thus, pathogenicity of *Vibrio* species in ballan wrasse is not
7 clear. Atypical strains of the bacterium *Aeromonas salmonicida* (*As*) have also been reported
8 during mortality events of ballan wrasse in Norway (Bornø and Gulla 2016). An additional,
9 outer membrane - the paracrystalline surface protein (A-layer protein) - plays an important
10 role in the infection of the host as well providing protection for the bacterium by resisting
11 host response processes (Udey & Fryer 1978, Munn et al. 1982, Kay & Trust 1991, Daly et
12 al. 1996). The gene that encodes this protein is known as the virulence array protein A (*vapA*)
13 and 23 A – layer (*vapA*) types of *As* were identified by sequencing the hypervariable region
14 of the gene (Gulla et al. 2016, Gulla et al. 2019). Furthermore, type V and VI found to be
15 related with cleaner fish species *L. bergylta* and *C. lumpus* in Scotland and Norway (Gulla
16 et al. 2016, Gull et al. 2019). Cohabitation and intraperitoneal (i.p.) injection with a*As* (one
17 strain of each type V and VI used) successfully induced disease and morbidities during
18 experimental conditions (Biering et al. 2016). Specifically, type V was found to cause the
19 highest morbidities, suggesting that atypical strains are virulent to the species *L. bergylta*
20 (Biering et al. 2016).

21 Information related to mortality events including causative agents / pathogens of cleaner fish
22 such as ballan wrasse in Scotland is limited (Treasurer 2012). Bacterial disease outbreaks
23 have been speculated to be related with a*As* on commercial sites in Scotland but there are
24 very few reports available. Prevention of disease outbreaks through vaccination is needed
25 for the species *L. bergylta* in order to improve their welfare in aquaculture and to enable
26 their efficient performance as cleaner fish in salmon pens. Health screening and

1 characterisation of these bacterial pathogens is essential for successful vaccine formulation.
2 Thus, in the current study, a real-time health survey was conducted to determine the bacterial
3 pathogens present in both farmed ballan wrasse hatcheries and Atlantic salmon cage sites
4 (wild and farmed fish) in Scotland between February 2016 and October 2018, in order to
5 identify the most prevalent bacterial pathogens of ballan wrasse.

6

7 **2. MATERIALS AND METHODS**

8 **2.1. Bacterial identification**

9 Healthy and / or moribund farmed and wild ballan wrasse (> 0.5 to 900 g, n= 133) were
10 sampled from hatcheries (n= 2) and Atlantic salmon cage sites (n= 8) in Scotland between
11 February 2016 and October 2018. Less than half of the sampled individuals (n= 43, 32.3%)
12 had been vaccinated with an autogenous polyvalent vaccine (Ridgeway Biologicals Ltd)
13 which included atypical furunculosis (type V and VI). From those, 42 individuals were
14 originating from site A and had been vaccinated by two immersions (prime; ca. 0.5 g and
15 booster vaccination; ca. 2 g) and / or injection at ca. 15 g and all the fish in the batches from
16 which these individuals originated from had been vaccinated with the same practice. There
17 was one more individual that had been vaccinated however, no information has been
18 provided and whether the rest of the cleaner fish on site had been vaccinated. Furthermore,
19 16 (12.0%) fish were unvaccinated and the rest of the individuals (n=74, 55.6%) were of
20 unknown vaccination status (farmed or wild origin). Swab samples from skin lesions, gills,
21 liver, and kidney were inoculated onto Marine Agar, Tryptone Soya Agar (TSA), and TSA
22 + 5% Defibrinated Horse Blood + 1.5% NaCl, and incubated at 22°C for 24 – 72h for primary
23 bacterial isolation. Pure colonies were then picked on the basis of morphology,
24 predominance and prevalence, streaked onto fresh plates and incubated, as described before,
25 for purification. Passaged isolates were then tested by Gram's staining (bioMerieux) and

1 Catalase (catalase reagents. VWR UK)/ Oxidase (oxidase strips, Oxoid UK) tests for purity
2 confirmation and primary identification.

3 **2.2. Molecular analysis**

4 Bacterial DNA was extracted using genesig® Easy DNA/RNA Extraction Kit (Genesis)
5 according to the manufacturer's instructions. Bacterial species identification was performed
6 on the samples by targeting the subunit B protein of DNA gyrase (topoisomerase type II) –
7 *gyrB* gene (Yamamoto et al. 2000) and V3-V4 hypervariable region of the *16S rRNA* gene
8 (Klindworth et al. 2013) (Table 1). PCR reactions consisted of each primer at 10 µM, 1 unit
9 of GoTaqG2 master mix (Promega), 5 µL of DNA sample and milliQ water to reach a final
10 reaction volume of 25 µL. The following thermal cycling conditions were used in G-storm
11 thermocycler: 1 cycle at 95°C for 5 min, 35 cycles at 95°C for 30 sec, 55°C (*gyrB*) and 44°C
12 (*16 rRNA*) for 30 sec and 73°C for 1 min, followed by 1 cycle at 73°C for 7 min. The PCR
13 product was then purified using QIAquick PCR Purification Kit (Qiagen, Germany) as
14 described by the manufacturer and 3.5 µL of the clean-up were mixed with 2.5 µL of each
15 of the forward and reverse primers in a separate nuclease free Eppendorf tube and 1.5 µL of
16 nuclease free water to reach a total volume of 7.5 µL. Products sent for sequencing to GATC
17 (Eurofins) and obtained sequences were compared to known sequences using an in silico
18 nucleotide alignment tool 'BLAST' (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Isolates that
19 were recognised as presumed *aAs* by the naked eye; small, friable colonies, non-motile
20 coccobacilli (prior to 16S confirmation) or by PCR testing (16S) were then confirmed to be
21 *aAs* using the A-layer membrane - *vapA* primer sets (Gulla et al. 2016) to determine the *vapA*
22 strain type (Table 1) as described by Gulla et al. (2016). The PCR product was then purified
23 using QIAquick PCR Purification Kit (Qiagen, Germany) and samples mixed with forward
24 and reversed primers as described above and sent for sequencing to GATC (Eurofins).
25 Sequences were analysed with Clustal Omega at EMBL-EBI (<https://www.ebi.ac.uk/>)
26 against the published type strain sequences.

1 3. RESULTS

2 Among 327 samples (n= 133 individual fish) collected from all sites, 192 (n= 117 individual
3 fish) had visible colonies which were identified using biochemical (Gram staining, catalase
4 and oxidase test) and molecular (*gyrB* and *16S rRNA* sequencing, *aAs vapA* assay) analysis
5 (Table 2).

6 Atypical *As* was detected in 70 (43/117 positive individual fish; 36.8%) out of 215 bacteria
7 isolates (32.5 % of bacteria samples). Following *vapA* gene screening the majority of the
8 *aAs* colonies belonged to *vapA* type V with the exception of 2 individuals that were positive
9 to type VI from sites E and J. Atypical *As* were the most prevalent of the pathogenic bacteria
10 species during this survey followed by *Vibrio* spp. and *Aliivibrio* spp.– *Vibrio ichthyoenteri*.
11 *Vibrio splendidus*, *Vibrio tasmaniensis* *Aliivibrio logei*, *Aliivibrio salmonicida*, *Allivibrio*
12 *finisterrensis*– (69/215; 32.1% vibrio isolates and 55/116; 47.4% positive individuals)
13 (Figure 1 and 2). The bacteria prevalence per site is shown in Figure 1 and 2. Atypical *As* is
14 most prevalent in sites A, C and E; note that site E is not presented in a pie chart as *aAs* (2
15 isolates *vapA* V) were the only bacteria recovered from a single individual in a single
16 sampling event, while *Vibrio* spp. were the most prevalent in sites B, I and J (Figure 1 and
17 2). The majority of *aAs vapA* type V had been isolated from liver (25) and kidney (32), while
18 the least *aAs* recovery was noted from fin (5), skin (4) and gill (2) samples. Also the *aAs*
19 *vapA* type VI isolates (2) were from skin, liver and kidney of deployed ballan wrasse. The
20 54.3% of the *aAs* (*vapA* type V and VI) isolates recovered were from vaccinated fish (21 /43
21 individuals; 47.7%) and the majority (20 / 21) were originating from site A. Nearly half of
22 the vaccinated individuals (20/42; 47.6 %) were positive for the bacterium (*aAs*).
23 Furthermore, 8.6% of the *aAs* were from non vaccinated individuals (4 / 43; 9.1%) and the
24 remaining *aAs* isolates (37.1%) were recovered from fish with unknown vaccination status
25 (19 / 43; 43.2%).

1 Apart from aAs another 100 (46.0 %, 82/117 individuals; 70.0%) isolates were identified
2 and could potential be pathogenic in farmed ballan wrasse as they are known fish pathogens.
3 These were *Aliivibrio finisterrensis*, *Aliivibrio* sp., *Aliivibrio salmonicida*, *V. anguillarum*,
4 *Vibrio atlanticus*, *V. ichthyoenteri*, *V. lentus*, , *V. splendidus*, *V. tasmaniensis*, *T.*
5 *ovolythicum*, *T. soleae*, *T. diecentrachi* and *Pseudomonas putida*, *Pseudomonas*
6 *psychrophila*, *Pseudoalteromonas* sp. and *M. viscosa*. The above were recovered from gills,
7 fins, liver, spleen and head kidney except *T. ovolythicum*, *T. soleae*, *T. diecentrachi*,
8 *Pseudoalteromonas* sp. and *Moritella viscosa* which were isolated only in at least one of the
9 following skin lesions, gills and / or fins.

10 No external disease signs were noted on the fish with a few exceptions. The majority of fish
11 sampled from site A had fin rot and fish were lethargic. Internally, in some cases, the
12 following clinical signs were observed: granulomas in the liver and/or kidney, ascites and
13 empty gut which in some individuals was red. A suspected atypical As outbreak was active
14 during the samplings on site A. Vaccination status of the fish did not significantly affect
15 external or internal gross pathology for site A. Furthermore, a single wild individual from
16 site C had a heavy skin ulcer in the flank and 3 individuals sampled at site D had pale gills,
17 empty guts and granulomas in the organs. Co-occurrence of aAs and *Vibrio* spp. was noted
18 for sites A, B and C in 5, 2 and 1 individual, respectively. Bacteriology analysis also showed
19 that the individual from site C was positive for *V. splendidus* in the liver and *P. indicum* on
20 the skin and kidney, while from three individuals (site D) *Vibrio* spp. and *Shewanella* sp.
21 was isolated from liver and *P. indicum* from kidney.

22 Non-pathogenic bacteria also present in the samples included: *Arthrobacter* sp., *Bacillus*
23 *simplex*, *Chryseobacterium* sp., *Colwellia* sp., *Glaciecola punicea*, *Leucothrix mucor*,
24 *Oleispira antartica*, *Pianococcus* sp., *Planococcus* sp., *P. indicum*, *Phot. phosphoreum*,
25 *Phot. profundum*, *Photobacterium* sp., *Polaribacter irgensii*, *Polaribacter* sp.,
26 *Pseudoalteromonas marina*, *Pseudomonas fragi*, *Psychrobacter marinicola*, *Psychrobacter*

1 *nivimaris*, *Psychrobacter glacincola*, *Shewanella* sp, *Staphylococcus warneri*, *Vibrio*
2 *tapetis*. *Photobacterium indicum* was also isolated from 4 locations, sites B, C, D and J with
3 prevalence of 21.4% (3 / 14 individuals), 26.3% (5 / 19 individuals), 20.0% (1 / 5 individuals)
4 and 22.2% (2 / 9) respectively. The sequencing data in comparison with BLAST searches
5 gave high species similarity (97-99%) for all the above sequences.

6 **4. DISCUSSION**

7 In this study a bacteriology health survey was conducted at ballan wrasse hatcheries (n= 2)
8 and Atlantic salmon sea sites (n= 8), where wild and farmed wrasse have been deployed in
9 Scotland, for more than 2.5 years. The majority of the sampled ballan wrasse did not have
10 external sign of diseases with few exceptions for fish from site A, a single wild individual
11 sampled at site C and 3 individuals sampled at site D. The predominant pathogenic bacterial
12 species identified after bacteriology assessment and sequencing analysis (16S rRNA and
13 *gyrB*) was *aAs vapA* type V. In corroboration with Gulla et al. (2015) *aAs* type V appears
14 here to be the most predominant strain in Scotland whereas strain type VI appears to be
15 mainly in Norway.

16 Atypical strains of *As* were isolated from 6 out of 10 sites that took part in this health
17 screening survey and the bacterium was the most prevalent in 4 out of 10 sites. The results
18 from this survey suggest that *aAs* was the most prevalent bacterial species at these sites
19 between February 2016 and October 2018. It is worth noting that the *aAs vapA* type VI
20 isolates in this survey originated from two deployed individuals in sea cages and were
21 speculated to be related to a secondary infection following immune suppression and /or be
22 indicative of virulence adaptation of type VI against the host. Although currently, antibiotic
23 treatments are successfully applied for controlling disease outbreaks in hatcheries and cage
24 sites, *As* is known carry plasmids linked with antibiotic resistance. For instance *As* resistance
25 to oxytetracycline, tetracycline and chlorafenicol has been previously reported (Adams et al.

1 1998, L'Abée-Lund & Sørum 2002, Sørum et al. 2003). Autogenous vaccines against
2 atypical furunculosis are also used in cleaner fish hatcheries as licenced vaccines are not
3 available. Further characterisation of these *vapA* types through partial and / or whole
4 sequencing (e.g. pulsed field electrophoresis; PFGE and next generation sequencing; NGS)
5 can be helpful on identifying differences within the *aAs* strains that belong to the same type.
6 This information can then be used to improve/update existing autogenous vaccines.

7 In addition, interestingly, 47.7% of vaccinated individuals (21 /43 individuals) were positive
8 for the bacterium (*aAs*). Given that the majority of the positive individuals (20 / 21) had been
9 vaccinated in the same site (site A), there is a strong suggestion that the vaccination did not
10 appear to prevent infection by *aAs* in these fish. Protection may be influenced by the
11 immunisation regime used as well as the isolates included in the vaccine. Both immersion
12 and injection vaccination were being used for ballan wrasse during the time frame of this
13 study but little is known about the efficacy of either administration routes of the vaccine.
14 These findings support the importance for assessing immunocompetence of ballan wrasse
15 and vaccinating the individuals at an appropriate size so that uptake and immune
16 responsiveness to vaccine antigens is optimal. Administration of vaccines at earlier life
17 stages of fish can lead to immunosuppression (Joosten et al. 1995, Covello et al. 2013). The
18 majority of the individuals sampled did not show external/gross signs of disease. However,
19 clinical signs and histopathological changes following infection by the bacterium in ballan
20 wrasse have not yet been described, even though experimental trials have been conducted.
21 For instance, Biering et al. (2016) showed mortalities (75 – 89 % and 51%, respectively) in
22 juvenile ballan wrasse (50 g) infected with *aAs* either through intraperitoneal injection or
23 cohabitation. Currently, there are not known reports of disease in farmed Atlantic salmon
24 related with these *aAs* strains (type V or VI) and co-infection did not occur during
25 cohabitation with diseased wrasse (Gravningen et al. 1996, Treasurer 2012). Moreover,

1 cultured Atlantic salmon are protected against typical *As* as routine vaccination takes place
2 (Sommerset et al. 2005, Midtlyng 2014).

3 Bacteria belonging to the *Vibrio* and *Aliivibrio* genus (*V. ichthyoenteri*, *V. splendidus*, *V.*
4 *tasmaniensis*, *Aliivibrio salmonicida*.) known to be pathogenic to other fish species were
5 recovered from tissue samples of ballan wrasse in this survey in 8 out of 10 sites. *V.*
6 *splendidus*, *A. logei*, *A. wodanis* and *V. tapetis* have also been isolated from cleaner fish in
7 Norway (Hjeltnes et al. 2018). However, *Vibrios* are universal marine bacteria and three
8 species, *V. splendidus*, *V. ichthyoenteri* and *V. pacinii* may be part of the gut flora of ballan
9 wrasse and goldshinny wrasse (*Ctenolabrus rupestris* L.) (Birkbeck & Treasurer 2014).
10 Thus, isolation of *V. splendidus* and *V. ichthyoenteri* during the survey may have been due
11 to accidental eruption of the gut wall, even though there is not such report. Furthermore, the
12 presence of *Vibrio* species in the organs (liver and kidney) may have occurred at low levels
13 that the immune system could cope with. Nonetheless these bacteria may still pose a threat
14 as opportunistic pathogens for ballan wrasse in commercial production or during stressful
15 events in cage sites. Similarly, ballan wrasse experienced low (10 – 20%) or no mortalities
16 from *V. anguillarum* isolated from ballan wrasse during bath and cohabitation challenge,
17 while i.p. injection of an Atlantic salmon strain was more virulent (50 – 60 %) (Biering et
18 al. 2016). On the other hand lumpstickers are known to be susceptible to *V. anguillarum*, *V.*
19 *ordalii* and *V. splendidus* (Bornø & Gulla 2016). Taking the above into consideration, it is
20 not known if ballan wrasse can act as carries of these bacteria and infect lumpfish during
21 cohabitation in sea pens and *vice versa*.

22 A range of non – pathogenic bacteria known to ballan wrasse were recovered during this
23 study. From those *V. tapetis*, *T. dicentrarchi* and *P. indicum/profundum* are worth
24 mentioning. *Vibrio tapetis* is a known pathogen for bivalves, clam species and Atlantic
25 halibut (Reid et al. 2003, Paillard, 2004). Although, juvenile ballan wrasse (approx. 30 g)
26 were not susceptible to these bacteria species during cohabitation challenge and only i.p.

1 injected shedder fish experienced mortalities (Gulla et al. 2017), it is not known if larvae or
2 younger age juvenile ballan wrasse (<30 g) can be susceptible to the bacteria under rearing
3 conditions. *Tenacibaculum dicentrachi* was isolated from ballan wrasse during this survey
4 and to the best of the author's knowledge this is the first time that *T. dicentrachi* was
5 recovered from ballan wrasse in Scotland. The bacterium belongs to the Family
6 *Flavobacteriaceae* and *Tenacibaculum* spp. are ubiquitous bacteria of the marine
7 environment with a few members of the genus related with fish diseases. For instance, *T.*
8 *dicentrachi* was first isolated from European sea bass (*Dicentrarchus labrax*) in Spain
9 (Piñeiro-Vidal et al. 2012) and is now a rapidly emerging pathogen of farmed Atlantic
10 salmon in Chile (Avendaño-Herrera et al. 2016). In Norway, isolates of the genus have been
11 recovered from skin ulcers from salmonids and non-salmonid species (Olsen 2017).
12 Understanding the pathogenicity of this bacterium in individual ballan wrasse is important
13 considering that the closely related species of the genus are an emerging bacteria pathogen
14 for salmonids. Cohabitation with diseased salmon can lead to disease transmission between
15 hosts.

16 *Photobacterium indicum/profundum*, also reported in this screening, has not previously been
17 associated with fish disease outbreaks but has been isolated from moribund lobster and
18 associated with stress (Basti et al. 2011). A number of isolates (7/151; 6/82 individuals) were
19 recovered from diseased ballan wrasse in this study which might be indicative of a secondary
20 infection after individuals had been infected with aAs. Recently, *Photobacterium* sp., were
21 recovered from lumpsuckers experiencing mortalities due to *Pseudomonas anguilliseptica*
22 under rearing conditions in Scotland (Treasurer & Birkbeck 2018). Further investigation is
23 needed regarding the pathogenicity and transmission between hosts in order to understand
24 the importance of this bacterium in cleaner fish hatcheries and deployment sites.

25 Overall, aAs was the most prevalent bacterial species isolated from ballan wrasse on the farm
26 sites considering the number of individuals sampled in total, followed by *Vibrio* species

1 which were the most geographically prevalent bacteria. Understanding the prevalence of
2 these pathogens is vital for mitigating disease outbreaks by optimising fish husbandry and
3 biosecurity practices. Furthermore, the collection of these bacterial isolates provides useful
4 information for disease management. Also, characterisation of the *aAs vapA* types could
5 provide important information for improving/updating existing autogenous vaccines.

6

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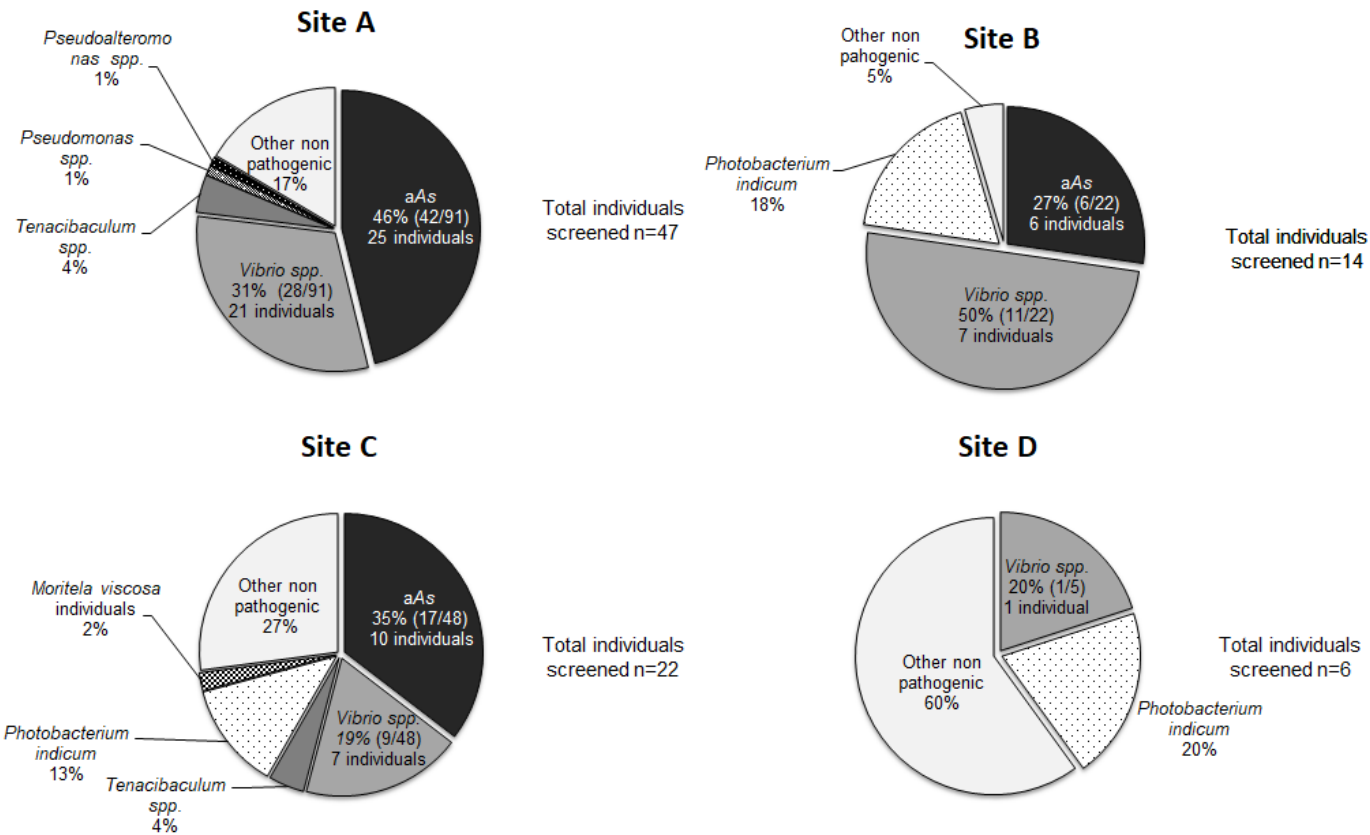
1 Table 1 List of primers used for bacteria species identification.

Primer	Primer name	Target gene	Annealing (°C)	Application	Reference
CAGGAAACAGCTATGACCA YGSN GG	UP -1E	<i>gyrB</i>	60	PCR	Yamamoto et al., 2000
NGGNAARTTYRA					
TGTAAAACGACGGCCAGTGCN GGRT	APrU	16S	44	PCR	Klintworth et al., 2013
CYTTYTCYTGRCA					
AGAGTTTGATCMTGGC	Bact-0008	16S	44	PCR	Klintworth et al., 2013
CCGTCAATTCMTT TGAGTTT	Bact-0907				
CTGGACTTCTCCACTGCTCA	F2	<i>vapA</i>	53	PCR and sequencing	Lund et al., 2003b
ACGTTGGTAATCGCGAAATC	R3				Gulla et al., 2016

1 Table 2. Standard bacteriology tests (Gram stain, shape, catalase, oxidase) on pathogenic
 2 bacteria isolated from skin lesions, gills, liver and kidney swabs of moribund or recovered
 3 ballan wrasse (*Labrus bergylta*; >0.5 to 900 g) during disease outbreaks in hatcheries and
 4 salmon sea cage sites in Scotland between February 2016 and October 2018. Sequencing
 5 similarity represents blasts results from *16S* and *gyrB* sequencing.

Bacteria species	Shape	Catalase (-/+)	Oxidase (-/+)	Sequencing similarity (%)
atypical <i>Aeromonas salmonicida</i>	Bipolar rods	-	+	99-100
<i>Vibrio spp.</i>	Curved rods	+	+	99-100
<i>Vibrio (Allivibrio) salmonicida</i>	Curved rods	+	+	99-100
<i>Vibrio tasmaniensis</i>	Rods	+	+	99
<i>Vibrio splendidus</i>	Short rods	+	+	99-100
<i>Vibrio logei</i>	Cigar like rods	+	-	99
<i>Vibrio splendidus</i>	Rods	+	+	99-100
<i>Vibrio ichthyoenteri</i>	Thin rods	+	+	96-100
<i>Vibrio sp</i>	Rods	+	+	100
<i>Vibrio anguillarum</i>	Bipolar rods	-	+	99
<i>Tenacibaculum dicentrachi</i>	Curved rods	+	+	99
<i>Tenacibaculum solea</i>	Slender rods	-	+	100
<i>Tenacibaculum ovoliticum</i>	Filamentous rods	+	-	100
<i>Pseudomonas spp.</i>	Short bipolar rods	+	+	99-100
<i>Pseudoalteromonas spp.</i>	Bipolar rods	-	+	100
<i>Moritella viscosa</i>	Slightly curved rods	+	+	99
<i>Flavobacterium frigidarium</i>	Chaining cocci-bacillus	+	-	97-100

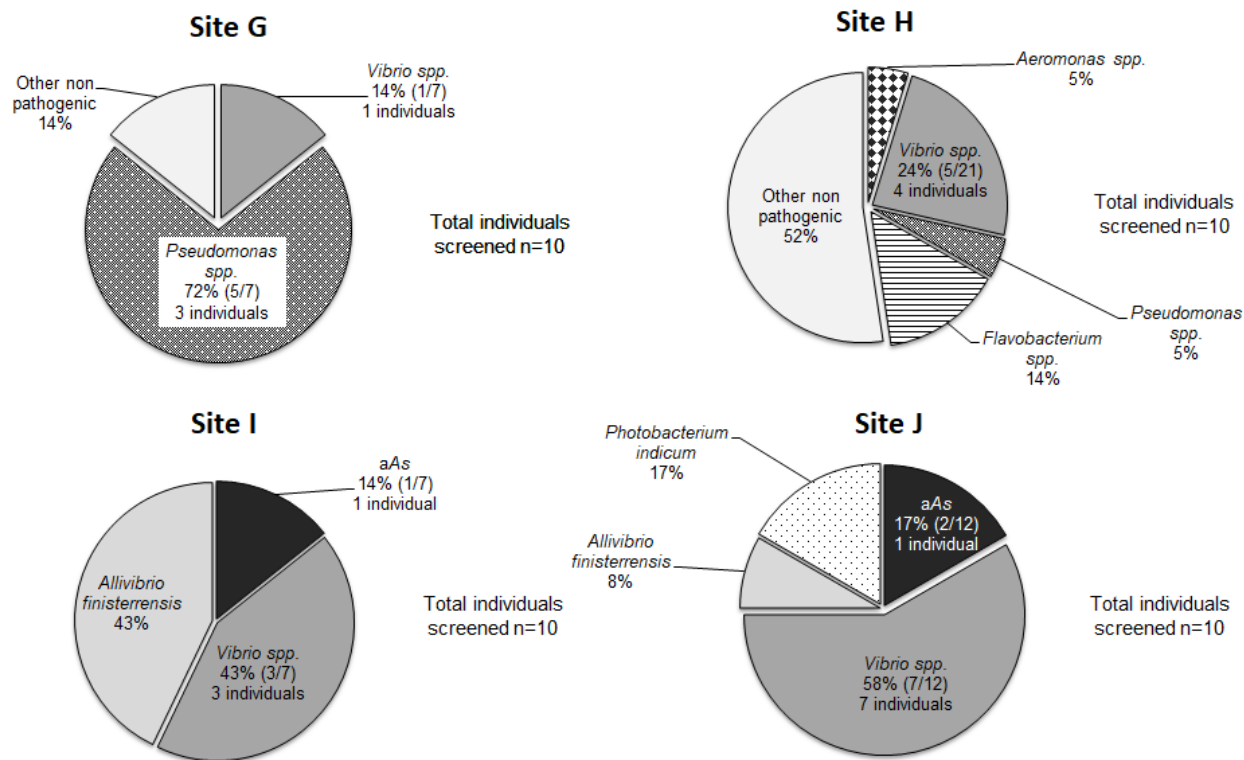
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2 **Figure 1.** Bacteria percentage recovery from 4 out of 10 sites during a health screening survey for ballan wrasse in Scotland between 2016 and 2018
 3 (Part 1). Swabs from skin lesions, gills, liver and kidney plated on variety of agar plates (Marine Agar, Tryptone Soya Agar (TSA), and TSA + 5%

- 1 Defibrinated Horse Blood + 1.5% NaCl) for phenotypic and sequencings identification. Pie chart for Site E is not shown as only atypical *Aeromonas*
- 2 *salmonicida* was isolated in a single sampling (2/2). Site F, single sampling point with no bacteria recovery from individuals sampled.



- 3
- 4 Figure 2. Bacteria percentage recovery from 4 out of 10 sites during a health screening survey for ballan wrasse in Scotland between 2016 and 2018 (Part
- 5 2). Swabs from skin lesions, gills, liver and kidney plated on variety of agar plates (Marine Agar, Tryptone Soya Agar (TSA), and TSA + 5% Defibrinated

- 1 Horse Blood + 1.5% NaCl) for phenotypic and sequencings identification. Pie chart for Site E is not shown as only atypical *Aeromonas salmonicida* was
- 2 isolated in a single sampling (2/2). Site F, single sampling point with no bacteria recovery from individuals sampled.