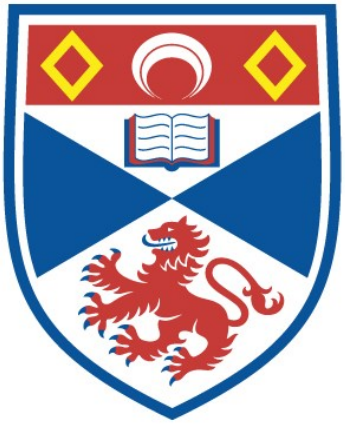
HYDROZOAN JELLYFISH AND THEIR INTERACTIONS WITH SCOTTISH SALMON AQUACULTURE

Anna Helen Kintner

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews

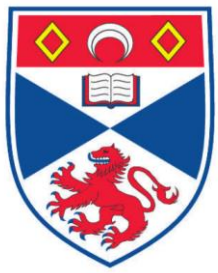


2016

Hydrozoan jellyfish and their interactions with Scottish salmon aquaculture

Anna Helen Kintner

Submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy



School of Biology, University of St Andrews

March 2016

Abstract

Medusozoan jellyfish (Classes Scyphozoa and Hydrozoa) have gained a degree of worldwide notoriety in the last fifteen years, particularly as anthropogenic influences such as climate change and overfishing push some ecosystems toward their advantage (Lynam et al. 2005, Purcell and Arai 2001, Purcell et al. 2007, Purcell 2012, Flynn et al. 2012, Dawson et al. 2014). Accordingly, both the lay and scientific media have paid a good deal of attention to jellyfish bloom phenomena and their impacts on human activities, but the bulk of this attention has been devoted to larger, visually obvious species of Class Scyphozoa. Only recently have their smaller cousins, the hydrozoans, come to be recognized as potentially problematic. This thesis examines population ecology of hydrozoan medusae (hydromedusae) and their implications for salmon aquaculture in Scotland.

My review of available literature has found hydrozoans to be a recognized – though under-studied – problem for Scottish salmon (Chapter 1, Prospective monitoring of hydromedusa populations at salmon aquaculture facilities). Typically, hydrozoan populations at salmon farms have been discussed in the scientific literature only in the context of extremely dense visible blooms or in the wake of major mortality incidents. This retrospective, rather than prospective, approach has left a dearth of knowledge pertaining to hydromedusan interactions with farmed fish, with both fish welfare and industry economics vulnerable to future blooms.

This thesis sought to build a basis for the goals of prediction, avoidance, and mitigation of harmful hydrozoan jellyfish blooms. First and foremost, this required the development of a prospective time-series dataset of hydromedusan occurrences at salmon farms (Chapter 2, Bacterial genera biodiversity in three medusozoan species in Shetland). To this end, four farms were recruited as participants across a three-year survey. Weekly plankton tow-based sampling at these sites identified which hydrozoan species could be expected to produce blooms, the seasonality of such blooms, and the pathological sequelae that could be expected in salmon after exposure to such blooms. Following one particularly dramatic bloom, a spike in gill pathologies in salmon was observed, followed by a spike in overall mortality and the eventual loss of up to £2.5 million value as the fish were humanely culled.

This survey also demonstrated that hydromedusan blooms are usually spatially and temporally patchy, limiting the opportunities for geographically-encompassing predictive power. Instead, individual aquaculture facilities may require site-specific risk assessment and planning frameworks to monitor and cope with blooms. Potential methods for continued basic monitoring and a mitigation strategy based on minimizing contact between fish and high-density blooms are suggested.

A second mitigation goal examined the theory that medusae may act as vectors for microbial pathogens, particularly *Tenacibaculum maritimum* (Ferguson et al. 2010, Delannoy et al. 2011; Chapter 3). Sampling methods designed to target *T. maritimum* were employed with the aim of determining its distribution and role as a symbiont in various life stages of medusozoan species. While *T. maritimum* itself was not observed, a number of other fish pathogens were found in close association with several species. This included *Aeromonas salmonicida*, known to cause furunculosis in aquaculture of both salmon and trout (Nomura et al. 1992). Further work is required to piece together the nature of these associations.

Finally, Chapter 2 identified a particular hydrozoan genus, *Obelia*, as a likely significant contributor to blooms at salmon aquaculture sites. One of its species, *O. geniculata*, has a widely distributed and well-recognized benthic colonial life stage (called the hydroid stage) in Scottish nearshore sublittoral environments. In attempting to sample these hydroids from previously well-colonized sites in Shetland in late 2012, it became apparent that a severe local reduction in the benthic population was taking place. This allowed for the opportunity to study phylogeographic population structure – i.e. the boundaries of its gene pool(s) in Scottish waters and its potential for dispersal during one seasonal reproductive period – using a molecular study of the mitochondrial cytochrome oxidase subunit I (mtCOI) gene (Chapter 4, Phylogeographic analysis of *Obelia geniculata* populations in the north of Scotland). In sampling immediately after the observed dieback, *O. geniculata* was found to follow a south-to-north pattern of genetic grouping, as well as a confirmed dieback. However, this pattern disappeared in samples collected after the population had recovered, probably due to the immigration of genetically novel individuals. This finding, in conjunction with the spatial-temporal patchiness found in the medusa bloom stage, suggests the importance of the larval stage as the primary stage for dispersal in the plankton. This study was also able to compare present population genetic data with a set of *O. geniculata* mtCOI

data collected between 1998 and 2002. The combined data potentially show a high degree of mixing across a number of North Atlantic regions, including Icelandic and North American sites. Further investigation will be required to discern whether this pattern is temporally based (i.e. artefact of 15 years' elapsed time in opportunities for population mixing), or whether ecological, anthropogenic, or combined mechanisms are facilitating rapid transport of propagules to yield a well-mixed population.

Further work in refining prediction and mitigation is still needed, as are effective veterinary interventions in the event of blooms. Continued study into the ecological patterns of colonization and dispersal may help to minimize exposure to blooms, by helping to assess site-based risks. This research forms the basis for such studies into hydrozoan interactions with salmon farms in Scotland, and how the industry might seek to minimize their impacts.

Declarations

1. Candidate's declarations:

I, Anna Kintner, hereby certify that this thesis, which is approximately 40,000 words in length, has been written by me, and that it is the record of work carried out by me, or principally by myself in collaboration with others as acknowledged, and that it has not been submitted in any previous application for a higher degree. I was admitted as a research student in October 2011 and as a candidate for the degree of PhD in May 2012 the higher study for which this is a record was carried out in the University of St Andrews between 2011 and 2016.

Date Signature

2. Supervisor's declaration:

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of PhD in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date Signature

3. Permission for publication:

In submitting this thesis to the University of St Andrews I understand that I am giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. I also understand that the title and the abstract will be published, and that a copy of the work may be made and supplied to any bona fide library or research worker, that my thesis will be electronically accessible for personal or research use unless exempt by award of an embargo as requested below, and that the library has the right to migrate my thesis into new electronic forms as required to ensure continued access to the thesis. I have obtained any third-party copyright permissions that may be required in order to allow such access and migration, or have requested the appropriate embargo below.

The following is an agreed request by candidate and supervisor regarding the publication of this thesis:

PRINTED COPY

b) Embargo on all or part of print copy for a period of 2 years (maximum five) on the following ground(s):

- Publication would preclude future publication

ELECTRONIC COPY

b) Embargo on all or part of print copy for a period of 2 years (maximum five) on the following ground(s):

- Publication would preclude future publication

Candidate's signature

Supervisor's signature

Contents

Abstract.....ii

Declarations.....v

Acknowledgements.....xvii

Chapter 1: Hydrozoan jellyfish and Scottish aquaculture: current state of knowledge and outlook for the future.....1

1.1 Medusozoan blooms in Scotland.....2

1.2 Medusozoan life cycle and taxonomy.....3

1.3 When and why do medusozoan blooms occur?.....6

1.4 Forecasting a cnidarian bloom: prior investigations and projected strategies.....8

1.5 During a bloom: nematocysts, venom, and infection.....10

1.5.1 Predatory ecology of the Cnidaria: the venom system.....10

1.5.2 Bacterial infection and symbiosis.....13

1.6 Investigations in this thesis.....15

Chapter 2: Prospective monitoring of hydromedusa populations at salmon aquaculture facilities.....17

2.1 Capsule findings.....18

2.2 Introduction.....19

2.3 Methods.....21

2.3.1 Sites.....21

2.3.2 Hydrozoan sampling methods.....25

2.3.3 Health monitoring methods.....26

2.3.4 Water quality conditions.....27

2.3.5 Primary productivity……27

2.3.6 Statistical analyses……27

2.4 Results……28

2.4.1 Hydromedusan population density……28

2.4.1.1 Commonly occurring species……28

2.4.1.2 Data distribution……30

2.4.1.3 Time-series comparisons……31

2.4.1.4 Defining a bloom……35

2.4.2 Environmental factors prior to and during blooms……37

2.4.2.1 Temperature……37

2.4.2.1.1 Missing values and hindcasts ……37

2.4.2.1.2 Bloom occurrence……39

2.4.2.2 Photoperiod ……41

2.4.2.3 Salinity……43

2.4.2.3.1 Missing values and hindcasts……43

2.4.2.3.2 Bloom occurrence……43

2.4.2.4 Water clarity……45

2.4.2.5 Primary productivity……48

2.4.2.6 Tide state ……50

2.4.3 Fish health outcomes……50

2.4.3.1 Data constraints……50

2.4.3.2 Portnalong 2013: qualitative impact of a two-species high-magnitude bloom……54

2.5 Discussion……58

2.5.1 Population dynamics of Obelia *sp. and* Lizzia blondina……58

2.5.2 Role of temperature……60

2.5.5 Role of geography……61

2.5.4 Impact on aquaculture……64

2.5.5 Recommendations for further work.....65

Chapter 3: Bacterial genera biodiversity cultured from three medusozoan species in Shetland.....68

3.1 Capsule findings.....69

3.2 Introduction.....70

3.3 Methods.....72

3.3.1 Procedural summary.....73

3.3.2 Sites.....73

3.3.3 Incubation of cnidarian samples.....75

3.3.4 Bacterial sampling.....76

3.3.4.1 Media.....76

3.3.4.2 Cultured tissues.....76

3.3.4.3 Sampling.....76

3.3.5 Culturing and identification.....77

3.3.6 DNA extraction and amplification.....78

3.3.7 Sequence analysis.....79

3.3.8 Statistics.....79

3.4 Results.....80

3.4.1 Limitations and yield.....80

3.4.2 Location-specific results.....87

3.4.3 Other cnidarian species.....88

3.5 Discussion 99

3.5.1 Observed bacterial flora 99

3.5.2 Diversity.....101

3.5.3 Further work.....101

3.6 Appendix: Control sequences.....104

Chapter 4: Phylogeographic analysis of *Obelia geniculata* populations in the north of Scotland.....108

4.1 Capsule findings.....110

4.2 Introduction.....111

4.3 Methods.....114

4.3.1 Summary in brief.....114

4.3.2 Sampling locations.....114

4.3.3 Notes on sampling.....115

4.3.4 Genetic extraction, amplification, and sequencing.....117

4.4 Results.....119

4.4.1 Sequence variation.....119

4.4.2 Taxonomy and distribution of haplotypes.....122

4.4.3 Haplotype richness.....128

4.4.4 Spatial-temporal analyses 130

4.5 Discussion.....136

4.5.1 Evidence for dieback and population recovery.....136

4.5.2 Potential for geographically broad mixing in the North Atlantic.....138

4.5.3 Summary findings.....143

4.6 Appendix I.....144

4.7 Appendix II.....147

Chapter 5: General discussion, future work, and conclusions.....148

5.1 Wider research contributions.....150

5.1.1 Investigative avenues: the benthic and the pelagic.....150

5.1.2 Plankton-induced gill pathology in salmon aquaculture.....154

5.2 Future work.....156

5.2.1 Next steps in monitoring, modelling, and mitigation.....156

5.2.2 Next steps in microbial ecology and aquaculture-relevant vectoring……160

5.2.3 Next steps in phylogeography of hydrozoans……161

5.3 Conclusions……163

References……164

List of figures

Chapter 1

1.1 Characteristics-based classification of significant cnidarian classes in UK waters……..4

1.2 Representative hydrozoan life cycle and colony diagram, adapted and drawn from *Phialella fragilis* (Boero 1987)……5

1.3 Venom systems in Phylum Cnidaria……11

1.4 Precedent for microbial endosymbiosis in the Cnidaria……14

Chapter 2

2.1 Map of study sites……24

2.2 Local area maps of each sampling site……24

2.3 Marine Harvest gill scoring method……26

2.4 Relative frequency of all species appearing in the survey……29

2.5 Most frequently occurring hydrozoan species appearing at the study sites……30

2.6 Relative frequency for all species appearing in the survey……31

2.7 Time series of hydromedusan populations appearing at all sites in 2012……32

2.8 Time series of hydromedusan populations appearing at all sites in 2013……33

2.9 Time series of hydromedusan populations appearing at sites in 2014……34

2.10 Observations of varying population density measures……35

2.11 Temperatures (˚C) measured at 5 m depth at Greshornish and Portnalong, 2014……38

2.12 Temperature records (including hindcast values) for all sites……39

2.13 *Lizzia blondina* and *Obelia* sp. medusae are most likely to occur in both spike and bloom-level population density measures……40

2.14 Temperature measured at 5 m depth in the 60 days prior to blooms……41

2.15 Mean photoperiod during the 7 days prior to *Obelia* sp. or *Lizzia blondina* population measurements……42

2.16 Salinity range in which populations of *Lizzia blondina* and *Obelia* sp. were observed……44

2.17 Salinity fluctuations in the 60 days prior to 4 moderate or high-magnitude blooms of *Lizzia blondina* and *Obelia* sp……45

2.18 Water clarity observed concurrently with hydromedusan population density……46

2.19 Water clarity in the 60 days prior to moderate to high-magnitude blooms……47

2.20 Area of chlorophyll-a measurements adjacent to sea cage sampling locations……48

2.21 Composite median chlorophyll-a measurement of the Isle of Skye for the period of August 2013……49

2.22 Hydromedusan population density and incidence of gill pathology, summer 2012-2014……52

2.23 Degradation and inflammation of gill lamellae following exposure to *Obelia* sp. and *Lizzia blondina* medusae at a cumulative population density of nearly 670 jm^{-3} ……55

2.24 Increase in PGD and AGD following bloom exposure……56

2.25 Mortality increased dramatically at Portnalong in 2013 following exposure to multiple hydromedusan species and a rise in gill pathology……57

Chapter 3

3.1 Locations of sampling sites……73

3.2 Areas sampled at each site……74

3.3a ClustalW alignment of 1-30 of 87 sequences of 16S rDNA obtained from bacteria cultured from *Obelia geniculata* hydroid, *Neoturris pileata* hydromedusae, C*yanea capillata* tentacle, *C. capillata* gut, and control samples……82

3.3b ClustalW alignment of 31-60 of 87 sequences of 16S rDNA obtained from bacteria cultured from *Obelia geniculata* hydroid, *Neoturris pileata* hydromedusae, C*yanea capillata* tentacle, *C. capillata* gut, and control samples……83

3.3c ClustalW alignment of 61-87 of 87 sequences of 16S rDNA obtained from bacteria cultured from *Obelia geniculata* hydroid, *Neoturris pileata* hydromedusae, C*yanea capillata* tentacle, *C. capillata* gut, and control samples……..84

3.4 Family-level 16S rDNA diversity across pooled samples, after elimination of poor sequences and controls……84

3.5 Genus level 16S rDNA biodiversity in all samples……86

3.6 Cladogram of 16S ribosomal sequences of bacterial cultures sampled from *Obelia geniculata* hydroids at Lunna, Shetland……..90

3.7 Cladogram of 16S ribosomal sequences of bacterial cultures sampled from *Obelia geniculata* hydroids at Redayre, Shetland……91

3.8 Cladogram of 16S ribosomal sequences of bacterial cultures sampled from *Obelia geniculata* hydroids at Roe Sound, Shetland……..92

3.9 Cladogram of 16S ribosomal sequences of bacterial cultures sampled from *Cyanea capillata* gut tissues at North of Papa, Shetland……93

3.10 Cladogram of 16S ribosomal sequences of bacterial cultures sampled from *Cyanea capillata* tentacles at North of Papa, Shetland.……94

3.11 Cladogram of 16S ribosomal sequences of bacterial cultures sampled from *N. pileata* hydromedusae at North of Papa, Shetland……95

3.12 Bacterial samples of each represented genus obtained from *Obelia geniculata* hydroids at Lunna, Redayre, and Roe Sound, Shetland……97

3.13 Comparison of bacterial genera sampled from *Cyanea capillata* gut vs tentacle from individuals collected at North of Papa……98

Chapter 4

4.1 *Obelia geniculata* hydroid colonies on *Laminaria digitata* fronds……112

4.2 The Scottish slope current……113

4.3 *Obelia geniculata* hydroids were collected from sites within three distinct regions……116

4.4 Regional sampling sites ……116

4.5 Frequency of observations of 41 haplotypes……121

4.6 Bayesian consensus cladogram of 41 mtCOI haplotypes ……123

4.7 Shift in haplotype diversity distribution in NWM sites, 2013-2014……..124

4.8 Shift in haplotype diversity distribution in Ork/N sites, 2013-2014……125

4.9 Shift in haplotype diversity distribution in Shet sites, 2013-2014……126

4.10 Shift in haplotype diversity distribution by region, 2013-2014……127

4.11 Estimated richness based on sample size in each region across 2013-2014……129

4.12 Multidimensional scaling plot showing F_{ST} pairwise comparison between sites 135

4.13 Cluster analysis of site similarity……135

4.14 Bayesian consensus tree including 24 unique haplotypes reported in Govindarajan et al. (2004a)……140

4.15 ClustalW alignment of 41 unique haplotypes recovered in 2013 and 2014….147

List of tables

Chapter 2

2.1 Sample coverage during the weekly survey period……..25

2.2 Terminology used to describe hydromedusan blooming events……35

2.3 Incidence of moderate and high-magnitude population density……36

2.4 Autocorrelation in *Obelia* sp., *Lizzia blondina*, and summed population data….37

2.5 Comparing salinity fluctuations between sites……43

2.6 Fish health data availability……51

Chapter 3

3.1 Family-level bacterial diversity found at each site according to successfully sequenced samples, after application of control sequences……89

3.2 Sequences represented in *O. geniculata* hydroids at Lunna, Shetland……..90

3.3 Sequences represented in *O. geniculata* hydroids at Redayre, Shetland……..91

3.4 Sequences represented in *O. geniculata* hydroids at Roe Sound, Shetland……..92

3.5 Sequences represented in *C. capillata* gut tissue from North of Papa, Shetland……..93

3.6 Sequences represented in *C. capillata* tentacle tissue from North of Papa, Shetland……..94

3.7 Sequences represented in *N. pileata* hydromedusae from North of Papa, Shetland……..95

3.8 Pairwise Tukey's HSD q-statistic significance comparisons between samples obtained from medusae at Lunna, Redayre, and Roe Sound……96

3.9 Pairwise Tukey's HSD q-statistic significance comparison between samples obtained from medusae at North of Papa……..99

3.10 Listing of recommended further identification approaches appropriate to each sampled genus……102

Chapter 4

4.1 Sampling sites for *Obelia geniculata* within three regions.……117

4.2 Site-by-site breakdown of successfully sequenced samples and diversity over 2013-2014, excluding Wari Geo……..119

4.3 Singly-occurring haplotypes……121

4.4 Thirteen haplotypes occurring in multiple sampling efforts, without frequency of occurrence given in each cell……122

4.5 χ^2 comparisons of richness as predicted by Chao 2 estimation vs richness observed by sampling……129

4.6 χ^2 temporal comparisons of observed richness and Chao 2 estimated richness between 2013-2014……130

4.7 AMOVA comparisons between 2013 groupings……131

4.8 AMOVA comparisons between 2014 groupings……132

4.9 AMOVA comparisons between 2013 and 2014……133

4.10 Pairwise F_{ST} comparisons between all sites……134

4.11 ANOSIM comparisons, ranked by increasing R-statistic……..136

4.12 Sites attempted in 2013 with no samples retrieved……146

Acknowledgements

Thank you to the Marine Alliance for Science and Technology Scotland (MASTS) for awarding me a Prize Studentship to carry out this research, and to both MASTS and the University of St Andrews School of Biology for the extra support that comes with educating an American. I am forever grateful. Thank you also for the Russell Trust Award and the MASTS Postgraduate and Early Career Researcher Exchanges (PECRE) funding most of the travelling and experience this research took, and for the North Atlantic Fisheries College (NAFC) in Scalloway, Shetland for generously hosting me during summer 2012. Thanks also to Marine Harvest, in particular Chris Wallace and the guys at every salmon site, for enthusiastic participation.

To my supervisors, Andrew Brierley (University of St Andrews) and Clive Fox (Scottish Association for Marine Sciences), thank you. Andy, without your stubbornness, I'd probably be in the US Navy right now dreaming jellyfish dreams. Just keep swimming – preferably with a thick enough wetsuit.

Thank you to April Blakeslee for a successful PECRE exchange and to Long Island University for helping to make that happen. April, it was just like old times, complete with critical beverage… except with even cuter and more delightful kids.

Axel Miller, Dave Patterson, Emma Defew, Mark Johnson – thank you for your encouragement, and for calling me out on impostor syndrome all the time. It's been a pleasure. To the rest of the MASTS postgraduates – especially Penne Donohue, Amy Scott-Murray, Ewan Edwards, thank you for the laughs, gin, and everything else.

To my friends in Shetland: Jim Tait and Fiona Grieve, you are two of the most generous souls I've ever met, and you and your whole family deserve the best of everything. Thank you. Rachel Shucksmith, you generously donated your time and brainpower to a whole slew of projects that weren't your job – I couldn't have done it without you. Kenny Gifford – your company and boat skills, not to mention tunes, are some of my favourite memories. I can't wait to visit all of you again.

To Neil Banas and Emily Doolittle, who rival for medals in generosity and general wonderfulness, thank you for helping me launch this ship (and then to pilot it across the finish line). To Kiran Garimella, who helped me stay sane across a long process – and for whose sanity I like to claim a little credit in return – thank you, and I hope we go on to yell more science at each other forever. To Mary Mackay, you were a fantastic collaborator for too short a time, and I hope you nail it in Tasmania. To Suzanne Gray – I've never laughed so hard during field work, or turned such dramatically failed fieldwork into such a success. I hope we get to do it again, though hopefully without driving from Skye and back twice in an afternoon. To Eileen Bell, I will always appreciate the time you spent reading this thesis for no purpose other than just being helpful as hell. To Annie Ritchie, you're amazing and deserve a Nobel for being one of the planet's best human beings. Thank you for all the support through the years I've been in Scotland – mummy backup has never been so cheerfully given. To Martin Stewart – you remain my favourite, and I'm so glad we can talk geekery at each other without either one of us understanding a thing. Plankton toes and farads indeed – I couldn't have done it without you.

To my family back in the United States – thank you for all of your encouragement and support, especially for tolerating yet another chuck-toys-out-of-pram-and-move-to-Scotland family incident. I hope you stay proud of me.

1

Hydrozoan jellyfish and Scottish aquaculture: current state of knowledge and outlook for the future

Impacts of jellyfish blooms on various coastal industries around the world are widespread and well-documented in numerous reviews (e.g. Seaton 1989, Purcell et al. 2007, Richardson et al. 2009, Nickell et al. 2010). In Scotland, marine industries are vulnerable to cnidarian blooms by way of stock losses in aquaculture, interference with coastal power generation, and competitive or predatory interactions with commercial finfish species. This thesis focuses on the interactions of hydrozoan jellyfish with Scottish salmon production.

1.1 Medusozoan blooms in Scotland

Two classes of jellyfish-producing cnidarians are represented in Scottish waters: the larger-bodied Scyphozoa, with 13 endemic species, and the less-recognized Hydrozoa, with about 90 species (Russell 1953, Nickell et al. 2010). Members of both classes have been associated with fish kills at aquaculture facilities, but lay awareness of "jellyfish blooms" in practice amongst aquaculture workers and management is almost exclusively limited to blooms by scyphomedusae. This is likely due to the publicity afforded large, easily-photographed species, and the relative difficulty in observing and identifying hydrozoan species. Nonetheless, numerous fish kill events associated with hydrozoan species have been reported in the scientific literature.

The first reported such damage took place in 1984, with a *Phialella quadrata* bloom that caused mass mortality of salmon smolts in Shetland in 1984 (Bruno and Ellis 1985). Since then, various species of scyphozoan and hydrozoan medusae including *Cyanea capillata* and *Aurelia aurita* (Seaton 1989, Baxter et al. 2011, Mitchell et al. 2011, Mitchell and Rodger 2011), *Solmaris corona* (Tørud and Håstein 2008), and *Pelagia noctiluca* (Doyle et al. 2008) have also been implicated, and other jellyfish-related fish kills have been reported without identification of the species involved (McKibben and Hay 2002). Outside of Scotland, but near enough to be of concern, the same

list of cnidarian species plus others such as *Apolemia uvaria* and *Muggaiea atlantica* have caused mortality of caged salmon in Norway and Ireland (Båmsted et al. 1998, Tørud and Håstein 2008).

It has been suggested that worldwide jellyfish populations, and associated deleterious events, may be on the rise (Purcell et al. 2007, Richardson et al. 2009), though this is under some dispute as a global phenomenon (Condon et al. 2013). However, many localities show trends toward cnidarian-dominated food webs, decreased finfish landings, and numerous blooms which interfere with power stations (Lynam et al. 2004, Purcell et al. 2007, Richardson et al. 2009, Miller 2011). Though the vast majority of this literature focuses on scyphozoan medusae, it would be unreasonable to overlook the potential for impact of such phenomena within Scottish aquaculture. This thesis seeks to increase the understanding of hydrozoan population ecology, and to characterize the threat to aquaculture posed by hydromedusae.

1.2 Medusozoan life cycle and taxonomy

Class Hydrozoa falls under the subphylum Medusozoa within Phylum Cnidaria, which also contains the Scyphozoa (true jellyfish), Cubozoa (box jellyfish), and Staurozoa (stalked jellyfish). The class is broadly – though not exclusively – characterized by an alternation of generations between an asexually-reproducing polyp phase and a sexually-reproducing medusa phase, though the medusa stage itself has been repeatedly lost in various clades, and a number of species are holoplanktonic with a lack of a benthic polypoid phase (Cornelius 1990, Cornelius 1992, Boero et al. 1995, Govindarajan et al. 2004a). The complete derivation of lineage within Phylum Cnidaria is still under some dispute (e.g. Berntson et al. 1999, Kayal et al. 2013), but characteristic non-cladistic divisions relevant to most species appearing in British waters are given below (Figure 1.1).

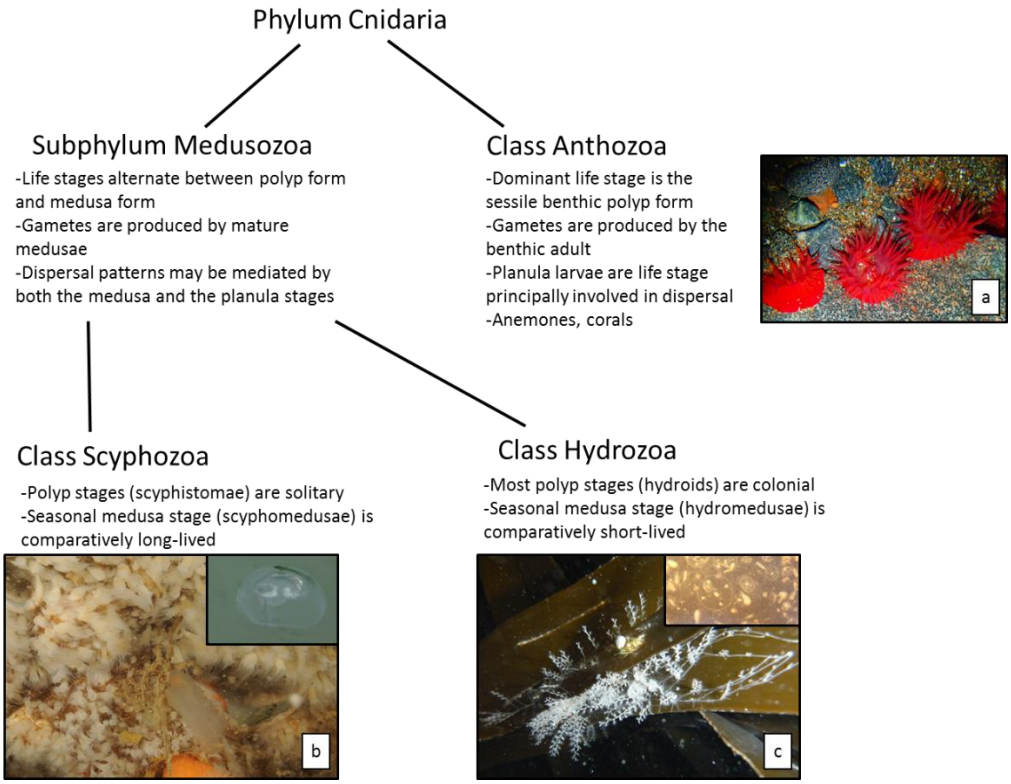


Figure 1.1 Characteristics-based general classification of significant cnidarian classes in UK waters. Phylum Cnidaria can be grouped broadly by their mode of sexual reproduction. (a) Members of Class Anthozoa, represented here by beadlet anemones (*Actinia equina*), lack a medusa stage of dispersal. (b) Members of Class Scyphozoa, such as moon jellyfish, *Aurelia aurita*, alternate generations between a solitary polyp stage (or scyphistoma, lower) which can asexually reproduce new polyps, and a free-swimming, sexually-reproducing medusa stage (scyphomedusa, upper right corner). (c) Members of Class Hydrozoa, such as *Obelia geniculata*, alternate generations between a colonial polyp phase (hydroids, lower), which grows laterally via asexual reproduction, and a sexually-reproducing medusa stage (hydromedusae, upper right corner). Notably, the Hydrozoa are a diverse class with many exceptions to this archetype, including several species which have lost the hydromedusa dispersal stage and/or those which have a solitary hydroid polyp stage. Additionally, certain members (particularly members of Class Siphonophorae) utilize a colonial life stage which is not sessile, but itself planktonic. Other members of the Medusozoa include the Staurozoa (stalked jellyfish) and Cubozoa (box jellyfish), which are not of concern in terms of blooms in the northern UK. All photographs by the author.

In examining the ecological context of jellyfish blooms, it is important to understand the life cycle features that contribute to bloom appearance. Broadly speaking, all medusozoan species undergo an alternation of generations between a benthic polyp stage and a free-swimming medusa stage. Benthic individuals may reproduce and grow laterally by budding, or by releasing medusae; once released, medusae mature and reproduced sexually to produce a motile planula larva, which can settle onto substrate to

begin a new benthic life stage. In many hydrozoan species, the benthic stage is colonial, with clonal growth yielding an interconnected network of individuals specialized as either feeding hydranths of as reproductive gonangia (Figure 1.1). The timing of this life cycle varies taxonomically, but within the UK assemblage of species, it is strongly seasonal, with hydromedusae and scyphomedusae appearing in the plankton from late spring to early autumn (Russell 1953).

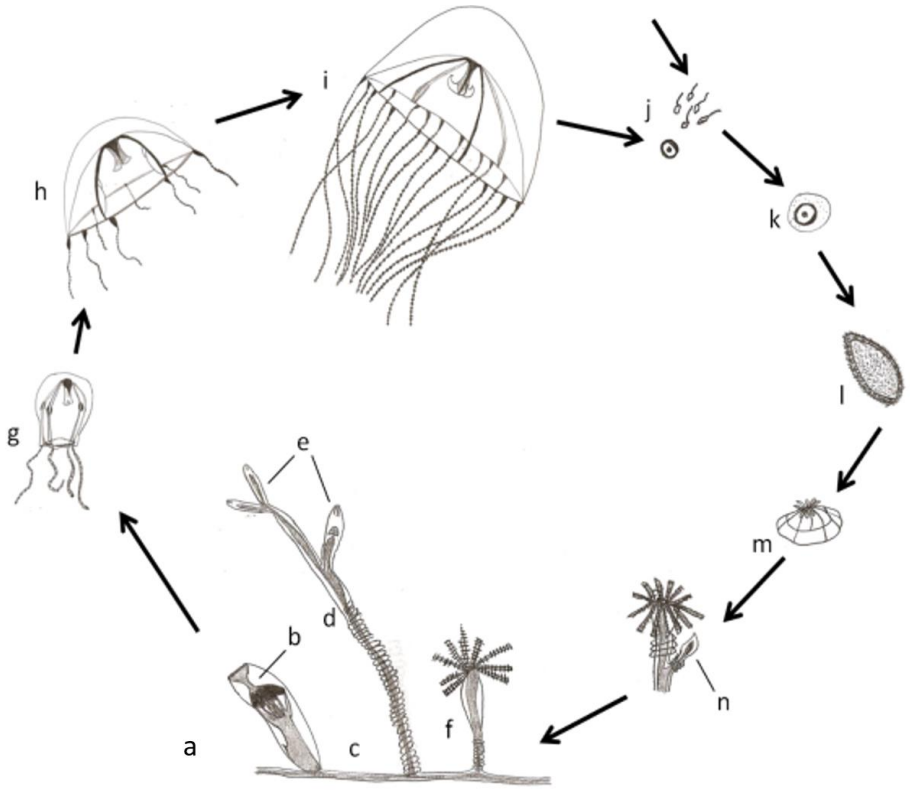


Figure 1.2 Representative hydrozoan life cycle and colony diagram, adapted and drawn from *Phialella fragilis* (Boero 1987). Hydroid colonies (a) consist of interconnected sessile polyps, of two distinct types: reproductive gonangia and feeding hydranths. Gonangia (b) produce the pelagic, sexually reproducing medusae, which are released into the plankton. Colonies grow asexually, forming both lateral creeping hydrorhizae (c) and branching stalks (d). Hydranth polyps are specialized for feeding only, and do not produce medusae (e: retracted hydranths; f: extended, actively feeding hydranth). After release from the gonangia, juvenile medusae develop in the plankton to maturity (g-i). Gametes are released from mature medusae and fertilized externally (j). Zygotes (k) develop in the plankton into ciliated planula larvae (l). Planulae attach by the anterior pole to an appropriate substrate and cells collapse around this point, eventually re-developing into the tissues of the primary polyp of a new colony (m), which increases in size by asexual budding (n). Drawn and adapted from Boero (1987).

Both the benthic and pelagic phases are important to understand in predicting medusa blooms. While it is the medusa life stage that causes damage to industry, it is the polyp stage that gives rise to medusae, necessitating an investigation of its reproductive dynamics and dispersal mechanisms.

1.3 When and why do medusozoan blooms occur?

The appearance and magnitude of blooms are dependent on the benthic biomass (i.e. the number and size of reproducing hydroid colonies) and the stimulus or stimuli to produce medusae. A wide variety of physical and biological conditions conducive to hydromedusa production and colony growth are cited for various medusozoan species, including light (Brinckmann-Voss 1985, regarding *Sarsia princeps*; Costello 1988, regarding *Cladonema californicum*), temperature (Werner 1958, regarding *Rathkea octopunctata,* and Werner 1961 and 1962 regarding *Bougainvillea superciliaris*, in Arai 1992; Widmer 2004, regarding *Mitrocoma cellularia*), salinity (Goy 1973, regarding *Scolionema suvaensis*), food availability (Roosen-Runge 1970, regarding *Clytia gregarium*; Miglietta et al. 2008 regarding numerous hydrozoan taxa), lunar phases (Elmhirst 1925, regarding *Obelia geniculata*; Goy 1973, regarding *Scolionema suvaensis*), salinity (Kawamura and Kubota 2008), upwelling (Miglietta et al. 2008), and various interactions amongst the above (Edwards 1978 and 1983, regarding *Sarsia occulta*, *S. tubulosa*, and *S. cliffordi*; Ma and Purcell 2005, regarding *Moerisia lyonsi*). *M. lyonsi* in particular showed increased gonangium ratios amongst the colonial polyps and a resulting increase of medusa release in response to rising temperatures, by as much as 25% per 1° C rise in temperature. Alternatively, some species release medusae with regularity without

apparent regard to particular physical stimuli (Kubota 2008, regarding *Eugymnanthea* sp.). In some cases, these conditions are met as a constant; that is, a particular species' colony held at 11° C will produce medusae regularly and continuously and will do so significantly less at over 15° C (Widmer 2004, regarding *Mitrocoma cellularia*); other conditions, such as a peak in prey abundance, may lead to similarly temporary peaks in medusa production (e.g. Arai 1987, regarding *Sarsia cliffordi*). This may represent an ecological situation in which resources are sufficiently flush that the hydroid colony can invest resources in sexual reproduction. In other cases, the stimulus for medusa production may be hormetic; that is to say, a short-term stressor such as rapidly dropping salinity, a damaging rise in temperature, or chemical toxicity appearing in the water column may cause a benthic colony to produce motile medusae which can escape unfavourable conditions, as well as increase diversity and population fitness through genetic recombination (e.g. Stebbing 1981a, Stebbing 1981b, Stebbing 2002, Widmer 2004).

Factors affecting scyphomedusan populations are also worth considering. In having a comparably longer life cycle (Russell 1953), scyphozoan species appear to be more heavily influenced by longer-duration stimuli than hydrozoans, with marked correlation with climatological and oceanographic effects. For example, scyphozoan medusa populations of *Cyanea capillata*, *C. lamarckii*, and *Aurelia aurita* have been found to correlate with the North Atlantic Oscillation, with positive NAO phases leading to cooler water in the North Sea and greater numbers of medusae (Lynam et al. 2004). This phenomenon has been borne out in laboratory cultures of this and similar species (e.g. Widmer 2015). Additionally, a number of coastal scyphozoan species have shown increased medusa population in response to eutrophication, in particular from nitrogen and phosphorus runoff, which

tends to favour nutrient pathways toward low-energy species such as jellyfish rather than higher-energy, more complex food webs (Greve and Parsons 1977, Purcell et al. 2007).

The role of these factors in producing demonstrable effects for industry is correlatively documented almost exclusively in scyphozoan species (e.g. Lynam 2006, Widmer 2015), and their spatial-temporal predictive power remains broad-scale in geographic resolution. Moreover, the infrequent nature of high-density blooms suggests that the conditions giving rise to blooms are not met constantly, or even often. Prospective investigation of blooms must focus on: (a) identifying species of interest; (b) narrowing environmental stimuli of interest; and (c) examining oceanographic conditions that might lead to high-density aggregations.

1.4 Forecasting a cnidarian bloom: prior investigations and projected strategies

Effective prediction of harmful jellyfish blooms is an obvious objective for marine industry, but requires a far greater data bank than is currently available. Prior to the past decade, jellyfish have been of little economic interest in UK waters, so few historical surveys exist on which to base estimates of population variability. Several recent strategies for gathering data on gelatinous zooplankton occurrence in Scotland have been tested and implemented, with varying degrees of success. First, in a 2008 Crown Estate-funded survey of jellyfish impacts, aquaculture facility managers were provided with a standardized questionnaire regarding frequency, severity, and causative species via the Scottish Salmon Producers Organisation (SSPO). Of 257 potential respondents, only 9 responded (Nickell et al. 2010). One

aquaculture syndicate in Shetland cited insurance concerns as a reason for withholding their data records. This limited response was insufficient for meaningful data analysis. In addition, such an approach also overlooks blooms that are difficult to notice, and fails to examine environmental conditions in the run-up to blooms (Nickell et al. 2010). Remote sensing has also been investigated as a cnidarian research tool (Lynam et al. 2005, Nickell et al. 2010). Acoustic detection of jellyfish is possible for large-bodied scyphozoan medusae, though has not yet been put into broader-scale use as would be required for long-term population dynamic study, and is not yet applicable to smaller hydromedusae. Aerial monitoring via spotter plane was trialled and found to be cost-prohibitive due to a combination of poor aerial visibility of problem species, and poor average weather conditions in the west of Scotland (Nickell et al. 2010). Direct visualization of blooms via satellite has been investigated but is restricted, again, by weather conditions and optical acuity (Nickell et al. 2010). Furthermore, satellite visualization of jellyfish is useful only in detecting blooms that develop elsewhere and are carried to aquaculture sites by current flow; spotting blooms developing in situ is likely too late for preventative measures to be implemented. Prior warning of an incoming bloom might apply, for example, to a 2007 bloom of *Pelagia noctiluca* that moved in toward Northern Ireland and western Scotland, which could easily be sighted from the air before arriving inshore (Doyle et al. 2008). However, at least two hydrozoan blooms by *P. quadrata* appeared to have developed in the immediate vicinity of a salmon farm, rather than arriving by advection (Bruno and Ellis 1985, Ferguson et al. 2010). If this is a common theme, aerial flyover or other remote-sensing strategies would need to observe proxy oceanographic conditions predicting blooms, rather than blooms themselves, in order to be useful in providing advance warning.

The use of such proxy conditions, whether remotely sensed or modeled locally, is contingent on gathering sufficient data to correlate bloom occurrence with environmental events. Again, surprisingly little historical data are available. A lack of awareness among the aquaculture workers most strongly affected by blooms appears to have prevented any self-monitoring by the industry itself. A prospective study, shared between industry and academia, would be useful in developing even basic insight into such phenomena, and is reported in this thesis (Chapter 2, Prospective monitoring of hydromedusa populations at salmon aquaculture facilities).

1.5 During a bloom: nematocysts, venom and infection

There are several ways in which hydrozoan medusa blooms may affect caged fish. While scyphozoan bloom biomass has occasionally been so great that localized anoxia and physical crushing have been sources of mortality in aquaculture (e.g. Doyle et al. 2008), hydrozoan blooms tend to cause harm via the prey-capture system of nematocysts and venom. The previously-mentioned 2008 bloom by the hydrozoan species *P. quadrata* strongly suggested that medusae may be acting as vectors for pathological microbes as well (Ferguson et al. 2010). These two separate means of pathology require separate consideration.

1.5.1 Predatory ecology of the Cnidaria: the venom system

All cnidarian classes, at some point in the life cycle, possess cnidocyte cells containing stinging organelles commonly referred to as nematocysts. Nematocysts comprise a variety of types, but broadly speaking, all consist of a hollow capsule containing an inverted tubule which everts rapidly when stimulated by prey or self-defense to do so (Gravier-Bonnet 1987, Hessinger

and Lenhoff 1988, Östman 2000). The predatory purpose of these tubules falls into two categories: those which entangle or stick to prey, and those which deliver venom (Hessinger and Lenhoff 1988). These were formerly termed astomocnidae and stomocnidae, in reference to a closed or open tubule tip that would allow for a flow of capsule contents, but more recent scanning electron microscopy images have found that nematocyst discharge often causes rupture of astomocnid tubules, and the terminology has been discarded (Östman 2000). However, for the purposes of discussing cnidarian predatory ecology, nematocysts can be viewed as penetrant or non-penetrant. Non-penetrants serve to tether or stick to microstructures on the outside of prey such as setae or exoskeletal plates, while penetrant nematocysts pierce prey integument either for venom delivery or for further tethering (Hessinger and Lenhoff 1988, Carrette et al. 2002). The arrangement and location of differing types of nematocyst varies according to species, ontogeny, and body location on a given medusa (Carrette et al. 2002, Underwood and Seymour 2007).

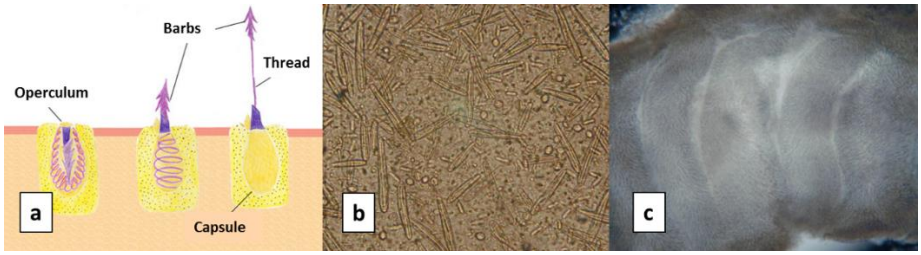


Figure 1.3 Venom systems in Phylum Cnidaria. (a) Nematocyst organelles consist primarily of an enclosed capsule and a coiled, inverted tubule. Appropriate stimuli will cause the tubule to evert rapidly, stinging or entangling prey. (Diagram by NOAA, reproduced with permission). (b) A single species may have numerous types of nematocysts, including both penetrant and non-penetrant types. (Numerous types of *Chiropsella bronzei* nematocysts removed from the tentacle, 100X magnification; photo by the author). (c) Nematocysts are arranged in densely-packed folded bands along the tentacles. Once the first nematocysts fire, writhing motions in the tentacle bring more nematocysts into contact with the prey, increasing the envenoming and tethering hold (Hessinger and Lenhoff 1988) (Nematocyst banding on a *Chironex fleckeri* tentacle at 40X magnification; photo by the author.)

Meanwhile, there is virtually no information regarding the dynamics of venom in any UK hydrozoan species, or venom contributions to fish kills during a bloom event. A wide gamut of pharmacological agents, including neurotoxins, muscle type-specific signaling or inhibitory peptides, digestive enzymes and cytolytic peptides, and even pain-producing hormones such as histamines and serotonin have been isolated from medusozoan species (e.g. Tamkun and Hessinger 1981, Hessinger and Lenhoff 1988, Mustafa et al. 1995, Torres-Ramos and Aguilar 2003). Predicting (and treating) the actions of any given venom is not presently possible, due to the variability of each species' array and potency of toxins. Even very closely related species may be wildly different in their venom complement, and may have equally varying effects on different prey targets (Kintner et al. 2005). Furthermore, the study of cnidarian venoms has historically been complicated, with debate ongoing into the preservation, extraction and purification of what can be considered a "true" representation of venom (Bloom et al. 1998, Carrette and Seymour 2004, Kintner et al. 2005). However, recent work has seen a number of studies which broaden the scope of cnidarian venoms investigation to include ecological rather than only pharmacological contexts, with many toxin pathways being linked to prey preference and niche (Carrette et al. 2002, Torres-Ramos and Aguilar 2003, Kintner et al. 2005). Many examples of neurotoxicity, for example, have been shown to operate most efficiently in crustacean models and can be reproduced only poorly in vertebrate models (Torres-Ramos and Aguilar 2003), demonstrating prey-specific targeting of venom compounds. Variation in prey targets may account for some intra-family variation in some species, as venom toxin assemblage evolves to suit prey habit (Carrette et al. 2002, Kintner et al. 2005).

While a detailed investigation of venom mechanisms and valid extraction techniques is outside the scope of this review, it should be emphasized that the precise actions and outcomes of any medusozoan venom cannot be

predicted without dedicated study. In all likelihood, envenoming and physical trauma inflicted by stinging nematocysts contribute strongly to deleterious outcomes seen in aquaculture.

1.5.2 Bacterial infection and symbiosis

A more recent line of investigation into bloom biology has raised the possibility of microbial pathogen transfer as a consideration. *Tenacibaculum maritimum* is a relatively well-known fish pathogen and is most often linked to skin conditions, particularly in farmed fish, though tenacibaculosis of the gills is by no means rare (Avendaño-Herrera et al. 2006a, 2006b). In 2008, *T. maritimum* was found in conjunction with a *P. quadrata* bloom at a salmon farm in Shetland. Occasionally the bacilli have been found living in fish dermal mucus, suggesting the possibility of their being endemic there (Avendano-Herrera et al. 2004a). Given this situation, it has been suggested that the jellyfish may be picking up the bacteria from fish integument and merely transferring them to gill tissue as the medusae are sucked in through the mouths of salmon; however, tenacibaculosis in any form is virtually unheard of in Shetland salmon, including as a dermal pathology (Sutherland pers. comm. 2012). This does not rule out the fish themselves as the origin of bacteria, but certainly argues against the idea. Moreover, no other natural, non-pathogenic reservoirs of *T. maritimum* are yet known (Avendaño-Herrera et al. 2004a). Scanning electron microscopy of *P. quadrata* medusae showed a heavy colonization of the bacteria on the mouth parts (Ferguson et al. 2010). The question of medusozoans as vectors for such pathogens should be considered.

Microbial symbiosis in cnidarians is well known, particularly in the cases of shallow-water hard corals and various scyphozoans such as the *Cassiopeia* and *Mastigias* genera, all of which harbour photosynthetic zooxanthellae.

However, the *P. quadrata* bloom in Shetland marked the first recorded instance of a medusa symbiosis with a prokaryotic organism (Ferguson et al. 2010). A similar symbiosis was found in 2011, with *T. maritimum* living on the mouth parts of *Pelagia noctiluca* medusae which had had no contact with farmed fish (Delannoy et al. 2011). This lends credence to the possibility that the relationship between medusa and bacteria is long term and not merely a case of pathogen transference from skin to gills.

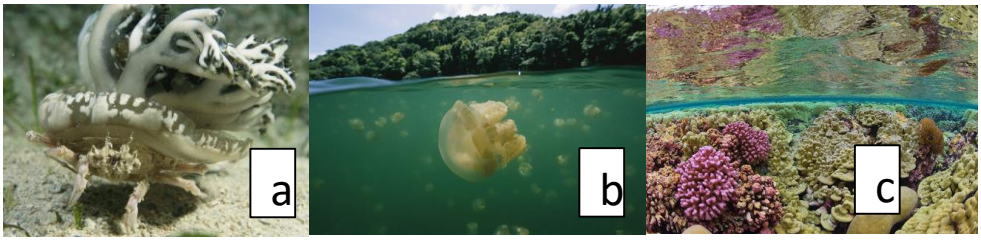


Figure 1.4 Precedent for microbial symbiosis in the Cnidaria. Microbial symbiosis is well-known in the cnidarian phylum, but until recently, only eukaryotic algal symbionts have been found. These include the photosynthesizing zooxanthellae of two scyphozoan genera: the "upside-down" jellyfish of the *Cassiopeia* genus (a) (photograph T. Laman), the *Mastigias* sp. medusae best known from enclosed lakes in Palau (b) (photograph T. Laman) and the perhaps best-known photosynthetic zooxanthellae of anthozoan hard corals (c) (photograph B. Skerry). In the scyphozoans, nematocyst complement may be reduced or altered in the presence of the zooxanthellae, with the jellyfish dependent on photosynthetically-derived energy.

If medusae are living symbiotically with marine bacteria, this raises the question of the nature of the symbiosis: parasitic, wherein the bacteria are pathogens of the medusae themselves; commensal, wherein the bacteria are incidental to the survival and success of the medusae but benefit from a free ride to whatever organisms the jellyfish may be preying on; or mutualistic, wherein the bacteria provide some kind of selective advantage to the jellyfish and vice versa. This latter possibility recalls the gut flora mutualism in higher animal taxa, wherein endosymbiotic bacteria would provide digestive and enhanced nutritional benefit to the medusae. Furthermore, if any form of symbiosis is long-term, it is not known at what point in the hydrozoan life cycle the bacteria become involved, whether at the colonial hydroid stage or

exclusively during the pelagic medusa stage. Bacterial contributions (or detriments) to hydrozoan fitness and survival and role in the various life stages should be pertinent lines of inquiry into further study of bloom ecology.

1.6 Investigations in this thesis

The present state of knowledge regarding hydrozoan blooms involvement is inadequate to begin developing mitigation strategies for the aquaculture industry. The investigations in this PhD thesis built information towards these strategies, and towards questions in ecology raised by bloom events.

In Chapter 2, "Prospective monitoring of hydromedusa populations at salmon aquaculture facilities," a working hypothesis suggested that caged salmon would encounter blooms of hydromedusae; that population fluctuations of blooming gelatinous zooplankton species could be observed and recorded; and that blooms' harm to fish could also be recorded. By keeping data on the hydromedusan fauna on a time-series basis, rather than ad hoc as blooms were noticed, this study assesses which species are likeliest to cause harm, what the health outcomes might be, and what environmental stimuli might serve as indicators of risk for blooms. A collaboration with an aquaculture industry partner was forged in order to monitor the development of blooms and their effects at aquaculture sites. This approach included tracking basic physical factors, such as salinity and temperature, as well as fish health indices. The investigation identifies various frequently-blooming species and develops associations between such blooms and declines in fish health, and identifies some seasonal or environmental parameters when these are most

likely to occur. Possible stimuli such as water temperature and freshwater runoff events are explored and discussed.

In Chapter 3, "Biodiversity of bacterial genera cultured from three medusozoan species in Shetland," a pilot study sought to document *T. maritimum* symbiosis with hydrozoan species, operating under the hypothesis that gill tenacibaculosis following medusozoan exposure could be a potential point of mitigative treatment in aquaculture. This was expanded into an investigation of potentially pathogenic microbial communities within several medusozoan species, including *Obelia geniculata*, *Cyanea capillata*, and *Neoturris pileata*, collected from four different sites in Shetland. A number of potential pathogens are described as close associates of these, providing further possible lines of inquiry and defense against the adverse sequelae of aquaculture-hydromedusan interaction.

Finally, in Chapter 4, "Phylogeographic analysis of *Obelia geniculata* populations in the north of Scotland," the genetic patterns of dispersal of *O. geniculata* are examined. This investigation uses mitochondrial cytochrome oxidase I markers collected from different sites across the Scottish northwest mainland, Orkney, and Shetland to discern population structure and haplotypic diversity, and estimates geographic trends in dispersal. In particular, the questions of whether whether population distribution followed trends of predicted current flow along the Scottish west coast, and whether population breaks are mediated by Orkney and Shetland, are examined. Geographically broad mixing of the Scottish *O. geniculata* population and temporal patterns of population diversity are assessed.

The results of these studies will form the basis for robust future academic work and effective planning within the aquaculture industry.

2

Prospective monitoring of hydromedusa populations at salmon aquaculture facilities

I estimate that I have contributed 85% of the total effort towards the eventual publication of this chapter, which can be broken down as follows:

- Plankton sample collection – 10%; conducted by Marine Harvest staff
- Plankton sample analysis – 50%; conducted by me
- Veterinary examinations – 5%; conducted by MH health staff or subcontractors where stated in text
- Environmental and health database aggregation – 10%; conducted by me
- Data analysis and writing – 25%; conducted by me and reviewed by ASB.

Material from this chapter is in preparation for submission to the *Journal of the Marine Biological Association* (putative title "Spatially and temporally variable cryptic hydrozoan blooms necessitate targeted monitoring in salmon aquaculture," by A. Kintner and A. Brierley). In addition, it has been accepted for presentation at the Fifth International Jellyfish Bloom Symposium in Barcelona, Spain in June 2016. A smaller version of this chapter containing 2012 data only was given as a poster presentation at the International Conference on Coelenterate Biology in 2013 in Eilat, Israel, under title "Cryptic hydromedusan blooms and caged salmon: challenges to aquaculture in identification and pathology."

2.1 Capsule findings

- Blooms by hydromedusan species tend to be spatially and temporally heterogeneous, with adjacent monitoring sites experiencing no correlation in either species blooming or population density of any given single species.
- Large blooms of *Obelia* sp. and *Lizzia blondina* medusae appear relatively frequently in Scottish west coast waters, often concurrently. The statistical relationship between these blooms and health in caged salmon is difficult to examine through analyses of observational data, but compelling anecdotal evidence exists to suggest that these blooms can lead to deteriorating gill health and economic losses.

- Localized, targeted sampling and skilled examination is required to discern the presence or absence of a bloom, due to the majority of hydrozoan species causing blooms being not readily visible without microscopic examination. It is likely that many blooms have historically caused damage in salmon aquaculture while going unrecognized as the root cause.
- Blooms by these species appear to be temperature-mediated, but temperature is clearly not the only stimulus for bloom production; photoperiod, salinity, and broad-measure turbidity were not found to be significantly associated.
- Use of satellite-based remote sensing to study this issue is of limited value, owing to the localized nature of blooms and the weather constraints of the region.

2.2 Introduction

Salmon aquaculture plays a major role in the Scottish economy, as a critical source of employment in rural areas and a worldwide retail value of over £1 billion (SSPO Annual Report 2014). Rearing salmon from smolts (fish aged 10-14 months, around 90-100 g in weight) to adult market size requires marine cultivation, and the industry typically relies on the use of pens placed in sea lochs or natural harbours for this purpose. Use of outdoor pens avoids a need to build large onshore marine aquaculture facilities with high energy costs and carbon footprint, but sea-caged fish are vulnerable to environmental threats, including blooms of medusozoan jellyfish.

The northeast Atlantic coastal region hosts two major classes of pelagic medusa-producing species, the Scyphozoa and the Hydrozoa. While the larger-bodied scyphozoan jellyfish species are easily recognizable by the lay worker or beachgoer,

hydrozoan medusae (hydromedusae) tend to be overlooked due to their small size (often between 0.5-10 mm) and lack of pigmentation. Both classes possess nematocyst stinging organelles on the tentacles and gut for prey capture. When nematocysts pass across the gill structures of salmon, or are swallowed, nematocysts may discharge into the tissues, causing both physical microtrauma and injecting small amounts of venom (Hessinger and Lenhoff 1988). An occasional encounter of a jellyfish by caged salmon is probably of limited concern, but when large numbers are encountered during a bloom, injuries may accumulate, as has been documented during scyphozoan jellyfish blooms (e.g. Doyle et al. 2008).

A review of the literature surrounding jellyfish impacts on aquaculture highlights that most investigations are retrospective rather than prospective in nature – that is, a dramatic major mortality event or visually obvious bloom prompts an investigation (e.g. Bruno and Ellis 1985, Seaton 1989, Tørud and Håstein 2008, Doyle et al. 2008, Baxter et al. 2011). This retrospective approach limits information in key ways. First, present understanding of the physical and biotic factors which contribute to blooms is insufficient to develop prediction or mitigation strategies: time-series data collection in the run-up to major bloom events is required. Second, with only high-profile acute incidents investigated, the long-term sub-lethal consequences of bloom exposure are not known. Possibly as a result, fish health issues related to jellyfish blooms – particularly blooms by the less visually obvious hydrozoan species – appear under-researched compared to day-to-day health hazards such as sea lice and communicable microbial pathogens.

A widely-held general hypothesis pertaining to large scyphozoan blooms is worth discussing when considering cnidarian medusozoan reproductive blooming (e.g. Lynam et al. 2004, Widmer 2015, Wang and Li 2015). As stated above, bloom/aquaculture interaction literature is skewed toward high-profile incidents and species which are visually obvious. In turn, this selects for attention paid to the larger-bodied scyphozoans, whose juveniles (ephyrae) spend months in

developmental stasis before metamorphosing into true medusae, which are also comparatively long-lived (Russell 1953). Numerous studies have examined reproductive cues for relevant scyphozoan species, many of which are geographically broad in their application (e.g. temperature) (Lynam et al. 2004). As a result, the working hypothesis for scyphozoan blooms is that a large seasonal population is the result of months of behind-the-scenes development, and the sudden appearance of such a population is due to a combination of universally-applied factors and current advection. The present study sought prospectively to evaluate this model and its application to hydromedusa populations, both in temporal and spatial scales, and to assess impacts on salmon aquaculture.

2.3 Methods

2.3.1 Sites

This investigation established a partnership with Marine Harvest Ltd., one of the largest producers of farmed salmon in Scotland, in order to share site and data access. Four salmon aquaculture sites owned by Marine Harvest volunteered as sentinel monitoring sites, two relatively exposed sites on the Isle of Skye and two within narrow sea lochs in Lochaber. All sites consisted of up to 24 sea cages of 32 m diameter surrounded by floating walkway structures, with net pens sinking to 11-18 m depth. Four cm net mesh was cleaned on a bi-weekly basis using an automated washer to prevent the accumulation of fouling. Floating structures such as walkways and feeding infrastructure were cleaned as necessary, normally on a 2-4 year basis. Farms were on a 17-21 month production cycle, with 14-18 months given over to raising salmon from smolt to harvest weight (normally 4.5 kg), and 3 months given over to a fallow period during which site maintenance was carried out. This study included sites through both active and fallow periods.

Specific sites are as follows, with map reference in Figures 2.1 and 2.2.

1) Linnhe is sited off the northern shore of Loch Linnhe southwest of Fort William (grid reference NN 02170 66184). Loch Linnhe is a mainland sea loch with a southwestern-exposed mouth, partially blocked by the islands of Mull, Colonsay, Jura, and Islay. Immediately to the southwest of the site, the loch passes through the Corran Narrows, a bottleneck of 200m with a sill shallowing to approximately 11 m from a basin depth of 150 m (Edwards and Sharples 1986). Twenty-two aquaculture cages are situated just off the northern shore of Loch Linnhe past the narrows, in a basin with a maximum depth of 155 m. The proximity to the narrows, and a 3.7 m tidal range, leads to typically brisk current flow through the cages, of up to 3.5 knots. Freshwater runoff averages 3.6 million m^3 per year via a watershed of 1820 km^2 (Edwards and Sharples 1986). No cnidarian blooms at this site stand out either in the published record or in anecdote.

2) Invasion Bay is sited off the south shore of Loch Sunart in the Ardgour peninsula (grid reference NM 80026 60355). Loch Sunart is also a narrow and deep loch, opening to the Minch at the west, with its mouth partially obscured by the islands of Mull and Coll. It is divided into a series of basins by 6 shallowing sills throughout the loch, with 18 sea cages situated in the uppermost basin near the loch head (Edwards and Sharples 1986). This basin reaches a maximum depth of 91 m. A watershed of 299 km^2 delivers annual runoff of 523.3 million m^3. This site is described anecdotally as being subject to annual blooms of scyphozoan medusae, particularly *Aurelia aurita*.

3) Portnalong is sited within Loch Harport, a narrow subsidiary of the larger bay of Loch Bracadale off the western coast of the Isle of Skye (grid reference NG 35729 35915). The bulk of Loch Bracadale is lacking in sills and is largely exposed to southwesterly winds and waves generated over the Minch, but Loch Harport itself is

somewhat sheltered from direct southwesterly influences by the island of Oronsay, a narrowing at the Ardtreck peninsula, and a shallowing slope within the loch (Loch Bracadale Aquaculture Framework Plan 2002). Compared to Loch Sunart and Loch Linnhe, Loch Harport is relatively shallow with a maximum of 47 m (Loch Bracadale Aquaculture Framework Plan 2002); 22 sea cages are situated in approximately 30 m depth. Runoff and watershed data are unavailable for Loch Harport, but historic data collected by Marine Harvest and anecdote show frequent flushing through the sea cages by peaty, acidic freshwater (McCauley 2007).

4) Greshornish is sited within Loch Greshornish, a small and narrow subsidiary of Loch Snizort at the north of the Isle of Skye (grid reference NG 34883 55125). Twelve sea cages are situated approximately 1/3 of the distance from the mouth of the loch, in 40 m depth. Loch Snizort is a broad embayment with no landmasses in obstruction to its opening onto the Minch. Loch Greshornish itself is moderately sheltered from the larger bay by the Greshornish and Lyndale Point peninsulas, but does not have any seafloor sills (Edwards and Sharples 1986). Like Loch Harport, it is relatively shallow in comparison to the Lochaber sites, with a maximum depth of 48 m. A watershed of 47 km^2 delivers 77 million m^3 runoff annually.

Figure 2.1 Map of study sites (UK Ordnance Survey 2015).

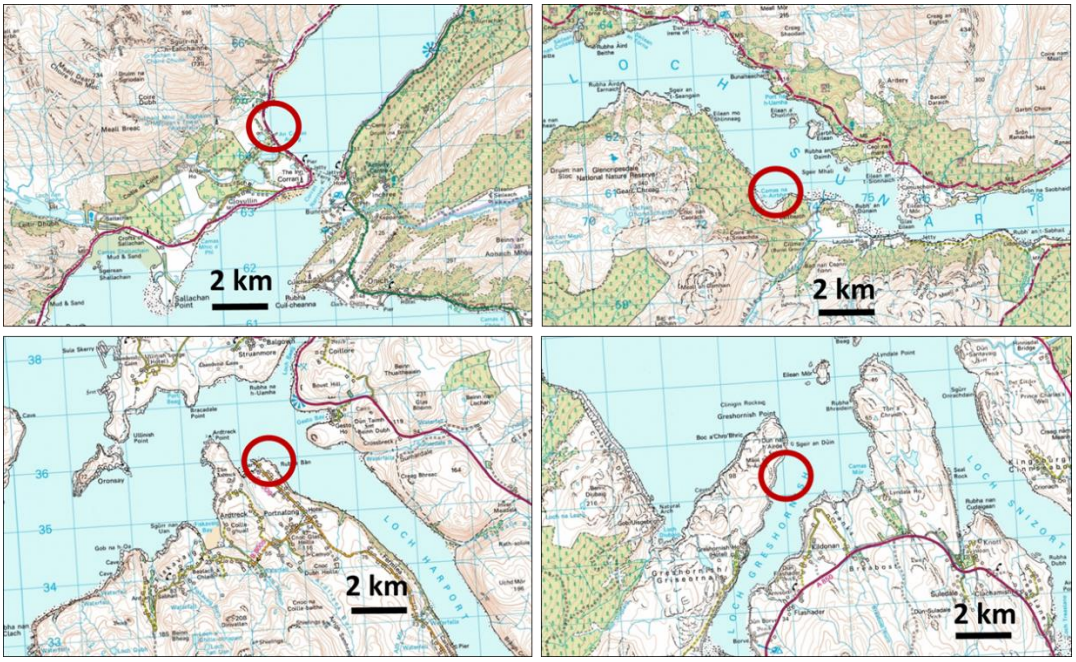


Figure 2.2 Local area maps of each sampling site (UK Ordnance Survey 2015, 1:25000). Sea cage positions are circled in red. Top left: Linnhe. Top right: Invasion Bay. Bottom left: Portnalong. Bottom right: Greshornish.

2.3.2 Hydrozoan sampling methods

Each site was provided with a 0.5 m ring \ plankton tow net with a 270 µm mesh cod end attached to a 5 m tow line. Using the formula $V_{water\ sampled} = \pi r^2 l$, where r = net radius and l= the vertical distance towed, each towed sample using this equipment would filter 3.9 m^3 seawater. Triplicate vertical tows were conducted from the seaward side of sea cages on a weekly summertime basis and a monthly wintertime basis (Table 1). Samples were placed in seawater with 4% buffered formalin. All gelatinous zooplankton, including hydrozoan medusae, scyphozoan ephyrae, and ctenophores were identified, counted, and recorded as medusae per cubic metre seawater (denoted hereafter as jm^{-3}) (Russell 1953, Conway 2012).

Due to an initially high rate of failure in terms of sample leakage or incorrect preservation, these numbers were pooled by date for statistical analysis. Sampling in 2012 spanned 11 June – 19 October (19 weeks). Sampling in 2013 began on 1 May and continued to 18 October (25 weeks). In 2014, only Greshornish and Invasion Bay participated regularly. Greshornish conducted sampling from 19 May to 6 October; Invasion Bay sampled from 5 May to 29 September. Table 1 gives details of sampling coverage. Missed or incorrectly preserved samples were often grouped in 2-3 week periods, causing gaps in time-series data. Monthly winter sampling was conducted with good compliance, though only extremely low cnidarian population density was in evidence during these periods.

Table 2.1 Sample coverage during the weekly survey period.

Year	Sampling (weeks)	Linnhe: dates sampled	Invasion Bay: dates sampled	Portnalong: dates sampled	Greshornish: dates sampled
2012	19	12	10	18	16
2013	25	15	14	20	19
2014	25	none	18	none	23

2.3.3 Health monitoring methods

Mortality rates of salmon were estimated as a percentage on a weekly basis by collection of dead fish from pens. (Stocking density is relatively constant and initial density constant across Marine Harvest sites, with a low level of mortality of around 0.005% per week to be expected.) Specific monitoring of gill health at each site was implemented beginning in late July and early August 2012, also on a weekly basis. Five to ten fish were randomly selected from each pen on site using a dip net and anesthetized using MS-222. Gills were examined by eye, and a small lamellar scraping transferred to a slide for microscopy. Healthy fish were returned live to the pen; moribund fish were euthanized. Proliferative gill disease (PGD) and amoebic gill disease (AGD) were then assessed and scored based on guidelines laid out in Mitchell et al. (2011), wherein a score of 0 indicates health and 5 indicates widespread disease across the gills (Figure 2.3). Incidence and degree of both PGD and AGD were reported on a per fish, per cage, site-by-site basis. When evaluating gill health across the site, these were converted to a unitless rate of severity by adding the total gill scores observed, then dividing by the number of fish sampled.

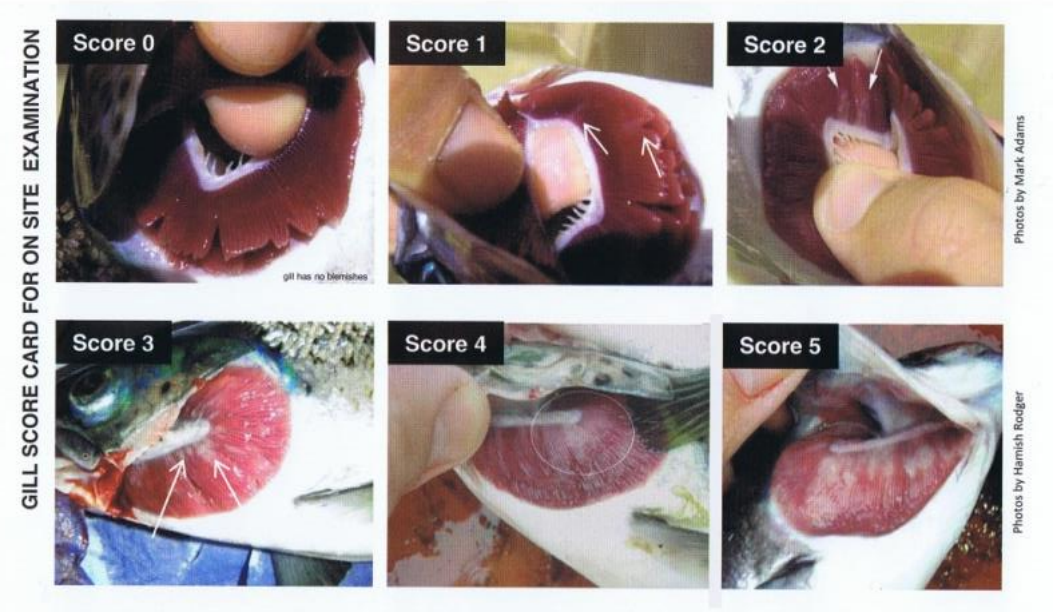


Figure 2.3. Marine Harvest gross examination of gill scoring. Adapted by Marine Harvest from Mitchell et al. 2011.

2.3.4 Water quality conditions

Automated monitoring of conditions at each site was carried out continuously at each site using a YSI EXO2 multi-parameter sonde submerged at 5 m depth at the seaward edge of the sea cage array at each site. This measured temperature, salinity, and light penetration (vertical metres to attenuation) during periods of active farming. It was removed during fallow periods.

2.3.5 Primary productivity

Productivity for each site was estimated using ocean colour reflectance data obtained from flyovers by the MODIS-enabled Aqua EOS satellite, downloaded to the NASA Goddard Spaceflight Center and accessed through the University of Strathclyde. Remote-sensed reflectances were used to calculate estimate chlorophyll-a concentration values using the standard chlor_a product distributed by Ocean Biology Distributed Active Archive Center (OB.DAAC 2014).

2.3.6 Statistical analyses

Data were collated and analysed using Microsoft Excel, R, and Matlab. Hydromedusan population data were plotted along a time series at each site. For certain analyses, raw data were normalized by adding a count of one individual medusa to each data point, then natural-log transforming the values. Site and regional means were compared using one-way ANOVA. Autocorrelation in time-series data, and the suitability of their use in linear modelling, was assessed using the Durbin-Watson test statistic. Regression analysis comparing proximate sites Greshornish and Portnalong was used to produce hindcasts of temperature during fallow periods between Greshornish and Portnalong, when the automated sensor equipment was removed from the water.

2.4 Results

Raw data for this section can be found in the directory "Chapter 2 Hydromedusan monitoring database," submitted in the metadata for this thesis. Guidance notes are provided therein.

2.4.1 Hydromedusan population density

2.4.1.1 Commonly occurring species

Of the 90+ species of medusa-producing hydrozoans reported in UK waters (Russell 1953), 44 identifiable species were found in this survey. The majority of these species appeared singly or very rarely; two (*Obelia* sp.* and *Lizzia blondina*) stood out as having considerably greater relative frequency (Figure 2.4). These contributed the majority of individuals in the pooled observations. A third species, *Muggiaea atlantica*, was also frequently present, though it must be considered with a different approach. While *Obelia* sp. and *L. blondina* are true medusozoan species, *M. atlantica* is a siphonophore, which exists as a pelagic colony of linked individuals (Cornelius 1995) (Figure 2.5). Its appearance in samples was always as individual body segments, particularly eudoxid segments that are specialized for prey capture (Cornelius 1995), and its presence in samples was quantified as *n* eudoxids. Where *Obelia* sp. and *L. blondina* can be regarded to be the result of medusa production, separate eudoxids represent the presence of a siphonophore colony that has been advected into the area and dissociated (Cornelius 1995).

Figure 2.4 Relative frequency of all hydromedusan species appearing in the survey (*n* observations of each species / *n* total observations.)
**Muggiaea atlantica* is a siphonophore occurring in coastal waters in individual segments called eudoxids; this relative frequency reflects *n* eudoxids appearing.
***Several species of leptomedusa lack identifying features at juvenile stages, and cannot be further classified from preserved specimens. Such individuals were grouped here.

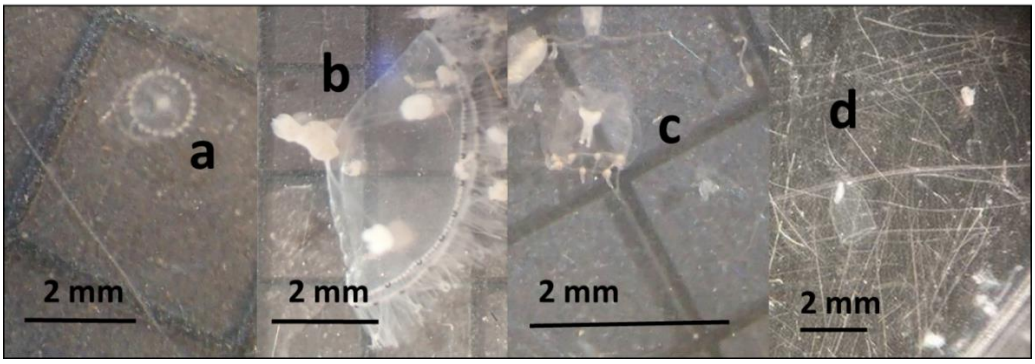


Figure 2.5 The most frequently occurring hydrozoan species appearing at the study sites. (a) small *Obelia* sp. medusa. (b) large *Obelia* sp. medusa. (c) *Lizzia blondina* medusa. (d) *Muggiaea atlantica* eudoxid.

2.4.1.2 Data distribution

Each of the four sites showed marked fluctuations in hydromedusan jellyfish population density over summer periods of weekly observation, ranging from 0 to nearly 700 jm^{-3}, with the majority of measures below 10 jm^{-3} and the probability of observing larger populations tapering off rapidly by mid-autumn. Every population density greater than 0.1 jm^{-3} was observed during weekly summer periods of observation rather than the monthly observations made over winter.

High-density populations were observed more frequently at Skye sites than mainland sites, and this difference was found to be statistically significant using ANOVA to compare means ($p<0.001$, $df=188$, $f=3.79$) (Figure 2.6). This pattern of population density held true for the three most frequently occurring taxa of *Obelia* sp. ($p<0.001$), *L. blondina* ($p<0.001$), and *M. atlantica* ($p<0.001$); the density across summed other species was not significantly different ($p=0.798$).

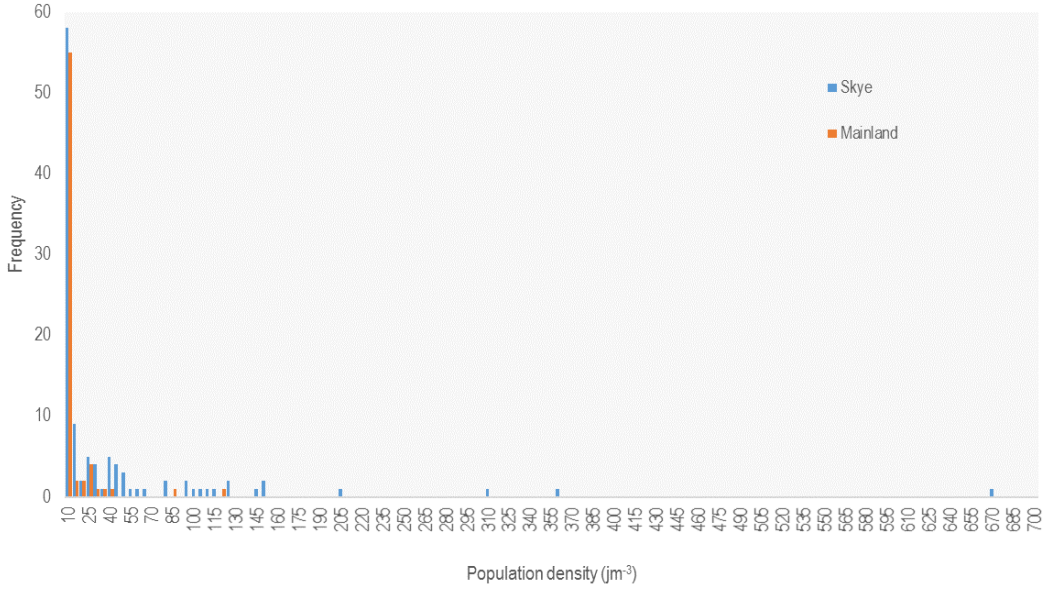


Figure 2.6 Relative frequency of all species appearing in the survey. Skye sites (Portnalong and Greshornish) experienced considerably higher population densities than did mainland sites (Invasion Bay and Linnhe).

2.4.1.3 Time-series comparisons

Figures 2.7-2.9 show the total hydromedusa from 2012-2013 at all sites observed, with the taxa of most common occurrence (*Obelia* sp., *L. blondina*, and *M. atlantica*) denoted. Temporally, all sites are strikingly different from one another in terms of both timing and magnitude of spikes and blooms. This is inconsistent with the hypothesis of a universal environmental mediator, such as lunar periodicity, being the primary stimulus for medusa production by hydroid colonies. Additionally, given the proximity of Portnalong and Greshornish sites, the temporal differences in species assemblage between these casts doubt on the hypothesis that geographically-broad advection is primarily responsible for the occurrence of *Obelia* sp. and *L. blondina* blooms.

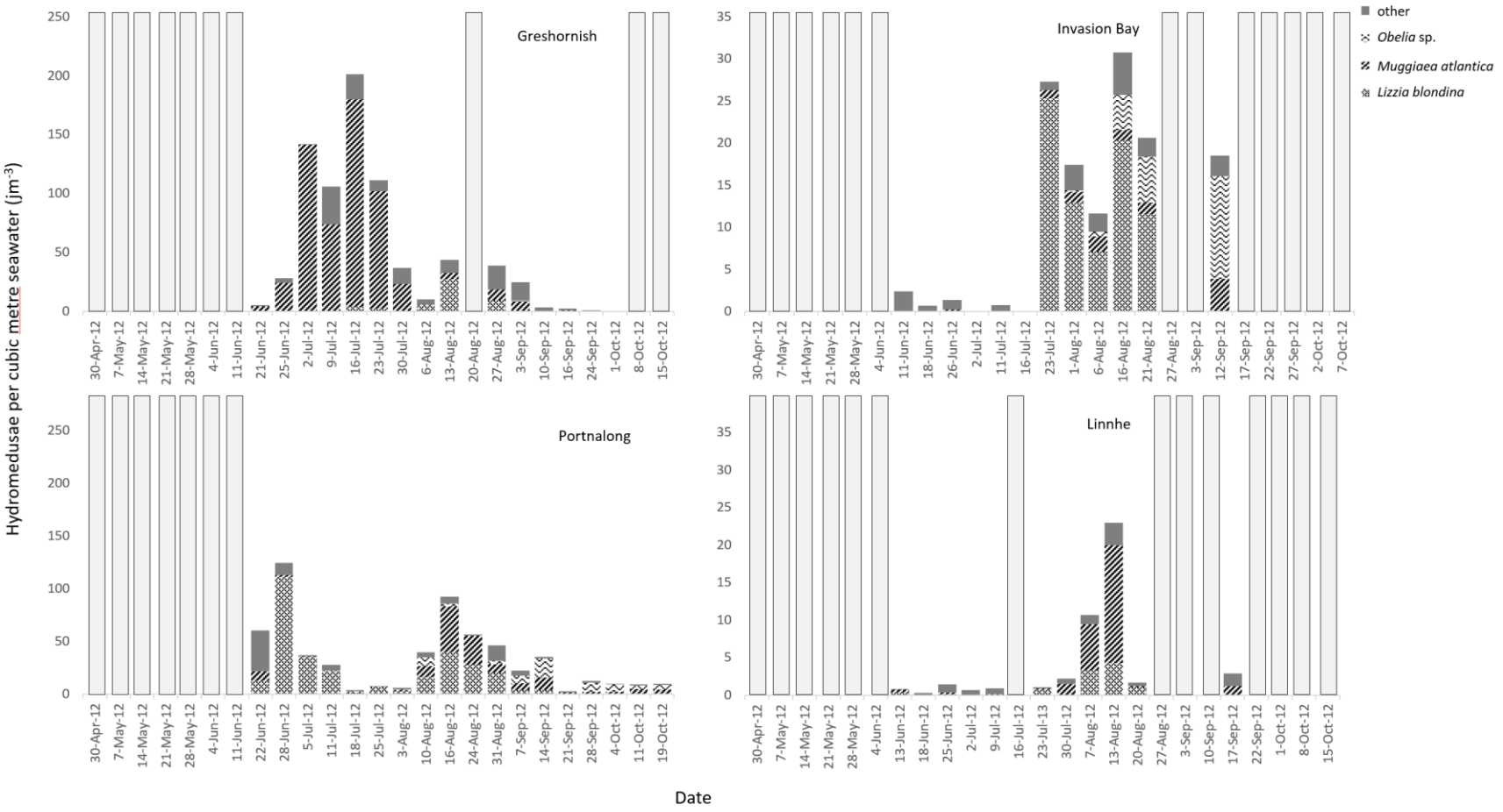


Figure 2.7 Time-series 2012 hydromedusa populations (jm^{-3} per date). Top left: Greshornish. Bottom left: Portnalong. Top right: Invasion Bay. Top left: Linnhe. Grey bars denote no sampling taking place on this date. Note that Y axes are not standard.

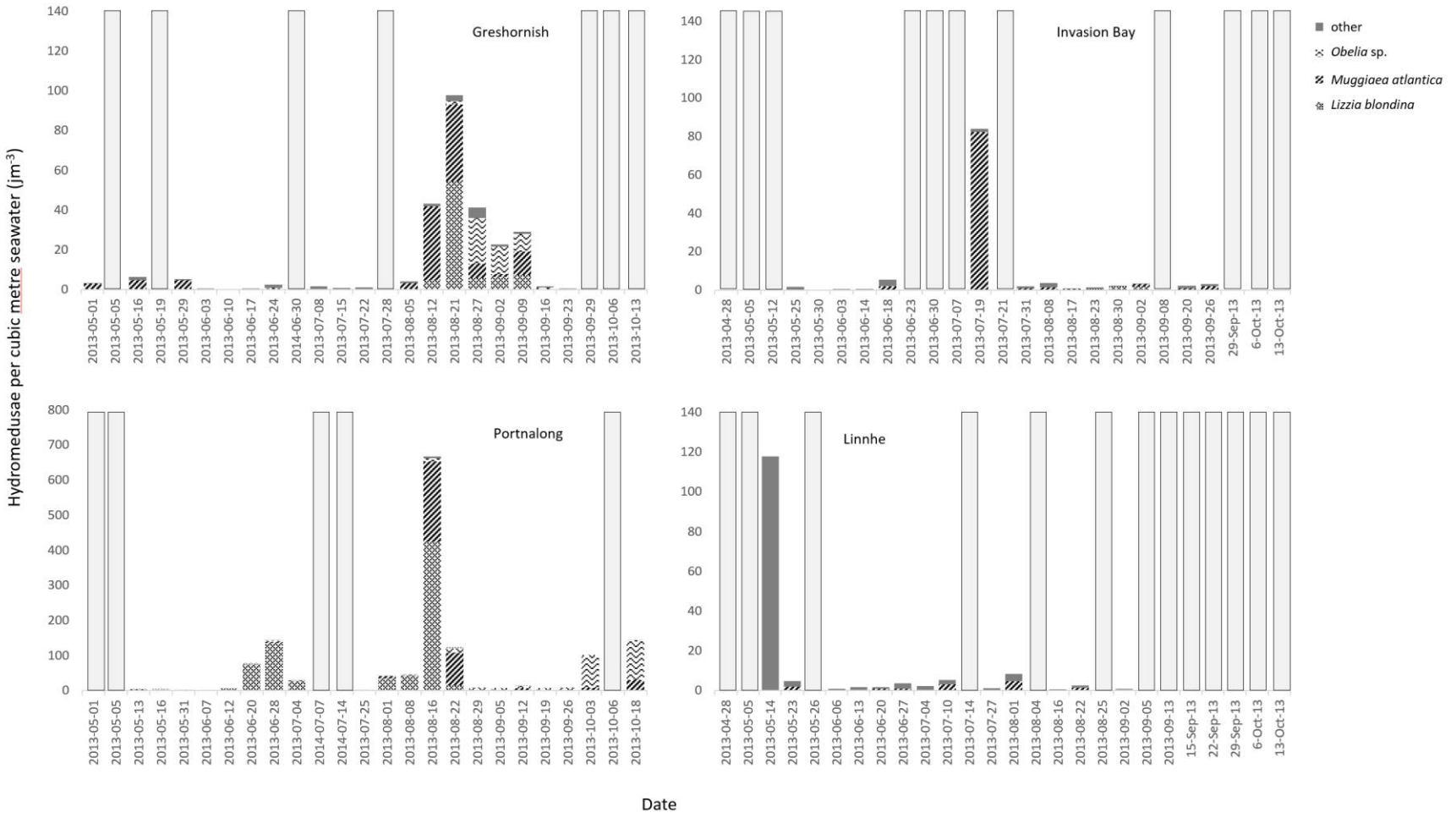


Figure 2.8. Time-series 2013 hydromedusa populations (jm^{-3} per date). Top left: Greshornish. Bottom left: Portnalong. Top right: Invasion Bay. Top left: Linnhe. Grey bars denote no sampling taking place on this date. Note that Y axes are not standard.

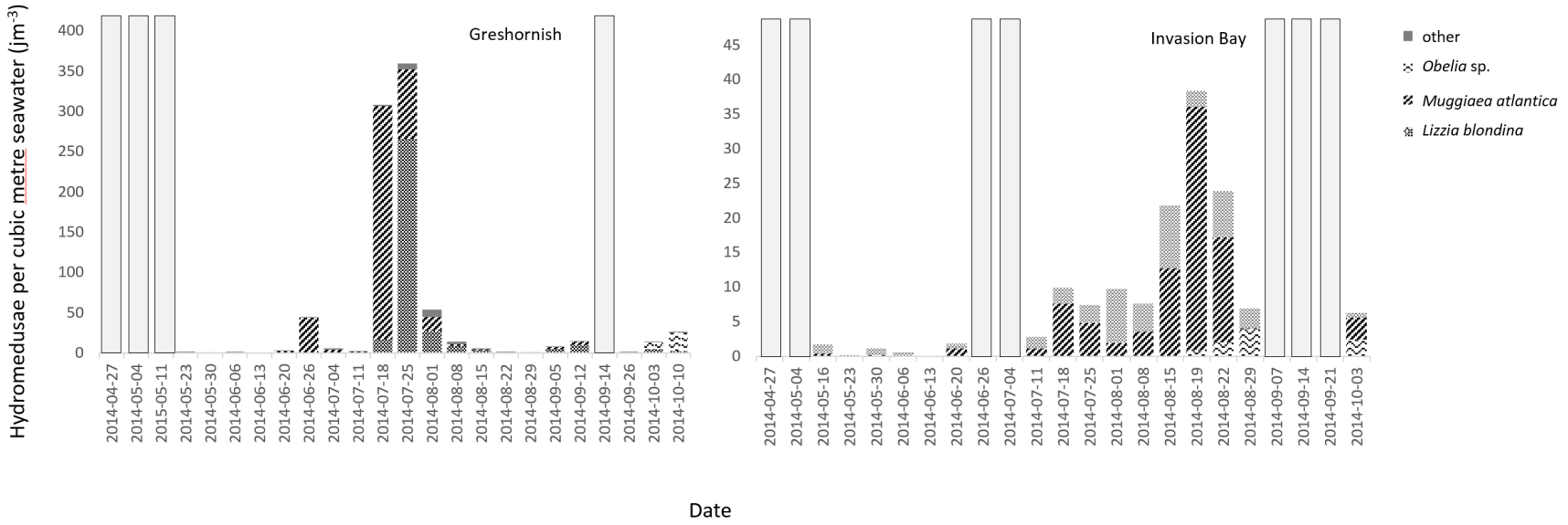


Figure 2.9 Time series of 2014 hydromedusa populations (jm^{-3} per date). Left: Greshornish. Right: Invasion Bay. Grey bars denote no sampling taking place on this date. Note that Y axes are not standard.

2.4.1.4 Defining a bloom

Population density of pooled species were arbitrarily grouped into four broad categories based on the frequency of occurrence: ≤ 10 (63.1% of measures); > 10 ≤ 70 (26.3% of measures), > 70 ≤ 150 (8.4% of measures), and > 150 (2.2% of measures). These parameters will be used to refer to populations as follows in Table 2.2.

Table 2.2 Terminology used to describe hydromedusan blooming events.

Population density	**Term**
≤ 10 j/m^{-3}	baseline
> 10 ≤ 70 j/m^{-3}	spike
> 70 ≤ 150 j/m^{-3}	moderate bloom
> 150 j/m^{-3}	high-magnitude bloom

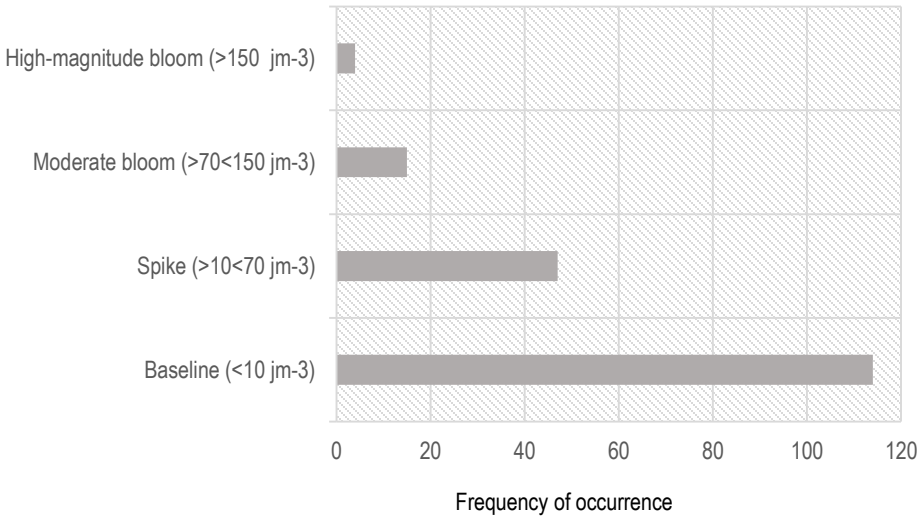


Figure 2.10 Observations of varying population density measures. Observations of baseline population density were far more common than observations of blooms.

Seventeen instances of moderate or high-magnitude hydromedusan population density were recorded (Table 2.3). Of these, 2 were attributable to the presence of eudoxid segments of *M. atlantica*, indicating advection of a colony into the study area. As such, these should not be considered a true bloom in the same manner as

understood for other hydrozoan species. Of the remaining 15 instances, one other stands out as particularly unusual: 14 May 2013 at Linnhe showed a bloom by *Hybocodon prolifer*, a species not seen blooming at any other times, and which is reported in New England waters to be primarily spring-blooming (Coestello and Mathieu 1995, Nakayama and Numakunai 2000). The remaining 14 instances were attributable to either *Obelia* sp. (50% incidence), *Lizzia blondina* (14% incidence), or a co-occurrence of both (36% incidence); all but one of these blooms occurred at Skye sites. Finally, several of these 14 incidents are attributable to a single blooming event spanning several weeks; these dates are grouped together in Table 2.3. In total, 8 blooms of *Obelia* sp., *L. blondina*, or both in combination were recorded.

Table 2.3 Incidence of moderate and high-magnitude population density. Long-duration single events occurring across multiple sampling dates are grouped by shading.

Date	Site	Species involved	Population density	Density scale	Grouping
2-Jul-12	Greshornish	*Obelia* sp.	142.08	moderate	A
9-Jul-12	Greshornish	*Obelia* sp.	105.82	moderate	
16-Jul-12	Greshornish	*Obelia* sp.	201.36	high-magnitude	
23-Jul-12	Greshornish	*Obelia* sp.	111.17	moderate	
28-Jun-12	Portnalong	*Lizzia blondina*	124.16	moderate	B
16-Aug-12	Portnalong	*Obelia* sp., *L. blondina*	92.23	moderate	C
19-Aug-13	Greshornish	*Obelia* sp., *L. blondina*	97.84	moderate	D
20-Jun-13	Portnalong	*Obelia* sp., *L. blondina*	79.32	moderate	E
28-Jun-13	Portnalong	*L. blondina*	145.73	moderate	
16-Aug-13	Portnalong	*Obelia* sp.	668.37	high-magnitude	F
22-Aug-13	Portnalong	*Obelia* sp., *L. blondina*	124.13	moderate	
3-Oct-13	Portnalong	*Muggiaea atlantica* (nectophores)	101.74	moderate	G
18-Oct-13	Portnalong	*Muggiaea atlantica* (nectophores)	145.31	moderate	H
14-May-13	Linnhe	*Hybocodon prolifer*	117.96	moderate	I
19-Jul-13	Invasion_Bay	*Obelia* sp.	84.08	moderate	J
18-Jul-14	Greshornish	*Obelia* sp.	308.11	high-magnitude	K
25-Jul-14	Greshornish	*Obelia* sp., *L. blondina*	359.98	high-magnitude	

Time-series analysis of population density using the Durbin-Watson test shows positive autocorrelation in *L. blondina*, *Obelia* sp. and summed population density (Table 2.4). This indicates that despite major week-to-week differences in population density, time-series measures are not entirely independent from one another, limiting the linear model analysis possibilities with the full dataset. Analysis of only

the dates showing moderate to high-magnitude blooms shows no statistically significant positive or negative autocorrelation, meaning these measures do not violate linear modelling assumptions (Table 4).

Table 2.4 Autocorrelation in *Obelia* sp., *Lizzia blondina*, and summed population data.

Species	D-statistic	D_L (critical lower limit)	D_U (critical upper limit)
Lizzia blondina (time-series)	1.70	1.75	1.77
Obelia sp. (times-series)	1.25	1.75	1.77
Summed (time-series)	1.26	1.75	1.77
Lizzia blondina (blooms only)	1.72	1.16	1.39
Obelia sp. (blooms only)	1.68	1.16	1.39
Summed (blooms only)	1.92	1.16	1.39

2.4.2 Environmental factors prior to and during blooms

2.4.2.1 Temperature

2.4.2.1.1 Missing values and hindcasts. Due to data logger equipment removal during fallow periods, two temperature record periods were hindcast based on the records of adjacent sites (Portnalong 28 April 2012 – 7 October 2012, Greshornish 10 March 2013 – 5 December 2013). Regression analysis calculated a relationship suitable for use in hindcasting values during periods of time when temperature observations were kept for only one site ($T_{Portnalong}$ = .9996 x $T_{Greshornish}$ - 0.2919; $p < 0.001$, $df = 1$, $r^2 = 0.9373$) (Figure 2.11). Temperature fluctuated annually between 5.5-6.5˚C in winter and 17˚C in summer (Figure 2.12).

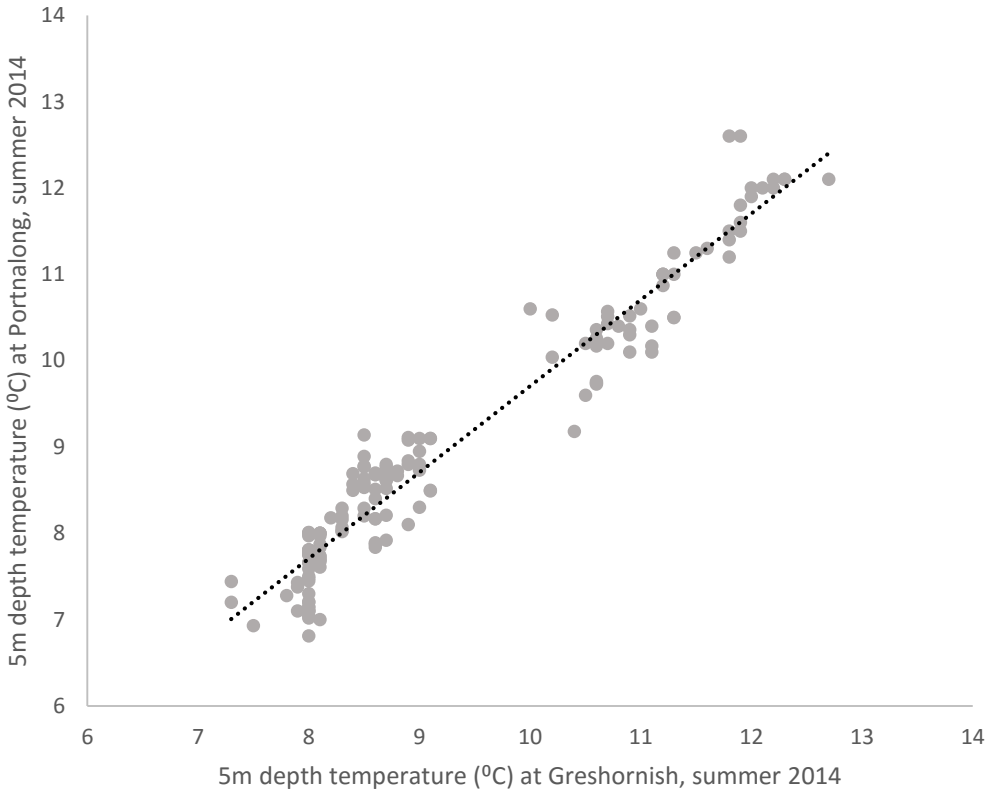


Figure 2.11 Temperatures (⁰C) measured at 5m depth at Greshornish and Portnalong, 2014.

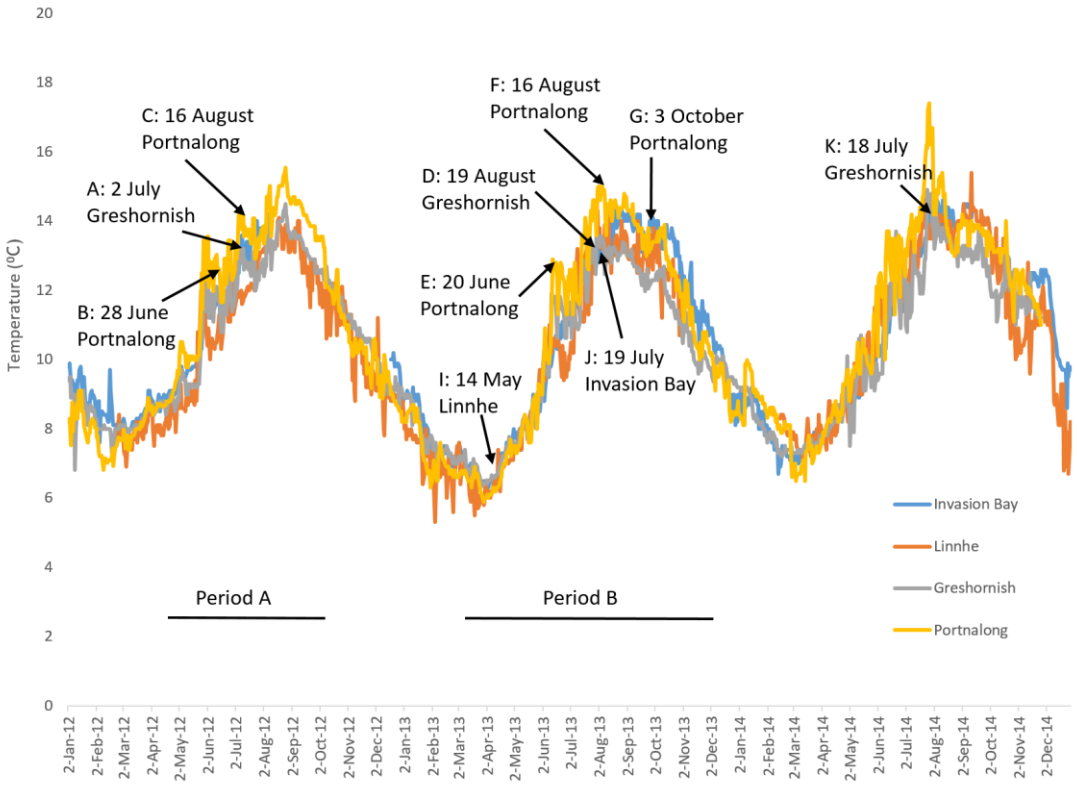


Figure 2.12 Temperature records (including hindcast values) for all sites. High magnitude bloom incidents, labeled as per Table 2.3, are indicated. Period A indicates values hindcast at Portnalong; Period B indicates hindcast at Greshornish. Blooms are labeled in accordance with Table 2.3.

2.4.2.1.2 Bloom occurrence. All blooms involving *Obelia* sp. and/or *Lizzia blondina* occurred in waters above 12.8°C measured at 5 m depth (Figure 2.13). (The single outlying moderate bloom in Figure 2.7, recorded in water of 8°C, involved *Hybocodon prolifer*.) In addition, 94% of population spikes took place in temperatures above 12°C. Temperatures in the 7 days prior to both moderate and high-magnitude blooms averaged 12.65°C degrees; during 14 days prior, it was 12.44°C; and during 28 days prior, it was 12.20°C.

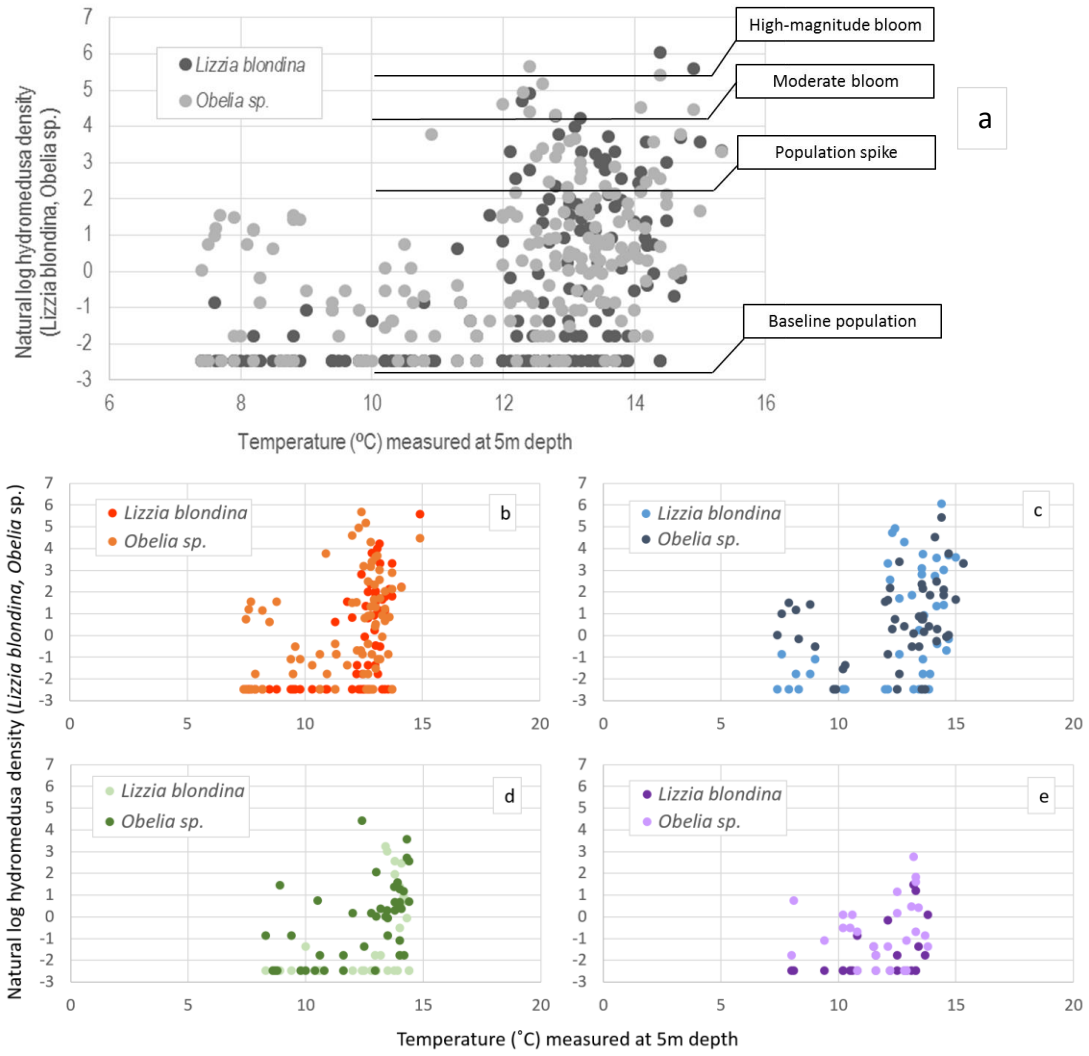


Figure 2.13 ***Lizzia blondina*** **and** ***Obelia*** **sp. medusae are most likely to occur in both spike and bloom-level population density measures in water greater than 12˚C.** (a) all samples pooled; (b) Greshornish; (c) Portnalong; (d) Invasion Bay; (e) Linnhe.

Temperature records in the 60-day periods prior to *Obelia* sp. and *L. blondina* blooms shared some general characteristics: in days 60-30 before blooms commenced, temperatures rose overall; in the 30 days prior to all except bloom J (Table 2.5), temperatures fluctuated around a central mean of 12^{0}C or above (Figure 2.14).

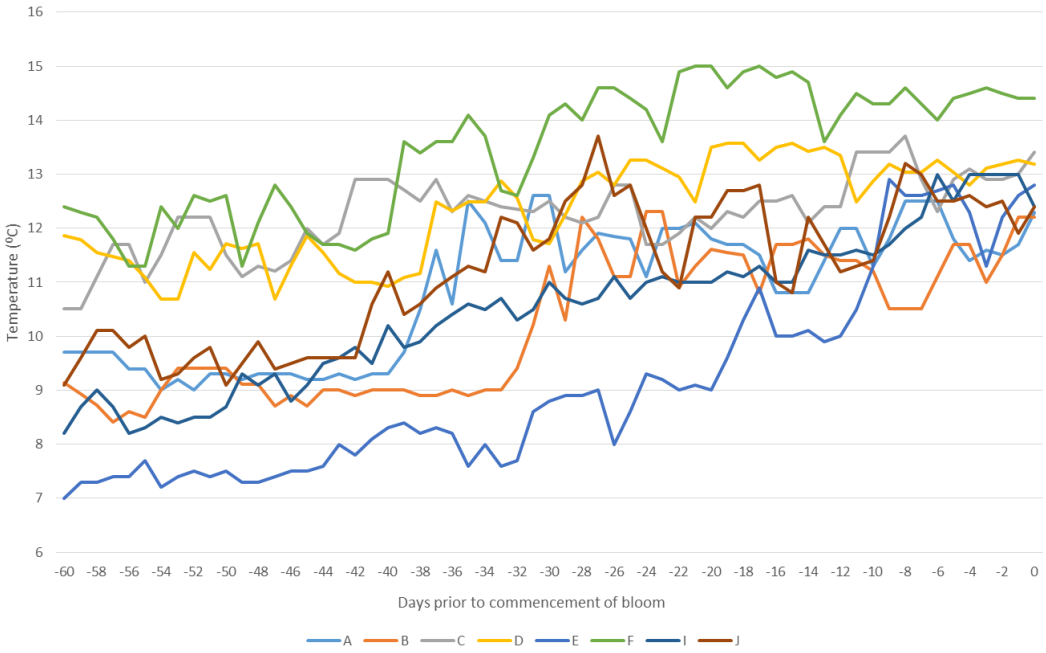


Figure 2.14 Temperature measured at 5 m depth in the 60 days prior to blooms.

2.4.2.2 Photoperiod

Most blooms took place in late summer, with photoperiod in the 7 days prior to bloom measures averaging 963 minutes (σ±136.4), with one outlier taking place in mid-October in Portnalong 2013 (Figure 2.15). It should be noted, however, that temperature measures at Portnalong during October 2013 were consistently a full degree warmer than those in October 2012. This suggests that temperature is a strong determinant, while photoperiod is merely an artefact of this.

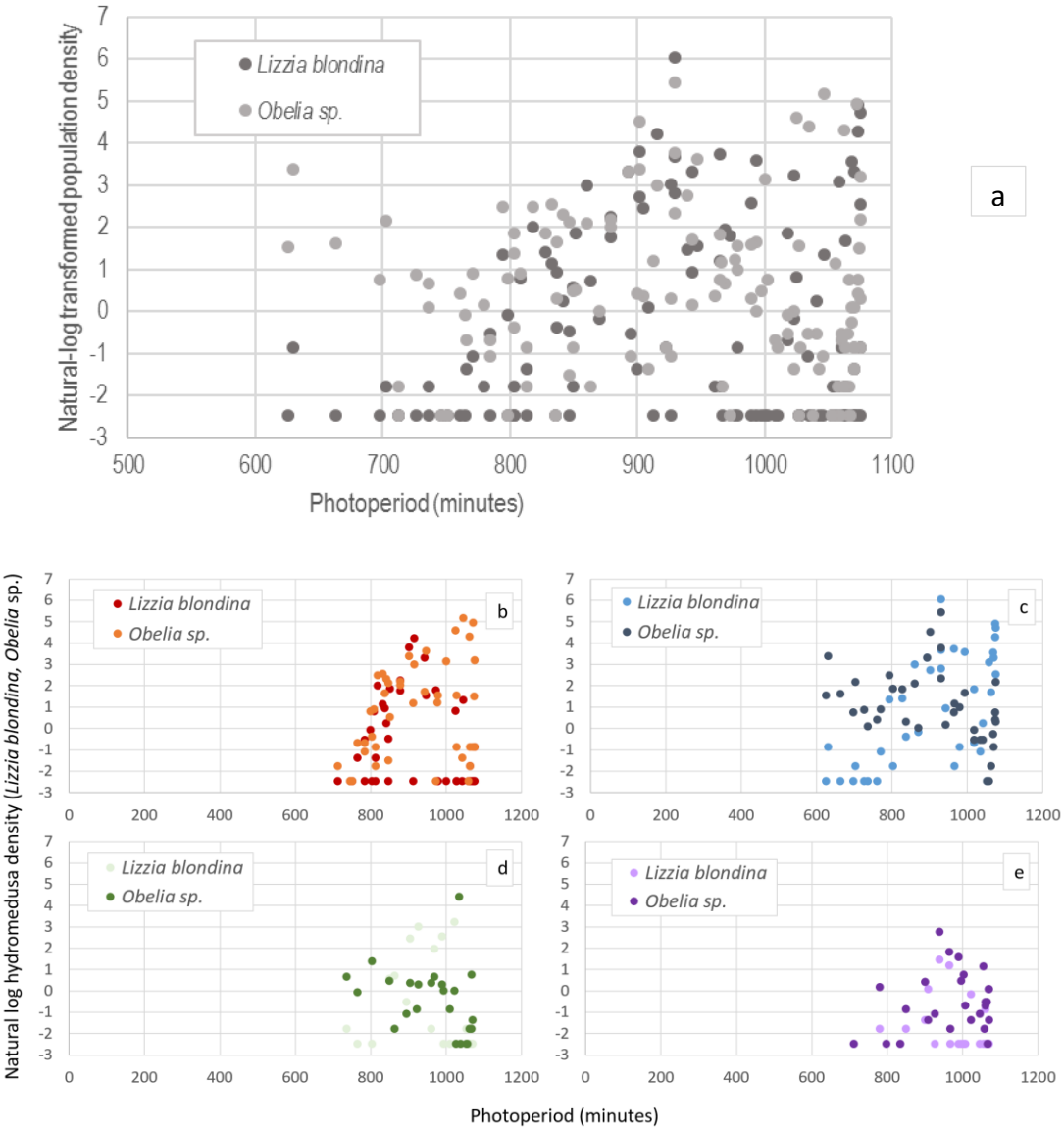


Figure 2.15 Mean photoperiod during the 7 days prior to *Obelia* sp. or *Lizzia blondina* population measurements. (a) all samples pooled; (b) Greshornish; (c) Portnalong; (d) Invasion Bay; (e) Linnhe.

2.4.2.3 Salinity

Overall, salinity measures changed independently between sites, with considerable fluctuations likely reflecting localized rainfall and runoff events (Table 2.5) (Loch Bracadale Framework Plan 2002).

Table 2.5 Comparing salinity fluctuations between sites.

	Portnalong	*Greshornish*	*Invasion Bay*	*Linnhe*
Mean (‰)	32.68	32.37	31.47	31.39
Standard Error	0.0830	0.0095	0.0716	0.2724
Range	10.3	2.4	7.9	29
Minimum	24	32.1	25.5	10
Maximum	34.3	34.5	33.4	39

2.4.2.3.1 Missing values and hindcasts. The expansion of data-gathering to include salinity measures was not implemented until 2013; therefore, blooms occurring in 2012 cannot be linked with a salinity record. In addition, due to equipment removal during fallow periods, the summer period in 2013 at Greshornish was not monitored. Regression analysis based on Portnalong values, while statistically significant, could not produce a reliable record of values ($p<0.001$, $df = 1$, $r^2 = 0.0747$), leaving a considerable gap in data. These limitations meant that only blooms E, F, I and J (Table 2.5) could be associated with salinity data.

2.4.2.3.2 Bloom occurrence. Blooms took place in salinity conditions ranging from 31-35, with no pattern in evidence of any influence on hydromedusa population density (Figure 2.16).

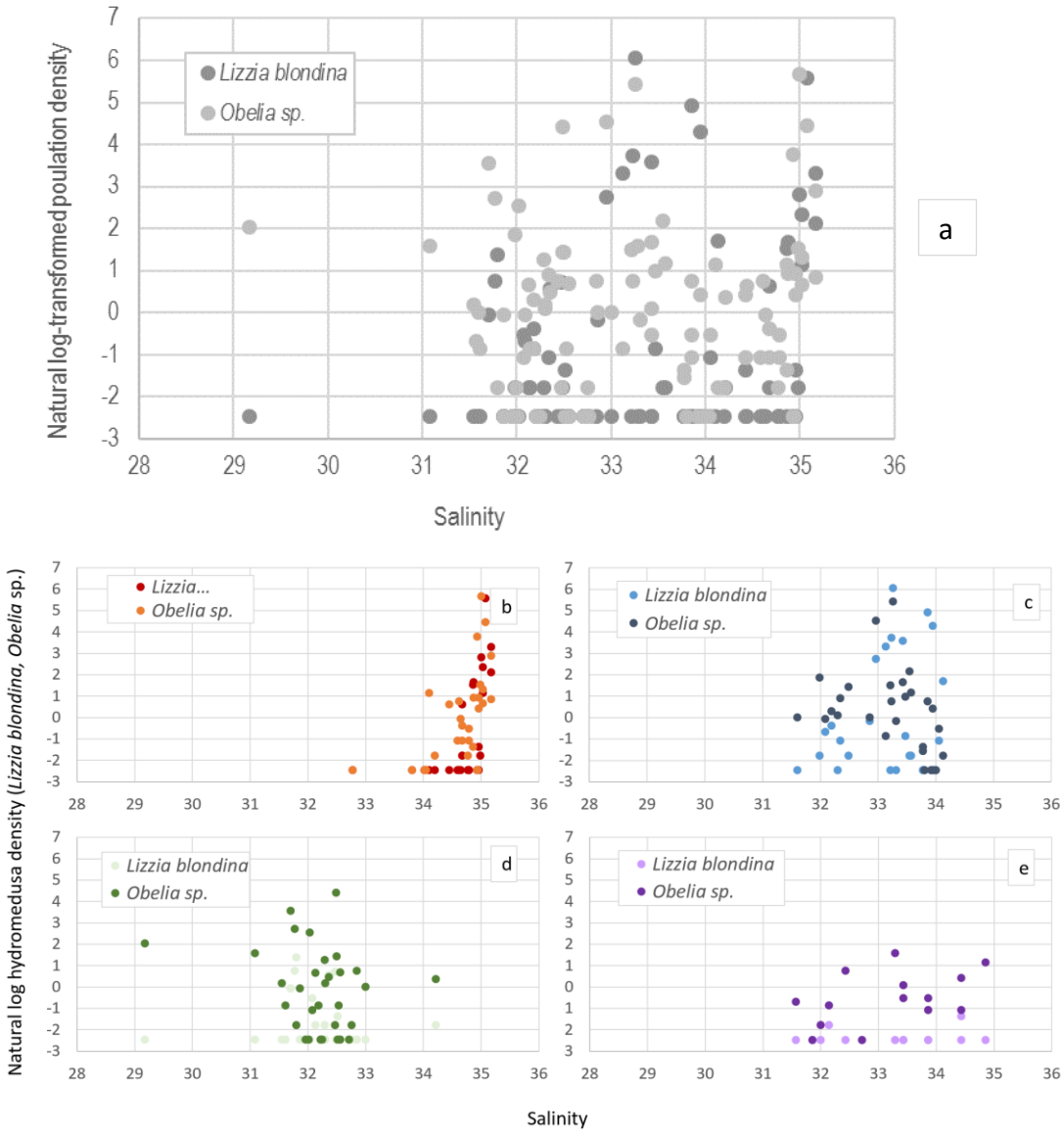


Figure 2.16 Mean salinity range over the 7 days prior to observed populations of *Lizzia blondina* and *Obelia* sp. were observed. (a) all samples pooled; (b) Greshornish; (c) Portnalong; (d) Invasion Bay; (e) Linnhe.

Furthermore, while range of salinity measures did vary considerably from site to site, this variability did not map to geographic probability of blooms: the site with the greatest range of salinity measures, Linnhe, had the least frequent incidence of blooms; the site with the second-greatest range of salinity measures, Portnalong, had the greatest incidence of blooms (Table 2.3). No common pattern in the 60- to 30-day periods prior to the four blooms with reliable salinity records could be discerned (Figure 2.17). It is notable, however, that no blooms took place during short-lived

periods of very low salinity at Portnalong. The significance of this could not be tested given the present dataset, but may be a useful point of future investigation.

Figure 2.17 Salinity fluctuations in the 60 days prior to 4 moderate or high-magnitude blooms of *L. blondina* or *Obelia* sp.

2.4.2.4 Water clarity

Turbidity measures obtained using the same data logger deployment as limited salinity measures, with no reliable means of hindcasting missing durations. Of the remaining 49 observations, no pattern was observable either in days on which blooms occurred or in the 60- to 30-day periods prior to blooms (Figure 2.18, 2.19). No observations at Greshornish were available during any of the recorded periods.

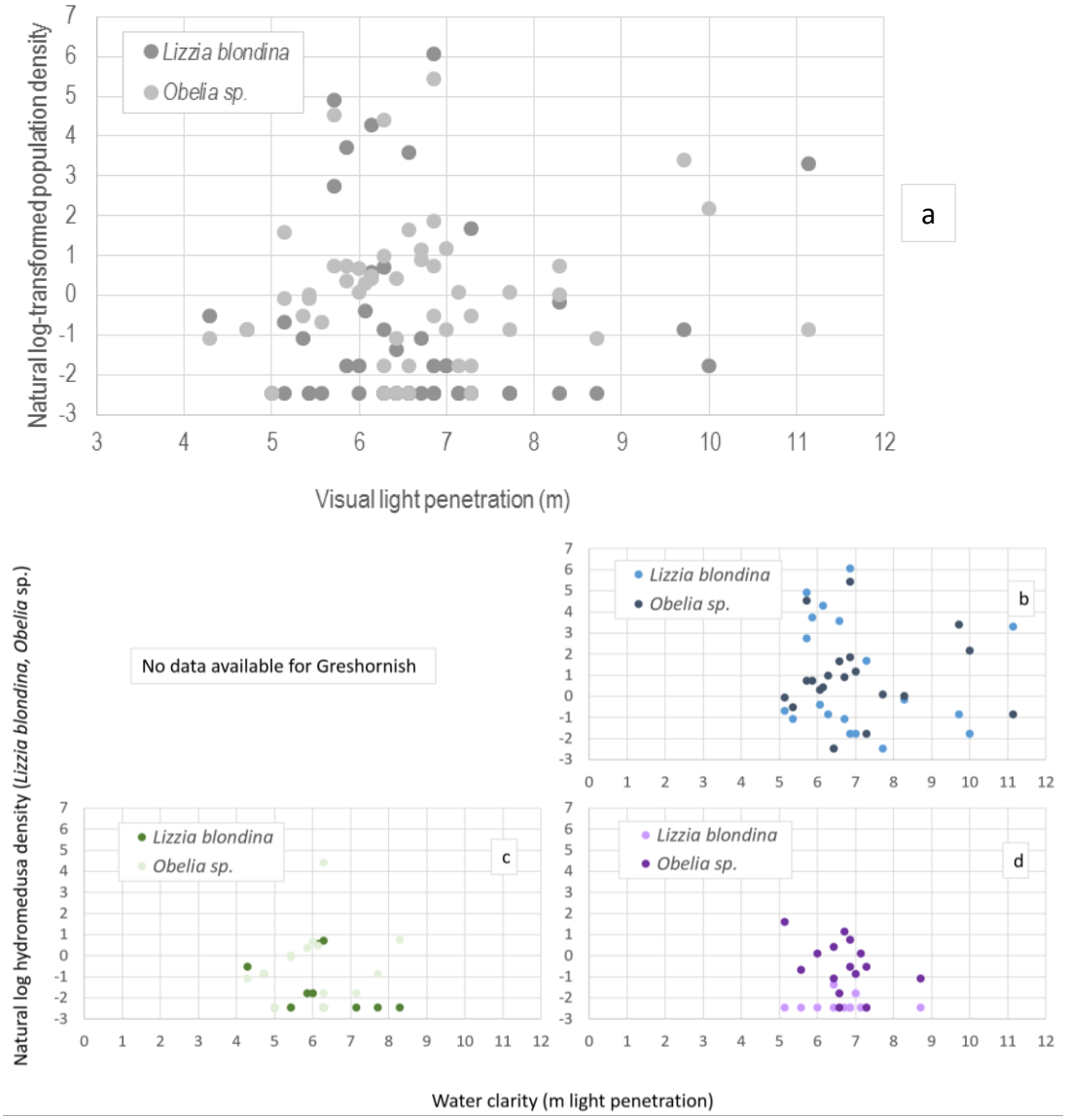


Figure 2.18 Mean water clarity (m to light penetration) observed in the 7 days prior to observations of *Obelia* sp. and *L. blondina* hydromeduse concurrently with hydromedusa population density. (a) all samples pooled; (b) Portnalong; (c) Invasion Bay; (d) Linnhe Bay. Data was unavailable at Greshornish.

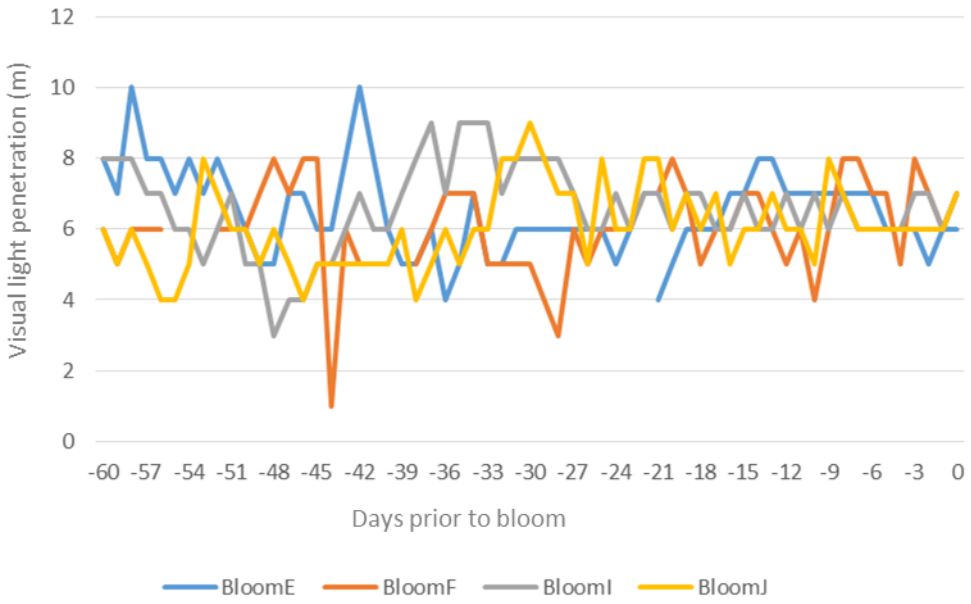


Figure 2.19 Water clarity in the 60 days prior to moderate to high-magnitude blooms. No obvious shared features are evident.

2.4.2.5 Primary productivity

Based on the dissimilarity of the hydromedusan population sizes and species assemblage between sites, it seems likely that blooms develop locally rather than being the result of advection (as is often the case with larger scyphozoans, e.g. Doyle et al. 2008). Therefore, geographically and temporally specific data are required to assess bottom-up trophic influences on hydromedusa populations. Two sites, Linnhe and Invasion Bay, had insufficient open water nearby to obtain reliable surface reflectance for this purpose, due to adjacency effects – i.e. light scattering by land surfaces causing the appearance of falsely high chlorophyll-a in nearshore areas (e.g. Zibordi et al. 2009). For Greshornish and Portnalong, 5x5 km grid cells were overlaid on the areas of open water nearest the sea cages, centred on 57.5495˚N,-6.4650˚W and 57.2078˚N,6.2150˚W respectively (Figure 2.20).

Figure 2.20 Area of chlorophyll measurements adjacent to sea cage sampling locations. 5x5 kilometre areas of ocean colour reflectance to be used for deriving median chlorophyll-a information are boxed in red. (UK Ordnance Survey 2015).

Median chlorophyll-a concentration was obtained from all cells containing reflectance information on a daily basis where possible; these values were averaged over the 7 to 14 days prior to hydrozoan population measures for comparisons. However, frequent cloud cover in the Isle of Skye region often completely obscures reflectance, and cloud edges during partially cloudy days also give false-positive adjacency effects (e.g. McKee et al. 2007, Zibordi et al. 2009). As a result, the vast majority of satellite over-passes returned no measurement at all, and partially cloudy days were apt to return extreme and obviously false outlier values. Finally, dissolved organic matter present during the frequent runoff events in the Skye region can also skew results (Figure 2.21), making even data from clear-sky flyovers suspect.

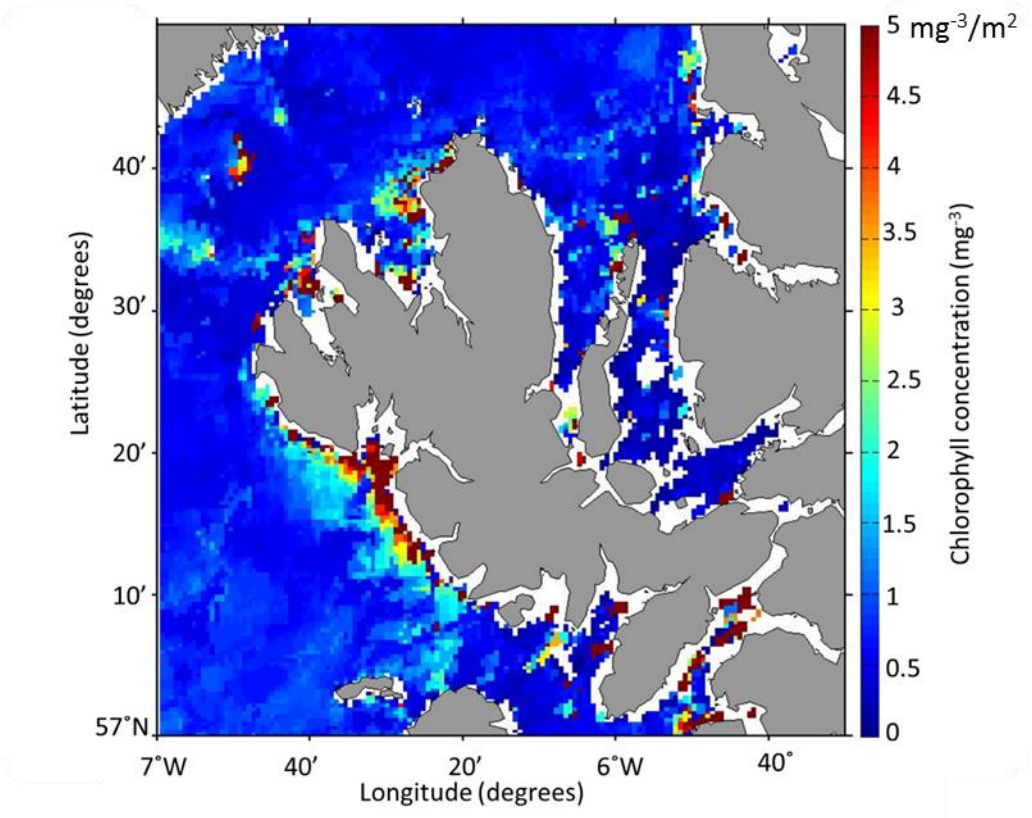


Figure 2.21 Composite median chlorophyll-a measurement of the Isle of Skye for the period of August 2013, created using Matlab analytical tools. The high measures in and around Loch Bracadale (nearby Portnalong sea cages) are likely false-positives due to frequent peaty runoff in the area (Loch Bracadale Aquaculture Framework Plan 2002). This figure was created in collaboration with Dr David McKee at the University of Strathclyde.

The remaining available composite chlorophyll-a values were aligned with the periods of time during hydromedusa population observation. In total, this left only 21 days (12 from Portnalong, 9 from Greshornish) during which both hydromedusan measures and preceding chlorophyll-a measures were observed. No temporal continuity in these measures prior to blooms was available. These observational constraints prevent any properly informed assessment of bottom-up trophic influence using this dataset, and likely this remote sensing approach. Instead, trophic influences might be better investigated using *in situ* chlorophyll-a monitoring equipment.

2.4.2.6 Tide state

Tide states were considered on the basis of tidal advection bringing medusae into the areas in question. Insufficient data on tide state or time of day during collection of samples were recorded at Linnhe and Invasion Bay sites. For Portnalong and Greshornish, population density data were pooled and sorted by temperature to remove those collected below a 12˚C threshold. These were then parsed as having been collected on either a falling or rising tide (defined as at least 60 minutes after slack water). Comparison of means between these two groups was carried out using one-way ANOVA, for population densities of *L. blondina*, *Obelia* sp., *Muggiaea atlantica* bracts, and remaining other species. No significant differences were found between population densities collected on rising or falling tides for any of these ($n = 55$, $df = 54$, $p = 0.652, 0.430, 0.562, 0.213$ respectively).

2.4.3 Fish health outcomes

2.4.3.1 Data constraints

Medusa population densities at or above 70 jm^{-3} were recorded on 17 dates, representing 10 discrete blooming events, 4 of which were high magnitude (Table 3). A further 47 instances measuring above 10 jm^{-3} were observed. However, not all of these periods could be matched to measures of fish health. Certain periods of

hydromedusan data collection corresponded to fallow periods; at other times, site staff did not conduct hydromedusa sampling. In total, 5 spatial-temporal summer periods had complete concurrent data collection of both fish health and hydromedusan data: Linnhe 2013, Invasion Bay 2013, Invasion Bay 2014, Portnalong 2013, and Greshornish 2014. Two further periods, Linnhe 2012 and Greshornish 2012, were subject to partial data collection, as regular gill examinations were implemented beginning in the middle of that time period. A total of 7 periods of time were logged during which health outcomes and hydromedusa population density were recorded concurrently (Table 2.6, Figure 2.22).

Table 2.6 Fish health data availability. Constraints of fallow periods and non-compliance limited the matched data available to assess the statistical relationship between hydromedusan population density and health outcomes at salmon farms. In total, concurrent data for both population and health outcomes were available for 5 spatial-temporal periods.

Site	Year	Medusa sampling	PGD, AGD	Mortality%	Missing data?
Linnhe	2012	✓	✓ **(partial)**	✓	Gill health checks implemented mid-summer
	2013	✓	✓	✓	Complete record
	2014	**X**	✓	✓	Hydromedusa sampling discarded
Invasion Bay	2012	✓	**X**	**X**	Fallow period
	2013	✓	✓	✓	Complete record
	2014	✓	✓	✓	Complete record
Portnalong	2012	✓	**X**	**X**	Fallow period
	2013	✓	✓	✓	Complete record
	2014		✓	✓	Hydromedusa sampling discarded
Greshornish	2012	✓	✓ **(partial)**	✓	Gill health checks implemented mid-summer
	2013	✓	**X**	**X**	Fallow period
	2014	✓	✓	✓	Complete record

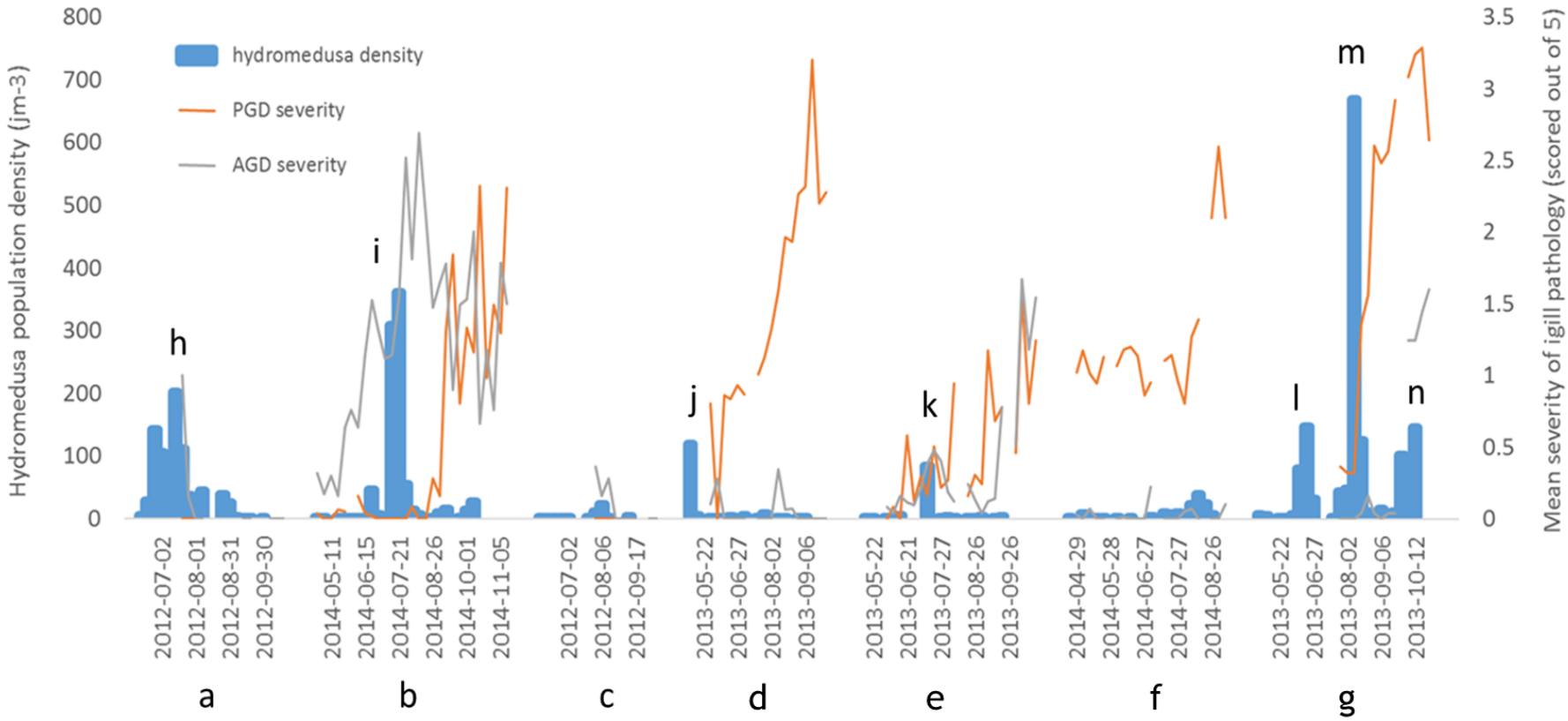


Figure 2.22 Hydromedusa population density and incidence of gill pathology, summer 2012-2014. Periods shown: (a) Greshornish 2012; (b) Greshornish 2014; (c) Linnhe 2012; (d) Linnhe 2013; (e) Invasion Bay 2013; (f) Invasion Bay 2014; and (g) Portnalong 2013. Specific blooms (see Table 2.3): (h) Bloom A, moderate to high magnitude and 4-week duration, involving *Obelia* sp.; (i) Bloom J, high magnitude and two-week duration, involving both *Obelia* sp. and *Lizzia blondina*; (j) Bloom H, moderate and 1-week duration involving *Hybocodon prolifer*; (k) Bloom I, moderate and 1-week duration involving *Obelia* sp.; (l) Bloom E, moderate and 2-week duration, involving both *Obelia* sp. and *Lizzia blondina*; (m) Bloom F, moderate to high magnitude and 2-week duration; involving both *Obelia* sp. and *Lizzia blondina*; and (n) Bloom G, representing not a true bloom but an incursion by the *Muggiaea atlantica* siphonophore.

It is important to note that in addition to cnidarian involvement, there are a number of physical factors and phytoplankton species that can affect gill health. Both AGD and PGD can and do occur independently of detectable hydrozoan blooming. This appears to have been the case in two of the periods of measurement: Linnhe in 2013 and Invasion Bay in 2014 experienced a persistently elevated population of *Chaetoceros* phytoplankton (Chris Wallace pers. comm.). (In both cases, fish recovered without intervention.) However, it is also worth noting that the largest bloom and the greatest incidence of poor gill health observed during the study period does coincide.

The potential for further statistical exploration of the relationships between gill pathology and hydromedusa density from in-situ observational data available here is limited. Two broad approaches are possible. First, data could be considered on an amalgamated site-by-site basis, with the incidence of gill pathology treated as a rate amongst sampled fish on any given day. Second, fish could be considered on an individual basis, with the gill condition of each fish sampled as a data point on any given date. However, both approaches suffer from significant positive autocorrelation in health outcome data, curtailing the linear model approach. Furthermore, of the 7 periods measuring both hydromedusan population density and gill health concurrently, 3 span a relatively short space of time (e.g. *l, m, n* of Figure 2.22); for purposes of considering health outcomes, the effects of these 3 are impossible to fully separate. This leaves 5 discrete bloom exposures to consider (e.g. *h, i, j, k*, and *l-n* of Figure 2.22). When considering that the rise in PGD severity at Linnhe in 2013 was attributed to exposure to harmful *Chaetoceros* phytoplankton, this effectively reduces the number of incidents of poor gill health with no alternative explanation to n = 4. This presents a severe limitation on separating signal from noise in drawing quantitative conclusions about the effects of hydromedusan blooms. In lieu of this, the highest-magnitude bloom observed in this study and its aftermath in terms of fish health is described qualitatively below.

2.4.3.2 Portnalong 2013: qualitative impact of a two-species high-magnitude bloom

In 2013, Portnalong was subject to the largest hydromedusan population density event recorded during the study period (Figure 2.8). *L. blondina* population density rose to the point of moderate bloom for two weeks, measured on 17 and 24 June (73 jm^{-3} and 136 jm^{-3}, respectively); three more spikes in *L. blondina* populations were recorded before the beginning of August. On 12 August, a combined population of *L. blondina* (424 jm^{-3}) and *Obelia* sp. (223 jm^{-3}) were recorded. Fewer *L. blondina* medusae (15 jm^{-3}) were recorded the following week on 19 August, but the *Obelia* sp. population remained at moderate bloom level at 124 jm^{-3}. August 12 constituted the highest population density measured during the study period.

Within days of this bloom, the severity of PGD measured in sampled fish increased rapidly (Figure 2.24). Gill health continued to deteriorate for the following several weeks (Figure 2.23), followed by increasing mortality at the site (Figure 2.25). Beginning September 12, moribund fish were sampled and histopathology analysed by FishVet Group. On September 12, severe acute gill changes "suggestive of a waterborne insult" were reported; secondary chronic amoebic pathology had also developed, with small clusters of amoeba colonies appearing (Cox 2013a). Changes in the hindgut epithelial tissues were found, suggesting that the causative agent may also have been ingested.

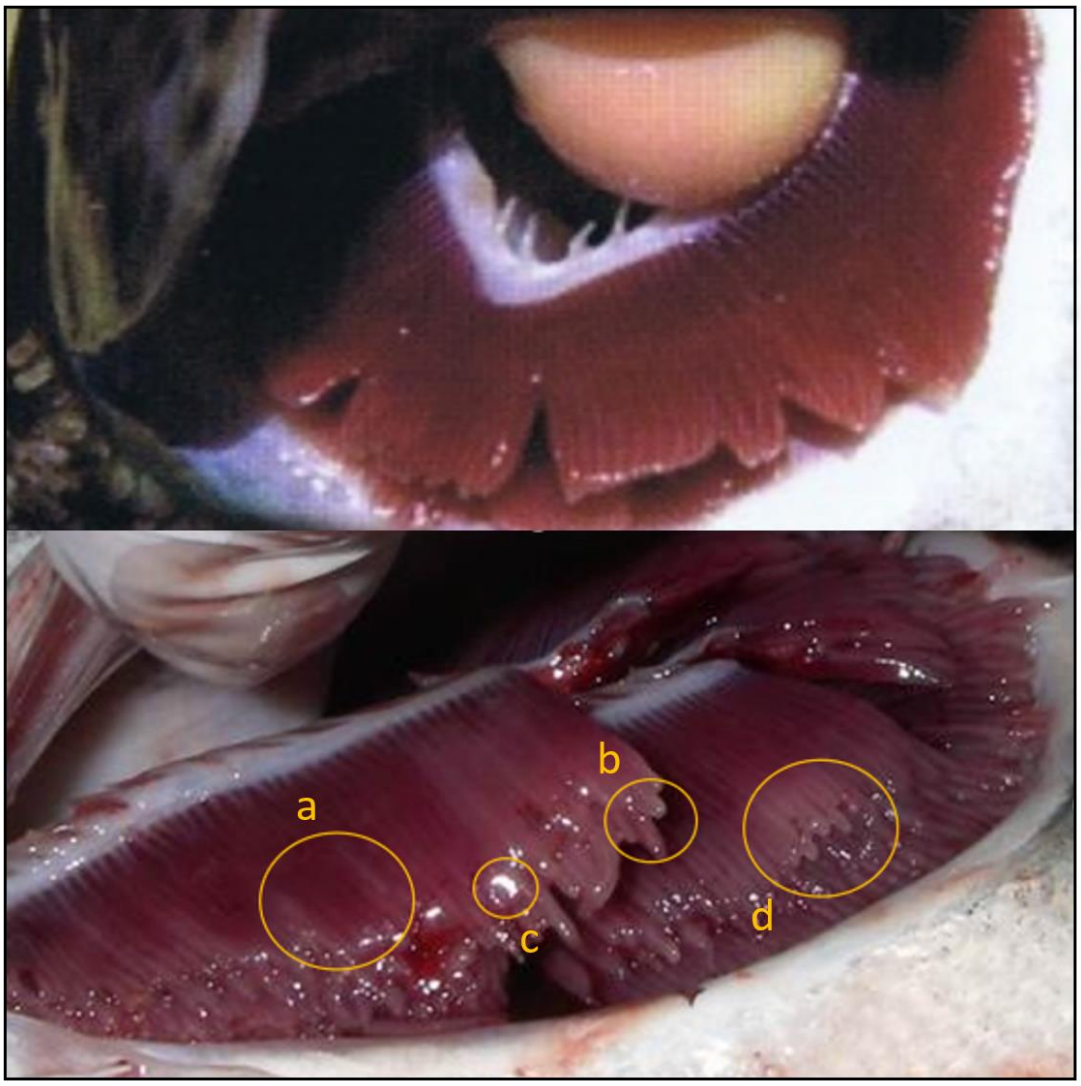


Figure 2.23 Degradation and inflammation of gill lamellae following exposure to a hydrozoan bloom. Top: normal healthy gill (photo by Mark Adams). Bottom: Gill exposed to *Obelia* sp. and *Lizzia blondina* medusae at a cumulative population density of nearly 670 jm^{-3}. Gills showed areas of both hyperhaemia (a) and anemia (b), overproduction of mucus (c), and thickening of the lamellar filaments (d).

By 24 September, overall mortality at the site had begun to rise as well (Figure 2.25), and a second histopathology analysis was conducted. This examination found consistently poor gill condition, and an increased incidence of secondary infection by amoebae (Cox 2013b). During early October, over 100 eudoxids/m^3 of *M. atlantica* were observed over two separate sampling days, indicating potentially as much as 3 weeks of exposure to this species (Figure 2.22, bloom n). Concurrently, a low-grade sea lice infestation had begun to take place (Cox 2013c), at a level not overly harmful

to fish, but which could increase rapidly as well as strongly affect market value. Under normal circumstances, this could be treated with hydrogen peroxide at a concentration of 1800 ppm, with no harm to fish and minimal environmental impact. However, because this treatment would further compromise damaged gills, the problem could not be adequately addressed.

Further histopathology examination on Oct 30 found significant chronic PGD with 50-80% of lamellar area involvement, and a further increased incidence of secondary AGD (Cox 2013d). A final examination on 12 November found amoebic infestation had increased further on compromised gill tissues, with no sign of resolving in PGD-affected areas (Cox 2013e). At this point the decision was taken to cull the remaining fish, at £2.5 million cost in lost product revenue, plus lost cost in treatment attempts.

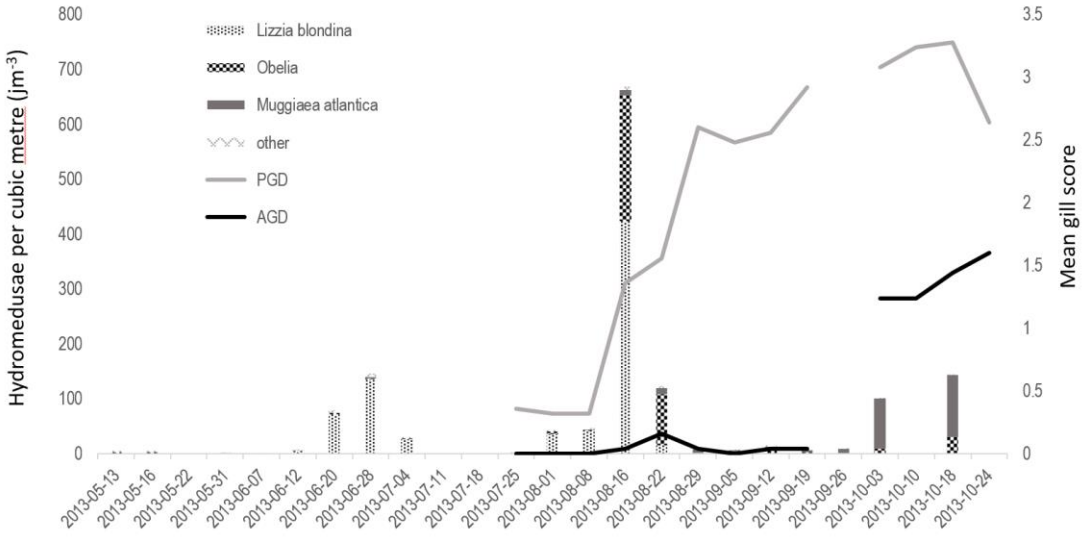


Figure 2.24 Increase in PGD and AGD following bloom exposure. A major increase in PGD followed a high-magnitude spike in populations of *Obelia* sp. and *Lizzia blondina*; AGD also increased with some lag.

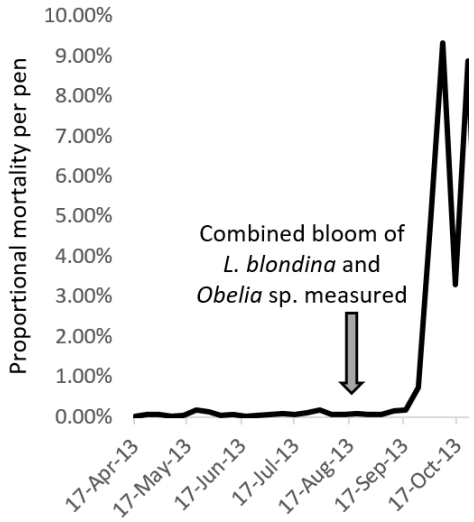


Figure 2.25 Mortality increased dramatically at Portnalong in 2013 following exposure to multiple hydromedusan species and a rise in gill pathology.

Other bloom incidents, of considerably lower magnitude, produced less-pronounced results that were nevertheless similar in pattern (Figure 2.22), suggesting that while a truly high-magnitude blooming event such as described here is highly likely to be a concern, lower-density exposures are also likely to be problematic. The precise threshold levels for exposure, in terms of time and density could not be estimated from these data.

2.5 Discussion

2.5.1 Population dynamics of *Obelia* sp. and *Lizzia blondina*

This investigation found that in spite of broad species richness represented in the hydromedusan fauna, *Lizzia blondina* and *Obelia* sp. medusae contributed the vast bulk of observed population density. Similar, if less dramatic, diversity dynamics were found during past investigations into North Atlantic gelatinous zooplankton, with *Obelia* sp. occurring occasionally in somewhat larger population magnitudes than other recorded species, though not to the degree found in this study (e.g. Ballard and Myers 2000, at Lough Hyne, Ireland; Primo et al. 2012, at Mondego estuary, Portugal). Previous studies did not involve the same degree of spatial-temporal resolution in their monitoring; as such, they may have missed short-lived high magnitude occurrences, or conducted sampling at sites not as prone to population fluctuations. Consequently, the boom-and-bust nature of many of the blooms reported here, as well as the tendency for the co-occurrence of *L. blondina* and *Obelia* sp. blooms, have not been previously reported in surveyed studies.

Complicating these observations are unavoidable problems of taxonomy. *Obelia* congeners can only be visually identified to species level at the hydroid colony life stage, leaving the medusae undifferentiated. These limitations make comparisons of medusae between and even within studies problematic. It is possible that the samples in this dataset, as well as in those previously published, reflect the presence of several different *Obelia* species, each with a different set of environmental parameters conducive to reproduction. Three species are frequently cited in UK waters: *O. geniculata*, *O. longissima*, and *O. dichotoma* (Cornelius 1995). The presence of thick settlement of *O. geniculata* colonies in the *Laminaria digitata* fouling communities at numerous aquaculture facilities is suggestive, but not conclusive, of a link to the medusae collected. Useful next steps might include

laboratory comparisons of reproductive behaviour amongst the congeners' hydroid colonies.

The complete lack of published information regarding the *L. blondina* hydroid colony is similarly confounding, though a different medusa production dynamic can also be considered. *L. blondina* is one of several hydrozoan species which deviates from the classic life cycle pattern in its ability to clone itself at the medusa stage, reproducing in the plankton by budding from the manubrium (Stibor and Tokle 2003, Hosia and Båmstedt 2007). Shucksmith et al. (in prep) found that *L. blondina* populations near Shetland flourished during periods of high microzooplankton abundance, suggesting that a response to favourable trophic conditions may account for the rapidly-developing large populations seen in this study.

The high-density, short-term presence of both of these species in the plankton may be an intriguing indicator of reproductive strategy. The medusa life stage represents a step in hydrozoan reproduction that is not strictly necessary: hydroid colonies can and do persist and grow without carrying out a planktonic dispersal phase, and a number of species, such as *Dynamena pumila*, lack a medusa stage altogether (Cornelius 1995). While the hydroid colony may be, theoretically, functionally immortal in its ability to continue to grow clonally, the medusa life stage is suited to recombination and/or longer-distance dispersal rather than longevity. In the case of *Obelia longissima,* Stepanjants (1998) found that medusae produced by a laboratory-maintained colony lived for only 7-30 days. Most species found in this study tended to occur in low numbers with relative evenness over time, suggesting that their hydroid stages are releasing few medusae at a steady rate. The boom-and-bust populations of *L. blondina* and *Obelia* sp. represent a major deviation from this pattern.

A number of possible explanations for similar behaviour in various medusozoan species have been put forward. First, some hydroid species may produce medusae during periods of plenty: metabolic resources can be devoted to reproduction and dispersal rather than individual survival (e.g. Schmid 1979). Alternatively, some species may produce medusae during periods of sub-lethal stress: although a sessile colony is incapable of escaping an environmental stressor, dispersing medusae can, continuing the genetic line in the event that the colony itself is eliminated (e.g. Stebbing 1981b). A third possibility might involve synchronized spawning as a strategy for maximizing the opportunities for sexual reproduction, as with gonochoric broadcast spawning in some coral species (e.g. Szmant 1986). It is not possible to completely discern reproductive strategy using this dataset, but its patterns are suggestive for future investigations using cultured hydroid colonies to test stimuli.

2.5.2 Role of temperature

The finding of 12°C as a threshold temperature for *Obelia* sp. or *L. blondina* blooms has not yet been reported in the literature. Cornelius (1995) reports *Obelia* sp. medusae as occurring off the south of England in March to late April, during seasonal temperatures of 9-10˚C and in eastern Denmark between late March and June, in water of 3-4˚C; Bruce at al. (1963) report *Obelia* sp. medusae as occurring as early as April off the Isle of Man, in water as cold as 8.5˚C. Baxter et al. (2011) and Shucksmith et al. (in prep) also found *L. blondina* present in summer periods. However, these publications do not specify the population density observed, and the data gathered in this study do show baseline low population incidence of *Obelia* sp. in the plankton at cooler temperatures. Based on the data in this study, warmer temperatures should be regarded as a threshold requirement for large population densities to be generated. Blackett et al. (2015) found a 10˚C temperature threshold for production of the eudoxid life stage of *M. atlantica* in the English Channel, with overall colder years associated with fewer cohorts produced due a temporal mismatch in zooplankton prey availability. Although threshold for observing large populations of

M. atlantica in this dataset was found to be higher at 12˚C, this may similarly be due to biotic cycles of its prey in Scottish waters.

This threshold would be a useful focus for further investigation, both in terms of focused observation and in terms of physical parameters to explore in laboratory-cultured colonies. One notable contradiction of this might be Elmhirst (1925), who describes an *O. geniculata* colony at a pier in Millport, Scotland producing medusae specifically in 10-day stretches "in the third week of the lunar cycles in July, August and September and not at other times." However, while often repeated in the literature, this phenomenon has not been reproduced in controlled studies or been clearly reflected in a published zooplankton record.

2.5.3 Role of geography

There was a substantial difference between Skye sites and Lochaber sites in the probability of large-scale blooms occurring, with Portnalong and Greshornish encountering considerably more frequent and larger population density increases of both *Obelia* sp. and *L. blondina*. There are a number of potential abiotic factors that could influence this. First, Loch Harport and Loch Snizort (where Portnalong and Greshornish are located, respectively) are far more exposed to the broad expanse of open water in the Minch than are Loch Sunart and Loch Linnhe (Invasion Bay and Linnhe). Some scyphozoan aggregations have been recorded as taking place as the result of surface current advection (e.g. Doyle et al. 2008), and hydrozoan blooms may be subject to similar influences. However, if bloom populations recorded at Portnalong and Greshornish were the result of advection alone, a temporal relationship between the two sites' hydromedusan populations might be expected, and this was not found to be the case; instead, species occurrence and population density between the two lochs were independent of one another. It seems likely that blooms are locally-arising phenomena, with local stimuli prompting the development and release of medusae to the plankton. Open-water exposure may be prompting

local medusa production through other means. Wave action has previously been put forward as one possibility for inducing investment in sexual reproduction in other hydrozoan species, particularly the intertidal species *Dynamena pumila* (e.g. Dayton 1975, Rossi et al. 2000), which might be consistent with the relative exposure of Greshornish and Portnalong to the broad sea surface fetch of the Minch. These studies consistently found that locations with high wave action were strongly associated with higher proportions of reproductive gonangia to feeding hydranths in the colonies. Ellyat (2015) found that in a time series assessment of *D. pumila* colonies in an intertidal rockpool, a period of high wave action was immediately followed by a large increase in gonangia. The limited amount of time over which this study was conducted prevented a replication of this observation, but it does suggest periods of wave exposure may trigger reproductive investment in a hydroid. Meanwhile, sea surface energy as a reproductive cue is well known in various neritic or intertidal limpet and abalone species (e.g. Orton et al. 1956, regarding *Patella vulgata*, Creese and Ballentine 1983, regarding *Cellana radians*, Sasaki and Sheperd 1995, regarding *Haliotis disc*us and *Tegula* spp., Shanks 1998 regarding *Lottia digitalis*). In all of these cases, strong onshore wind-driven waves were associated with coastal downwelling, which in coupling with coastal currents, swept propagules out of the surf zone but maintained them in near the shoreline with appropriate settlement substrate (Shanks 1998). An association between similar oceanographic phenomena and neritic hydroid fauna found in Scotland may be worth investigation.

Other interlinked physical differences amongst the sites should also be considered as possible stimuli. A number of factors cited as stimuli for other species, such as photoperiod and lunar cycle (e.g. Elmhirst 1925, Arai 1992), are unlikely to be applicable, as these do not differ considerably across the sites observed. Fluctuations in salinity were temporally variable between sites, but all sites' mean salinity were within one standard deviation of one another, and rapid changes in salinity were not associated with subsequent major changes in hydromedusan population density.

The temperature parameter established above is certainly striking, but while both Linnhe and Invasion Bay experienced sustained temperatures over the 12.5˚C mark throughout the summer periods investigated, neither experienced the blooms characterized at Portnalong and Greshornish. Difference in character in terms of the sea lochs' geography and bathymetry could play a role. Linnhe and Invasion Bay are situated within deep, steep-sided, narrow lochs of 155 and 124 m maximum depths, respectively. These lochs are also bathymetrically complex, with multiple sills distinguishing discrete basins (Edwards and Sharples 1986). The bathymetric profiles of Loch Harport and Loch Greshornish, where Portnalong and Greshornish are situated, are much simpler: Loch Greshornish possesses only one minor sill and Loch Harport none, and neither are deeper than 50 m. These factors may affect the residence time of the lochs, with the Lochaber sites requiring considerably longer for complete flushing. This could limit bloom likelihood by restricting the availability of critical nutritional resources. Alternatively, while freshwater runoff is itself unlikely to be a main causative factor, soluble chemicals associated with geographically specific runoff might also have a dampening effect on medusa production over long-term exposure.

Finally, this study did not quantitatively consider the distribution of the hydroid stage of *Obelia geniculata* or its congeners, and the hydroid colony (or its absence) of *Lizzia blondina* has never been discovered or described. The presence and density of the parent colonies will almost certainly have a bearing on the magnitude of bloom incidence in a given area, particularly if the theory of local medusa production is accurate. Anecdotal evidence suggests patchiness of *Obelia* sp. across the west of Scotland, with some areas showing thick colonization across *Laminaria digitata* fronds and others showing few, if any, colonies present. *Obelia* sp. hydroids were confirmed to be part of the fouling communities at all four sites in this study, but the degree of colonization was not examined. A quantitative study of geographic

distribution of both medusa and colony density would be useful in assessing the impact of the colonial stage.

2.5.4 Impact on aquaculture

This study was unable to link statistically mortality and gill pathology of sea-caged salmon with their exposure to hydrozoan blooms, owing to a small overall sample size and the inconsistent compliance of sites involved. It may be that the only conclusive approach to forging this link will be the use of challenge trials, e.g. the intentional exposure of salmon to dose-calibrated population density levels of hydromedusae in a controlled setting. However, Baxter et al. (2011) demonstrated the serious and enduring effects of jellyfish nematocysts on salmon gills. The magnitude of both *L. blondina* and *Obelia* sp. population density immediately prior to a period of sustained and ultimately lethal gill pathology seems unlikely to be coincidental.

Several mechanisms explaining the outcomes seen in this study are possible. First, not all nematocysts contain venom; many are geared primarily toward puncturing integument or tethering prey (Östman 2000). These may cause considerable physical microtrauma to lamellae when numerous jellyfish tentacles are passed across the gill structures. The tubular threads associated with nematocysts, once extruded, also remain embedded in lamellar tissue. Both of these factors could elicit inflammatory responses from fish. Second, the venom contained in some nematocysts is worth considering. *Obelia* sp., *L. blondina*, and *Muggiaea atlantica* venoms have not been characterized, and with cnidarian venoms varying widely across taxa (e.g. Kintner et al. 2005), generalizations are hard to make. Broadly, however, animal venoms are usually evolved to suit a particular target (e.g. Carrette 2002, Kintner et al. 2005). With hydromedusae as predators of small zooplankton, the venom components are unlikely to be especially potent to vertebrates, but the cumulative effects of numerous stings may become clinically relevant. Cytolytic and haemolytic protein

and peptide components, as well as phospholipase enzymes, are frequently reported in hydrozoan and scyphozoan venoms (Hessinger and Lenhoff 1988, Nevalainen et al. 2004), potentiating considerable tissue damage. It is likely that the combination of physical punctures by nematocysts, the embedding of nematocyst threads, and the pharmacological actions of lytic toxins in the venoms all contribute to produce the degree of proliferative gill disease seen in this study.

Some degree of gill damage is likely to self-resolve, when given opportunity to heal without further insult. However, sea-caged fish do not live in the proverbial vacuum, and are subject to a highly non-sterile environment. *Neoparamoeba perurans*, the species implicated in amoebic gill disease, are frequently present in very low numbers in farmed fish populations (Mitchell and Rodger 2011). An insult producing sustained PGD and inflammation, as seen in this investigation, is likely catalysing the overgrowth of amoebae and establishment of clinically relevant AGD. Complicating this further is the consideration of sea lice. Of the treatments for low-grade sea lice infestation, hydrogen peroxide baths have the least environmental impact, and are becoming comparatively less costly than pyrethroid pesticide treatments due to the development of parasitic resistance (Chris Wallace pers. comm., Aaen et al. 2015). These peroxide treatments have no appreciable side effects on healthy salmon, but produce considerable mortality when used on highly compromised gills (Chris Wallace pers. comm.). These knock-on health effects of exposure to hydromedusan blooms should put aquaculturists on their guard.

2.5.5 Recommendations and future work

On the basis of the understanding developed in this investigation, it is apparent that dedicated hydromedusan monitoring could be a useful part of the weekly or even daily environmental monitoring conducted for salmon health and welfare. Effects of medusozoan blooms on salmon aquaculture have historically been reported retrospectively in the wake of a fish kill (e.g. Doyle et al. 2008, Ferguson et al. 2010);

samples are either taken after morbidity and mortality are noticed, or are presumptively associated with the visible presence of a medusa bloom. This dataset demonstrates that such an approach is highly likely to miss relevant information. First, the short-lived nature of many blooms may cause the original causative species to be missed with retrospective sampling. Second, most of the species represented in this study, and particularly the two most responsible for bloom events, are too small to be seen without dedicated sampling and microscopic examination. Many idiopathic fish kills or morbidity events in the past are likely to have been the result of such cryptic blooms. A proactive, real-time approach to hydromedusa monitoring could be very useful in recognizing the source of serious problems as well as increasing options for early mitigation. This method would also help in future data-gathering, in terms of pinpointing the thresholds of bloom size and exposure duration that may be of concern to aquaculturists, as well as providing opportunity for more in-depth histopathological investigations. Finally, an increased awareness of the environmental threats to which the fish are exposed might help in avoiding or minimising dilemmas such as that described for Portnalong in 2013, wherein poor gill health precluded effective treatment for sea lice infestation.

This continuous-monitoring approach would be beneficial to environmental and ecological research interests as well. Daily or near-daily, industry-wide monitoring activities would help to make up the difference in site non-compliance, with sites potentially able provide one another with early warnings and data interpolation. Educational outreach and industry partnership can help to accomplish this goal, and to transfer the monitoring skills necessary for sharing high-quality information.

Controlled experiments on the hydroid stages of *L. blondina* and *Obelia* congeners would also be a logical next step. Difference in medusa production patterns between *O. geniculata*, *longissima* and *dichotoma* may help to account for spatial-temporal difference in *Obelia* sp. medusa blooms. *L. blondina* reproduction is even

less well understood, with the hydroid colony as yet undiscovered. An array of stimuli operating on various hydroid species has been reviewed in Arai (1992); many of these are worthy of investigation, particularly in conjunction with the temperature-based patterns seen here. Finally, a quantitative examination of colony presence and density at each site, matched with its bloom record, would be useful to help assess the risk of hydroid presence in the fouling community.

This study identified *Obelia* sp. and *Lizzia blondina* medusae as being key (and often concurrent) contributors to potentially problematic gelatinous zooplankton blooms in Scottish aquaculture situations, with dissociated eudoxid segments of *Muggiaea atlantica* also posing concern. Blooms by these species were found to occur with spatial and temporal heterogeneity, where adjacent sites did not show related blooms between one another. This heterogeneity makes region-wide environmental changes such as photoperiod and lunar cycles unlikely to be bloom stimuli, and also limits the information available from satellite remote sensing platforms due to proximity to land and the frequency of cloud cover in Scotland. While blooms do appear to be temperature-mediated in terms of a minimum threshold requirement, other specific environmental stimuli for blooms are still unknown, though this study ruled out changes in salinity and turbidity (associated with freshwater runoff events) as being directly linked. Blooms were found to precede outbreaks of amoebic gill disease and proliferative gill disease, both of which are rising areas of salmon welfare and economic concern in Scottish aquaculture. All of the species most frequently occurring in this study are virtually invisible to the naked eye, and generally go unnoticed without targeted sampling. They are likely to have caused problems in the past, but been unrecognized. This is the first study to thoroughly document the occurrence and effects of blooms in multiple sites in time-series.

*The *Obelia* genus contains three relatively common hydrozoan species in Scotland: *O. longissima*, *O. dichotoma*, and *O. geniculata*. While the medusozoan stage cannot be used to differentiate to species level without use of molecular techniques, the *O. geniculata* hydroid stage is especially common and was observed on the fouling communities at the participating aquaculture sites during this study.

3

Biodiversity of bacterial genera cultured from three medusozoan species in Shetland

I estimate that I contributed 85% of the total effort to towards the material reported in this chapter, which can be broken down as follows:

- Sterile aquarium system construction 5% (by me)
- Cnidarian sample collection 15% (me 10%, assistance of Rachel Shucksmith and Kenny Gifford of North Atlantic Fisheries College, Scalloway 5%)
- Bacterial culturing 30% (me 25%, assistance of Christopher Delannoy of the Moredun Research Institute, Roslin 5%)
- Genetic extraction and PCR 20% (by me)
- Sequencing of 16S rDNA outsourced to Eurofins Genomics (5%)
- Data analysis and writing 25% (by me and reviewed by ASB)

Publication of this material is pending on further sequencing to confirm species-level identification of samples, using the reverse sequence of the 16S subunit gene as well as others, as laid out in the Discussion. Working title: "Diversity of medusa-associated bacteria with implications for salmon health in aquaculture." A. Kintner, M. Clinton, A. Brierley, D. Ferrier, C. Delannoy.

3.1 Capsule findings

- Targeted culturing of bacteria obtained from medusozoan species shows some association with potential pathogens, including *Aeromonas salmonicida*. Further work would be required to clarify bacterial species, and to pin down whether any of these are long-term symbionts.
- Previous findings of *Tenacibaculum maritimum* were not reproduced here in any samples, suggesting that *T. maritimum* vectoring by jellyfish may not be a frequent occurrence. Studies re-examining the *Phialella quadrata* and *Pelagia noctiluca* species, species in which *T. maritimum* has previously been reported, (Ferguson et al. 2010, Delannoy et al. 2011), could not be undertaken during this investigation.

3.2 Introduction

A major bloom of the hydrozoan jellyfish species *Phialella quadrata* lasting several weeks took place at Green Organics Seafarms in Shetland in 2008, resulting in up to 90% mortality of caged salmon (Ferguson et al. 2010). Investigations found that fish were suffering from bacterial infection in the gills by *Tenacibaculum maritimum*, a species not unknown in aquaculture, but more frequently associated with integumentary conditions in turbot and sole than in salmon (Powell et al. 2005, Avendaño-Herrera et al. 2006b) and previously unreported in Shetland aquaculture (Sutherland pers. obs. 2012). *T. maritimum* colonization was found on the mouth parts of the *P. quadrata* hydromedusae sampled during the bloom (Ferguson et al. 2010), leading these authors to suggest the possibility of medusae acting as vectors for aquacultural pathogens.

Some debate remains as to whether jellyfish are acting as original-source vectors or merely transmitting bacteria from fish to fish within an already-infected caged population. *T. maritimum* is often found in the dermal mucus of healthy sole and turbot (Avendaño-Herrera et al. 2006a), and Steinum et al. (2009) found *T. maritimum* appearing occasionally and without causing pathology in the gills of healthy farmed salmon. A possible scenario for the observations made in Shetland in 2008 might be that *T. maritimum* had been part of the non-pathological gill flora among some of the salmon at Green Organics Seafarms, with the *P. quadrata* bloom causing sufficient inflammation and immune compromise via nematocyst injury and envenoming as to permit the pathological development and spread of tenacibaculosis. The finding of *T. maritimum* bacteria on the gut tissues of *P. quadrata* might reflect a transmission from fish to jellyfish, rather than vice versa. Fringuelli et al. (2012) found somewhat equivocal further evidence, wherein *T. maritimum* was identified from *P. quadrata* and *Muggiaea atlantica* individuals, but at low titers and almost exclusively in individuals sampled from within or immediately adjacent to salmon sea cages. *T. maritimum* has been found associated with *Pelagia*

noctiluca scyphozoan jellyfish outwith any aquaculture context (Delannoy et al. 2011), but only once, leaving the hypothesis of jellyfish playing an original-source vectoring role still unconfirmed. However, the ecology of *T. maritimum* itself encourages speculation: even if the bacterium is commonly found in non-pathogenic situations, it does not survive well in the absence of a host or substrate (Avendano-Herrera et al. 2006b). A low level of endemism in a caged fish population, as reported by Steinum et al. (2009), might give rise to colonization of jellyfish mouth parts during a bloom; this, in turn, might lead to transfer of the bacteria between fish.

A working expectation that tenacibaculosis – or any other microbial pathogenic disease - could be a likely outcome of jellyfish blooms at salmon farms would be useful in devising applied treatment strategies. To this end, this study sought to investigate the occurrence of *T. maritimum* in three key situations:

1) In *P. quadrata* blooms outwith co-occurrence at salmon farms, in the absence of possible transfer from infected fish;
2) In *P. quadrata* hydroid stages (refer to Figure 1.2);
   and
3) In other opportunistically sampled hydromedusae and scyphomedusae besides *P. quadrata.*

A positive finding in the first situation would provide strong evidence of endosymbiosis between *P. quadrata* medusae and *T. maritimum* bacteria. A positive finding in the second situation would also suggest that *T. maritimum* is vertically transferred from parent hydroid colony to the medusa offspring as a long-term part of the species' life cycle. Finally, a positive finding in the third situation would suggest widespread incidence of this phenomenon across the taxon, with the application that tenacibaculosis should be treated prophylactically in the incidence of any species' bloom at a salmon farm.

Two serious drawbacks hampered the intended investigation. First, during the field time available, *P. quadrata* did not undergo an observable bloom, preventing

sampling of live adult medusae. *P. quadrata* hydroid colonies were also not in evidence, preventing an examination of the benthic life stage. As a result, the decision was made to change focus to *Cyanea capillata* scyphomedusae and *Neoturris pileata* hydromedusae, which could be sampled opportunistically. Partway through the proposed sample collection schedule, these medusae also disappeared from the plankton assemblage, necessitating another change; *Obelia geniculata* hydroid colonies were selected on the basis of year-round presence in the benthic fauna.

These field-necessitated changes to sampling protocol forced an expansion of study goals, from simply searching for *T. maritimum* symbiosis to characterizing the diversity of bacterial species found in the cnidarian species sampled. Sequence of bacterial small ribosomal subunit 16S rDNA is a widely-recognized tool for this purpose (Woese et al. 1990, Ludwig and Schleifer 1994, Clarridge 2004, Rajendhran and Gunasekaran 2011). 16S rDNA is highly conserved in size and function across the prokaryotes, but exhibits sufficient species-level variability as to be the current standard for multi-level taxonomic comparison (Clarridge 2004). As the 16S identification approach is also recommended as diagnostic for *T. maritimum* in aquaculture settings (Cepeda et al. 2003), this method was well-suited to the expanded focus of this investigation.

3.3 Methods

3.3.1 Procedural summary

Live medusae and hydroids were identified and collected by hand (3.2.2 Sites) and rinsed in UV-treated seawater to remove as much environmental source contamination as possible (3.2.3 Incubation of cnidarian samples). They were then incubated overnight in flowing, UV-treated seawater before relevant bacterial sampling was conducted (3.2.4 Bacterial sampling; Lee et al. 2009). Control samples

were also collected from each seawater site and from the incubation system. Each sample was transferred to two separate broth media, incubated, and streaked to corresponding agar media plates. Multiple colonies occurring on single plates were then selected out individually to monoculture (3.2.5 Culturing and identification; Lee et al. 2009). DNA from each monoculture was extracted and the 16S rDNA region amplified using PCR, and the forward-strand sequenced and used for phylogenetic comparison (3.2.6 Extraction and amplification; 3.2.7 Sequence analysis).

3.3.2 Sites

Four separate sites were used to source hydroids, hydromedusae, and scyphomedusae: Redayre, Roe Sound, North of Papa, and Lunna (3.1).

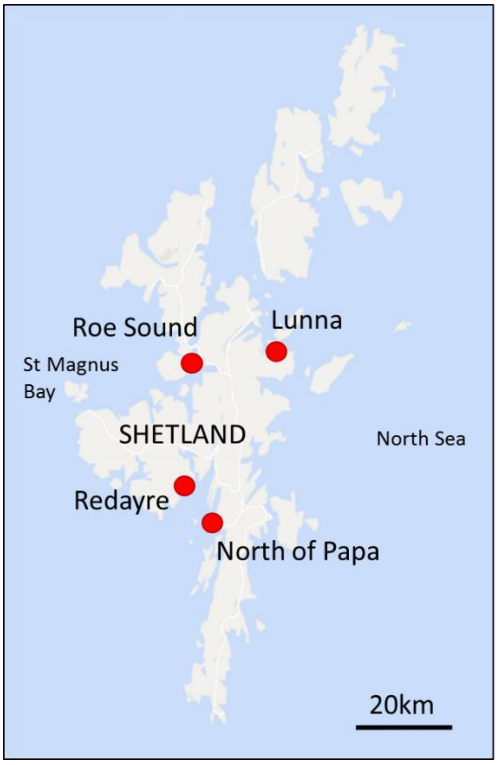


Figure 3.1 Locations of sampling sites.

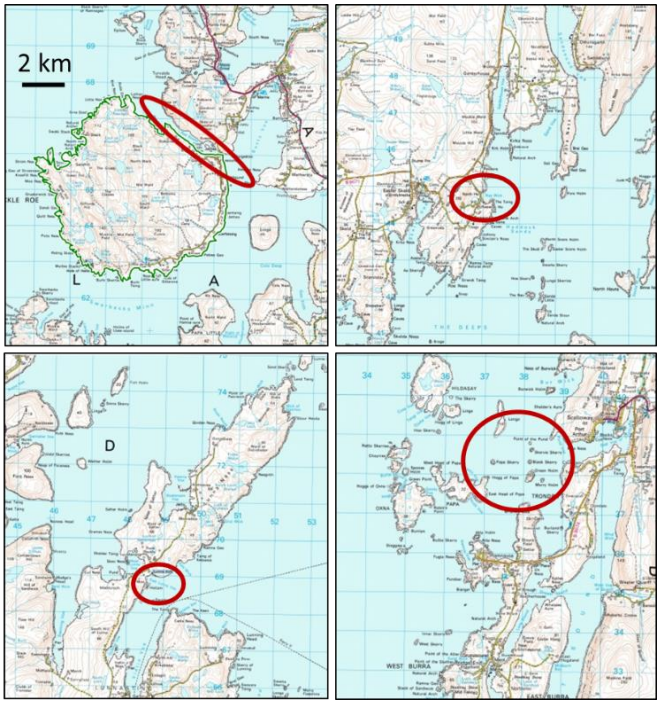


Figure 3.2 Areas sampled at each site. Top left: Roe Sound. Top right: Redayre. Bottom left: Lunna. Bottom right: North of Papa.

North of Papa. The area between Scalloway Harbour and the island of Papa to the southwest hosts a salmon aquaculture site operated by Hjaltland Seafarms, consisting of 8 sea cages in the lee of Papa. Eight *C. capillata* and 7 *N. pileata* adult medusae were collected in this area.

Following sampling at North of Papa, several attempts were made to collect *C. capillata* medusae at other sites on various dates, without success. It was concluded that the seasonal appearance of this species had ended in the Shetland region, and sampling effort was shifted to collection of benthic hydroids.

Roe Sound. Roe Sound site consists of a 250 m wide strait of 10m depth between the Shetland main island and the island of Muckle Roe, in the broader area of St Magnus Bay (UKHO 1991a). A disused floating walkway, abandoned since 2005, is positioned on the mainland side of the strait, to the west of a bridge connecting Muckle Roe to the main island. A fouling community has developed along the walkway, providing

ideal kelp substrate for hydroid growth. No salmon aquacultural sites are present within 8 km. Nine hydroid samples of species *O. geniculata* were collected from the walkway and shallow subtidal kelps along the shoreline.

Redayre. Redayre is a sheltered, east-facing beach area on the western side of the Shetland main island, with a maximum 10 m sandy benthos (UKHO 2001) and several large rocky outcrops providing substrate for growth of kelps. The nearest aquaculture site is North of Papa, 16 km distant. Ten *O. geniculata* hydroid samples were collected from the kelp fronds along the outcrops.

Lunna. This site consists of a southeast-facing, sheltered cobble beach, part of the larger fjord of Vidlin Voe. A salmon aquaculture site, operated by Hjaltland Seafarms, is positioned approximately 500 m southwest of the sampling area. Vidlin Voe deepens to approximately 30 m near the sampling area, reaching a maximum depth of 40m near its mouth (UKHO 1991b). Sampling was conducted on the kelps growing on the shallow subtidal cobbles. Six *O. geniculata* hydroids were collected from this site.

3.3.3 Incubation of cnidarian samples

A two-step process of sterile rinsing, followed by 12-hour incubation, was conducted in order to maximize the probability that any bacteria cultured were endogenously associated with the cnidarian individuals sampled. An aquarium system was built for this purpose at the North Atlantic Fisheries College in Scalloway, Shetland. All system components were hand-cleaned using Virkon disinfectant solution (Day-Impex Ltd.) then soaked for 24 hours in a 200 ppm free chlorine solution (OIE 2009) prior to aquarium assembly. Twelve individual tanks and separate pipe components were prepared in this manner. NAFC made available its system of UV-sterilized seawater (OIE 2009), which was used to thoroughly rinse all materials once aquarium construction was complete. Each cnidarian individual collected was brought to NAFC,

gently rinsed in UV-treated seawater, and placed for 12 hours in its own tank. All tanks were supplied with aerated, UV-treated seawater at a median 33‰ and 11˚C.

3.3.4 Bacterial sampling

After incubation, each individual was removed to an ethanol-cleaned fume extraction cupboard for bacterial culture.

3.3.4.1 Media

Two different sterile broth media were prepared for culturing. *Flexibacter maritimus* media (FMM) is a semi-selective medium designed for the purpose of *T. maritimum* culture (Pazos et al. 1996; Conda Laboratories). Bovine brain-heart infusion (BHI) is a more general medium suitable for culture of fastidious aerobic and anaerobic bacteria, which would cover a number of species of potential aquaculture-veterinary relevance. Each cnidarian sample was transferred to both FMM and BHI broth tubes (1 mL broth medium per tube) for incubation (see section 3.3.5).

3.3.4.2 Cultured tissues

In the case of *Cyanea capillata* scyphomedusae, 1cm length samples of fishing tentacle tissue was removed and transferred to each of the broth media. Sterile swabs were used to sample bacteria in the gut. In the case of the much smaller hydroids, 1cm length sections of colony stalks were placed directly into both broth media. *Neoturris pileata* medusae, each of 1cm in bell height, were halved; each half went to separate broth media.

3.3.4.3 Control samples

A water sample from each site was collected in a 1L sterile container and allowed to settle at room temperature for 1 hour; a 0.5 mL sample from the bottom of this container (including a small amount of sediment, if any, that may have settled) was placed in broth media and incubated alongside medusozoan samples. An additional

water sample of 0.5 mL was collected during each incubation period in the sterile tank system. All cultures produced from controls were identified in order to rule out non-endogenous bacterial contamination.

3.3.5 Culturing and identification

Each individual sample in broth media was transferred by overnight post to the Moredun Research Institute in Roslin, Midlothian for continued culturing. After arrival, all samples were placed in a static 25˚C incubator for 24 hours. Broths were then streaked to either FMM or BHI agar plates as appropriate and incubated a further 24 hours. Each sample typically developed multiple distinct colonies on each plate. These polycultures were visually discerned and sub-sampled based on colony morphology, e.g. shaped form, elevation, margin, and colour, with subsampling repeated to monoculture (Lee et al. 2009). Determination of polyculture was made conservatively, with any minor variation in the morphology triggering further differentiation of culture. Nomenclature of each sample was recorded as:

- a capital letter designating cnidarian source species (J for *C. capillata*, Ob for *O. geniculata*, N for *N. pileata*; or 'ew' for environmental water and 'tw' for tank water controls
- a number designating which individual from which the culture was sourced, e.g. *C. capillata* individual number 4
- the culture medium in which it was grown (a for BHI; b for FMM)
- if gut or tentacle was sampled, this was designated with a "g" or a "t"
- a capital letter designating the site from which the cnidarian individual was sourced (NP for North of Papa, L for Lunna, RA for Redayre, RS for Roe Sound)
- a final number designating the subculture from which the monoculture was derived if originating from a polyculture, e.g. 1 for the first monoculture, 2 for the second, etc. If multiple steps were required to reach monoculture, this was sub-labeled as 2a, 2b, etc. These were frequently duplicates of one another due to conservative sub-culturing.

Hence, J6atNP2a designates a bacterial culture sourced from *C. capillata* individual number 6, in BHI medium from a tentacle sample, from North of Papa, with sample 2a derived from polyculture. Meanwhile, Ob3aRS2 designates a bacterial culture sourced from *O. geniculata* individual number 3, in BHI medium, from Roe Sound, culture number 2 derived from polyculture.

3.3.6 DNA extraction and amplification

Extraction. Colony samples from each plate monoculture were placed in 2mL corresponding broth media and incubated for 24 hours at 25˚C. Cultures from this broth were then used to obtain DNA from each sample by a crude boiling approach, wherein 1 mL of broth cultures were centrifuged to settle out bacterial material, and the supernatant discarded. The pellet was then re-suspended in 0.5 mL tris-EDTA (TE) buffer and heated at 95˚C for ten minutes. Samples were then centrifuged a final time, at 12,000 rpm for ten minutes, and stored at -80˚C. Supernatant material was used in PCR amplification, as follows.

PCR amplification. Two universal primers for bacterial 16S rDNA, 20F (5'-AGAGTTTGATCATGGCTCAG-3') and 1500R (5'-GGTTACCTTCTTACGACTT-3') (Weisburg et al. 1991) and the Bioline MyTaq kit were used to carry out PCR amplification, wherein 1 µL extracted DNA and 0.5 µL of each primer was added to 5 µL MyTaq buffer + 17.75 RNAse-free water + 0.25 µL Taq polymerase. These were amplified using a thermal cycle as follows: 95˚C/5 min preheating step, then 30 cycles of denaturation at 95˚C/30 s + 57˚C/30 s annealing + 72˚C/60 s extension, then 72˚C/5 min final extension step (Cepeda et al. 2003).

Purification of amplified DNA. PCR products were electrophoresed using 1% agarose gel and ethidium bromide staining, for 45 min at 100 V, using GeneRuler 1kb DNA

Ladder (ThermoFisher Scientific) for reference. Bands were removed and amplified DNA extracted using QIAquick extraction kit (QIAGen).

Sequencing. Sequencing of the forward (5'-3') strand was carried out by Eurofins Genomics (Ebersberg, Germany). Sequencing of the reverse strand was not undertaken.

3.3.7 Sequence analyses

All sequence analysis and manipulation was conducted in Geneious versions R8 and R9 (www.geneious.com, Kearse et al. 2012). After trimming and annotation, sequences with clear base pair calls over sufficient sequence length (>300 bp) were parsed using pairwise alignment of those from common sources (e.g, from a single hydroid, or a single control source such as tank samples). Alignments were made using ClustalW global alignment with no free end gaps (Larkin et al. 2007), and single nucleotide polymorphism (SNP) calls were checked visually using closely-paired samples as reference (Kearse et al. 2012). Duplicate sequences coming from a single cnidarian source, and sequences that were duplicates of environmental or tank system control samples, were eliminated from further analysis. The remaining sequences were used to construct location-specific phylogenies to better visualize diversity, using Tamura-Nei neighbour-joining analysis of the Clustal W alignments based on 1000 bootstrap resamplings (Tamura and Nei 1993). Sequences of insufficient quality were set aside for future analysis. Those remaining were identified using the Basic Local Alignment Search Tool (BLAST) to compare sequences deposited in the GenBank database operated by the United States National Institutes of Health for comparison (Camacho et al. 2009).

3.3.8 Statistics

All statistics were carried out either within Geneious R9 or using the Real Statistics Resource Pack Software 4.3 (Zaiontz 2015, www.real-statistics.com).

3.4 Results

Raw data for this section can be found in the directory "Chapter 3 Bacterial Sequences," submitted in the metadata for this thesis. Guidance notes are provided therein.

3.4.1 Limitations and yield

Resource and time limitation prevented the sequencing of reverse 16S sequencing. As a result, some sequences were of insufficient length or quality for complete species identification and phylogenetic analysis beyond the genus level. Follow-up investigation using the reverse sequence and/or secondary ribosomal coding regions (Table 3.10) could be used to strengthen these results to species and subspecies, yielding finer-resolution results.

Of 194 samples amplified, 151 yielded forward sequences sufficient for this analysis (defined as clear calls over 300 bp in length), inclusive of control samples. 23 samples went unsequenced due to poor PCR amplification: 17 from *O. geniculata* and 2 from environmental controls at Roe Sound, and 4 from *O. geniculata* at Redayre. A further 43 samples were amplified successfully but returned poor sequence data; all of these samples should be re-visited in the forward sequence as well as the reverse sequence, as described above. Many of these will certainly be duplicates of sequences already run from the same source animal, but some may indicate the presence of genera not yet represented in this dataset. Remaining unidentified control sequences may serve to remove some genera represented, but not so many as to strongly disrupt the patterns described below.

Within successfully-run sequences, within-source (e.g., obtained from the same hydroid, medusa, medusa tissue, or control sample of origin) duplicates were removed, leaving 87 unique sequences. These were aligned using ClustalW alignment with no free end gaps (Figure 3.3a-c). Samples were further parsed by

using ten control samples (3.6 Appendix) to remove sequences duplicated in either environmental water samples or incubation tank samples, representing potential artefactual contaminants. 77 samples remained after this step: 10 from *O. geniculata* hydroids at Lunna, 12 from *O. geniculata* at Redayre, 20 from *O. geniculata* at Roe Sound, 24 from *C. capillata* tentacle and gut samples at North of Papa, and 11 from *N. pileata*.

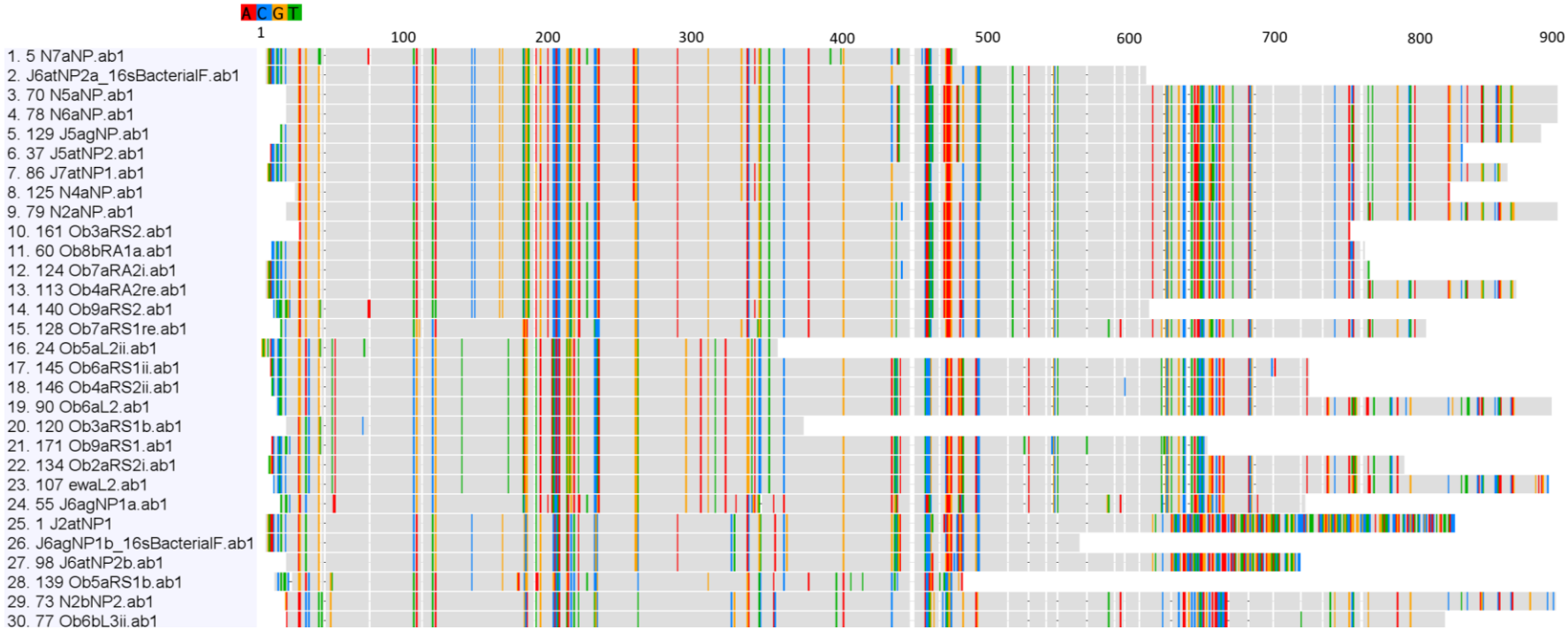


Figure 3.3a ClustalW alignment of 1-30 of 87 sequences of 16S rDNA obtained from bacteria cultured from *Obelia geniculata* hydroid, *Neoturris pileata* hydromedusae, C*yanea capillata* tentacle, *C. capillata* gut, and control samples. Grey show areas of agreement; polymorphic areas are displayed in base pair colours. Labels are given as the numeric designate used in sequencing followed by the culture label encoded as described in section 3.3.5. An expanded view of this alignment can be viewed in the file "ClustalW alignment of all 87 sequences.fasta," provided in the metadata for this thesis.

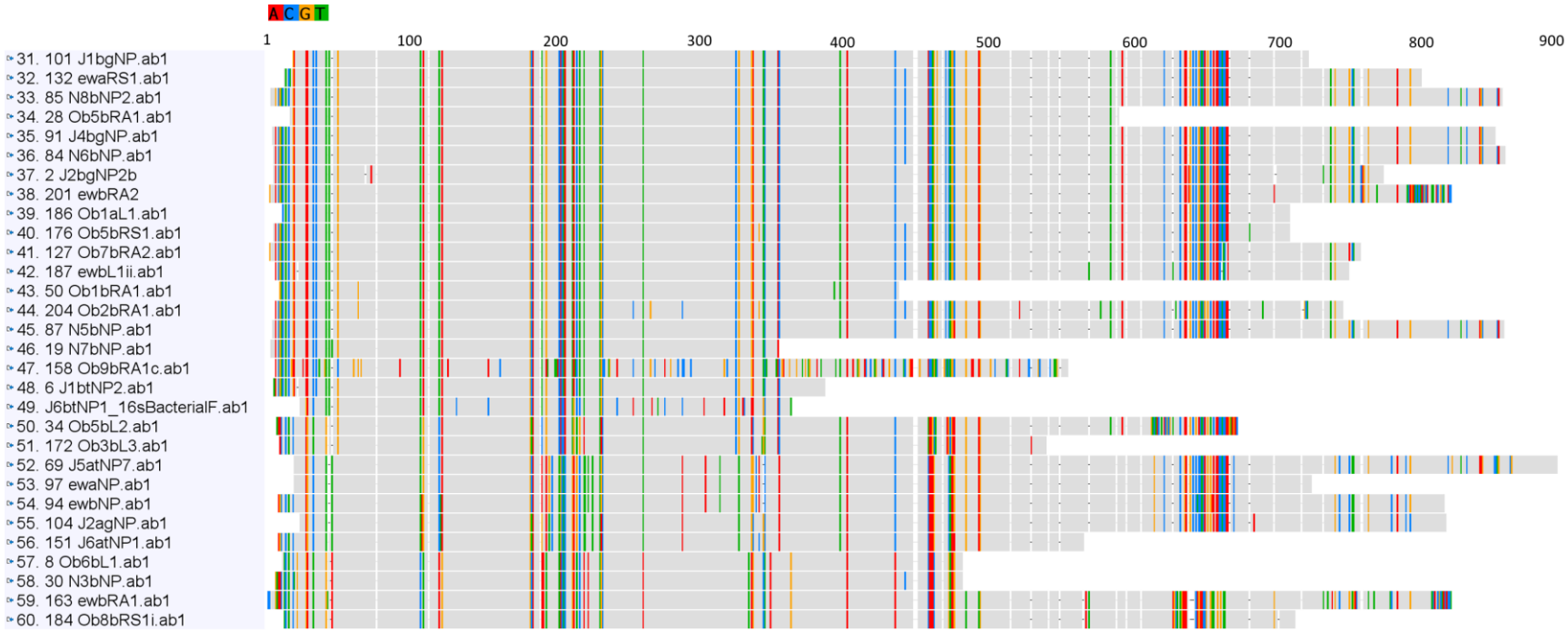


Figure 3.3b ClustalW alignment of 31-60 of 87 sequences of 16S rDNA obtained from bacteria cultured from *Obelia geniculata* hydroid, *Neoturris pileata* hydromedusae, C*yanea capillata* tentacle, *C. capillata* gut, and control samples. Grey show areas of agreement; polymorphic areas are displayed in base pair colours. Labels are given as the numeric designate used in sequencing followed by the culture label encoded as described in section 3.3.5. An expanded view of this alignment can be viewed in the file "ClustalW alignment of all 87 sequences.fasta," provided in the metadata for this thesis.

Figure 3.3c ClustalW alignment of 61-87 of 87 sequences of 16S rDNA obtained from bacteria cultured from *Obelia geniculata* hydroid, *Neoturris pileata* hydromedusae, C*yanea capillata* tentacle, *C. capillata* gut, and control samples. Grey show areas of agreement; polymorphic areas are displayed in base pair colours. Labels are given as the numeric designate used in sequencing followed by the culture label encoded as described in section 3.3.5. An expanded view of this alignment can be viewed in the file "ClustalW alignment of all 87 sequences.fasta," provided in the metadata for this thesis.

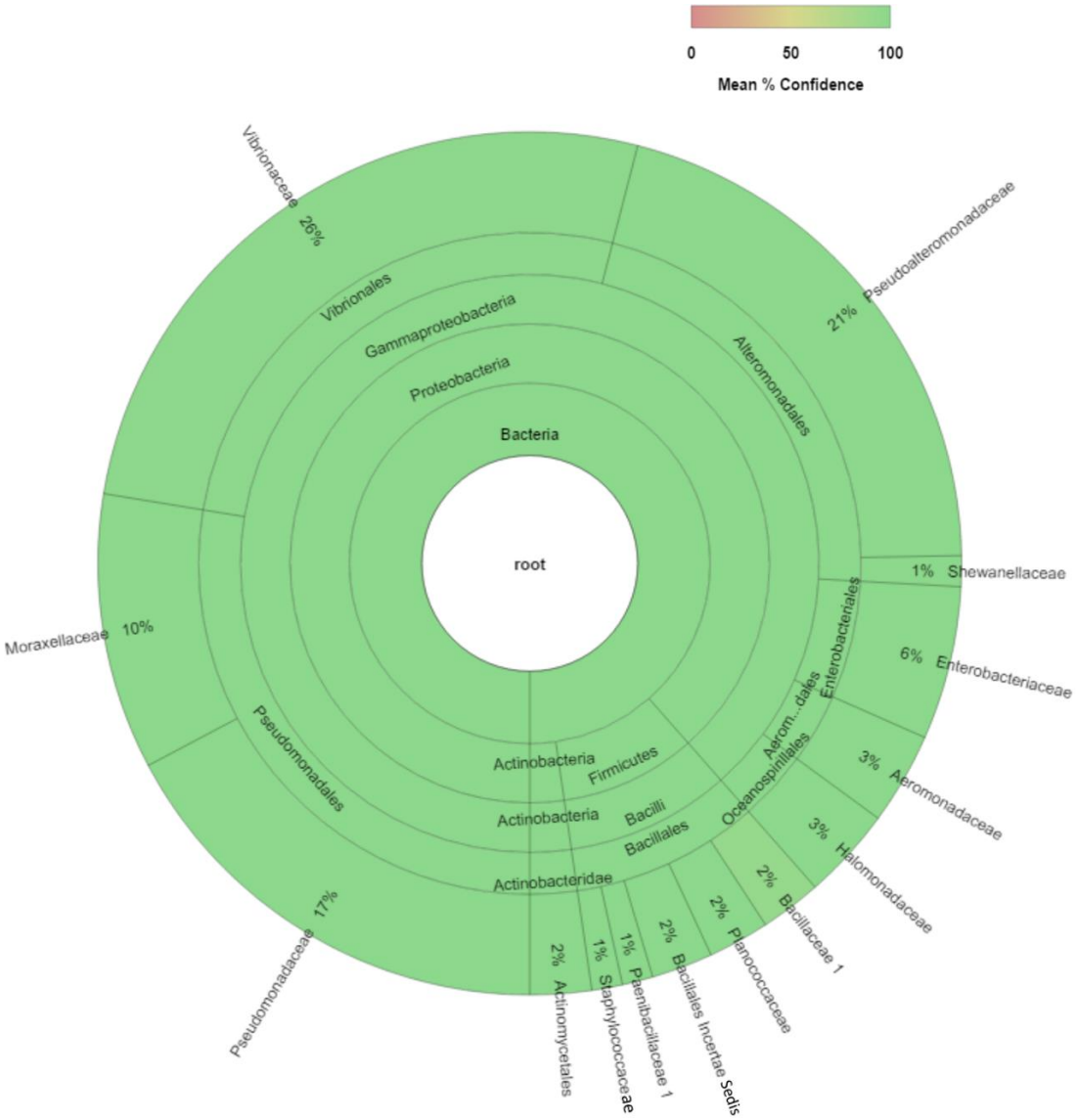


Figure 3.4 Family-level 16S rDNA diversity across 87 pooled samples, inclusive of controls, after elimination of poor sequences. Percentages indicate approximate proportion of all sequences falling within each family.

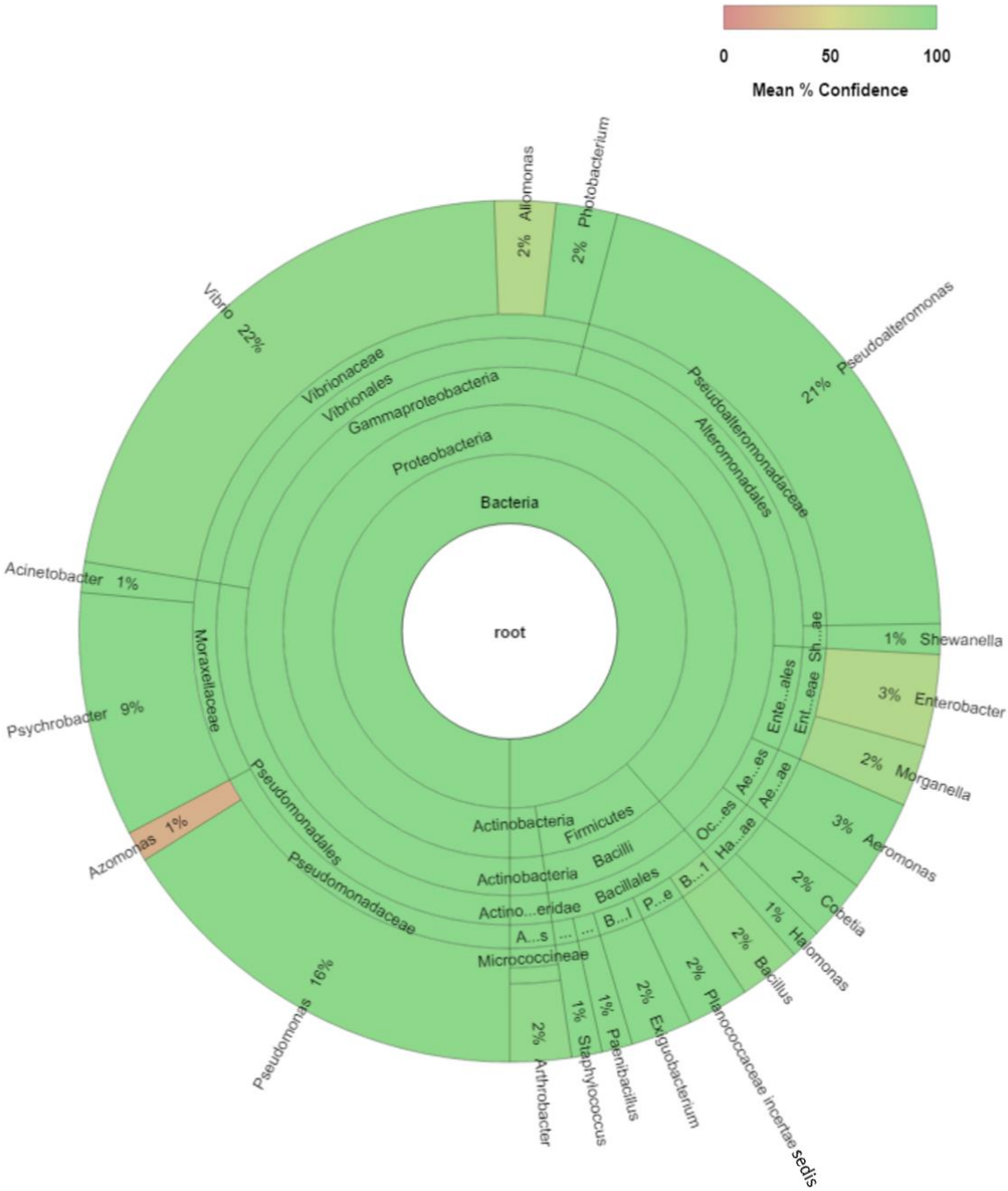


Figure 3.5 Genus-level 16S rDNA diversity across 87 pooled samples, inclusive of controls, after elimination of poor sequences. Percentages indicate approximate proportion of all sequences falling within each genus.

14 bacterial families were represented in the pooled analysis (including controls) of all 86 remaining samples with high confidence, with 49 unique haplotypes amongst these (Figure 3.5, Table 3.1). Genus-level differentiation showed 20 genera, though confidence in identification drops below 50% in one of these (Figure 3.6).

3.4.2 Location-specific results

Lunna. After controls were applied and duplicate sequences removed, ten distinct bacterial 16S samples were obtained from *O. geniculata* hydroids collected at Lunna. Within these ten sequences, 6 genera were represented (Figure 3.6, Table 3.2): *Arthrobacter*, *Psychrobacillus*, *Photobacterium*, *Vibrio*, *Pseudoalteromonas*, and *Psychrobacter*.

Redayre. Twelve distinct 16S samples were obtained from hydroids collected at Redayre, representing six different genera (Figure 3.7, Table 3.3): *Arthrobacter*, *Staphylococcus*, *Psychrobacillus*, *Exiguobacterium*, *Pseudomonas*, and *Vibrio*.

Roe Sound. Twenty distinct 16S samples were obtained from hydroids collected at Roe Sound, representing ten different genera (Figure 3.8, Table 3.4): *Pseudomonas*, *Azomonas*, *Paenibacillus*, *Bacillus*, *Exiguobacterium*, *Psychrobacter*, *Cobetia*, *Pseudoalteromonas*, *Halomonas*, and *Vibrio*. It should be noted that the *Pseudomonas* classification is not monophyletic, and the *Azomonas* genus is contained therein.

3.4.3 Other cnidarian species

Gut and tentacle tissues of adult medusae of *Cyanea capillata* were sampled separately. Ten samples were obtained from gut tissue, representing six different genera (Figure 3.9, Table 3.5): *Pseudoalteromonas*, *Acinetobacter*, *Pseudomonas*, *Aeromonas*, *Morganella*, and *Vibrio*. Fourteen samples were obtained from tentacle

tissues, representing 8 genera (Figure 3.10, Table 3.6): *Aeromonas*, *Pseudomonas*, *Bacillus*, *Pseudoalteromonas*, *Enterobacter*, *Morganella*, *Vibrio*, and *Shewanella*. *Neoturris pileata* adult medusae yielded 11 samples representing 3 genera: *Pseudomonas*, *Pseudoalteromonas*, and *Vibrio* (Figure 3.11, Table 3.7).

Table 3.1 Family-level bacterial diversity found at each site according to successfully sequenced samples, after application of control sequences. Fourteen families were represented in total. The rightmost column of this table, 'n unique sequences at all sites,' reflects the number of unique haplotype sequences of each family represented in the dataset across all sites (49 unique haplotypes in total).

Site	Lunna	Redayre	Roe Sound	North of Papa	North of Papa	North of Papa	All	*n* unique
Cnidarian source	*O. geniculata* hydroid	*O. geniculata* hydroid	*O. geniculata* hydroid	*C. capillata* tentacle	*C. capillata* gut	*N. pileata* hydromedusa	environmental sample	all sites
Bacterial family								
Vibrionaceae	4	5	1	2	3	5	3	10
Pseudoalteromonadaceae	2	0	5	3	3	1	4	7
Shewanellaceae	0	0	0	1	0	0	0	1
Enterobacteriaceae	0	0	0	2	1	0	2	3
Aeromonadaceae	0	0	0	2	1	0	0	3
Halomonadaceae	0	0	3	0	0	0	0	2
Bacillaceae	0	0	1	1	0	0	0	2
Planococcaceae	1	1	0	0	0	0	0	1
Bacillales Incertae Sedis	0	1	1	0	0	0	0	1
Paenibacillacae	0	0	1	0	0	0	0	1
Staphylococcacae	0	1	0	0	0	0	0	1
Actinomycetales	1	1	0	0	0	0	0	2
Pseudomonadacae	0	3	3	3	1	5	0	10
Moraxellacae	2	0	5	0	1	0	1	5
n families represented	5	6	8	7	6	3	4	49

Table 3.2 Sequences represented in *O. geniculata* hydroids at Lunna, Shetland

Genus	Exemplar sequence	*n* occurrences	Duplicate occurrences
Arthrobacter	72 Ob2aL1ii1a	1	
Psychrobacter	90 Ob6aL2	2	24 Ob5aL2ii
Pseudoalteromonas	93 Ob2bL2ii	2	8 Ob6bL1
Vibrio	77 Ob6bL3ii	2	186 Ob1aL1
Photobacterium	34 Ob5bL2	2	172 Ob3bL3
Psychrobacillus	112 Ob2aL1iireb	1	

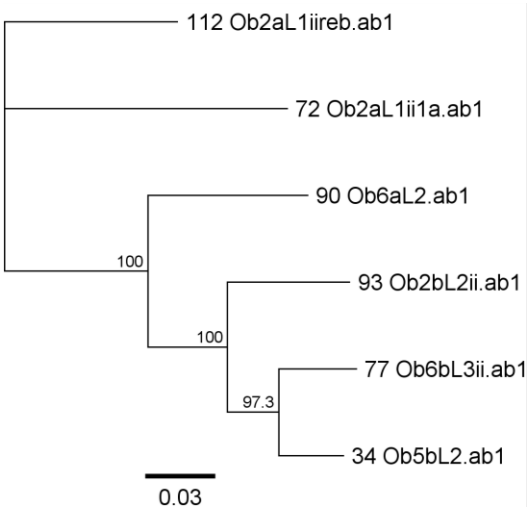


Figure 3.6 Tamura-Nei neighbor-joining tree based on 1000 bootstrap resamplings of ClustalW alignment of bacterial 16S sequences obtained from *O. geniculata* hydroids at Lunna, Shetland. Bootstrap support values are given at each node.

Table 3.3 Sequences represented in *O. geniculata* hydroids at Redayre, Shetland

Genus	Exemplar sequence	*n* ocurrences	Duplicate occurrences
Pseudomonas	113 Ob4aRA2re	1	
Pseudomonas	60 Ob8bRA1a	1	
Pseudomonas	124 Ob7aRA2i	1	
Vibrio	127 Ob7bRA2	2	28 Ob5bRA1
Vibrio	50 Ob1bRA1	1	
Vibrio	204 Ob2bRA1	1	
Vibrio	158 Ob9bRA1c	1	
Arthrobacter	12 Ob1aRA3	1	
Psychrobacillus	148 Ob9aRA1	1	
Exiguobacterium	131 Ob2aRA2	1	
Staphylococcus	63 Ob3aRA3	1	

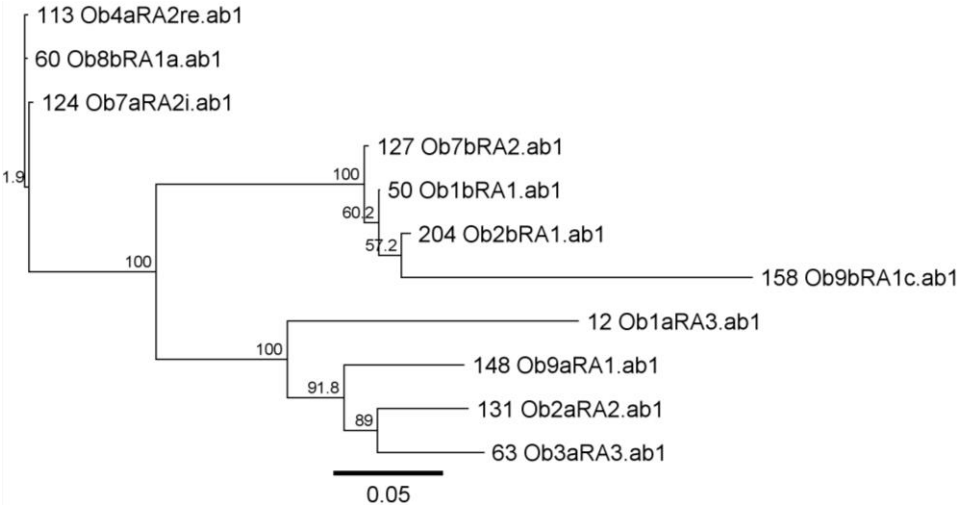


Figure 3.7 Tamura-Nei neighbor-joining tree based on 1000 bootstrap resamplings of ClustalW alignment of bacterial 16S sequences obtained from *O. geniculata* hydroids at Redayre, Shetland. Bootstrap support values are given at each node.

Table 3.4 Sequences represented in *O. geniculata* hydroids at Roe Sound, Shetland

Genus	Exemplar sequence	*n* occurrences	Duplicate occurrences
Pseudomonas	161 Ob3aRS2	1	
Azomonas	140 Ob9aRS2	1	
Pseudomonas	128 Ob7aRS1re	1	
Paenibacillus	156 Ob6aRS1i	1	
Bacillus	136 Ob1aRS1i	1	
Exiguobacterium	147 Ob4aRS1	1	
Psychrobacter	134 Ob2aRS2i	2	120 Ob3aRS1b
Psychrobacter	171 Ob9aRS1	1	
Psychrobacter	145 Ob6aRS2ii	1	
Psychrobacter	146 Ob4aRS2ii	1	
Cobetia	150 Ob3bRS3	2	185 Ob8bRS1ii
Pseudoalteromonas	162 Ob3bRS2	1	
Pseudoalteromonas	184 Ob8bRS1i	4	170 Ob7bRS1, 165 Ob5bRS2a, 141 Ob2bRS2
Halomonas	139 Ob5aRS1b	1	
Vibrio	176 Ob5bRS1	1	

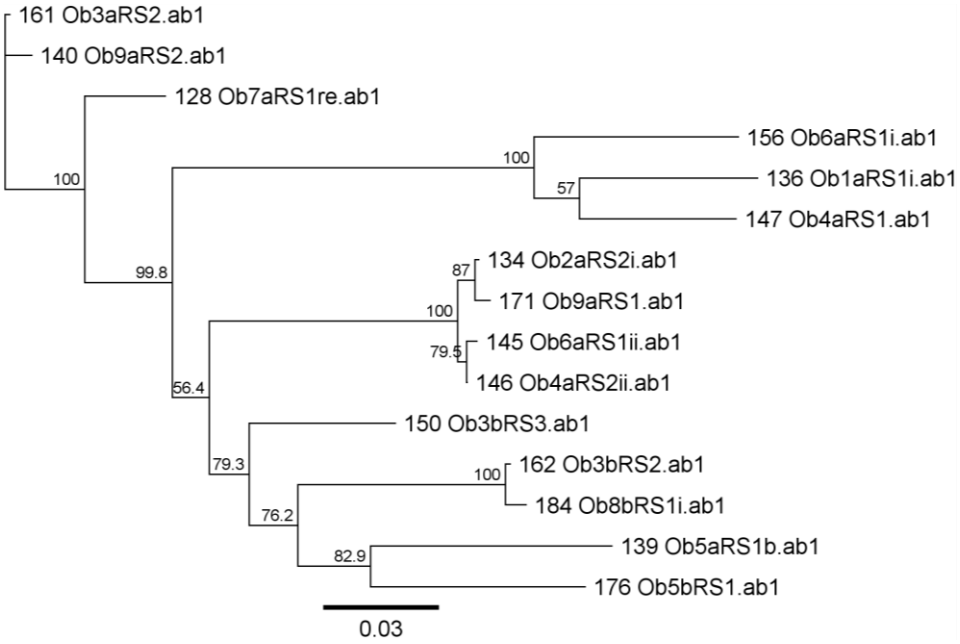


Figure 3.8 Tamura-Nei neighbor-joining tree based on 1000 bootstrap resamplings of ClustalW alignment of bacterial 16S sequences obtained from *O. geniculata* hydroids at Roe Sound, Shetland. Bootstrap support values are given at each node.

Table 3.5 Sequences represented in *C. capillata* gut tissue from North of Papa, Shetland

Genus	Exemplar sequence	*n* occurrences	Duplicate occurrences
Vibrio	91 J4bgNP	3	101 J1bgNP
			2 J2bgNP2b
Morganella	104 J2agNP	1	
Pseudoalteromonas	45 J5bgNP	2	103 J5bg7NP2
Pseudoalteromonas	J8bgNP2	1	
Aeromonas	J6agNP1b	1	
Acinetobacter	55 J6agNP1a	1	
Pseudomonas	129 J5agNP	1	

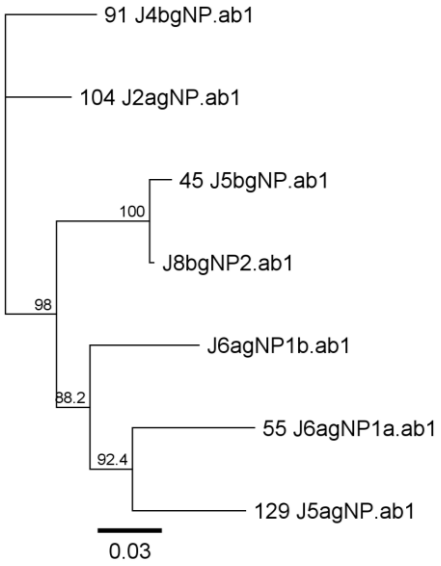


Figure 3.9 Tamura-Nei neighbor-joining tree based on 1000 bootstrap resamplings of ClustalW alignment of 16S ribosomal sequences of bacterial cultures sampled from *C. capillata* gut tissue at North of Papa, Shetland. Bootstrap support values are given at each node.

Table 3.6 Sequences represented in *C. capillata* tentacle tissue from North of Papa, Shetland

Genus	Exemplar sequence	*n* occurrences	Duplicate occurrences
Aeromonas	1 J2atNP1	1	
Aeromonas	98 J6atNP2b	1	
Vibrio	6 J1btNP2	1	
Vibrio	J6btNP1	1	
Enterobacter	69 J5atNP7	1	
Morganella	151 J6atNP1	1	
Pseudoalteromonas	81 J1btNP1	2	J6btNP2
Pseudoalteromonas	89 J8btNP2	1	
Shewanella	65 J1atNP1b	1	
Bacillus	64 J3atNP	1	
Pseudomonas	37 J5atNP2	1	
Pseudomonas	86 J7atNp1	2	J6atNP2a

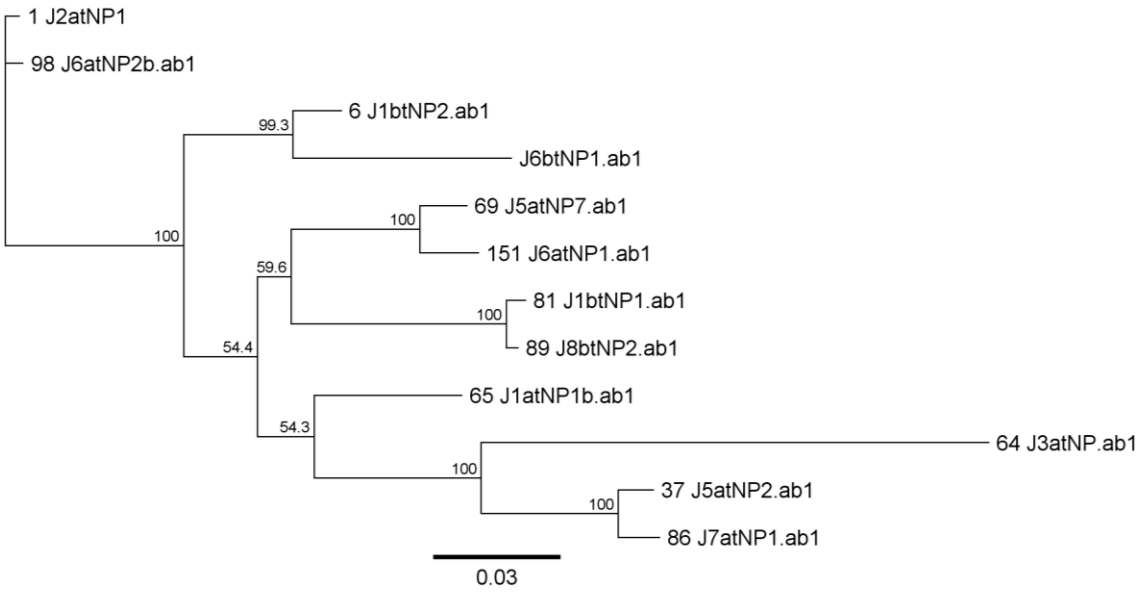


Figure 3.10 Tamura-Nei neighbor-joining tree of 16S ribosomal sequences of ClustalW alignment of bacterial cultures sampled from *C. capillata* tentacle tissue at North of Papa, Shetland. Bootstrap support values are given at each node.

Table 3.7 Sequences represented in *N. pileata* medusae from North of Papa, Shetland

Genus	Exemplar sequences	*n* occurrences	Duplicate occurrences
Vibrio	84 N6bNP	1	
Vibrio	85 N8bNP2	1	
Vibrio	87 N5bNP	2	19 N7bNP
Vibrio	73 N2bNP2	1	
Pseudoalteromonas	30 N3b	1	
Pseudomonas	79 N2aNP	1	
Pseudomonas	125 N4aNP	1	
Pseudomonas	5 N7aNP	1	
Pseudomonas	70 N5aNP	1	
Pseudomonas	78 N6aNP	1	

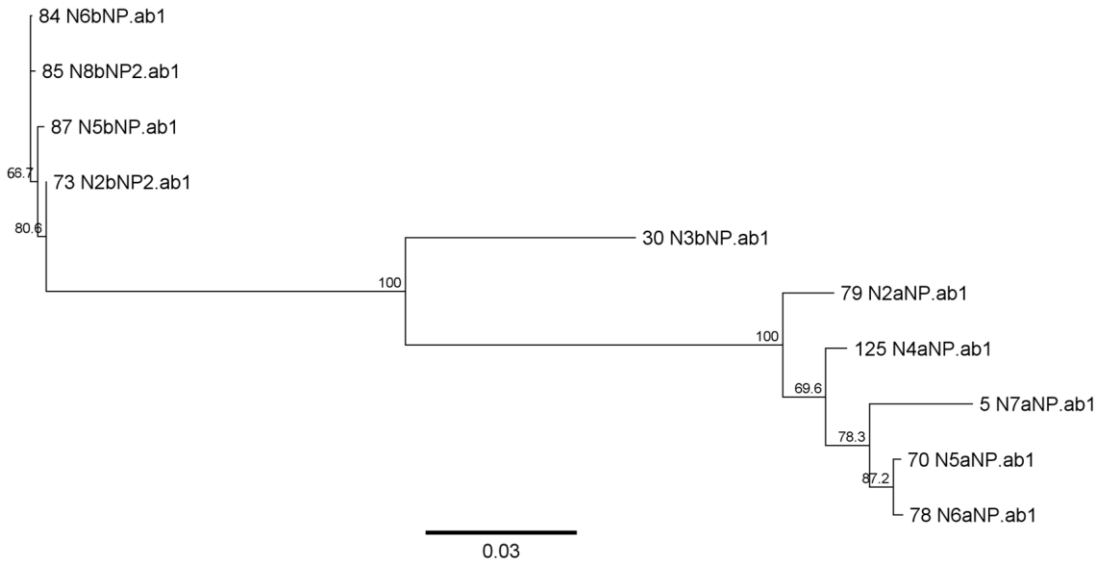


Figure 3.11 Tamura-Nei neighbor-joining tree of 16S ribosomal sequences of ClustalW alignment of bacterial cultures sampled from *Neoturris pileata* at North of Papa, Shetland. Bootstrap support values are given at each node.

Of the three sites where O. geniculata was sampled, Roe Sound showed the greatest diversity (Figure 3.13), though not significantly so (ANOVA, p = 0.131, df = 23; Table 3.8).

Table 3.8 Pairwise Tukey's HSD q-statistic significance comparisons between samples obtained from hydroids at Lunna, Redayre, Roe sound. Q-crit = 3.565. Values above this indicate a significant difference in the comparison; values below do not.

	Lunna	**Redayre**	**Roe Sound**
Lunna		0.9974	-1.66227
Redayre	0.9974		-2.974
Roe Sound	-1.66227	-2.974	

The largest number of hydroids was obtained at Redayre (10 hydroids sampled), which showed only 6 genera as opposed to the 10 represented at Roe Sound (8 hydroids sampled). The overall small sample size may have artefactual effect.

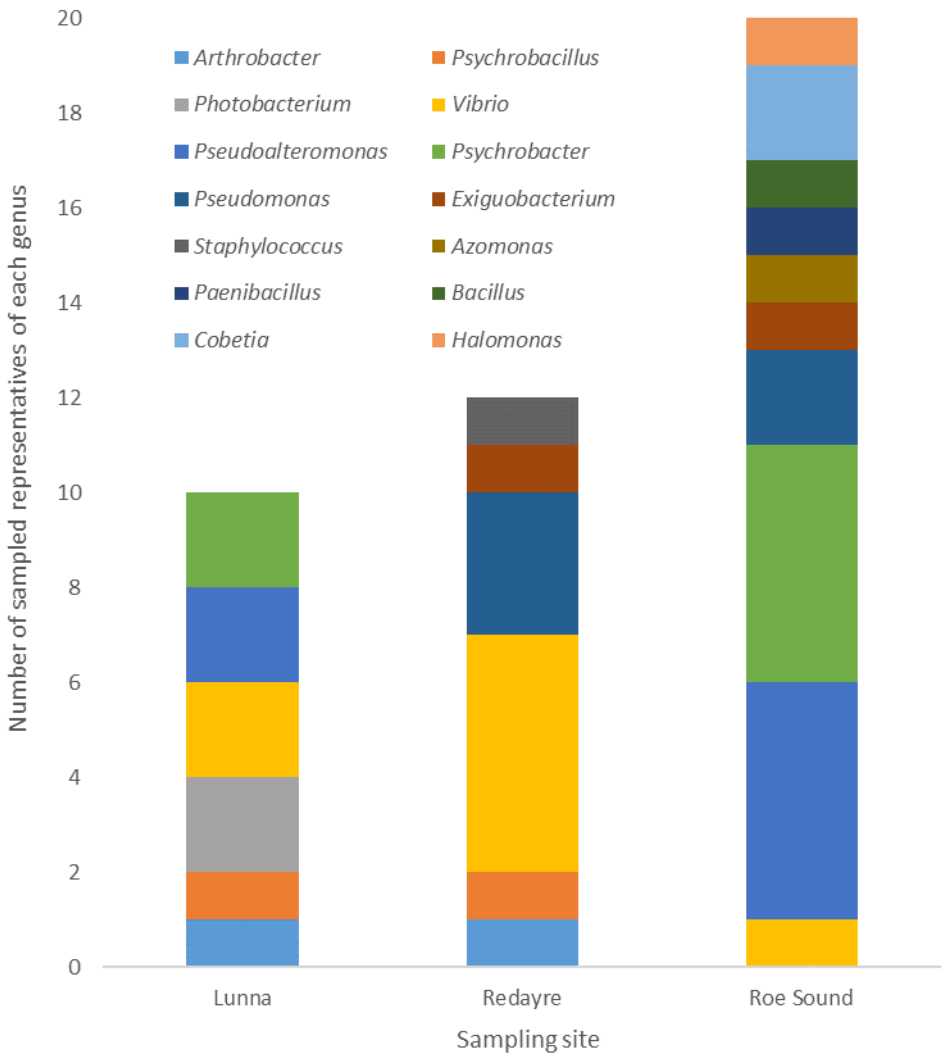


Figure 3.12 Bacterial samples of each represented genus obtained from *Obelia geniculata* hydroids at Lunna, Redayre, and Roe Sound, Shetland.

A pairwise comparison of bacterial diversity using PERMANOVA between *C. capillata* individuals' tentacle vs. gut tissues found no significant difference ($p = 0.528$, $df = 7$). Interestingly, comparisons also do not seem to indicate significant pairwise similarity. Based on these eight individuals, it does not appear that bacterial species isolated from tentacle tissue are significantly correlated with bacterial species isolated from gut tissue, though this conclusion would benefit from a larger sample size (Figure 3.13).

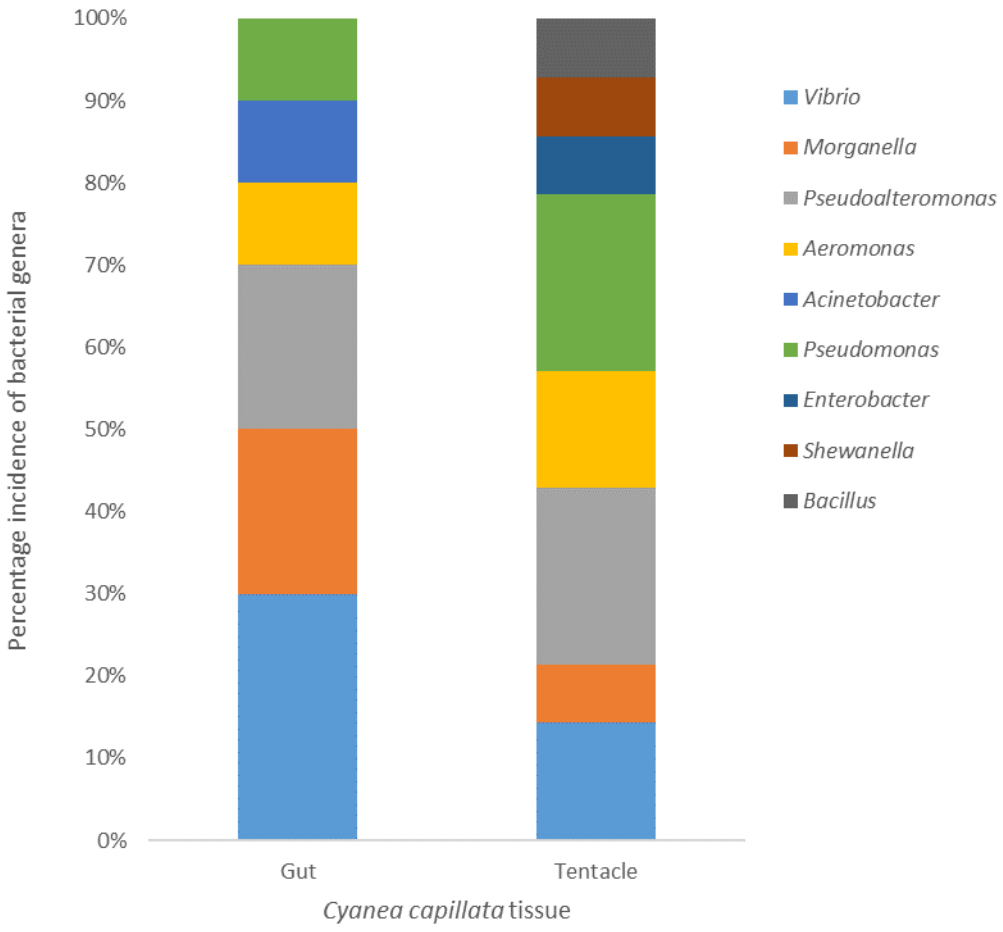


Figure 3.13 Comparison of bacterial genera sampled from *Cyanea capillata* gut vs tentacle from 8 individuals collected at North of Papa. While there is some overlap in genera sampled, this does not always correspond to overlap within a single individual medusa ($p = 0.528$).

A comparison between the two species collected at North of Papa, *N. pileata* and *C. capillata*, was also made. Pooled (tentacle and gut) bacterial diversity found greater diversity in bacteria obtained from *C. capillata* pooled samples, though this was not significantly so given the successfully-run sequences available (ANOVA, p = 0.120, df = 14). There was also no significant difference in diversity between gut and tentacle samples (Table 3.9).

Table 3.9 Pairwise Tukey's HSD q-statistic significance comparisons between samples obtained from medusae at North of Papa. Q-crit = 3.901. Values above this indicate a significant difference in the comparison; values below do not.

	All *C. capillata* samples	***C. capillata* gut**	***C. capillata* tentacle**	***N. pileata***
All *C. capillata* samples		2.348	1.838	2.626
***C. capillata* gut**	2.348		-0.570	0.163
***C. capillata* tentacle**	1.838	-0.570		0.763
N. pileata	2.626	0.163	0.763	

3.5 Discussion

The original questions set out for this investigation could not be answered directly due to limitations in fieldwork: namely, the target medusozoan species did not appear, either in an adult medusa-stage bloom or in a benthic hydroid stage. Questions as to *T. maritimum* distribution in the medusozoa remain. However, this study does provide a basis for further investigation of bacterial symbiont community in *O. geniculata*, *C. capillata*, and *N. pileata*, particularly by clinically-relevant species, as well as demonstrating that *T. maritimum* symbiosis is likely not widely occurring in medusozoan species

3.5.1 Observed bacterial flora

Of the genera observed at this stage, several are of aquacultural or environmental interest. *Aeromonas*, found in two *C. capillata* individuals' gut and tentacle tissue, is

familiar as the genus of *A. salmonicida*, the pathogenic agent of furunculosis in farmed salmon (Ellis et al. 1981), and indeed was shown as likely to be *salmonicida. A. salmonicida* has been found in association with sea lice (*Lepeophtheirus salmonis*) in Norwegian salmon farming and suggested as a likely vector (Bruno and Stone 1990, Nese and Enger 1993) and has been suspected as associated with marine zooplankton. A definitive identification as to bacterial species and further confirmation of its incidence in a common medusozoan species might open useful lines of inquiry in epidemiology of furunculosis. *Photobacterium damselae piscicida* is known as the pathogenic agent of pasteurellosis in cultured yellowfin tuna, seabream, striped bass, and white perch (Romalde 2002), and is considered an emerging pathogen in marine aquaculture (Labella et al. 2011, Rivas et al. 2011). As of yet, it has not been found in salmon or in fish farms at the temperatures observed in Shetland, with this finding as a bellwether for potential future problems. *Vibrio* contains some 75+ species and is near-ubiquitous in the marine environment, but the genus does include several species which are pathogenic in aquaculture, and some of which are required for planular metamorphosis by *Cassiopeia* jellyfish species (Neumann 1979, Bruno 1996). Bacteria of genus *Alteromonas* have also been found to induce planular metamorphosis in the hydrozoan *Hydractinia echinata* (Leitz and Wagner 1993, Leitz 1997). The broad diversity of *Vibrio*, and its commonality in marine environments, may offer opportunity for comparison of the *Vibrio* species occurring in benthic hydroids vs free-swimming medusae using this data set, contingent upon further sequencing of the samples available (see section 3.4.3, and Table 3.10). Alternately, *Pseudoalteromonas*, one of the most commonly found bacterial genera found in this study, is often found in symbiosis with a number of eukaryotes, including macroalgae and corals, and produces compounds that inhibit fouling by algal epiflora (Holmström and Kjelleberg 1999, Vynne et al. 2011). Of the genera observed in this study, *Pseudoalteromonas* seems a likely candidate for a mutualistic relationship with medusozoan hosts as well, in potentially conferring similar anti-fouling benefits.

3.5.2 Diversity

Qualified inferences, bearing in mind the limitations of available sequences, can be made as to the bacterial community diversity found in these cnidarian species. In terms of bacterial genera found in *O. geniculata* hydroids, the lack of consistency is interesting – each individual hydroid had, as mean, 1.6 successfully cultured and sequenced bacterial species in close association, compared to 11 total genera represented in pooled *O. geniculata* sources. Even assuming a substantial increase in this mean to account for those samples not yet successfully sequenced, this demonstrates considerable variability in what may be expected in any given hydroid. A completed dataset with 16S reverse sequences and/or any other identifiers with species-level identification of all samples would be useful in observing what common overlap, if any, exists.

Similarly, the pairwise comparison of genera incidence in gut vs tentacle tissues in *C. capillata* found no strong link. These findings may be artefactual due to the complexities of bacterial culturing – i.e., dominant species may successfully exclude the growth of others in a polyculture (e.g. Hibbing et al. 2010) – or to bacterial competition for substrate within a eukaryote symbiont, or even spatial distribution within the tissues of the medusae sampled. Completed bacterial species and strain identification may help answer these questions by enabling a clearer discernment of each genus' distribution across medusozoan species hosts.

3.5.3 Further work

Several of the study limitations described could be ameliorated with straightforward further analyses. Inherent drawbacks due to the difficulties of sampling cnidarian species, described in the introductory section, are unavoidable: in the absence of *Phialella quadrata* medusae, focus was shifted to other medusozoan species (*C. capillata* and *N. pileata*); when seasonal limitations prevented sufficient collection of

medusae, focus was shifted to such benthic hydroid species as could be obtained (*O. geniculata*). However, such sampling has amassed a DNA library with interesting potential. This material is ripe for further sequencing, largely in terms of obtaining simply the 16S rDNA reverse sequence for most of the samples represented. For most of the samples discussed, a complete or near-complete 1400 bp 16S amplicon will be sufficient to identify species; others will require some secondary sequence analysis, including species-specific probes for the 16S region, sequence of the 23S ribosomal region, ribosomal intergenic spacer analysis (RISA), and sequencing of the rpoβ gene region (Table 3.4).

Table 3.10 Listing of recommended further identification approaches appropriate to each sampled genus.

16s reverse sequence	**Species-specific probes**
Arthrobacter (Dorsch et al. 1994)	*Aeromonas* (Sen 2005, Graf 2015)
Cobetia (Inbakandan et al. 2010)	*Tenacibaculum maritimum* (Cepeda et al. 2003)
Enterobacter (Woo et al. 2001)	
Exiguobacterium (Tan et al. 2009)	
Morganella (O'Hara et al. 2000)	**Ribosomal intergenic spacer-analysis (RISA)**
Photobacterium (Zhao et al. 2009)	*Halomona* s (Jan-Roblero et al. 2004)
Pseudoalteromonas (Vynne et al. 2011)	*Pseudomonas* (Guasp et al. 2001)
Psychrobacillus (Krishnamurthi et al. 2010)	*Vibrio* (Hoffman et al. 2010, Larsen et al. 2010)
Shewanella (Todorova and Costello 2006)	*Staphylococcus* (Mendoza et al. 1998)
Psychrobacter (Ringø et al. 2008)	
23S sequence	**rpoβ**
Acinetobacter (Yoon et al. 2007, Visca et al. 2011)	*Bacillus* (Ki et al. 2009)
Vibrio (Hoffman et al. 2010)	*Paenibacillus* (da Mota et al. 2004)

These secondary steps to identify these samples to species and subspecies would be useful in terms of maximizing data value generated in this study. Furthermore, once clear and complete sequences are available across all remaining samples, a dedicated 16S database such as the Ribosomal Database Project (RDP) hosted by the Center for Microbial Ecology at Michigan State University. This will be of further help in identifying ambiguous sequences in the dataset.

Findings were also limited by bacterial culturing methods. While the culture media selected target a broad spectrum of bacteria pathogenic in aquaculture, not all species within a bacterial community are covered by these, or indeed easily cultured

at all (Wade 2002, Vartoukian et al. 2010, Stewart 2012). A more comprehensive method for studying this bacterial community might be terminal-restriction fragment length polymorphism (TRFLP), which can amplify 16S sequences without the need for a lengthy culturing and DNA extraction stage (Osborn et al. 2000, Vengatasen 2010, Rajendhran and Gunasekaran 2011), or use of second-generation sequencing platforms such as Illumina MiSeq (Caporaso et al. 2012, Manzari et al. 2014). This approach would constitute an appropriate next step in characterizing the bacterial communities associated with medusozoan species.

This study provided a first look into the bacterial symbiont community in several medusozoan species. Of interest to aquaculturists, several potentially pathogenic bacterial genera were observed in *O. geniculata*, *C. capillata*, and *N. pileata*. *T. maritimum* was not documented, suggesting that its vectoring by medusozoans is relatively rare. Future work may wish to use broader techniques for sampling bacteria independent of culturing steps in order to further document these communities.

3.6 Appendix: Control sequences

Control sequences included representatives of the *Pseudoalteromonas*, *Vibrio*, *Enterobacter*, and *Psychrobacter* genera. These have congeners remaining in the main dataset; only those sequences which were a 100% match to the relevant controls with no polymorphism were removed from further analysis. This approach precluded the erroneous removal of sequences based o n common genera, given the taxonomic overlap.

Sample 68/twbNP2 (*Pseudoalteromonas*)
Source: incubation system water during North of Papa sampling
Length: 813 bp
Sequence:
TCCCGAGGGTTAGACTATCTACTTCTGGAGCAACCCACTCCCATGGTGTGACGGGCGGTGT
GTACAAGGCCCGGGAACGTATTCACCGCGTCATTCTGATACGCGATTACTAGCGATTCCGA
CTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTAAGTGATTCGCTT
ACTCTCGCGAGTTCGCAGCACTCTGTATGCGCCATTGTAGCACGTGTGTAGCCCTACACGT
AAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCTT
AGAGTTCTCAGCATTACCTGCTAGCAACTAAGGATAGGGGTTGCGCTCGTTGCGGGACTTA
ACCCAACATCTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTATCAGAGCTCCCGA
AGGCACCAAACCATCTCTGGTAAGTTCTCTGTATGTCAAGTGTAGGTAAGGTTCTTCGCGT
TGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTT
TAACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTAATGCGTTAGCTTTGAAAAACAGAAC
CGAGGTTCCGAGCTTCTAGTAGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCT
GTTTGCTCCCCACGCTTTCGTACATGAGCGTCAGTGTTGACCCAGGTGGCTGCCTTCGCCAT
CGGTATTCCTTCAGATCTCTACGCATTTCACCGCTACACCTGAAATTCTACCACCCTCTATCA
CACTCTAGTTTGCCA

Sample 94/ewbNP (*Enterobacter*)
Source: environmental water sample from North of Papa
Length: 779 bp
Sequence:
AGCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGG
GCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTCTGATCTACGATTACTAGCG
ATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTATGAGG
TCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCT
ACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGT
CTCCTTTGAGTTCCCGGCCTAACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGG
GACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAGT
TCCCGAAGGCACCAAAGCATCTCTGCTAAGTTCTCTGGATGTCAAGAGTAGGTAAGGTTCT

TCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTT
GAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGC
CACTCCTCAAGGGAACAACCTCCAAGTCGACATCGTTTACGGCGTGGACTACCAGGGTATC
TAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCT
TCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTAC

Sample 97/ewaNP (*Enterobacter*)
Source: environmental water sample from North of Papa
Length: 679 bp
Sequence:
GAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTAC
AAGGCCCGGGAACGTATTCACCGTGGCATTCTGATCCACGATTACTAGCGATTCCGACTTC
ATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTC
TCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGG
GCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAG
TTCCCGGCCTAACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCC
AACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAGTTCCCGAAGGC
ACCAAAGCATCTCTGCTAAGTTCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCA
TCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAAC
CTTGCGGCCGTACTCCCCAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGCCACGCCTCAA
GGGCACAACCTCCAAGTCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTT
TGCTCCC

Sample 107/ewaL2 (*Psychrobacter*)
Source: environmental water sample from Lunna
Length: 849
Sequence:
CGCCTCCCCGAAGGTTAAGCTATCCACTTCTGGTGCAATCAACTCCCATGGTGTGACGGGC
GGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATTCTGATCCGCGATTACTAGCGA
TTCCTACTTCATGGAGTCGAGTTGCAGACTCCAATCTGGACTACGATAGGCTTTTTGAGATT
CGCATCACATCGCTGTGTAGCTGCCCTCTGTACCTACCATTGTAGCACGTGTGTAGCCCTGG
TCGTAAGGGCCATGATGACTTGACGTCGTCCCCGCCTTCCTCCAGTTTGTCACTGGCAGTAT
CCTTAGAGTTCCCGGCTTAACCCGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTGCGGG
ACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTATTCTAATT
CCCGAAGGCACTCCCGCATCTCTGCAGGATTCTAGATATGTCAAGACCAGGTAAGGTTCTT
CGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTG
AGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTATTGCGTTAGCTGCGTCACTAA
GTCCTCAAGGGACCCAACGACTAGTAGACATCGTTTACGGCGTGGACTACCAGGGTATCT
AATCCTGTTTGCTACCCACGCTTTCGAGCCTCAGTGTCAGTATGATGCCAGGAAGCTGCCTT
CGCCATCGGTATTCCTTCAGATCTCTACGCATTTCACCGCTACACCTGAAATTCTACTTCCCT
CTCACCTACTCTAGCCTAACAGTTTCAGATGCAGTTCCCAGGGTAAAGCCC

Sample 174/ewbL3 (*Pseudoalteromonas*)
Source: environmental water sample from Lunna
Length: 804 bp
Sequence:
CTAAGGTTAAGCTACCTACTTCTGGAGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTA
CAAGGCCCGGGAACGTATTCACCGCGGCATTCTGATCCGCGATTACTAGCGATTCCGACTT
CATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTAAGTGATTCGCTAACC
TTCGCAGGCTCGCAGCACTCTGTATGCGCCATTGTAGCACGTGTGTAGCCCTACACGTAAG
GGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCTTAGA
GTTCCCACCATTATGTGCTGGCAACTAAGGATAGGGGTTGCGCTCGTTGCGGGACTTAACC
CAACATCTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTATCAGAGTTCCCGAAG
GCACCAAACCATCTCTGGTAAGTTCTCTGTATGTCAAGTGTAGGTAAGGTTCTTCGCGTTGC
ATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAA
CCTTGCGGCCGTACTCCCCAGGCGGTCTACTTAATGCGTTAGCTTTGAAAAAGTTGTCCGA
AGACCCCAGCTTCTAGTAGACATCGTTTACGGCGTGGACTACCGGGGTATCTAATCCCGTT
TGCTCCCCACGCTTTCGTACATGAGCGTCAGTGTTGACCCAGGTGGCTGCCTTCGCCATCG
GTATTCCTTCAGATCTCTACGCATTTCACCGCTACACCTGAAATTCTACCACCCTCTATCACA
CTCTAGT

Sample 187/ewbL1ii (*Vibrio*)
Source: environmental water sample from Lunna
Length: 719 bp
Sequence:
AGCGTCCTCCCCGAAGGTTAAACTACCCACTTCTTTTGCAGCCCACTCCCATGGTGTGACGG
GCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGACATTCTGATTCACGATTACTAGCG
ATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTTTGGGA
TTCGCTCACTATCGCTAGCTTGCTGCCCTCTGTATGCGCCATTGTAGCACGTGTGTAGCCCT
ACTCGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGT
CTCCCTGGAGTTCCCGACATTACTCGCTGGCAAACAAGGATAAGGGTTGCGCTCGTTGCGG
GACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAGC
TCCCGAAGGCACACCTGCGTCTCCGCTGGCTTCTCTGGATGTCAAGAGTAGGTAAGGTTCT
TCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTTCATT
TGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGTCTACTTAACGCGTTTAGCTCCGAAAG
CCACGGCTCAAGGCCACAACCTCCAAGTAGACATCGTTTACGGCGTGGACTACCAGGGTA
TCTAATCCTGTTTGCTCCCCACGCTTTCGCATCTGAGTGTCAGT

Sample 132/ewaRS1 (*Vibrio*)
Source: environmental water sample from Roe Sound
Length: 760 bp
Sequence:
CTCCTCGAAAGGTTAAACTACCCACTTCTTTTGCAGCCCACTCCCATGGTGTGACGGGCGG
TGTGTACAAGGCCCGGGAACGTATTCACCGTGACATTCTGATTCACGATTACTAGCGATTC
CGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTTTGGGATTCG

CTCACTATCGCTAGCTTGCTGCCCTCTGTATGCGCCATTGTAGCACGTGTGTAGCCCTACTC
GTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCC
CTGGAGTTCCCGACATTACTCGCTGGCAAACAAGGATAAGGGTTGCGCTCGTTGCGGGAC
TTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAGCTCC
CGAAGGCACACCTGCGTCTCCGCTGGCTTCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCG
CGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAG
TTTTAATCTTGCGACCGTACTCCCCAGGCGGTCTACTTAACGCGTTAGCTCCGAAAGCCACG
GCTCAAGGCCACAACCTCCAAGTAGACATCGTTTACGGCGTGGACTACCAGGGTATCTAAT
CCTGTTTGCTCCCCACGCTTTCGCATCTGAGTGTCAGTGTCTGTCCAGGGGGCCGCCTTCGC
CACTGGTATTCCTTCAGATCTCTA

Sample 175/twbRS1 (*Pseudoalteromonas*)
Source: incubation system water during Roe Sound sampling
Length: 675 bp
Sequence:
GTCCTCCCGAGGGTTAGACTATCTACTTCTGGAGCAACCCACTCCCATGGTGTGACGGGCG
GTGTGTACAAGGCCCGGGAACGTATTCACCGCGTCATTCTGATACGCGATTACTAGCGATT
CCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTAAGTGATTC
GCTTACCTTCGCAGGTTCGCAGCACTCTGTATGCGCCATTGTAGCACGTGTGTAGCCCTAC
ACGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCT
CCTTAGAGTTCTCAGCATTACCTGCTAGCAACTAAGGATAGGGGTTGCGCTCGTTGCGGGA
CTTAACCCAACATCTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTATCAGAGTTC
CCGAAGGCACCAATCTATCTCTAGAAAGTTCTCTGTATGTCAAGTGTAGGTAAGGTTCTTC
GCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGA
GTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTAATGCGTTAGCTTTGAAAAACA
GAACCGAGGTTCCGAGCTTCTAGTAGACATCGTTTACGGCGTGGACTACCGGGGTATCTA
ATC

Sample 163/ewbRA1 (*Pseudoalteromonas*)
Source: environmental water sample from Redayre
Length: 1014 bp
Sequence:
GAATCACACCTCCGTGGTAACGTCCTCCCGAGGGTTAGACTATCTACTTCTGTGAGCAACC
CACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGTCATT
CTGATACGCGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGA
CTACGACGCACTTTAAGTGATTCGCTTACTCTCGCGAGTTCGCAGCACTCTGTATGCGCCAT
TGTAGCACGTGTGTAGCCCTACACGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTC
CTCCGGTTTATCACCGGCAGTCTCCTTAGAGTTCTCAGCATTACCTGCTAGCAACTAAGGAT
AGGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACAACACGAGCTGACGACAGCC
ATGCAGCACCTGTATCAGAGTTCCCGAAGGCACCAAACCATCTCTGGTAAGTTCTCTGTAT
GTCAAGTGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGT
GCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCTACT
TAATGCGTTAGCTTTGAAAAACAGAACCGAGGTTCCGAGCTTCTAGTAGACATCGTTTACG

GCGTGGACTACCAGGGGTATCTAATCCTGTTTGCTCCCCACGCTTTTCGTACATGAGCGTCA
GTGTTGACCCAGGTGGCTGCCTTCGCCATCGGTATTCCTTCAGATCTCTACGCATTTTCACC
GCTACACCTGGAAATTCTACCACCTCTATCACACTCTAGTTTGCCAGTTCGAAATGCAGTTC
CCAGGTTGAGCCCGGGGCTTCACATTCTCGCTGACAACCGCCTGGCGTACGCTTTACGCCA
AGTAATTTCCGATTAGCGTCCTCGGCACCCTCCGGCATATTACCGGCGACTGTCTGGCCCC
GGAAATTAGCTCCGGGGTGCTCTCTTCTGCGTTC

Sample 201/ewbRA2 (*Vibrio*)
Source: environmental water sample from Redayre
Length: 1042 bp
Sequence:
CCCCCCTTAGCCAAAGTGGTGAGCGTCCTCCCCGAAAGGTTAAACTACCCACTTCTTTTGCA
GCCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGAC
ATTCTGATTCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCG
GACTACGACGCACTTTTTGGGATTCGCTCACTATCGCTAGCTTGCTGCCCTCTGTATGCGCC
ATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCGTCCCCACCT
TCCTCCGGTTTATCACCGGCAGTCTCCCTGGAGTTCCCGACATTACTCGCTGGCAAACAAG
GATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACA
GCCATGCAGCACCTGTCTCAGAGTTCCCGAAGGCACACCTGCGTCTCCGCTGGCTTCTCTG
GATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCT
TGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGTCT
ACTTAACGCGTTAGCTCCGAAAAGCCACGGCTCAAGGCCACAACCTCCAAGTAGACATCGT
TTACGGCGTGGACTACCAAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCATCTGAGT
GTCAGTGTCTGTCCCAGGGGGGCCGTCTTCGCCACTGGTATTCCTTTCAGATCTCTACGCAT
TTTCACCGCTACACCTTGAAATTCTACCCCCCCTCTACAGCACTCTAGTTTCCACCAGTTTCA
AATGCAGTTTCCGAGGGTTGAGCCCCCGGGGCCTTTCACATTCTGAACTTTAAATGAAACC
ACCTTGCAATGCGCTTTTTACGCCCCAGTAATTTCCGAATTTAACGCCTTCGCACACCCTCC
GTAATTACCGGCGACTGCTGCAACGGAGATAGGCCCGGTGCCTTCCTATCTGGTGGCTC

4

Phylogeographic analysis of *Obelia geniculata* populations in the north of Scotland

I estimate that I contributed 86% of the total effort towards the eventual publication of this paper, which can be broken down as follows:

- 2013 sampling 10% (5% by me, 1% assistance from Kiran Garimella of Oxford University, 1% assistance from Mary Mackay of the University of St Andrews, 1% assistance from Ewan Edwards of the University of Aberdeen, 2% assistance from Rachel Shucksmith of North Atlantic Fisheries College, Scalloway)
- 2014 sampling 10% (6% by me, 2% by April Blakeslee of Long Island University, 1% by Rachel Shucksmith, 1% by Tara Beeny of the University of St Andrews)
- Sample DNA extraction, purification and amplification 20% (18% by me, 2% by April Blakeslee)
- Sample sequencing outsourced to Macrogen USA (5%)
- Data analysis 30% (25% by me, 5% by April Blakeslee). Analyses produced by Dr Blakeslee are noted in text. These included as shared responsibility for AMOVA analyses, and Dr Blakeslee's responsibility for multidimensional scaling and cluster analysis.
- Writing 25% (by me, with review and suggestions by Dr Blakeslee, and review by ASB).

The material in this chapter is in preparation for publication in *PeerJ* (www.peerj.com) in current form using single-gene analysis, in the near future, under title "Phylogeographic analysis of *Obelia geniculata* hydroids in the North of Scotland shows population boom-and-busts within a well-mixed haplotypic assemblage," by A. Kintner, A. Brierley, and A. Blakeslee. Additionally, I aim to add further samples to the analyses (as laid out in the Discussion) and to use further genomic markers, so as to aim for updated publication in *PLoS One Ecology* or *Diversity and Distributions* (Blackwell Publishing).

4.1 Capsule findings

- The gene pool of *Obelia geniculata* hydroid colonies is weakly defined between the Scottish northwest mainland and the northern islands, as

evidenced by phylogenetic analysis of the mitochondrial cytochrome oxidase I gene.

- Local sites within the regions of the northwest mainland, Orkney and Shetland are not substantially different to one another, suggesting that local populations frequently intermix.
- There is strong evidence for localized colony dieback, but the lack of substantial interannual change in genotypic assemblage suggests that repopulation occurs readily from a well-mixed population.
- Based on comparison with previously published haplotypic assemblages, mixing may be more geographically broad than previously assumed possible.

4.2 Introduction

The hydrozoan archetype for reproduction in the North Atlantic follows an alternation of sexual-asexual reproduction: benthic colonies produce clonal individuals that live interdependently, and seasonally produce free-swimming medusae which release gametes into the water (Russell 1953; illustrated in Figure 1.2 and Figure 4.1). After fertilization, planula larvae develop in the plankton until a suitable substrate allows for settlement and development into a new colony. Theoretically, these colonies are immortal, capable of continuously growing clonally and producing medusae as long as environmental conditions remain favourable, and if colonies are lost to senescence, resettlement by new planulae could be expected.

The regional colonial population of a common species, *Obelia geniculata* (Figure 4.1 below), appeared to die back *en masse* from previously established nearshore settlements in Shetland in 2012 (Kintner and Shucksmith pers. obs. 2012). Most sites previously surveyed as containing substantial populations became devoid, and any sites remaining showed very few, small colonies. Population rebound after this condition could come from one of two sources: first, remaining local colonies might

grow clonally rapidly enough to recover lost settlement substrate, in spite of competition for space. *O. geniculata* is unusual in its ability to propagate clonally in the plankton via tissue capsules (Berrill 1948, Panteleeva et al. 1999, Slobodov and Marfenin 2004). Second, re-colonization might come from immigration and settlement of planulae from other regions. Medusae produced by hydrozoan colonies spend time in the plankton during the maturation process; in addition, larvae produced during the spawning of these medusae spend a second period of time in the plankton before metamorphosing and settling out to produce a new hydroid colony (Russell 1953). Both of these life stages offer opportunity for relocation according to prevailing currents.

Figure 4.1 ***Obelia geniculata*** **hydroid colonies on** ***Laminaria digitata*** **fronds in North Uist, Scotland.**

A comparison of genetic markers before and after such a dieback, and with the diversity of adjacent regional populations, can provide insight into this type of migration. Govindarajan et al. (2004a, 2004b) found genetic differentiation associated with geography using the barcoding marker mitochondrial cytochrome oxidase I (mtCOI) across the North Atlantic in *O. geniculata* populations, making it an ideal target for this investigation (see also Ortman et al. 2010). In waters off northwestern Scotland and the northern islands, the dominant non-tidal flow shows a northward drift of up to 0.16 m s^{-1} (Booth and Ellett 1983) (Figure 4.2); *O. geniculata* gene flow might be expected to mirror this drift.

Figure 4.2 The Scottish Slope Current. Northeasterly movement of water along the coast takes place at up to 0.16 m^{-1} (Booth and Ellett 1983).

This investigation sought to make this comparison using *O. geniculata* hydroids collected from Shetland, the Scottish northern coast and Orkney, and the Scottish northwest mainland, sampling prior to and after a hypothesized population recovery predicted to take place in the summer season of 2013.

Broadly, outcomes along two different dimensions can be predicted. First, in terms of spatial differentiation, the regional populations may be shown to be panmictic, with no gene pool definition across the north of Scotland. Alternatively, populations may be defined on a regional or even local scale. Panmixis would seem to indicate a longer time spent in the plankton, with survival and recruitment at distant sites, as demonstrated in the common acorn barnacle species *Semibalanus balanoides* by Flight et al. (2011). Differing degrees of this, such as found in scallops (*Placopecten magellanicus*) on the North American east coast (Kenchington et al. 2006), may indicate limited dispersal distance, with small amounts of regional differentiation embedded amongst shared lineage showing dispersal along geographic "stepping stones" that facilitate longer distance dispersal over multiple seasons. Meanwhile, strong differentiation at regional or local levels might indicate a strategic

minimization of time spent in the plankton in favour of increased odds for successful recruitment. This was found to be the case in a number of reef-building coral species in Australia, particularly in those that brooded offspring (Ayre and Hughes 2000). Second, in terms of temporal differentiation, populations may show no year-to-year change in their haplotype assemblage. This outcome would seem to indicate that local populations, in spite of an apparent dieback in 2012, retain sufficient genet to allow for recovery to come from local sources. Alternatively, a strong year-to-year change in assemblage might indicate both extensive local dieback as well as frequent migration from distant sources.

4.3 Methods

4.3.1 Summary in brief

This study aimed to observe patterns of distribution of *O. geniculata* haplotypes in space and time, before and after summertime dispersal and settlement in 2013. Therefore, colonies from a range of geographic areas in the north of Scotland were sampled in early spring 2013 (prior to summertime dispersal) and again in 2014.

4.3.2 Sampling locations

Three regional areas of northern Scotland – the northwest mainland (NWM), Orkney/North (Ork/N), and Shetland (Shet) – were identified as sampling zones for *O. geniculata* hydroids (Figure 4.3). Sampled sites within these regions are laid out in Table 4.1 and Figure 4.4 below.

All *O. geniculata* hydroid colonies were accessed via surface snorkeling and collected from macroalgae – usually *Laminaria digitata* – growing at each of these sites, with each colony sampled treated as one single clonal individual. Up to 20 individuals

were collected from each site and removed from their substrate and placed in separate 1.5mL tubes containing 95% ethanol preservative (as per Blakeslee et al. 2010). Two such sampling efforts were carried out: once in early 2013, prior to potential summertime sexual reproduction and resettlement, and once in 2014 after the summer season of 2013 provided a sufficient period for medusa production and dispersal.

4.3.3 Notes on sampling

Sampling within Orkney/North was considerably limited by availability of appropriate sampling sites, partially owing to the paucity of safe entry points along the Orkney Atlantic coast and the northern coast of the Scottish mainland (see Appendix). While sampling was attempted at numerous sites with *L. digitata* beds in this region, very few sites hosted healthy – if any – *O. geniculata* colonies, limiting both the number of sites sampled and the number of individuals collected. In Shetland, a final site, Wari Geo (Figure 4.4, site *y*), was sampled for spatial comparison from the North Sea-exposed eastern coast of Shetland, in 2014 only. This yielded a total of 23 sampled spatial-temporal groups across all sites and both years.

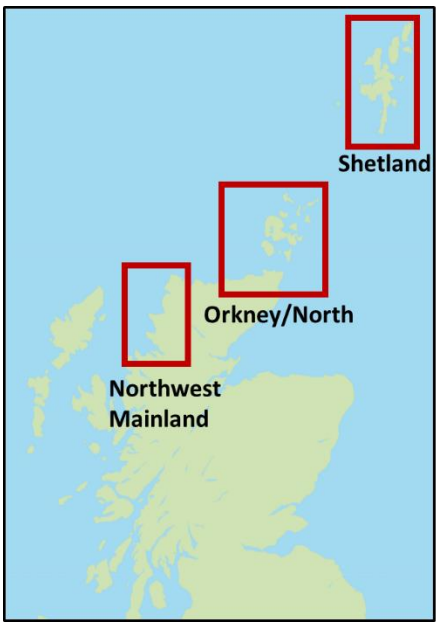


Figure 4.3 *Obelia geniculata* hydroids were collected from sites within three defined regions.

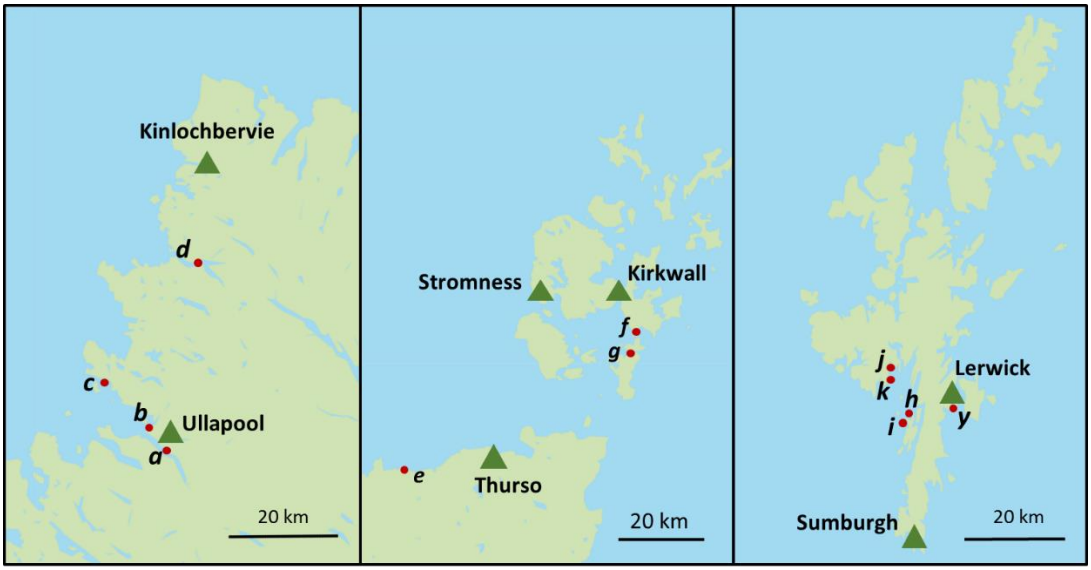


Figure 4.4 Regional sampling sites. Regions left to right: Scottish northwest mainland, Orkney/North, Shetland. Northwest mainland (NWM) sampling sites: (a) Loch Broom, (b) Polbain, (c) Ardmair, (d) Kylesku. Orkney/North (Ork/N) sampling sites: (e) Portskerra, (f) First Churchill Barrier, (g) Fourth Churchill Barrier. Shetland (Shet) sampling sites: (h) Setter, (i) Hamnavoe, (j) Redayre, (k) Reawick. Site (y) in Shetland, Wari Geo, was not included in significance tests during phylogeographic analyses. Triangles mark local towns for reference.

Table 4.1 Sampling sites for *O. geniculata* within three regions. All island sites in the main study were Atlantic-exposed sites. A final site, Wari Geo, was sampled on the east coast of Shetland in 2014 only; as it represents a distinct predicted biome from the Shetland west coast, it was not included in statistical significance tests during phylogeographic analyses. Letters represent site identifications in genetic analyses and positions of sites in Figure 4.4. Letters a-k are sites sampled in 2013 and letters l-y are sites sampled in 2014. Sites sampled in 2013 and 2014 only use the 2013 letter designations in Figure 4.4.

Region	Sites	Latitude/Longitude
Northwest Mainland (NWM)	(a, l) Loch Broom (b, m) Polbain (c, n) Ardmair (d, o) Kylesku	57.892059, -5.152613 58.030187, -5.371440 57.936778, -5.195932 58.253002, -5.032795
Orkney/North (Ork/N)	(e) Portskerra (f, q) 1st Churchill Barrier (g, r) 4th Churchill Barrier	58.569847, -3.944564 58.894690, -2.898607 58.842175, -2.906477
Shetland (Shet)	(h, s) Setter (I, t) Hamnavoe (j, u) Redayre (k, w) Reawick	60.106963, -1.320703 60.105527, -1.338615 60.193509, -1.405769 60.184734, -1.403736
Shetland East	(y) Wari Geo	60.147883, -1.149424

4.3.4 Genetic extraction, amplification, and sequencing

As part of the Marine Alliance for Science and Technology Scotland's Postgraduate and Early Career Research Exchange (MASTS/PECRE) program, samples were then transported to Long Island University in New York, USA, where they were processed in the laboratory of Dr April Blakeslee (co-recipient of the PECRE grant). DNA was extracted from each *O. geniculata* sample using a standard cetyl trimethyl ammonium bromide (CTAB)-chloroform extraction, followed by precipitation in 100% ethanol (as per France et al. 1996). A 70% ethanol wash was used to remove small organic contaminants from the remaining DNA pellet, and the purified DNA suspended in 50µL molecular-grade water. All samples were stored at -20˚C prior to PCR amplification.

MtCOI universal primers (forward sequence GGTCAACAAATCATAAAGATATTGG, reverse complement TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994, used previously for *O. geniculata* by Govindarajan et al. 2004a) were used to carry out PCR amplification for each sample, where one sample reaction included 1.5 µL primer F, 1.5 µL primer R, 8.5 µL pure water volume, and 12.5 µL mastermix containing 5 unit/µL Taq DNA polymerase and dNTP (PCR Master Mix, Promega, Madison, WI). PCR amplification was conducted using the following thermocycler profile: an initial denaturation at 95˚C for 2 min, then 30 cycles of 95˚C for 30 s, 55˚C for 30 s, 72˚C for 1 min, followed by a final extension at 72˚C for 5 min. Successful amplicons were identified using a 1% TAE agarose gel, and products purified using 2 µL of ExoSap-IT (Affymetrix, Inc., Santa Clara, CA). Cleaned up PCR products were sent to Macrogen USA (Silver Spring, Maryland) for forward sequencing.

Sequence data were visualized using Geneious R8-R9 (Kearse et al. 2012). Primer binding regions and regions from the 3' end with poor coverage were trimmed to give as much length of clear calls as possible. All sequences were checked to confirm accurate sequence identification using the BLAST search tool to compare sequences deposited in the GenBank database operated by the United States National Institutes of Health for comparison (Camacho et al. 2009). Alignments were created using ClustalW global alignment (Larkin et al. 2007). Phylogeny was inferred in MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001), using a GTR inverse gamma substitution model with 4 gamma categories, 11,000,000 chain length, 4 heated chains at 0.2 heated chain temperature, 100,000 burn-in number, and a subsampling frequency of 200. Individual haplotypes were identified and exported to Arlequin v. 3.5.2.2 for AMOVA analyses of regional and pairwise site comparisons (Excoffier and Lischer 2010). Multi-dimensional scaling (MDS) plots and ANOSIM analyses were made by April Blakeslee using Primer-E (Plymouth Marine Laboratory, UK). Rarefaction analyses were conducted using EstimateS (Colwell 2004).

4.4 Results

Raw data for this section can be found in the directory "Chapter 4 Hydroid Sequences," submitted in the metadata for this thesis. Guidance notes are provided therein.

4.4.1 Sequence variation

A total of 165 samples returned analyzeable mtCOI sequence results. These were annotated visually and aligned using the ClustalW algorithm (Thompson et al. 1997), then trimmed to a final length of 607 base pairs. Of these, 129 sites were found to be variable, yielding 41 distinct haplotypes, with the distribution of successful sequences and site-by-site haplotype richness given in Table 4.2 below. (Two haplotypes were unique to Wari Geo, on the east side of Shetland outside the main study areas. Contributions from this site are excluded from the main biogeographic analyses.)

Table 4.2 Site-by-site breakdown of successfully sequenced samples and diversity over 2013-2014, excluding Wari Geo. Haplotype diversity (h) is calculated in terms of haplotype frequencies and sample size per population; a higher number indicates a more diverse population. Nucleotide diversity (π) measures the degree of polymorphism within a population, or the average number of nucleotide differences between any two sequences (Nei and Li 1979).

Year	Region	Site	N sequences	N haplotypes	h	π
2013	**Northwest Mainland**	Loch Broom	16	6	0.7167 +/- 0.0988	0.002403 +/- 0.001722
		Polbain			no sequences returned	
		Ardmair	8	7	0.9643 +/- 0.0772	0.028889 +/- 0.016395
		Kylesku	9	5	0.8611 +/- 0.0872	0.003844 +/- 0.002623
	Orkney/ North	Portskerra	2	2	1.0000 +/- 0.5000	0.004942 +/- 0.005707
		1st Barrier	6	3	0.7333 +/- 0.1552	0.003295 +/- 0.002480
		4th Barrier	8	4	0.7857 +/- 0.1127	0.003471 +/- 0.002457
	Shetland	Setter	6	4	0.8667 +/- 0.1291	0.005821 +/- 0.003971
		Hamnavoe	10	7	0.9111 +/- 0.0773	0.004064 +/- 0.002708
		Redayre	9	3	0.7222 +/- 0.0967	0.002563 +/- 0.001909
		Reawick	2	2	1.0000 +/- 0.5000	0.004942 +/- 0.005707
2014	**Mainland**	Loch Broom	8	7	0.9643 +/- 0.0772	0.003883 +/- 0.002689
	Mainland	Polbain	7	6	0.9524 +/- 0.0955	0.004707 +/- 0.003219
		Ardmair	7	4	0.8095 +/- 0.1298	0.002824 +/- 0.002135
		Kylesku	8	7	0.9643 +/- 0.0772	0.032361 +/- 0.018290
	Orkney/ North	Portskerra			no sequences returned	
		1st Barrier	7	4	0.8571 +/- 0.1023	0.003609 +/- 0.002590
		4th Barrier	9	4	0.6944 +/- 0.1470	0.003766 +/- 0.002623
	Shetland	Setter	8	6	0.9286 +/- 0.0844	0.003766 +/- 0.002623
		Hamnavoe	11	5	0.8182 +/- 0.0826	0.027557 +/- 0.015009
		Redayre	6	2	0.5333 +/- 0.1721	0.002636 +/- 0.002084
		Reawick	7	4	0.8095 +/- 0.1298	0.004864 +/- 0.003309

Sites showed a mean of 4.6 distinct haplotypes per site per year (± 1.72 σ). Of the 41 haplotypes observed, 26 were observed only once; the remaining 15 haplotypes were observed between 2 and 43 times (Figure 4.5). The 26 singletons plus two further haplotypes appeared in only one sampling effort (i.e. only in one region in one year): 7 in NWM, 3 in Ork/N, and 4 in Shetland in 2013, and 7 in NWM and 7 in Shetland in 2014 (Table 4.3). This left 13 haplotypes occurring in multiple regions and times (Table 4.4).

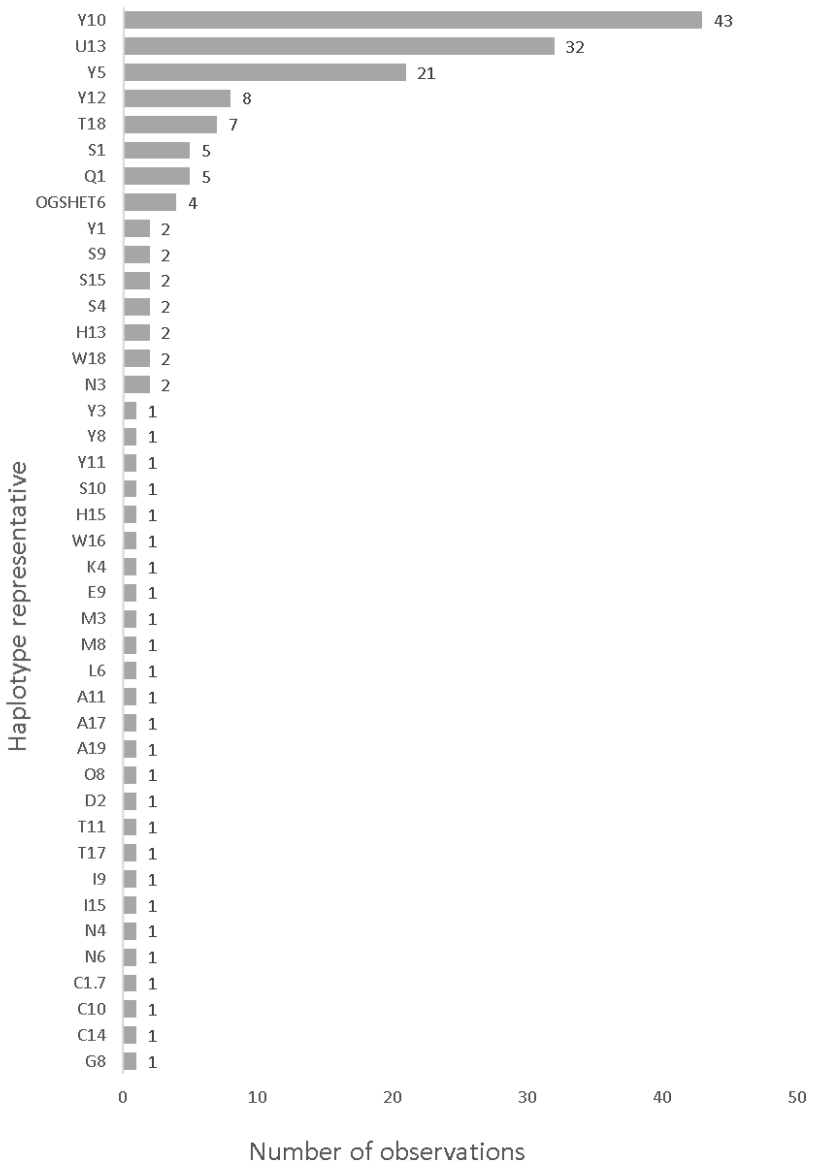


Figure 4.5 Frequency of observations of 41 haplotypes.

Table 4.3 Singly occurring haplotypes. Twenty-eight haplotypes were observed in only one sampling effort, shown here distributed by region and year. Twenty-six of these were altogether unique; the final two were observed twice, but in only one sampling effort (e.g. at one site, on one occasion).

	2013	2014
NWM	7	7
Ork/N	3	0
Shetland	4	7

Table 4.4 Thirteen haplotypes occurring in multiple sampling efforts, with frequency of occurrence given in each cell. These haplotypes were observed in more than one sampling effort, whether at the same site during both years, at multiple sites, or both.

	2013			2014		
Haplotype	**NWM**	**Ork/N**	**Shetland**	**NWM**	**Ork/N**	**Shetland**
Y12	0.00%	6.25%	0.00%	10.00%	18.75%	0.00%
Y10	9.09%	37.5%	37.04%	10%	43.75%	31.25%
Y5	15.15%	6.25%	11.11%	13.33%	18.75%	9.38%
U13	39.39%	37.50%	3.704%	23.33%	18.75%	6.25%
T18	0.00%	0.00%	3.70%	6.67%	0.00%	12.5%
S4	0.00%	0.00%	3.70%	0.00%	0.00%	3.13%
S1	0.00%	0.00%	11.11%	3.33%	0.00%	3.13%
Q1	9.09%	0.00%	0%	3.33%	0.00%	3.13%
OGSHET6	3.03%	0.00%	11.11%	0.00%	0.00%	0.00%
N3	3.03%	0.00%	0.00%	3.33%	0.00%	0.00%

4.4.2 Taxonomy and distribution of haplotypes

Bayesian consensus taxonomy broadly suggests a dominant clade (Figure 4.6, blocked in blue) plus two outliers: one comprising the T11 haplotype alone, and one comprising N6 and C1. Each of these three haplotypes were recorded only once. The dominant clade was represented across all sample regions, and contains two major subclades (A, blocked in red, and B, blocked in green) and five minor subclades (C in purple, D in navy, E in amber, F in grey, G in yellow) are contained therein. Clade B includes Y5, commonly found across all regions; a single subclade comprising T18 and M8 (found mostly in Shetland); S9, found only in Shetland; and H13, C10, and A11, found only on in the northwest mainland. Altogether, 38 observations of Clade B haplotypes took place, 14 of which (37%) were in northwest mainland sites; 8 of which (21%) were in Orkney/North sites, and 16 of which (42%) were in Shetland. In the absence of the most common haplotype (Y5), this would yield 10 of 15 haplotype observations (or 67%) of Clade B observations in Shetland and 5/15 (33%)

observations in the northwest mainland, suggesting a slight skewing of Clade B prevalence in Shetland over the two southern regions surveyed. Haplotype occurrences are illustrated in Figures 4.7-4.10.

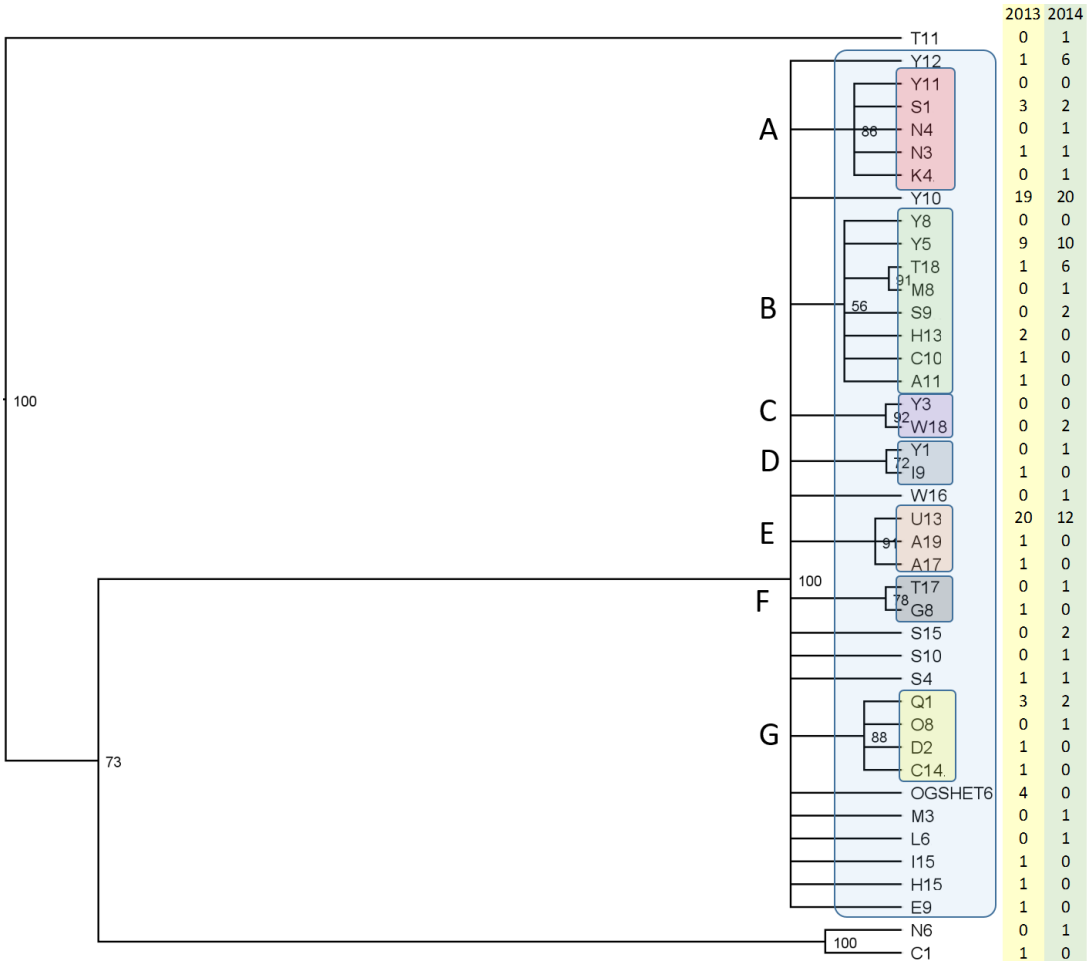


Figure 4.6 Bayesian consensus tree of 41 *Obelia geniculata* mtCOI haplotypes, constructed using 11,000,000 chain length with GTR substitution model and gamma rate variation, 100,000 burn-in rate and 4 heated chains with subsampling frequency of 200. Node annotations reflect percent simulated trees in which clustered samples remain clustered during bootstrap resampling. Coloured columns show the frequency of haplotype occurrence in both years sampled.* Colour overlay indicates the dominant clade, containing two major subclades and 5 minor subclades: A in red, B in green, C in purple, D in navy, E in amber, F in grey, G in yellow.

*Two haplotypes, Y11 and Y3, were found only in Wari Geo, outside the main study regions; their occurrence is not included in year-to-year consideration.

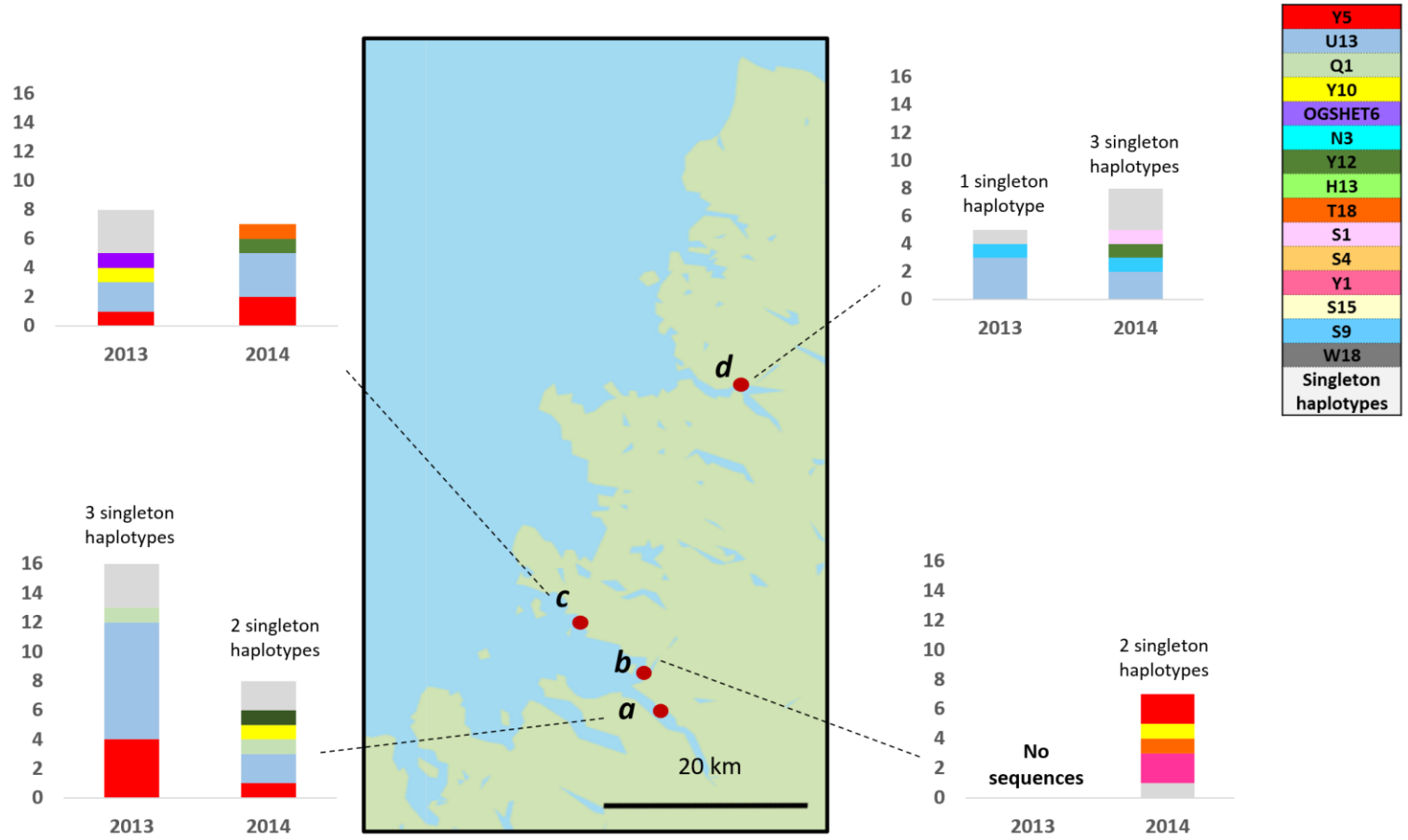


Figure 4.7. Shift in haplotype diversity distribution in Northwest Mainland sites, 2013-2014. Multiply-occurring haplotypes are shown in colour (see legend, top right); singly-occurring haplotypes are pooled and shown in grey. (a) Loch Broom; (b) Polbain; (c) Ardmair; (d) Kylesku. All assemblages successfully sequenced in 2013 were dominated by the U13 haplotype, with this type also appearing dominant in 3 out of 4 sites in 2014.

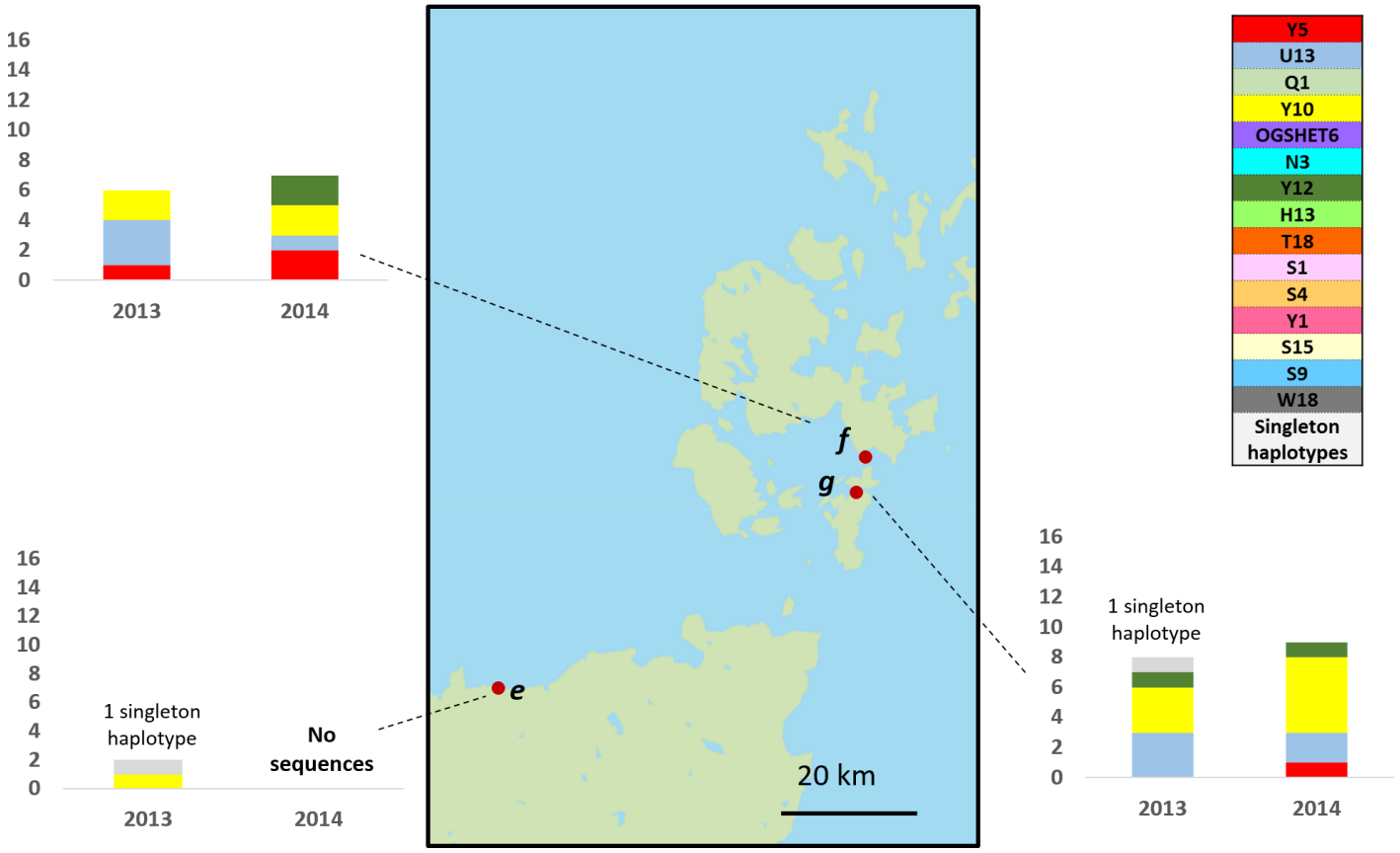


Figure 4.8 Shift in haplotype diversity distribution in Orkney/North sites, 2013-2014. (e) Portskerra; (f) 1st Churchill Barrier, (g) 4th Churchill Barrier. Multiply-occurring haplotypes are shown in colour (see legend, top right); singly-occurring haplotypes are pooled and shown in grey. In both years, the U13 haplotype is still present in Orkney sites, though not Portskerra; Y10 appears with greater frequency than in NWM sites to the south.

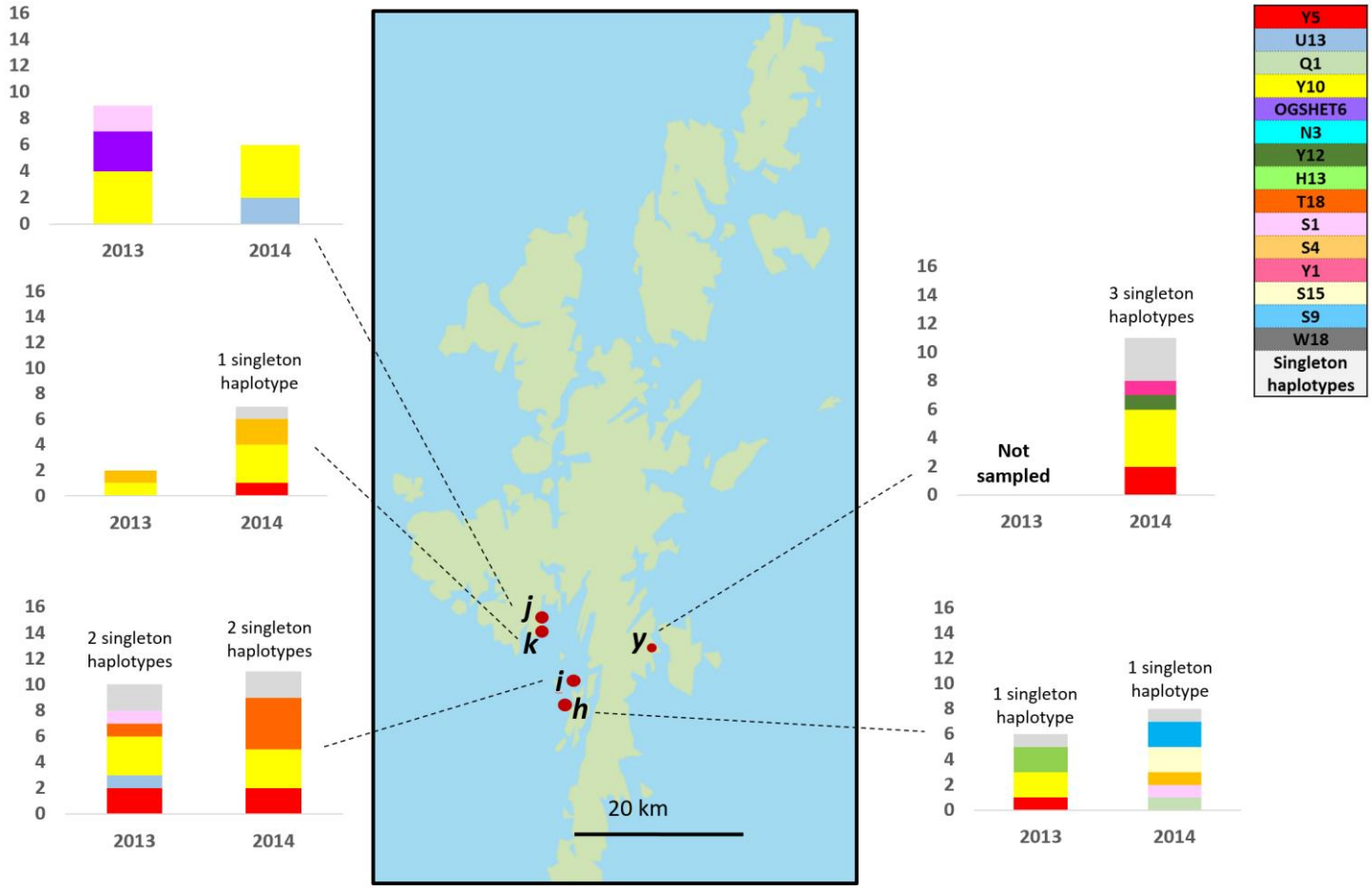


Figure 4.9 Shift in haplotype diversity distribution in Shetland sites, 2013-2014. (h) Setter; (i) Hamnavoe; (j) Redayre; (k) Reawick. Multiply-occurring haplotypes are shown in colour (see legend, top right); singly-occurring haplotypes are pooled and shown in grey. In both years, the Y10 haplotype is found most frequently, while the U13 haplotype common to the NWM is present in much lower numbers.

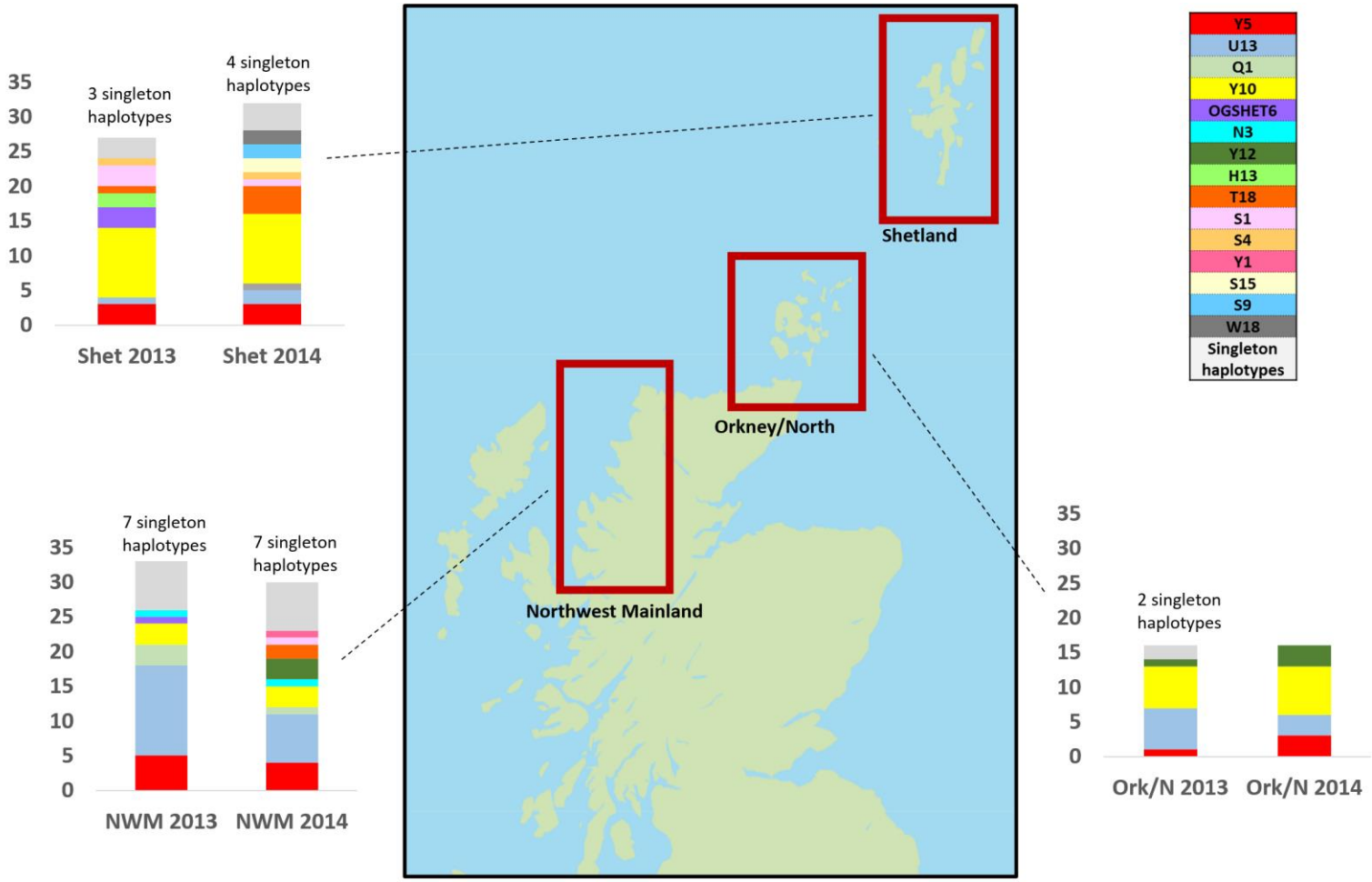


Figure 4.10 Shift in haplotype diversity distribution by region, 2013-2014. Multiply-occurring haplotypes are shown in colour (see legend, top right); singly-occurring haplotypes are pooled and shown in grey.

4.4.3 Haplotype richness

Rarefaction analysis, which is used to estimate true richness from the richness observed in sampling, was applied in order to assess whether sampling captured a representative snapshot and to compare the predicted richness estimates across regions and years. Rarefaction of observed haplotype richness, considered by region-year grouping, shows that this sampling probably did not capture the totality of richness that could be observed with expanded sampling methods such as SCUBA (Figure 4.11, Table 4.5). (Methodological limits on sampling access, especially in the Ork/N region, are detailed in the Appendix to this chapter.) However, comparison of rarefaction curves for the Northwest Mainland and Shetland in 2013 and 2014 show similar patterns of sampled richness; that is, while some richness has probably been missed, any "missing" richness is likely to be similar between these groups, demonstrating that the two can be compared accurately. Meanwhile, a significant increase in estimated richness occurs in all three regions in 2014 (Table 4.6). Chao 2 estimation (Chao 1987) predicts consistently lower true richness in 2013 than in 2014, particularly in NWM and Shet regions. In 2013, they are close to asymptote, with very little increased return of richness per sampling effort predicted; in 2014, predicted richness is much higher, with return of richness per sampling effort continuing to increase. This is consistent with an overall lower haplotypic diversity present in 2013.

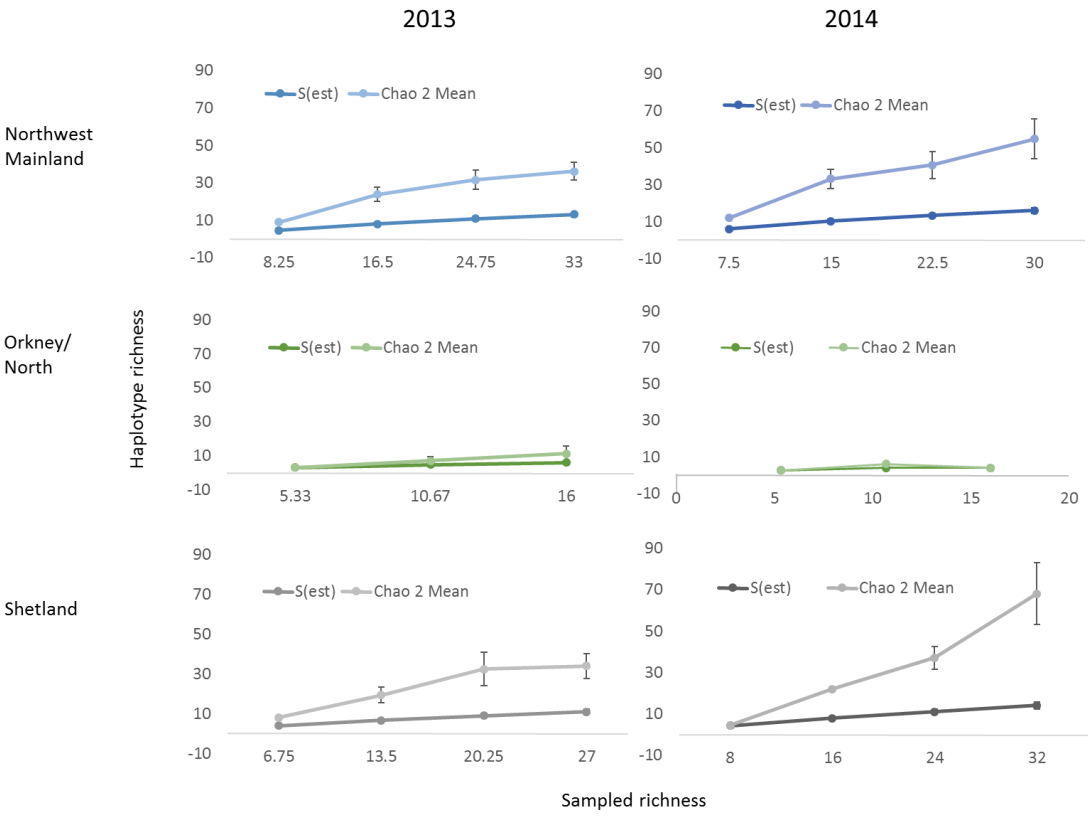


Figure 4.11 Estimated richness based on sampled sites in each region, across 2013 and 2014. S(est), +/e 1 SE, gives the accumulated haplotype richness across sampled individuals observed using the methods described. Chao 2 (Chao 1987), +/- 1 SE, is an incidence-based estimator giving predictions of expected haplotype richness across sampled individuals. Chao 2 estimation in both 2013 at NWM and Shet regions approached asymptote, suggesting that increased sampling would not have increased predicted diversity; this implies an overall lower haplotypic diversity present at that time, particularly as compared to 2014.

Table 4.5 χ^2 comparisons of richness as predicted by Chao 2 estimation vs richness as observed via sampling. Observed richness via our sampling was consistently significantly below the richness as predicted by Chao 2, except in Shet 2013 and NWM 2014.

Year	Region	p	df
2013	NWM	0.044	3
	Ork/N	0.064	2
	Shet	0.356	3
2014	NWM	0.141	3
	Ork/N	<0.0001	2
	Shet	0.010	3

Table 4.6 χ^2 temporal comparisons of observed richness and Chao 2 estimated richness between 2013 and 2014. While observed richness does not show significant year-to-year differences, estimated richness is significantly and consistently greater in 2014 than in 2013 in all regions.

2013:2014	S(est)				Chao2			
NWM : NWM	p =	0.861	df =	3	p =	0.015	df =	3
Ork/N : Ork/N	p =	1.000	df =	2	p =	0.001	df =	2
Shet : Shet	p =	0.582	df =	3	p =	0.000	df =	3

4.4.4 Spatial-temporal analyses

Analysis of molecular variance (AMOVA) showed considerable – and unexpected – overlap between various temporal-spatial populations, but with some significant difference between comparisons. These are highlighted in Table 4.7. It should be noted that not all direct comparisons could be made in both 2013 and 2014, due to failed DNA amplification in all samples from the Portskerra sampling site in 2014. This left the Ork/N grouping without the full statistical requirement of 3 sites for 2014; comparisons have been made by grouping Ork/N + NWM vs Shet, and Ork/N + Shet vs NWM.

The fixation indices from these AMOVA comparisons give several pieces of key information. All F values represent a measure of population differentiation as estimated from nucleotide polymorphisms within the haplotypes observed. F_{CT} gives a regional-level comparison, wherein the degree and statistical significance of regional groupings are estimated. F_{SC} compares all sites within a single region. Finally, F_{ST} makes comparisons of the haplotypes occurring on a site-by-site basis across all regions. A fixation index of 0 indicates panmixis of haplotypes; that is, the two compared populations occupy one gene pool, while a fixation index of 1 indicates a well-defined population structure with completely discrete gene pools.

Table 4.7 AMOVA comparisons between 2013 groupings. Asterisk* indicates significant or near significant ($p < 0.05$) difference in F_{CT} between groups being compared. These were produced in collaboration with April Blakeslee.

All regions 2013			***p***	***df***	***% variation***
Fixation indices	F_{CT} =	0.0309	0.0772*	2	6.47
	F_{SC} =	0.6680	0.1163	8	3.09
	F_{ST} =	0.0956	0.0821	65	90.44
NWM : Ork/N 2013					
Fixation indices	F_{CT} =	-0.0124	0.5523	1	-1.24
	F_{SC} =	0.0830	0.1075	5	8.40
	F_{ST} =	0.0716	0.0990	42	92.84
NWM : Shet 2013					
Fixation indices	F_{CT} =	0.0500	0.0274*	1	5.00
	F_{SC} =	0.0886	0.0987	6	8.42
	F_{ST} =	0.1342	0.0567	52	86.58
Ork/N : Shet 2013					
Fixation indices	F_{CT} =	0.0360	0.2160	1	3.59
	F_{SC} =	-0.0139	0.5415	5	-1.34
	F_{ST} =	0.0255	0.4203	36	97.75
NWM + Ork/N : Shet 2013					
Fixation indices	F_{CT} =	0.0530	0.0059*	1	5.30
	F_{SC} =	0.0609	0.0987	11	5.77
	F_{ST} =	0.1107	0.0860	76	88.93
NWM : Ork/N + Shet 2013					
Fixation indices	F_{CT} =	0.0526	0.0088*	1	5.26
	F_{SC} =	0.0597	0.1114	6	5.66
	F_{ST} =	0.1091	0.0948	65	89.09

Significance values for 2013 show some degree of geographical structuring in the *O. geniculata* population. Analysis of all regions together shows a *p* value just under statistical significance, and significant difference between NWM and Shetland. Ork/N shows comparative similarity to both NWM and Shetland, and its inclusion in analysis with either NWM or Shetland would yield significant results. However, F_{CT} values are

not large enough for any comparison, suggesting that the geographic structuring is not well-defined and has considerable overlap.

Table 4.8 AMOVA comparisons between 2014 groupings.

All regions 2014			***p***	***df***	***% variation***
Fixation indices	**F_{CT} =**	-0.0081	0.7850	2	-0.81
	F_{SC} =	0.0189	0.2800	7	1.9
	F_{ST} =	0.0109	0.2053	68	98.91
NWM + Ork/N : Shet 2014			***p***	***df***	***% variation***
Fixation indices	**F_{CT} =**	-0.0048	0.7116	1	-0.48
	F_{SC} =	0.0158	0.1652	8	1.58
	F_{ST} =	0.0110	0.2072	68	98.90
NWM : Ork/N + Shet 2014					
Fixation indices	**F_{CT} =**	0.0007	0.4956	1	0.07
	F_{SC} =	0.0128	0.2239	8	1.28
	F_{ST} =	0.0136	0.2043	68	98.65
NWM : Shet 2014					
Fixation indices	**F_{CT} =**	-0.0058	0.8524	1	-0.58
	F_{SC} =	0.0133	0.2630	6	1.34
	F_{ST} =	0.0075	0.2688	54	99.25

NWM combined with Ork/N sites in 2014 were not significantly different to Shet sites, nor were NWM sites significantly different to Ork/N combined with Shet sites. Comparison of NWM sites with Shet sites also did not show significant difference, suggesting a lack of geographic structuring in 2014. Fixation indices, meanwhile, show complete mixing of haplotypes.

Table 4.9 AMOVA comparisons between 2013 and 2014.

All sites 2013 : all sites 2014			***p***	***df***	***% variation***
Fixation indices	F_{CT} =	-0.004	0.7371	1	-0.4
	F_{SC} =	0.0281	0.0772	18	2.82
	F_{ST} =	0.0242	0.1202	134	97.58
NWM 2013 : NWM 2014	F_{CT} =	-0.023	0.7488	1	-2.3
Fixation indices	F_{SC} =	0.0316	0.0557	5	3.23
	F_{ST} =	0.0093	0.1417	56	99.07
Ork/N 2013 : all sites 2014			***p***	***df***	***% variation***
Fixation indices	F_{CT} =	0.0066	0.5132	1	0.66
	F_{SC} =	-0.0690	0.7752	3	-6.86
	F_{ST} =	-0.0066	0.8153	27	106.19
Shet 2013 : Shet 2014					
Fixation indices	F_{CT} =	-0.0072	0.9169	1	-0.72
	F_{SC} =	0.0231	0.1633	6	2.32
	F_{ST} =	0.0160	0.1769	51	98.40

Inter-annual comparisons, strangely, also do not show significant difference or large fixation indices, suggesting that the haplotype complement measured in 2013 did not differ from that measured in 2014, on any regional scale. Year-to-year shifts in observed haplotype complement on a site-by-site basis are illustrated in Figures 4.7-4.9; Figure 4.10 shows year-to-year shifts in haplotypes when grouped by region. Many haplotypes found to be common (e.g. U13 at sites in the NWM region) persisted from year to year, while many singly-occurring haplotypes in 2013 did not recur in 2014.

No individual site showed significant year-to-year difference in pairwise F_{ST} comparison, but comparison from site to site revealed the strongest pairwise differences appearing between the most southerly sites and the more northerly sites (Table 4.10, Figure 4.12). This is consistent with F_{CT} comparisons above demonstrating some genetic division with a degree of overlap between regions.

Table 4.10 Pairwise F_{ST} comparisons between all sites. Gradient colour indicates larger comparative differences. Boxed cells indicate statistically significant *p* values ($p < 0.001$).

	A	C	D	E	F	G	H	I	J	K	L	M	N	O	Q	R	S	T	U	W
A	0.00000																			
C	0.08743	0.00000																		
D	0.14543	0.02418	0.00000																	
E	0.34438	-0.24409	-0.05882	0.00000																
F	0.05464	-0.03044	-0.04878	-0.06849	0.00000															
G	0.16636	0.01478	-0.03203	-0.12142	-0.12102	0.00000														
H	0.25008	-0.02524	0.10940	-0.12048	0.04231	0.08583	0.00000													
I	0.19051	0.02412	0.03103	-0.14338	-0.03759	-0.01838	-0.02222	0.00000												
J	0.43039	0.06382	0.13462	-0.08359	0.13634	0.08056	0.14526	0.05909	0.00000											
K	0.34438	-0.25652	-0.05882	-0.50000	-0.06849	-0.12142	-0.12048	-0.14338	-0.08359	0.00000										
L	0.08058	-0.00406	-0.05529	-0.74010	-0.07026	-0.05042	0.04140	-0.02690	0.13439	-0.07401	0.00000									
M	0.22359	-0.01671	0.09064	-0.09789	0.03296	0.04454	-0.03772	-0.07437	0.13844	-0.09789	0.01542	0.00000								
N	-0.03288	-0.02051	0.11444	0.22408	0.02708	0.09854	0.99500	0.07030	0.37038	0.22408	-0.00301	0.05085	0.00000							
O	0.09960	-0.11501	0.00543	-0.26755	-0.03067	-0.00288	-0.02086	0.02189	0.04194	-0.27909	-0.00144	-0.00915	-0.00896	0.00000						
Q	0.16205	-0.01490	0.02285	-0.10321	-0.04871	-0.04604	-0.02143	-0.06759	0.11308	-0.10321	-0.07967	-0.03922	0.01375	-0.02001	0.00000					
R	0.30279	0.04740	0.04282	-0.17011	-0.04335	-0.06416	0.06546	-0.01926	0.00874	-0.17011	0.03368	0.06201	0.22882	0.02945	-0.02367	0.00000				
S	0.19635	0.00270	0.08616	0.05296	0.10984	0.13228	0.06715	0.04839	0.24416	0.00328	0.00952	0.04231	0.07885	0.00325	0.03546	0.19159	0.00000			
T	0.11637	-0.02898	0.04954	-0.22871	-0.00941	0.02187	-0.03932	0.00438	0.05631	-0.22871	0.01498	-0.04902	-0.00933	-0.01653	-0.01666	0.03747	0.01402	0.00000		
U	0.38014	0.02148	0.07216	-0.11864	-0.01250	-0.04025	0.12830	0.04531	0.03271	-0.11864	0.11078	0.14066	0.34230	-0.00237	0.09096	-0.11710	0.28358	0.02140	0.00000	
W	0.30128	0.01060	0.06916	-0.18713	0.03184	0.02638	0.02255	-0.01205	0.05235	-0.18713	0.03694	0.01839	0.18913	0.00052	0.00000	-0.00114	0.10859	0.00231	0.03596	0.00000

Cluster analysis of similarity between sites also shows overlap in regional comparisons, indicating a largely non-discrete gene pool with a few pockets of isolation (Figure 4.13). As shown in earlier AMOVA results (Table 4.7-4.8), 2013 shows a significantly greater degree of geographic demarcation than does 2014.

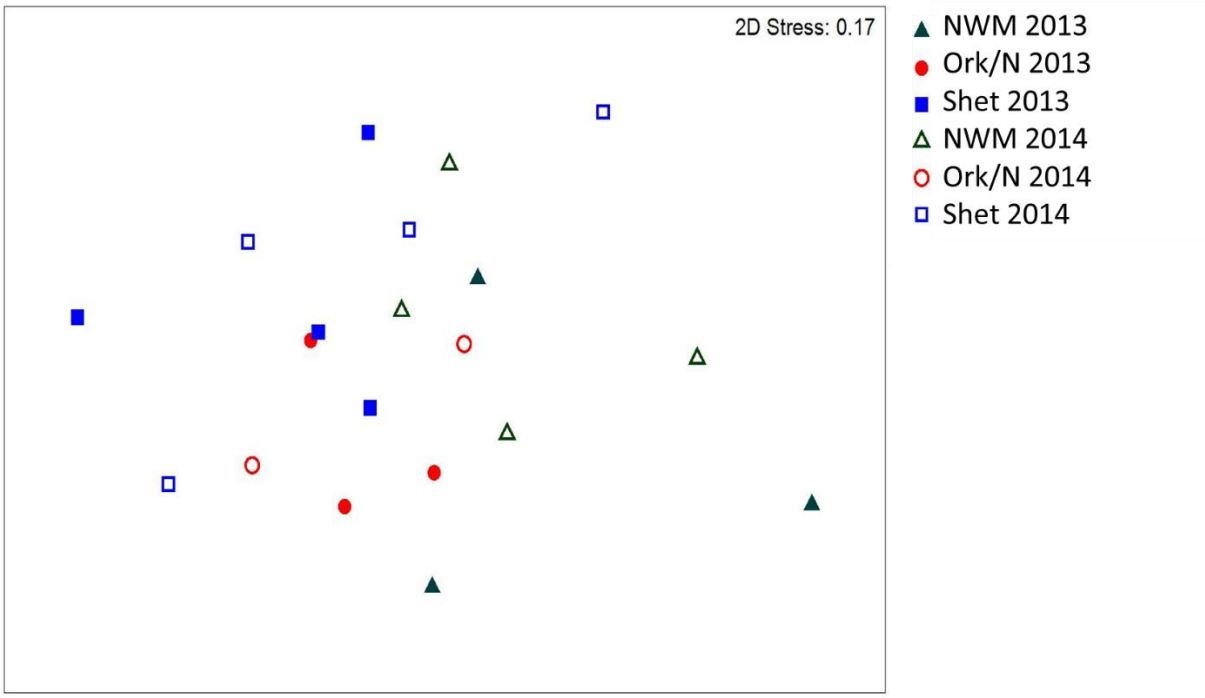


Figure 4.12 Multidimensional scaling plot showing F_{ST} pairwise comparison between sites. Northwest mainland sites show some separation from Shetland sites, but with considerable overlap; Orkney/North sites fall in the middle. This figure was produced by Dr April Blakeslee.

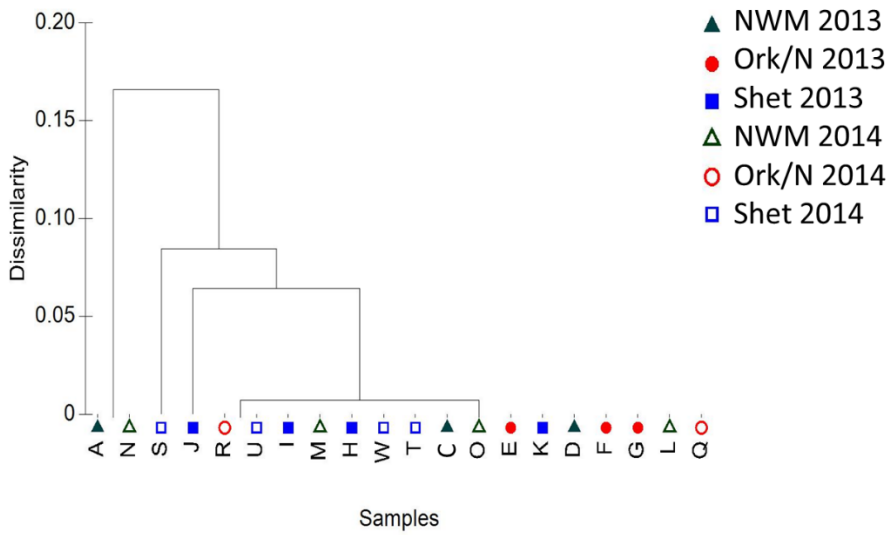


Figure 4.13 Cluster analysis of site similarity. Again, northwest mainland sites show some separation from Shetland sites, but with overlap; Orkney/North sites fall in the middle. This figure was produced by Dr April Blaskeslee.

Analysis of similarity (ANOSIM) across the same regional groupings likewise found close similarity in the majority of comparisons, with the greatest differences observed in comparisons between NWM 2013 and Shetland 2013, and between NWM 2013 and Shetland 2014 (Table 4.11).

Table 4.11 ANOSIM comparisons, ranked by increasing R statistic.

Pairs compared		R statistic	Significance level (%)
NWM 2013	Shet 2013	0.315	5.7
Shet 2013	NWM 2014	0.177	17.1
NWM 2013	Ork/N 2014	0.167	40
Ork/N 2013	Ork/N 2014	0.167	40
NWM 2013	Ork/N 2013	0.148	40
Shet 2013	Ork/N 2014	0.143	46.7
NWM 2014	Ork/N 2014	0.143	40
Ork/N 2013	NWM 2014	0.13	34.3
Ork/N 2013	Shet 2013	0.102	40
NWM 2014	Shet 2014	0.094	31.4
NWM 2013	Shet 2014	0.074	28.6
Shet 2013	Shet 2014	-0.01	48.6
Ork/N 2013	Shet 2014	-0.037	57.1
NWM 2013	NWM 2014	-0.13	71.4
Ork/N 2014	Shet 2014	-0.179	86.7

4.5 Discussion

Our data shows statistical evidence for dieback followed by robust population recovery. In addition, local sites within each region do not show significant difference from one another, suggesting well-mixed regional populations. Moreover, the potential for genotypic mixing across the North Atlantic may be considerably wider than previously assumed.

4.5.1 Evidence for dieback and population recovery

Statistical analyses of phylogeographic population structure amongst nearshore *Obelia geniculata* colonies across the north of Scotland indicates low-grade distinction amongst regional groupings in 2013, wherein the northwest mainland is distinct from Shetland, and Orkney/North sites can be significantly included in either mainland or Shetland sites. Taken together, this suggests a slight northward drift in line with oceanographic predictions, wherein the distance of dispersal along the northern coast of Scotland and the Fair Isle Channel logically represent barriers to complete panmictic dispersal. This is also in keeping with phylogeographic dispersal patterns seen in European marine invertebrates with a similar length of time (5-21 days for the planula) spent in the plankton during larval development (e.g. Jolly et al. 2005, regarding the polychaete *Pectinaria koreni*) and in numerous patterns of invasion shown by non-native benthic species, such as in red algae species *Dasysiphonia japonica* and ascidian species *Corella eumyota* (Collin et al. 2015).

However, significant phylogeographic structuring did not persist into 2014, largely due to the appearance of previously unobserved haplotypes (Table 4.2). Common haplotypes – e.g. U13 in northwest mainland sites, Y5 and U13 in Orkney/North sites, and Y10 in Shetland sites – tended to recur in 2014, but with slightly less proportional contribution to the overall number of individuals sampled (Table 4.2, 4.4). Less-common haplotypes in 2013 – e.g. OGSHET6, N3, and myriad singly-occurring haplotypes – often disappeared altogether in 2014. 2014 saw an influx of novel haplotypes to both northwest mainland (7 haplotypes) and Shetland sites (8 haplotypes). (Limited sampling in Orkney/North sites, as described in the Appendix, makes this region difficult to judge.) Moreover, a significant increase in estimated richness via rarefaction occurred in all three regions (Table 4.6): return in observed richness for sampling effort in 2013 was shown to be approaching asymptote, while 2014 suggested considerable richness went unobserved.

This pattern, wherein a large proportion of common haplotypes is reduced by an addition of novel haplotypes, is a common signature of a genetic bottleneck having taken place. Keller et al. (2001) found a similar pattern after a 95% dieback of song sparrows (*Melospiza melodia*) reduced diversity substantially, with a return to pre-dieback allelic diversity taking place within 2-3 years via low-level immigration. Likewise, Doerner et al. (2005) found a similar resurgence in diversity during rapid population recovery of white-tailed deer (*Odocoileus virginianus*). Spatially, Blakeslee et al. (2008) found this pattern in periwinkle (*Littorina littorea*) invasion of North American regions from Europe. All told, these findings are suggestive of a late 2012 dieback across the *O. geniculata* population which would have reduced rare haplotypes below the sampling threshold and led to a disproportionately large representation of common haplotypes as seen in spring 2013; the subsequent population rebound over the summer of 2013 would have been an opportunity for newcomer haplotypes to colonize substrate vacated by colonies of both common and rare haplotypes, as observed in spring 2014.

This raises the question as to the geographic source of haplotypes observed for the first time in 2014. The prevalence of previously undetected haplotypes in 2014 in both of these regions indicates migration from regions not observed during this study. These sources might include more southerly west coast regions of the UK, the Scottish Western Isles, or simply colonies found more frequently offshore and/or at greater depth than could be sampled here. Sequencing and analysis of *O. geniculata* individuals sampled opportunistically at Western Isles sites in 2015, and greater access to deeper-living samples, may help shed light on the matter.

4.5.2 Potential for geographically broad mixing in the North Atlantic

Interestingly, a combined analysis of the 41 haplotypes observed in this analysis plus 24 haplotypes observed in a previous investigation (Govindarajan et al. 2004a), sampled between 1998 and 2002, suggests a pattern of migration that includes

Western Atlantic regions as well. The previous study found that Pacific hydroids from Japan and New Zealand were phylogeographically distinct both from one another and from North Atlantic samples. However, North Atlantic samples showed phylogenetic overlap. Samples from France, Iceland, New Brunswick, and Massachusetts were not found to be genetically isolated; in fact, several haplotypes showed up at more than one of these locations. When compared to the present dataset, these haplotypes appear embedded amongst many sampled in Scotland (Figure 4.14).

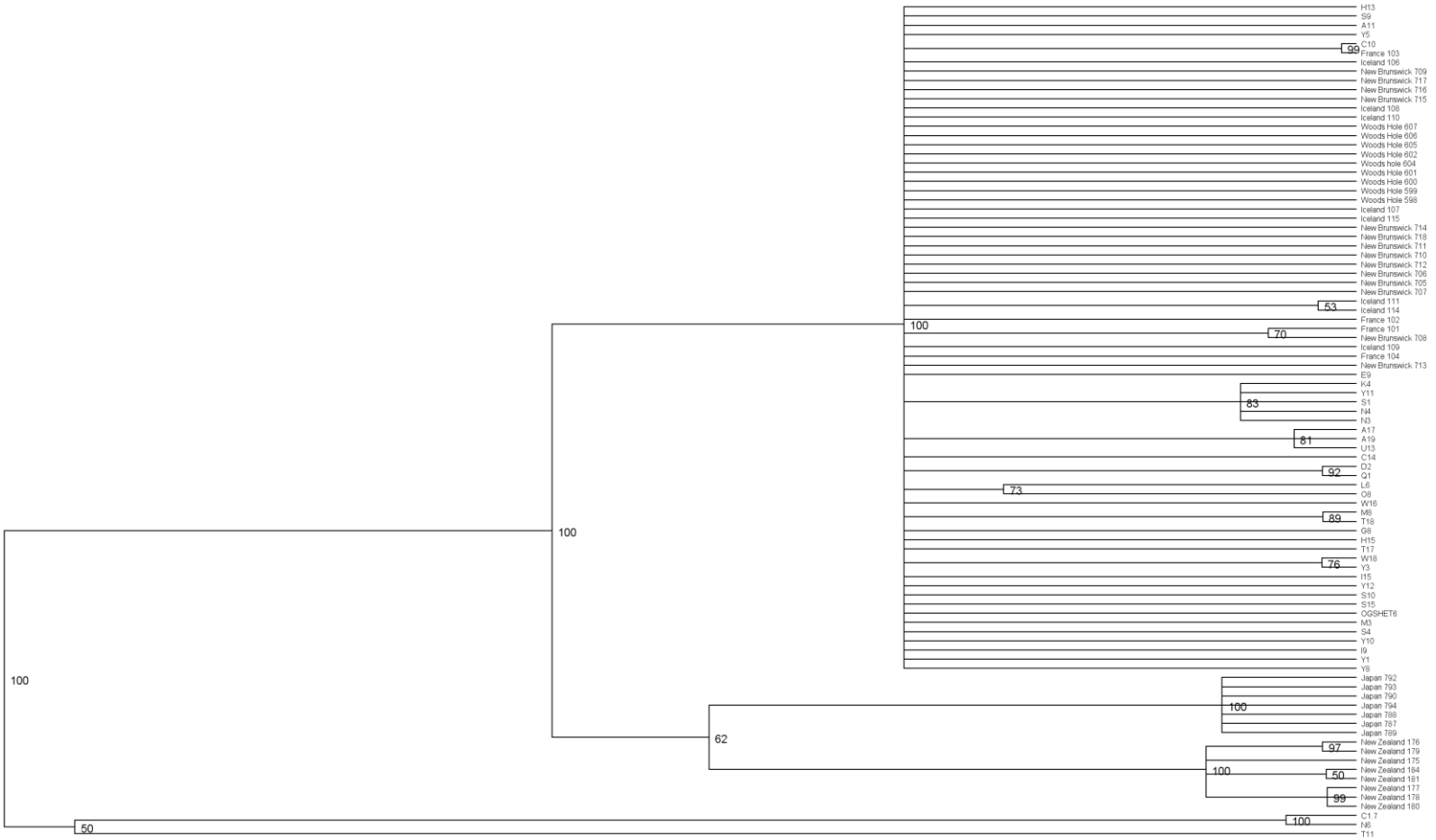


Figure 4.14 Bayesian consensus tree of combined *Obelia geniculata* mtCOI haplotypes from this study and from Govindarajan et al. (2004a), constructed using the run parameters of Figure 4.6. Node annotations reflect percent bootstrap support during resampling.

This phylogeny should not yet be taken as conclusive. First, alignment inference is limited by some lack of overlap: namely, the historical haplotypes cited here are 20 bp longer at the 5' end, and the Scottish haplotypes investigated here are 40 bp longer at the 3' end. As a result, some of the subcladic diversity described in Figure 4.6 is lost. Second, the 2004 study was unable to examine details of North Atlantic population structure, owing to a geographically and numerically limited sampling regime. Only 35 *O. geniculata* individuals were examined from North Atlantic sites, with no sub-regional sampling or temporal analysis; moreover, the small sample size prevented comparison of distributions of common vs. uncommon haplotypes. With present data showing differences between sites and a degree of temporal shift over one year, a combined analysis may be muddied by 11-15 years' opportunities for mixing. Furthermore, a significant geographic demarcation is not at all out of the question: the use of a small sample size artificially inflates the influence of common – and possibly more widely dispersed – haplotypes on the results. More in-depth sampling may well reveal marked trans-Atlantic population structuring by dint of increasing the odds of observing rare haplotypes.

If one assumes this phylogeographic embedding to be permanent, rather than temporally artefactual, this suggests the possibility of much more rapid long-distance dispersal than previously assumed possible. A natural means for pan-Atlantic dispersal might include passage along the North Atlantic drift, with Iceland and Greenland coasts serving as colonial refugia prior to colonization of Canada's Labrador Coast and the northeast United States. If such a migration is indeed taking place, two distinct mechanisms may be at work. First, rafting via broken macroalgae may permit the transport of *O. geniculata*'s colonial adult life stage (e.g. Thiel and Gutow 2005). Second, if rafting or similar transport is taking place at northerly locations, an interesting alternative dispersal strategy by *O. geniculata* may be coming into play – namely, Slobodov and Marfenin (2004) found that colonies in the White Sea dispersed in the plankton not by medusa release and sexual reproduction,

but by asexual frustule propagules. These are pluripotent clonal tissues encased in protective theca which can be carried on currents and settle on distant substrates, and which were the primary dispersal mechanism for a population of *O. geniculata* that rarely saw water temperatures warm enough to facilitate medusa maturation and planula development. Finally, there is the possibility of *O. geniculata* being more rapidly dispersed via anthropogenic means such as ballast water or movement of intact algal biomass. If the observed phylogeographic embedding is found to have persisted over the 15-year timespan described, this possibility should be examined as potentially having implications for facilitating invasions by non-native species, as present biosecurity measures potentially being insufficient to prevent invasions by plankton-dispersed marine invertebrates with physical tolerances similar to *O. geniculata*.

These questions could be addressed by expanded North Atlantic sampling and genetic analysis. As mentioned, a number of Western Isles and Minch samples (specifically from North and South Uist, Skye, and Lochalsh sites) were collected opportunistically in 2015. An additional series of samples were collected in 2014 at Rhode Island, Massachusetts, New Hampshire, and Maine sites. Completed extraction and analysis of these samples would help to resolve the question of whether North Atlantic *O. geniculata* populations are indeed panmictic, or whether this finding is artefactual due to the time elapsed since the sampling of non-Scottish haplotypes.

This study highlights several interesting features of *O. geniculata* population dynamics. The benthic colonial population has been demonstrated to die back, but also shows robust recovery potential. The cause of the initial dieback is unknown, but the fact of the recovery encourages an exploration of the links between this phenomenon and the possibility of its affecting bloom phenomena in the following summer reproductive season. The suggestion of common lineage with other North Atlantic conspecifics also requires further work, in order to shed light on the

mechanisms for population mixing, particularly if these are anthropogenic in nature. Future work utilizing second-generation techniques targeting multi-locus nuclear DNA markers may be particularly effective in examining some of these issues.

4.5.3 Summary findings

North-to-south definition of O. geniculata hydroid populations is present, though weakly defined based on these data; a dieback in 2012 followed by a late 2013 recovery may obscure some population structure via new settlement and recolonization. Sites with sampling regions of Scotland are not substantially different to one another, indicated well-mixed regional populations. When compared to previously-studied *O. geniculata* haplotypes, the data obtained in this study suggest unexpectedly broad North Atlantic mixing, which may have implications in considering marine biosecurity.

4.6 Appendix I: Notes on sampling.

Sampling methods proceeded on the assumption of pairwise year-to-year comparisons between sites. Therefore, initial sampling in 2013 not only collected the necessary *O. geniculata* individuals, it identified sites for comparative sampling in 2014. It is necessary to note that access to sampling sites in 2013 was considerably limited by a number of factors (Table 4.12). First, the project objectives necessitated sampling prior to the summer months – in this case, April 2013. While summer in northern Scotland is no guarantee of favourable conditions, it does provide an advantage of longer days, marginally warmer weather, and a greater likelihood of low wind and calm water. April 2013 conditions were a patchwork of high wind with rain and hail, with bright sunshine and occasional drops in wind to light breezes. Many sites were inaccessible due to wind-driven wave action making surface sampling potentially unsafe. Second, the project methods called for near-surface sampling only, either by snorkelling, wading, or collection from easily-accessible fouling communities. Water temperature at this time varied between 7-9˚C, forcing use of drysuits rather than wetsuits for personal safety. While drysuits ensure sufficient in-water time for a thorough search of a given area without developing hypothermia, their buoyancy prevents any breath-hold duck diving below the surface; with a few minor exceptions, this necessitated that all hydroids collected be within approximately 1.5 m of low tide level. This meant that sampling time was limited to low tides taking place during daylight hours. Finally, sampling was limited by road access. A number of sites which may look promising from the comfort of a paper map are either prohibitively distant from roads, or are below escarpments without straightforward ingress and egress.

Choice of sampling sites was further limited by seemingly arbitrary occurrence of *O. geniculata* colonies. A number of sites with appropriate macroalgal substrate were searched, but found to lack *O. geniculata* entirely. For some, such as Laxford Bay or Bridge of Walls, explanatory hypotheses such as low salinity or heavy sedimentation

were readily available (Table 4.12). For others, such as Handa Sound, Birsay Bay, Yesnaby Geo, 2nd and 3rd Churchill Barriers, etc., there was no obvious reason for the absence of *O. geniculata*. (A thorough study of this phenomenon with access to expanded spatial community sampling methods, especially SCUBA, would be advisable in order to examine the factors driving this phenomenon.) This posed particular constraint on sampling in Orkney, with the result that the study area was expanded to include the northern coast of the Scottish mainland east of Bettyhill. However, this expansion yielded a number of sites suffering the same constraints already discussed, with the exacerbating factor that a considerable weather deterioration took place during sampling. In the end, only Portskerra could be added as a site, and colonization by *O. geniculata* was found to be thin.

The observation of a dieback in Shetland in 2012 is supported by the rarefaction analyses and evidence for genetic bottleneck presented in the body of this report. These same analyses would support a dieback in NWM sites as well, suggesting that some of the difficulties in sampling may be a reflection of this phenomenon being more widespread than previously realised. Future work in sampling or surveying *O. geniculata* may wish to reexamine many of these sites, as they may have been successfully recolonized during the 2013 dispersal season.

Table 4.12 Sites attempted in 2013 with no samples retrieved.

Site	Geographic coordinates	Reason for non-sampling
Northwest Mainland		
Morefield Campground, Ullapool	57.905020, -5.179639	Ample *L. digitata* substrate, but no *O. geniculata* colonization
Reiff	58.070329, -5.450985	No safe entry point
Achnahaird Bay	58.070959, -5.364378	Heavy surf
Inverkirkaig	58.123576, -5.265030	No kelps seen from shore; moderate surf
Lochinver	58.147828, -5.250547	No likely spots with shallow-growing kelp
Baddidarrach	58.151000, -5.252923	No safe entry point
Stoer Bay/Clachtoll	58.188809, -5.336979	Heavy surf
Bay of Culkein	58.243735, -5.339934	Dense colonization by different hydroid species
Achnacarnin	58.226148, -5.306839	Moderate surf; kelp too deep
Culkein of Drumbeg	58.236505, -5.167674	No safe entry point
Badcall	58.321074, -5.139621	
Scourie Pier	58.354207, -5.154867	No hydroids seen, high turbidity
Handa Sound/Tarbet	58.389315, -5.145279	Ample *L. digitata* substrate, but no *O. geniculata* colonization
Laxford Bridge	58.382092, -5.037933	No *O. geniculata* colonization; very peaty water with likely low salinity
Ardmore/Skerricha	58.409671, -5.034244	No shallow kelp growth
Loch Inchard/Kinlochbervie	58.382092, -5.037933	No safe entry point
Orkney/North		
Scrabster	58.613663, -3.540525	Heavy surf, safety question of Dounreay runoff
2nd Churchill Barrier	58.884243, -2.901540	Ample *L. digitata* substrate, but no *O. geniculata* colonization
3rd Churchill Barrier	58.871840, -2.915922	Ample *L. digitata* substrate, but no *O. geniculata* colonization
Burwick, South Ronaldsay ferry terminal	58.739945, -2.972544	Ample *L. digitata* substrate, slightly too deep/no shallow colonization
Yesnaby Geo	59.021806, -3.359331	Ample *L. digitata* substrate, but no *O. geniculata* colonization
Skaill Bay/Skara Brae	59.049322, -3.345701	Heavy surf
Birsay Bay	59.129736, -3.317389	Ample *L. digitata* substrate, but no *O. geniculata* colonization
Shetland		
St Ninian's tombola	59.972466, -1.334191	Moderate surf
Scalloway/NAFC	60.132419, -1.287007	No shallow-growing kelp
Sandness East cove	60.302358, -1.598979	No shallow-growing kelp in safely-accessible areas
Culswick	60.181385, -1.514522	No safe entry point; rough water
Vaila Sound	60.218406, -1.605843	Light colonization by different hydroid species; low salinity
Wats Ness	60.236068, -1.684306	No safe entry point; rough water
Bridge of Walls/Gruting Voe	60.242953, -1.526918	Light colonization by different hydroid species; low salinity
Mu Ness	60.254343, -1.687665	No safe entry point; rough water
Skinhoga	60.305702, -1.600625	Ample *L. digitata* substrate, but no *O. geniculata* colonization
Muckle Roe	60.377346, -1.384541	No hydroids in fouling community; no shallow-growing kelp
Eshaness peninsula	60.478162, -1.622289	No safe entry point; rough water

4.7 Appendix II: Alignment of 41 haplotypes

Figure 4.15 ClustalW alignment of 41 unique haplotypes recovered in 2013 and 2014. An expanded view of this alignment can be viewed in the file "Alignment of all 41 haplotypes.fasta," provided in the Chapter 4 metadata for this thesis.

5

General discussion, future work, and conclusions

Hydrozoan biology is considerably under-researched as compared to scyphozoan biology, with many questions as to their impacts on human interests going unaddressed in mainstream scientific discourse. This thesis aimed to investigate some basic questions in this field, particularly as they pertain to aquaculture. Major findings included:

- Unlike many scyphozoan blooms, many hydrozoan blooms appear to be localized and geographically specific, complicating the prospect of a jellyfish 'early-warning system' as is often proposed for large scyphomedusae. As a result, high resolution spatial-temporal sampling and attendant environmental observation will be necessary in further studies. The local geography of such nearshore environments precludes the use of satellite-based remote-sensing platforms.
- Hydromedusae have the potential to heavily impact the health of caged fish, particularly through gill injury and the attendant sequelae, including the emerging threat of amoebic gill disease. Steps for mitigating this impact should be taken by interested industry members.
- Hydromedusae are also suspected to act as vectors for pathogenic bacteria in an aquacultural setting. Several publications have documented a low-incidence occurrence of *Tenacibaculum maritimum*, the causative agent of tenacibaculosis in salmon gills and in a number of dermal conditions in other farmed fish, in medusozoan species. This study did not find *T. maritimum* in sampling of a number of medusozoan individuals of three separate species. This suggests either that long-term symbiosis between *T. maritimum* and gelatinous zooplankton is rare, or that *T. maritimum* vectoring is more likely to occur after medusae encounter infected fish.
- In spite of its apparently localized bloom development, *Obelia geniculata* (a genus closely associated with detriment to salmon aquaculture) appears to

be genetically well-mixed, with only weak demarcation between regions of colonization in Scotland.

- Genetic evidence was found for a population dieback in *O. geniculata*, followed by recovery indicating a broad dispersal of propagules, over a single reproductive season.

These findings open a number of new hypotheses for future investigations.

5.1 Wider research contributions

Cnidarian biology, as a whole, presently faces a number of research fronts to which this thesis contributes. A key issue investigated worldwide involves prediction, avoidance, and mitigation of many medusozoan species' interactions with human economic interests, particularly in fisheries (e.g. Lynam et al. 2005, Uye 2008), power generation (Masilamoni et al. 2000), and tourism (e.g. Macrokanis et al. 2004). Therefore, the bulk of the work worldwide has rightly focused on large-bodied hydromedusan species, scyphozoans, and cubozoans, with smaller British hydrozoan species going unnoticed as concerns from major ecological and economic standpoints. While my work has focused on these lower-profile taxa, the findings in this thesis can be scaled upward to contribute to broader questions.

5.1.1 Investigative avenues: the benthic and the pelagic

One perennial question in terms of predicting blooms of almost any medusozoan species concerns whether to focus investigation on the benthic stage or the medusa stage. Study of hydroids' medusa production and scyphozoan strobilation can be tempting on the basis of controlled environmental settings enabling experimentation with laboratory-reared individuals or colonies (e.g. Stebbing 1981a and 1981b, Wang and Li 2015, Widmer 2015). Assuming access to adequate immersion equipment,

study of *in situ* polyps in the wild may be similarly straightforward, yielding information as to colony ecology and population health that can be intrinsic in understanding the seasonal dynamics of medusae.

Nonetheless, focusing on only one life stage may miss critical information. An experimental approach can be blind to stochastic or chaotic events which stimulate or accelerate production, and studies of wild populations of polyps may not provide temporal power in predicting a bloom's appearance. An investigative focus on observed species assemblage and density time-series data, particularly in fine scale spatial-temporal resolution, can be simultaneously useful in refining the experimental questions to be asked and in testing the predictive power of experimentally-based bloom hypotheses (e.g. Chapter 2 of this thesis). However, observational data on their own are equally weak in their ability to probe the organism-level physiological responses to bloom stimuli. Historically, synthesis of the two appears to yield the most robust results in terms of predictive power and ecological insight (e.g. Lynam 2004 and Widmer 2015, regarding *Aurelia aurita* North Sea populations). Other recent and ongoing studies have recognized and examined the role of scyphozoan polyp populations in contributing to medusa population loads (e.g. Chae 2015, Wang and Feng 2015), but few have taken the same combined approach in hydrozoan species. This thesis took complementary lines of investigation in order to address bloom questions at both the pelagic and benthic life stages of medusozoan species.

This approach has raised an interesting question in the synthesis of findings in Chapters 2 and 4, regarding evidence pertaining to reproductive ecology in *Obelia*. Investigations in Chapter 2 found large-scale population spikes and blooms in *Obelia* sp. with surprisingly local specificity, suggesting that mediators for blooms apply only over small geographic scales. Meanwhile, the localized production of high-density blooms of medusae would seem to maximize the opportunities for successful gamete fertilization, by increasing the odds of fertile individuals encountering conspecifics.

Therefore, incidence of localized blooming by *Obelia* medusae would seem to predict localized haplotypic diversity, in keeping with other marine invertebrates with similar pelagic dispersal (Hedgecock 1986). As found in Chapter 4, this was not the case for *Obelia geniculata*.

Two phases of life in hydromedusae are spent in the plankton, both of which might facilitate the panmictic haplotype diversity observed in *O. geniculata*. The medusa stage might disperse a considerable distance from its point of origin before undergoing sexual reproduction; later, once a fertilized zygote is produced, the larval planula stage might disperse over long distance. Consideration of both is worthwhile in assessing which is the main driver of a panmictic population.

In laboratory observation, the medusa stage of *O. geniculata* is recorded as having a lifespan of between 7-31 days (Stepanjants 1998), though no mention as to when during this period sexual reproduction took place. In a species with mass spawning, as is suggested by the population fluctuations described in Chapter 2, a notable disadvantage of maximizing the period of time prior to gamete release would be that population density of conspecifics is likely to drop as hydrographically-driven dispersal takes place. That is, the point of greatest likelihood of encountering fertile conspecifics would take place not long after medusa liberation during a population bloom; if the medusae are slow to reproduce, their gametes might not have a fertilization opportunity. It would therefore seem most advantageous that reproduction by *O. geniculata* should take place at the earliest possible point of a medusa bloom. If resettlement of resulting planula larvae were to take place rapidly following development, this should lead to a far greater degree of site-by-site isolation than was observed in the present study.

Bodo and Bouillon (1968) give the time in the plankton for *Obelia* sp. planulae as 5-21 days. Such a distance is comparable to other hydromedusa species' planula dispersal time as reported by Sommer (1992). Without accounting for tidal and nearshore eddies, the Slope Current alone may move propagules in the plankton up

to nearly 14 km/day (Booth and Ellett 1983). Applied to the medusa phase, this becomes a disadvantage in driving conspecific dilution, reducing the likelihood of successful reproduction. Applied to the planula phase, this becomes a dispersal advantage, yielding distance traveled of up to 294 km before settlement. If the assumption of advantageous sexual reproduction during periods of high population density (e.g. early in a bloom) is valid, the 5-21 days spent by the planula in the plankton may be sufficient to facilitate the dispersal patterns observed in these investigations.

[Interestingly, Shanks' (2009) model of dispersal distance suggests that many non-cnidarian species with larval propagule duration of greater than 1 day may be subject to overestimation of dispersal distance based on genetic data, due to the effects of rare individuals which disperse farther and have a smoothing effect on resultant haplotypic datasets. However, this does not wholly explain the panmictic phenomenon described in Chapter 4.]

The question of whether the medusa stage or the planula stage is the major driver of dispersal could best be answered by choosing several likely "source" hydroid colonies at a given location and tracking their offspring. This could be done by identifying their haplotypes – possibly using several mitochondrial genes in addition to mtCOI as well – and then observing the haplotypic assemblages of medusae and planulae at increasing distances. The respective mean distances traveled by medusae and planulae related to the colony of origin could either be statistically similar, or skewed towards either the medusa stage or the planula stage.

A further interesting line of investigation may be the possibility that reproductive seasons following a dieback, such as was recorded in the Scottish Northwest Mainland and Shetland in late 2012 in Chapter 4, could be linked to more robust fecundity as part of a recovery strategy. Unfortunately, sites sampled during this investigation did not overlap geographically with sites sampled during the investigation into blooms detailed in Chapter 2, so no direct correlations between

blooms and benthic colonies can be made at present. However, if bloom-driven dispersal is part of a population-wide strategy after dieback, it may imply risks to aquaculture during some years more than others. Future studies discerning whether this is the case could be undertaken by correlating summertime *Obelia* sp. medusa density with year-round hydroid population density. If it proves to be the case that some seasons can be expected to be "worse" in terms of medusa production, observation of further changes in mtCOI haplotypic diversity over time may be useful in discerning the commonality and causes of such diebacks in order to identify risky years.

5.1.2 Plankton-induced gill pathology in salmon aquaculture

As mentioned previously, medusozoan species have been recognized as having potential to cause morbidity and mortality in caged fish. With the bulk of such studies focusing on visually obvious blooms and high-profile fish kills, the effects of small hydromedusan species and sub-lethal pathologies have been neglected. The temporal studies laid out in this thesis have highlighted the importance of prospective rather than retrospective examination of medusozoan-mediated insults. Consultation with salmon producers in Scotland and Norway has confirmed that many farm sites are plagued by idiopathic gill pathology; the data presented in this thesis strongly suggests that all salmon-producing regions would benefit from expansion of their investigative focus to include medusozoan blooms.

Microbial involvement as mediated by medusozoans is also worth further investigation. This thesis discussed two different means by which medusae may affect this. First, as suggested by Ferguson et al. (2010) and Delannoy et al. (2011) with regards to the notion of medusae potentially introducing non-endogenous *Tenacibaculum maritimum* to a population of caged salmon, direct vectoring by medusae is a potential hazard. Chapter 3 enumerates other potential bacterial pathogens which may be similarly carried by medusae. Second, as suggested in

Chapter 2, the physical and venom-based injuries caused by stings during hydromedusan bloom conditions may permit the establishment and overgrowth of secondary infection by bacteria and such emerging problems as *Neoparamoeba perurans* amoebae. Norwegian workers have recently noticed the emergence of salmon poxvirus and begun to document its endemism in farmed salmon population (Gjessing et al. 2015); its potential exacerbation by exposure to harmful gelatinous zooplankton is also not known. These disease interactions should be worthy of interest in both countries' aquaculture industries.

Interestingly, harmful algal bloom (HAB) studies may benefit from similar investigative approaches. Both HABs and hydromedusae are sampled and examined relatively straightforwardly at aquaculture sites (e.g. Burridge et al. 2010 and present industrial practice at Marine Harvest sites; see also Kent et al. 2995), with the potential for skills transfer to on-site aquaculture staff enabling pro-active treatment of fish. The methods used in this thesis can form a useful basis for this. In addition, both would benefit from similar future research approaches. First, a 'windows of risk' approach would be useful in further developing predictive power. Second, spatially-based risk assessments can help to judge whether sites with particular geographic or oceanographic characteristics are more or less vulnerable to blooms. Third, the combined bulk of data on both medusozoan and harmful algal plankton taken daily at numerous sampling sites across Scotland could provide powerful insight in assessing and refining oceanographic tools such as the newly-published Scottish Shelf Model (Marine Scotland 2016), and eventually using them to produce hindcasts of where blooms have appeared. In turn, this should enable expanded attempts at future predictions. This thesis can form the basis for work proceeding on this front.

5.2 Future work

A number of new study angles are suggested by the information developed in this thesis and its position in the wider context of medusozoan biology and aquacultural applications. Specific lines of investigation are suggested on the basis of the three data chapters: hydromedusan monitoring and mitigation in aquaculture, bacteriology and microbial symbiosis, and phylogeographic connections and their potential insights into dispersal and connectivity.

5.2.1 Next steps in monitoring, modelling, and mitigation

While the above findings shed some light on bloom phenomena at salmon farms, they do not yet provide a fully-developed mitigation solution. A reliable predictive model is not possible given the data resolution in this study, and attempts to produce one will likely require greater detail in time, space, and observed environmental variables than was available here. In particular, the degree of spatial-temporal heterogeneity seen in blooms necessitates that hydromedusan data should be carried out on a local, not geographically broad, scale. It also necessitates that monitoring be carried out daily. Similarly, trophic influences on hydromedusan blooming are best investigated at small spatial-temporal scales.

Early work to this end has begun in 2015. With the threat of hydromedusa blooms having been recognized, Marine Harvest Ltd. initiated daily monitoring at all of its sites in northwest Scotland, generating an archive of hydromedusan samples for the entirety of the 2015 season. Early analysis of these samples has supported the hypothesis of short-lived, spatially discrete blooms that could easily be missed in a weekly study using broad geographic monitoring. Several observed blooms have already been linked temporally to periods of poor gill health in fish. Remaining analysis of this archive is projected to be completed by the end of 2016, hugely increasing both spatial and temporal detail about blooms. While daily records of

hydromedusan populations are of great value in contributing to bloom analyses after the fact, the eventual goal is to rapidly detect blooms before they cause problems and to take steps toward minimizing fish contact with medusae. A protocol is already in place which minimizes contact with certain harmful algal species, wherein a fine-mesh phytoplankton tow sample is obtained in the early morning and examined for a high density of harmful species; if the density is above a set threshold, feeding of the fish is suspended until the density drops. This reduces fish presence at the surface of the pens (when normally, they would spend more time at the surface to feed) and minimizes the amount of harmful algae to which they are exposed. If staff can be trained to carry out basic hydromedusa population density analyses, there is no reason to think these protocols couldn't be adapted similarly to provide some relief during hydromedusan blooms. Other steps such as freshwater baths and temporary suspension of peroxide sea lice treatments are also under discussion for future investigation and use.

Continued daily investigation with aquaculture partners can also contribute to improved understanding of bloom development, particularly in conjunction with local hydrodynamic modelling of the sea lochs in question. Details such as how blooms are laid out spatially (including depth profile) within a single sea loch, and whether certain areas may be exposed to greater bloom magnitudes than others, may help to risk-assess new aquaculture facilities to minimize their medusozoan encounters. Knowledge of dispersal rates and patterns in a given state of currents or eddies may be useful in predicting the duration of risk, and could be applied to modelling of other problem plankton species. Spatial analyses can also apply to the benthic hydroid stage. Two complementary datasets are presently under analysis which examine substrate preference and distribution of hydroids, particularly *Obelia geniculata*, in Shetland, with results expected in summer 2016.

Some rather basic questions on the taxonomy of blooms should also be investigated. At present, the links between *Obelia* sp. medusae in blooms and *Obelia geniculata*

hydroids – or any other *Obelia* species' hydroid stage – is speculative. A genetic confirmation of the species involved in large blooms would be useful in directing experimental investigations into bloom stimuli. Also needed is a complete life cycle study of *Lizzia blondina* in order to identify and investigate its hydroid stage. These findings could be used to experimentally investigate aspects of bloom ecology such as temperature threshold and any other hypothesized physical or biotic stimuli for medusa production. They could also be used to investigate links with benthic ecology, such as whether local population density of hydroid colonies is strongly associated with bloom risks of that species; if this is the case, this may inform risk assessment of aquaculture site decisions.

While an ounce of prevention – in this case minimizing contact between caged salmon and hydromedusan blooms – is worth a pound of cure, it is not likely that avoiding blooms altogether is possible. Therefore, new investigations into bloom aftermath, diagnostic tools, and veterinary interventions should be undertaken. These might include challenge trials, in which a sample group of salmon are exposed to bloom conditions; this will help to discern the threshold bloom density and duration of exposure that can be safely tolerated by salmon. A secondary outcome of a challenge trial might be the development of immunological markers to diagnose blooms. Immunological assays which flag post-exposure markers of blooms may be useful in pinpointing medusozoan exposure as the source of pathologies. This could take the form of examining alterations in protein expression, as per Valdenegro-Vega et al. (2014), or in identifying antibodies raised in direct response to venom or nematocyst exposure.

An intriguing study seeking to disentangle the physiological reactions caused by nematocyst physical trauma versus those caused by venoms might also follow on from a challenge trial. Obviously a trial seeking to be representative of natural blooms would not need to make a distinction between the two, but discerning these differences might be useful in the long term in devising pharmacological

interventions. As noted in this thesis, and by previous authors (Carrette et al. 2002, Kintner et al. 2005, Underwood and Seymour 2007), substantial variability exists amongst the venoms of closely related species, and sometimes even within one species of medusozoan. However, penetrant nematocysts are common to all. An approach which addresses this distinction might proceed as follows:

- Nematocysts might be extracted, lyophilised and stored as per Bloom et al. (1998).
- Density-gradient centrifugation might be used to separate venom-containing penetrant nematocysts from other types of penetrant nematocysts.
- Fish might be exposed to a calibrated density of non-venomous penetrant nematocysts – i.e. those capable of causing trauma, but without envenoming capabilities – while a separate group of fish might receive calibrated doses of venom extracted (as per Jouiaei et al. 2015) from venom-containing nematocyst types. Physiological and immunological outcomes of the two groups could be compared.

Use of this extraction approach could also be used to characterize and compare venoms between species, e.g. between *Obelia* sp. medusae and *Lizzia blondina* medusae, and even between *Obelia* sp. medusae and *O. geniculata* hydroids.

Other clinically relevant future work might include explorations of the links between hydromedusan blooms and other recognized pathologies associated with salmon aquaculture. For example, the study described in Chapter 2 found that amoebic gill disease became prevalent in the wake of a serious bloom. The cause and interactions implied therein are worth investigating. It would be similarly beneficial to better understand the difficulties observed in treating for sea lice after bloom-induced gill pathology. While public demand has imposed something approaching a zero-tolerance policy for sea lice, cost-benefit analysis might favour a more judicious and

strategic use of sea lice treatments during periods of gill damage in order to allow for healing.

Finally, this thesis focused on the health of caged salmon, but the inclusions of Ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*) in salmon pens in order to control sea lice populations is becoming widely implemented. Future work will be required to discern whether medusozoan blooms have any detrimental effect on the health of these species.

5.2.2 Next steps in microbial ecology and aquaculture-relevant vectoring

The question of microbial pathogen vectoring by jellyfish has not been settled by this thesis. The two prominent species previously referenced as potential vectors for *Tenacibaculum maritimum – Phialella quadrata* (Ferguson et al. 2010) and *Pelagia noctiluca* (Delannoy et al. 2011) – were not obtained over this course of study, but I was able to investigate *T. maritimum* presence in *Cyanea capillata* scyphomedusae, *Neoturris pileata* hydromedusae, and *O. geniculata* hydroids, none of which showed *T. maritimum* association. While certainly not conclusive, this does suggest that *T. maritimum* association may be uncommon across the cnidarian taxa. Alternatively, given the findings put forward in Fringuelli et al. (2012), wherein *T. maritimum* association was found almost exclusively in cnidarian individuals sampled in close proximity to salmon sea cages, this may support a hypothesis wherein many different types of jellyfish species represent suitable substrate for *T. maritimum* to colonize, but are only colonized after contact with a sea farm. Spatial comparative study of newly developing blooms upstream and downstream of an affected salmon farm might be useful in clarifying this possibility.

Meanwhile, the presence of other potentially pathogenic bacteria besides *T. maritimum* raises basic questions about their prevalence, ecological roles, and distributions across taxa. Conclusive, final-stage sequencing of the samples archived

during this investigation would be useful in order to pinpoint species and strain of bacteria cultured from *O. geniculata* hydroids, *Cyanea capillata* medusae, and *Neoturris pileata* medusae. This might also enable better comparative statistics on which bacterial species are likely to be found, and where they might pose risks. Suggested techniques for this are laid out in Chapter 3, Table 3.4.

Future microbial community investigation may also wish to employ updated methods for sampling. This study used targeted growth media and conservative visual assessment in the culture stage to seek aquaculture-relevant bacteria, particularly *T. maritimum*. Broader microbiome studies could use TRFLP-based techniques (as per Osborn et al. 2000, Vengatasen 2010) or second-generation deep sequencing techniques (as per Manzari et al. 2014) in order to eschew the necessarily-selective culturing steps altogether, and ensure that "unculturable" species are taken into account (Gram et al. 2010).

Though the possibility of bacterial vectoring of *T. maritimum* by hydrozoan medusae has not been conclusively settled by this thesis, the role played by medusae – and indeed other zooplankton – is worthy of continued investigation, particularly as it pertains to aquacultural applications. A well-considered strategy for mitigating the effects of jellyfish blooms at sea farms would be advised to take into account the possibility of secondary infections mediated by the jellyfish themselves.

5.2.3 Next steps in phylogeography of hydrozoans

As with bacteriology, technological upgrade in methods approach could be applied in further investigations of phylogeography. Use of high-throughput techniques examining genome-level variability, rather than Sanger sequencing of targeted barcode genes such as were used in this study, might be able to answer some fundamental questions. For example, studying a broad array of genes to assess diversity might be useful in matching blooms to colonies on a more local scale than

was possible here. This could enable investigations pinpointing blooms' benthic sources within a single sea loch, assessing whether rates of medusa production (and therefore propensity to bloom) has any genetic basis, and defining the range limits for harmful blooms, which could be useful in devising spatial strategies to avoid large blooms.

Further study of North Atlantic *O. geniculata* populations may also be useful in examining geographic demarcations and the potential for genetic mixing across broad distances. As stated in Chapter 4, samples from Scottish sites in the Hebrides, Skye, Lochalsh, and from United States sites in Connecticut, Rhode Island, Massachusetts, and Maine were collected in 2014 and 2015 and have not yet been analyzed. These may help shed light on the time scale of population mixing, and the involvement of anthropogenic mechanisms for rapid movement of propagules. This work is expected to be undertaken in late spring 2016, in collaboration with colleagues in the United States.

Second-generation molecular techniques for this study are also likely to be useful. Restriction-site associated DNA sequencing (RADseq) may be a good candidate tool for future investigations, as it permits targeting multiple marker genes within pooled sample populations (Davey and Blaxter 2010). This approach would enable more powerful statistical comparisons than are available from single gene haplotyping, while also requiring less bench time given over to multiple PCR amplification stages. This would enable multi-site nuclear DNA comparisons as well, permitting observations of population dynamics on a much smaller spatial scale. For example, a comparison of benthic hydroids with blooming medusae within a given sea loch space would give insights into whether blooms tend to be generated by a single clonal genotype, or whether they are due to a within-loch spatially universal stimulus of all local colony genotypes. A comparison of these results with newly settled colonies would also help to reveal whether the majority of dispersal took place in the planula stage rather than the medusa stage. A second technique option might be whole

transcriptome shotgun sequencing (WTSS or RNA-seq) of the RNA transcriptome, which may be useful in comparing markers that are under selection (such as those associated with blooming) between populations (Wang et al. 2009, Zhong et al. 2011).

5.3 Conclusions

The importance of hydrozoan blooms as agents of harm at salmon farms should become widely recognized, particularly given the importance of aquaculture to the Scottish economy. This research has focused on improving understanding of the risks posed by hydrozoan species by investigating blooms from a number of angles, including: (a) prospective study of hydromedusan populations at salmon farms, which species may be implicated, their correlations with environmental factors, and their clinical effects; (b) examination of microbial symbionts found in close association with several medusozoan species; and (c) examination of *Obelia geniculata* hydroid phylogeny and geographic dispersal. Results suggest that visually cryptic hydromedusan blooms may pose considerable threat to salmon farms, particularly from frequently-blooming taxa such as *Obelia* sp., *Lizzia blondina*, and *Muggiaea atlantica*, with clinical sequelae that include exacerbation of known threats such as amoebic gill disease, sea lice infestation, and microbial pathogens. Additionally, dispersal of *O. geniculata* has been shown to be potentially widespread, raising questions as to whether anthropogenic influences are involved. It is hoped that this research has developed a solid basis for predicting, avoiding, and mitigating threats to industry posed by hydrozoan blooms.

References

Aaen, S. M., Helgesen, K. O., Bakke, M. J., Kaur, K., & Horsberg, T. E. (2015). Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends in Parasitology*, *31*(2), 72–81.

Affymetrix. (n.d.). ExoSAP-IT For PCR Product Cleanup | Affymetrix. http://www.affymetrix.com/catalog/131310/USB/ExoSAP-IT+For+PCR+Product+Cleanup#1_2

Arai, M. (1992). Active and passive factors affecting aggregations of hydromedusae: A review. *Scientia Marina*, *56*(2-3), 99–108.

Avendaño-Herrera, R., Irgang, R., Magariños, B., Romalde, J. L., & Toranzo, A. E. (2006a). Use of microcosms to determine the survival of the fish pathogen *Tenacibaculum maritimum* in seawater. *Environmental Microbiology*, *8*(5), 921–8.

Avendaño-Herrera, R., Magariños, B., López-Romalde, S., Romalde, J. L., & Toranzo, A. E. (2004a). Phenotypic characterization and description of two major O-serotypes in *Tenacibaculum maritimum* strains from marine fishes. *Diseases of Aquatic Organisms*, *58*(1), 1–8.

Avendaño-Herrera, R., Rodríguez, J., Magariños, B., Romalde, J. L., & Toranzo, a. E. (2004b). Intraspecific diversity of the marine fish pathogen *Tenacibaculum maritimum* as determined by randomly amplified polymorphic DNA-PCR. *Journal of Applied Microbiology*, *96*(4), 871–877.

Avendaño-Herrera, R., Toranzo, A. E., & Magariños, B. (2006b). Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: A review. *Diseases of Aquatic Organisms*, *71*(3), 255–266.

Ayre, D. J., & Hughes, T. P. (2000). Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution2*, *54*(5), 590–1605.

Ballard, L., & Myers, A. (2000). Observations on the seasonal occurrence and abundance of gelatinous zooplankton in Lough Hyne, Co. Cork, South-West Ireland. *Biological and Environmental Proceedings of the Royal Irish Academy*, *100*, 75–83.

Båmstedt, U. B., FossÅ, J. H., Martinussen, M. B., & Fosshagen, A. (1998). Short Communication Mass Occurrence of the Physonect Siphonophore. *Sarsia*, *83*, 79–85.

Baxter, E. J., Sturt, M. M., Ruane, N. M., Doyle, T. K., McAllen, R., Harman, L., & Rodger, H. D. (2011). Gill damage to Atlantic salmon (*Salmo salar*) caused by the common jellyfish (*Aurelia aurita*) under experimental challenge. *PLoS ONE*, *6*(4), e18529.

Berrill, N. (1948). A new method of reproduction in *Obelia*. *Biological Bulletin*, *95*, 94–99.

Berntson, E. A., France, S. C., & Mullineaux, L. S. (1999). Phylogenetic relationships within the class Anthozoa (phylum Cnidaria) based on nuclear 18S rDNA sequences. *Molecular Phylogenetics and Evolution*, *13*(2), 417–33.

Blackett, M., Lucas, C., Harmer, R., & Licandro, P. (2015). Popuation ecology of *Muggiaea atlantica* (Cnidaria, Siphonophora) in the Western English Channel. *Marine Ecology Progress Series*, *535*, 129–144.

Blakeslee, A. M. H., Byers, J. E., & Lesser, M. P. (2008). Solving cryptogenic histories using host and parasite molecular genetics: the resolution of *Littorina littorea's* North American origin. *Molecular Ecology*, *17*(16), 3684–96.

Blakeslee, A. M. H., McKenzie, C. H., Darling, J. A., Byers, J. E., Pringle, J. M., & Roman, J. (2010). A hitchhiker's guide to the Maritimes: anthropogenic transport facilitates long-distance dispersal of an invasive marine crab to Newfoundland. *Diversity and Distributions*, *16*(6), 879–891.

Bloom, D. a, Burnett, J. W., & Alderslade, P. (1998). Partial purification of box jellyfish (*Chironex fleckeri*) nematocyst venom isolated at the beachside. *Toxicon : Official Journal of the International Society on Toxinology*, *36*(8), 1075–85.

Bodo, F., & Bouillon, J. (1968). Étude histologique du développement embryonnaire de quelques hydroméduses de Roscoff: *Phialidium hemisphaericum* (L.), *Obelia* sp. Péron et Lesueur, *Sarsia eximia* (Allman), *Podocoryne carnea* (Sars), *Gonionemus vertens* Agassiz. *Cahiers de Biologie Marine*, (1).

Boero, F. (1987). (Cnidaria , Leptomedusae , Phialellidae) from central California, 465–480.

Boero, F., Bouillon, J., Piraino, S., & Schmid, V. (1995). Diversity of hydroidomedusan life cycles: ecological implications and evolutionary patterns. In *Proceedings of the 6th International Conference on Coelenterate Biology* (pp. 53–62).

Booth, D. A., & Ellett, D. J. (1983). The Scottish continental slope current. *Continental Shelf Research*, *2*(2-3), 127–146.

Brinckman-Voss, A. (1985). Hydroids and medusae of *Sarsia apicula* (Murbach and Shearer, 1902) and *Sarsia princeps* (Haeckel, 1879) from British Columbia and Puget Sound with an evaluation of their systematic characters. *Canadian Journal of Zoology*, *63*, 673–681.

Bruce, J., Colman, J., & Jones, N. (1963). *Marine fauna of the Isle of Man*. Liverpool: Liverpool University Press.

Bruno, D. W. (1996). Cold Water Vibrioisis caused by *Vibrio salmonicida*. *Aquaculture Information Series*.15, 1-8.

Bruno, D. W., & Ellis, A. E. (1985). Mortalities in farmed Atlantic salmon associated with the jellyfish *Phialella quadrata* [jellyfish toxin, *Cyanea capillata*]. *Bulletin of the European Association of Fish Pathologists*, *5*(3), 64–65.

Burridge, L. E., Martin, J. L., Lyons, M. C., & LeGresley, M. M. (2010). Lethality of microalgae to farmed Atlantic salmon (*Salmo salar*). *Aquaculture*, *308*(3-4), 101–105.

Caporaso, J. G., Lauber, C. L., Walters, W., Berg-Lyons, D., Huntley, J., Fierer, N., Owwns, S.N., Baur, M., Gormley, N., Gilbert, J., Smith, G., Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, *6*(8), 1621–1624.

Carrette, T., Alderslade, P., & Seymour, J. (2002). Nematocyst ratio and prey in two Australian cubomedusans, *Chironex fleckeri* and *Chiropsalmus* sp. *Toxicon*, *40*(11), 1547–1551.

Carrette, T., & Seymour, J. (2004). A rapid and repeatable method for venom extraction from cubozoan nematocysts. *Toxicon : Official Journal of the International Society on Toxinology*, *44*(2), 135–139.

Cepeda, C., Garcia-Marquez, S., & Santos, Y. (2003). Detection of *Flexibacter maritimus* in fish tissue using nested PCR amplification. *Journal of Fish Diseases*, *26*(2), 65–70.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics*, *10*, 421.

Chae, J. (2015). To what extent a polyp population of *Aurelia* sp. 1 contributes to jellyfish blooms? In *China-Korea Joint Workshop on Jellyfish Research* (pp. 10–11).

Chao, A. (1987). Estimating the population size for capture-recapture data with unequal catchability. *Biometrics*, *43*(4), 783–91.

Clarridge, J. E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews*, *17*(4), 840–62.

Coestello, J. H., & Mathieu, H. W. (1995). Seasonal abundance of medusae in Eel Pond, Massachusetts, USA during 1990-1991. *Journal of Plankton Research*, *17*(1), 1204–1999.

Collin, S. B., Tweddle, J. F., & Shucksmith, R. J. (2015). Rapid assessment of marine non-native species in the Shetland Islands, Scotland. *Bioinvasions Records*, *4*(3), 147–155.

Colwell, R. K., Mao, C. X., & Chang, J. (2004). Interpolating, extrapolating and comparing incidence-based species accumulation curves. *Ecology*, *85*(10), 2717–2727.

Condon, R. H., Steinberg, D. K., del Giorgio, P. A., Bouvier, T. C., Bronk, D. A., Graham, W. M., & Ducklow, H. W. (2011). Jellyfish blooms result in a major microbial respiratory sink of carbon in marine systems. *Proceedings of the National Academy of Sciences*, *108*(25), 10225–10230.

Condon, R. H., Duarte, C. M., Pitt, K. A., Robinson, K. L., Lucas, C. H., Sutherland, K. R., … Graham, W. M. (2013). Recurrent jellyfish blooms are a consequence of global oscillations. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(3), 1000–5.

Conway, D. V. P. (2012). *Marine zooplankton of southern Britain part 1: Radiolaria, Heliozoa, Foraminifera, Ciliophora, Cnidaria, Ctenophora, Platyhelmonthes, Nemertea, Rotifera and Mollusca.* (A. John, Ed.). Marine Biological Association of the United Kingdom.

Cornelius, P. (1992). The Azores hydroid fauna and its origin, with discussion of rafting and medusa suppression. *Arquipelago Ciencias Da Natureza*, *10*, 75–99.

Cornelius, P. F., Barnes, R. S. K., & Crothers, J. H. (1995). *North-west European Thecate Hydroids and Their Medusae (Synopses of the British Fauna) Paul F.S. Cornelius, Richard. S. K. Barnes, J.H. Crothers: 9781851532551: Books*. Field Studies Council.

Cornelius, P. F. S. (1990). Evolution of leptolid life-cycles (Cnidaria: Hydroida). *Journal of Natural History*, *24*, 579–594.

Costello, J. (1988). Laboratory culture and feeding of the hydromedusa *Cladonema californicum* (Anthomedusa: Cladonemidae). *Journal of Experimental Marine Biology and Ecology*, *123*, 177–188.

Cox, D. (2013a). *Histology Report FishVet Group (c): Marine Harvest, Portnalong*. Inverness, Scotland.

Cox, D. (2013b). *Histology report, FishVet Group (a): Marine Harvest, Portnalong*. Inverness, Scotland.

Cox, D. (2013c). *Histology Report, FishVet Group (b): Marine Harvest (Portnalong)*. Inverness, Scotland.

Cox, D. (2013d). *Histology Report, FishVet Group (d): Marine Harvest, Portnalong*. Inverness, Scotland.

Creese, R. G., & Ballantine, W. J. (1983). An assessment of breeding in the intertidal limpet, *Cellana radians* (Gmeline). *Journal of Experimental Marine Biology and Ecology*, *67*, 43–59.

da Mota, F. F., Gomes, E. A., Paiva, E., Rosado, A. S., & Seldin, L. (2004). Use of rpoB gene analysis for identification of nitrogen-fixing *Paenibacillus* species as an alternative to the 16S rRNA gene. *Letters in Applied Microbiology*, *39*(1), 34–40.

Davey, J. W., Davey, J. L., Blaxter, M. L., & Blaxter, M. W. (2010). RADSeq: next-generation population genetics. *Briefings in Functional Genomics*, *9*(5-6), 416–23.

Dawson, M. N., Cieciel, K., Decker, M. B., Hays, G. C., Lucas, C. H., & Pitt, K. A. (2014). Population-level perspectives on global change: genetic and demographic analyses indicate various scales, timing, and causes of scyphozoan jellyfish blooms. *Biological Invasions*, *17*(3), 851–867.

Dayton, P. (1975). Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecological Monographs*, *45*(2), 137–159.

Delannoy, C. M. J., Houghton, J. D. R., Fleming, N. E. C., & Ferguson, H. W. (2011). Mauve Stingers (*Pelagia noctiluca*) as carriers of the bacterial fish pathogen *Tenacibaculum maritimum*. *Aquaculture*, *311*(1-4), 255–257.

Doerner, K., Braden, W., Cork, J., Cunningham, T., Rice, A., Furman, B. J., & McElroy, D. (2005). Population genetics of resurgence: white-tailed deer in Kentucky. *Journal of Wildlife Management*, *69*(1), 345–355.

Dorsch, M., Ashbolt, N. J., Cox, P. T., & Goodman, A. E. (1994). Rapid identification of *Aeromonas* species using 16S rDNA targeted oligonucleotide primers: a molecular approach based on screening of environmental isolates. *The Journal of Applied Bacteriology*, *77*(6), 722–6.

Doyle, T. K., De Haas, H., Cotton, D., Dorschel, B., Cummins, V., Houghton, J. D. R., Davenbort, J., Hays, G. C. (2008). Widespread occurrence of the jellyfish *Pelagia noctiluca* in Irish coastal and shelf waters. *Journal of Plankton Research*, *30*(8), 963–968.

Edwards, A., & Sharples, F. (1986). *Sea lochs: a catalogue*. Scottish Marine Biological Association/Nature Conservancy Council. 1-250.

Edwards, C. (1978). The hydroids and medusae *Sarsia occulta* sp. nov., *Sarsia tubulosa* and *Sarsia loveni*. *Journal of the Marine Biological Association of the United Kingtom*, *58*, 291–311.

Edwards, C. (1983). The hydroids and medusa *Sarsia piriforma* sp. nov. and *Sarsia striata* nov. from the west coast of Scotland, with observations on other species. *Journal of the Marine Biological Association of the United Kingtom*, *63*, 49–60.

Ellis, A. E., Hastings, T. S., & Munro, A. L. S. (1981). The role of *Aeromonas salmonicida* extracellular products in the pathology of furunculosis. *Journal of Fish Diseases*, *4*(1), 41–51.

Ellyat, C. (2015). *Dissertation: environmental factors influencing abundance and reproduction of the common marine hydroid* Dynamena pumila. University of St Andrews.

Elmhirst, R. (1925). Lunar periodicity in Obelia. *Nature*, *116*, 358–359.

EstimateS: Biodiversity Estimation. (n.d.). Retrieved February 9, 2016, from http://viceroy.eeb.uconn.edu/estimates/

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suit ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567.

Ferguson, H. W., Delannoy, C. M. J., Hay, S., Nicolson, J., Sutherland, D., & Crumlish, M. (2010). Jellyfish as vectors of bacterial disease for farmed salmon (*Salmo salar*). *Journal of Veterinary Diagnostic Investigation : Official*

Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, *22*, 376–382.

Flight, P. A., O'Brien, M. A., Schmidt, P. S., & Rand, D. M. (2011). Genetic structure and the North American postglacial expansion of the barnacle, *Semibalanus balanoides*. *The Journal of Heredity*, *103*(2), 153–65.

Flynn, B., Richardson, A., Brierley, A., Boyer, D., Axelsen, B., Scott, L., Moroff, N.E., Kainge, P.I., Tjizoo, B.M., Gibbons, M. (2012). Temporal and spatial patterns in the abundance of jellyfish in the northern Benguela upwelling ecosystem and their link to thwarted pelagic fishery recovery. *African Journal of Marine Science*, *34*(1), 131–146.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*(5), 294–9.

Fringuelli, E., Savage, P. D., Gordon, A., Baxter, E. J., Rodger, H. D., & Graham, D. A. (2012). Development of a quantitative real-time PCR for the detection of *Tenacibaculum maritimum* and its application to field samples. *Journal of Fish Diseases*, *35*(8), 579–90.

FMM broth Conda Labs. (n.d.). http://www.condalab.com/pdf/1264.pdf

Genzano, G., Mianzan, H., Díaz-Briz, L., & Rodriguez, C. (2008). On the occurrence of *Obelia* medusa blooms and empirical evidence of unusual massive accumulations of *Obelia* and *Amphisbetia* hydroids on the Argentina shoreline. *Latin American Journal of Aquatic Research*, *36*(2), 301–307.

Gili, J., & Hughes, R. (1995). The ecology of marine benthic hydroids. *Oceanography and Marine Biology: An Annual Review*, *33*, 351–426.

Gili, J.-M., Orejas, C., & Robson, E. (2008). Hydrozoan biology: the view from a workshop. *Journal of the Marine Biological Association of the United Kingdom*, *88*(08), 1515.

Gjessing, M. C., Yutin, N., Tengs, T., Senkevich, T., Koonin, E., Rønning, H. P., Ylving, S., Lie, K., Saure, B., Tran, L., Moss, B., Dale, O. B. (2015). Salmon Gill Poxvirus, the Deepest Representative of the Chordopoxvirinae. *Journal of Virology*, *89*(18), 9348–67.

Govindarajan, A. F., Boero, F., & Halanych, K. M. (2004b). *Obelia longissima* voucher 235NZ cytochrome oxidase subunit I (COI) gen - Nucleotide - NCBI.

Govindarajan, A. F., Halanych, K. M., & Cunningham, C. W. (2004a). Mitochondrial evolution and phylogeography in the hydrozoan *Obelia geniculata* (Cnidaria). *Marine Biology*, *146*(2), 213–222.

Govindarajan, A. F., & Waterbury, J. (2005). PhD Thesis: Life Cycle Evolution and Systematics of a Campanulariid. *Archives*, Massachusetts Institute of Technology.

Goy, J. (1973). *Gonionemus suvaensis*: structural characters, developmental stages and ecology. *Publications of the Seto Marine Biological Laboratory*, *20*, 525–536.

Graf, J. (University of C. (2015). *Aeromonas*. (J. Graf, Ed.)*Caister Academic Press* (First Edit). Hartford, CT: Caister Academic Press.

Gram, L., Melchiorsen, J., & Bruhn, J. B. (2010). Antibacterial Activity of Marine Culturable Bacteria Collected from a Global Sampling of Ocean Surface Waters and Surface Swabs of Marine Organisms. *Marine Biotechnology*, *12*(4), 439–451.

Gravier-Bonnet, N. (1987). Nematocysts as taxonomic discriminators in thecate hydroids. *Modern Trends in the Systematics, Ecology and Evolution of Hydroids and Hydromedusae*.

Greve, W., & Parsons, T. (1977). Photosynthesis and fish production: hypothetical effects of climatic change and pollution. *Helgol Wiss Meeresunters*, *30*, 666–672.

Guasp, C., Moore, E., Lalucat, J., & Bennasar, A. (2000). Utility of internally transcribed 16S--23S rDNA spacer regions for the definition of *Pseudomonas stutzeri* genomovars and other *Pseudomonas* species. *International Journal of Systematic and Evolutionary Microbiology*, *50*(4), 1629–1639.

Hedgecock, D. (1986). Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bulletin of Marine Science*, *39*(2), 550–564.

Hessinger, D., & Lenhoff, H. (1988). *The Biology of Nematocysts*. San Diego: Academic Press Inc.

Hibbing, M. E., Fuqua, C., Parsek, M. R., & Peterson, S. B. (2010). Bacterial competition: surviving and thriving in the microbial jungle. *Nature Reviews. Microbiology*, *8*(1), 15–25.

Hoffman, J. I., Peck, L. S., Linse, K., & Clarke, A. (2010). Strong population genetic structure in a broadcast-spawning Antarctic marine invertebrate. *The Journal of Heredity*, *102*(1), 55–66.

Holmström, C., & Kjelleberg, S. (1999). Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. *FEMS Microbiology Ecology*, *30*(4), 285–293.

Hosia, A., & Båmstedt, U. (2007). Seasonal changes in the gelatinous zooplankton community and hydromedusa abundances in Korsfjord and Fanafjord, western Norway. *Marine Ecology Progress Series*, *351*, 113–127.

Huelsenbeck, J., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics Applications Note*, *17*(8), 754–755.

Inbakandan, D., Murthy, P. S., Venkatesan, R., & Khan, S. A. (2010). 16S rDNA sequence analysis of culturable marine biofilm forming bacteria from a ship's hull. *Biofouling*, *26*(8), 893–9.

Jan-Roblero, J., Magos, X., Fernández, L., Hernández-Rodríguez, C., & Le Borgne, S. (2004). Phylogenetic analysis of bacterial populations in waters of the former Texcoco Lake, Mexico. *Canadian Journal of Microbiology*, *50*(12), 1049–1059.

Jolly, M. T., Jollivet, D., Gentil, F., Thiébaut, E., & Viard, F. (2005). Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the North coast of France. *Heredity*, *94*(1), 23–32.

Jouiaei, M., Casewell, N. R., Yanagihara, A. A., Nouwens, A., Cribb, B. W., Whitehead, D., … Fry, B. G. (2015a). Firing the sting: chemically induced discharge of cnidae reveals novel proteins and peptides from box jellyfish (*Chironex fleckeri*) venom. *Toxins*, *7*(3), 936–50.

Kawamura, M., & Kubota, S. (2008). Influences of temperature and salinity on asexual budding by hydromedusa *Proboscidactyla ornata* (Cnidaria: Hydrozoa: Proboscidactylidae). *Journal of the Marine Biological Association of the United Kingdom*, *88*(08), 1601.

Kayal, E., Roure, B., Philippe, H., Collins, A. G., & Lavrov, D. V. (2013). Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology*, *13*(1), 5.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647–1649.

Kenchington, E. L., Patwary, M. U., Zouros, E., & Bird, C. J. (2006). Genetic differentiation in relation to marine landscape in a broadcast-spawning bivalve mollusc (*Placopecten magellanicus*). *Molecular Ecology*, *15*(7), 1781–96.

Keller, L. F., Jeffery, K. J., Arcese, P., Beaumont, M. A., Hochachka, W. M., Smith, J. N., & Bruford, M. W. (2001). Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. *Proceedings. Biological Sciences/The Royal Society*, *268*(1474), 1387–94.

Kent, M. L., Whytel, J. N. C., & Latrace, C. (1995). Gill lesions and mortality in seawater pen-reared Atlantic salmon *Salmo salar* associated with a dense bloom of *Skeletonema costatum* and *Thalassiosira* species. *Diseases of Aquatic Organisms*, *22*, 77–81.

Ki, J.-S., Zhang, W., & Qian, P.-Y. (2009). Discovery of marine *Bacillus* species by 16S rRNA and rpoB comparisons and their usefulness for species identification. *Journal of Microbiological Methods*, *77*(1), 48–57.

Kintner, A. H., Seymour, J. E., & Edwards, S. L. (2005). Variation in lethality and effects of two Australian chirodropid jellyfish venoms in fish. *Toxicon : Official Journal of the International Society on Toxinology*, *46*(6), 699–708.

Krishnamurthi, S., Ruckmani, A., Pukall, R., & Chakrabarti, T. (2010). Psychrobacillus gen. nov. and proposal for reclassification of *Bacillus insolitus* Larkin & Stokes, 1967, *B. psychrotolerans* Abd-El Rahman et al., 2002 and *B. psychrodurans Abd*-El Rahman et al., 2002 as *Psychrobacillus insolitus* comb. nov., *Psychrobacillus*. *Systematic and Applied Microbiology*, *33*(7), 367–73.

Kubota, S. (2008). Constant timing of medusa release in bivalve-inhabiting hydrozoans of the genus *Eugymnanthea* (Hydrozoa: Leptomedusae: Eirenidae). *Journal of the Marine Biological Association of the United Kingdom*, *88*(08), 1607.

Labella, A., Berbel, C., Manchado, M., Castro, D., & Borrego, J. (2011). *Photobacterium damselae* subspp. *damselae*, an emerging pathogen affecting new cultured marine fish species in southern Spain. In D. Faruk, Aral, Zafer (Ed.), *Recent Advances in Fish Farms* (pp. 135–152). New York, NY: InTech.

Laman, T. (2011). Photograph: crab carrying an upside down jellyfish in the Philippines. National Geographic.

Larkin, M., Blackshields, G., Brown, N., Chenna, R., McGettigan, P., McWilliam, G., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thomnson, J.D., Gibson, T.J., Higgins, D. (2007). ClustalW and ClustalX version 2. *Bioinformatics*, *23*(21), 2947–2948.

Larsen, A. M., Tao, Z., & Arias, C. R. (2010). Use of Ribosomal Spacer Analysis (RISA) to study bacterial communities associated to marine fish. In *Vibrios in the Environment*, conference proceedings 2010.

Lee, L. E. J., Dayeh, V. R., Schirmer, K., & Bols, N. C. (2009). Applications and potential uses of fish gill cell lines: examples with RTgill-W1. *In Vitro Cellular and Developmental Biology - Animal*, 1–8.

Leitz, T., & Wagner, T. (1993). The marine bacterium *Alteromonas espejiana* induces metamorphosis of the hydroid *Hydractinia echinata*. *Marine Biology*, *115*, 173.

Leitz, T. (1997). Induction of settlement and metamorphosis of Cnidarian larvae: signals and signal transduction. *Invertebrate Reproductive Development*, *109*.

Ludwig, W., & Schleifer, K. H. (1994). Bacterial phylogeny based on 16S and 23S rRNA sequence analysis. *FEMS Microbiology Reviews*, *15*(2-3), 155–73.

Lynam, C. P. (2006). Thesis: Ecological and Acoustic Investigations of Jellyfish (Scyphozoa and Hydrozoa), University of St Andrews. 256 pages.

Lynam, C. P., Brierley, A. S., & Axelsen, B. E. (2004). *Pinging down the food web : acoustic discrimination of jellyfish and fish*. *ICES CM Documents*, pp. 1-21.

Lynam, C. P., Heath, M. R., Hay, S. J., & Brierley, A. S. (2005). Evidence for impacts by jellyfish on North Sea herring recruitment. *Marine Ecology Progress Series*, *298*, 157–167.

Ma, X., & Purcell, J. E. (2005). Temperature, salinity, and prey effects on polyp versus medusa bud production by the invasive hydrozoan *Moerisia lyonsi*. *Marine Biology*, *147*(1), 225–234.

Macrokanis, C., Hall, N., & Mein, J. (2004). Irukandji syndrome in northern Western Australia: an emerging health problem. *Medical Journal of Australia*, *181*, 699–702.

Manzari, C., Fosso, B., Marzano, M., Annese, A., Caprioli, R., D'Erchia, A. M., … Pesole, G. (2014). The influence of invasive jellyfish blooms on the aquatic microbiome in a coastal lagoon (Varano, SE Italy) detected by an Illumina-based deep sequencing strategy. *Biological Invasions*, *17*(3), 923–940.

Mariottini, G. L. (2008). The Mauve Stinger *Pelagia noctiluca* (Forsskål, 1775). Distribution, Ecology, Toxicity and Epidemiology of Stings. A Review. *Marine Drugs*, *6*(3), 496–513.

Masilamoni, J., Jesudoss, K., Nadakumar, K., Satpathy, K., Nair, K., & Azariah, J. (2000). Jellyfish ingress: A threat to the smooth operation of coastal power plants. *Current Science*, *79*(5), 567–569.

McCauley, S. (2007). *Environmental Statement: Development Consent Renewal and Modification of a Marine Finfish Farm at Portnalong, Loch Harport*.

McKee, D., Cunningham, A., Wright, D., & Hay, L. (2007). Potential impacts of nonalgal materials on water-leaving Sun induced chlorophyll fluorescence signals in coastal waters. *Applied Optics*, *46*(31), 7720.

McKibben, M. A., & Hay, D. W. (2002). Planktonic distribution of sea lice *Lepeophtheirus salmonis* larvae in inter-tidal plankton samples in Loch Shieldaig , Western Scotland in relation to local salmon farm production cycles. *ICES*, *6*, 1–14.

Mendoza, M., Meugnier, H., Bes, M., Etienne, J., & Freney, J. (1998). Identification of *Staphylococcus* species by 16S-23S rDNA intergenic spacer PCR analysis. *International Journal of Systematic Bacteriology*, *48 Pt 3*, 1049–55.

Michael, M., Tamkun, A., & Hessinger, D.A. (1981). Isolation and partial characterization of a haemolytic and toxic protein form the nematocyst venom of the Portuguese man-o'-war, *Physalia physalis*, *667*, 87–98.

Miglietta, M. P., Rossi, M., & Collin, R. (2008). Hydromedusa blooms and upwelling events in the Bay of Panama, Tropical East Pacific. *Journal of Plankton Research*, *30*, 783–793.

Miller, D. (2011, June 30). Jellyfish force Torness nuclear reactor shutdown. *BBC Scotland*.

Mitchell, S. O., Baxter, E. J., & Rodger, H. D. (2011). Gill pathology in farmed salmon associated with the jellyfish *Aurelia aurita*. *The Veterinary Record*, *169*(23), 609.

Mitchell, S. O., & Rodger, H. D. (2011). A review of infectious gill disease in marine salmonid fish. *Journal of Fish Diseases*, *34*(6), 411–32.

Mulford, A. L., Salman, A., Ingvarsdottir, A., John, A., Crisp, C., Reid, C., Yau, C., Lear, D., Stevens, D., Conway, D., Lefkaditou, E., Boxshall, G., Pierce, G., Rasmussen, J., Fraser, J., Cook, K., Hastie, L., Pan, M., Wootton, M., Angel, M., Shaw, M., Jones, N., Fielding, S., Katsenevakis, S., Gasparini, S., Milligan, S., Hay, S., Chambers, S., Jonas, S., Sakurai, Y. *Muggiaea atlantica* (live), ZIMNES - Zooplankton Identification Manual for North European Seas.

Murray, A. G., Smith, R. J., & Stagg, R. M. (2002). Shipping and the spread of infectious salmon anemia in Scottish aquaculture. *Emerging Infectious Diseases*, *8*(1), 1–5.

Mustafa, M., White, E., Hongo, K., Othman, I., & Orchard, C. (1995). The mechanism underlying the cardiotoxic effect of the toxin from the jellyfish *Chironex fleckeri*. *Toxicology and Applied Pharmacology*, *133*, 196–206.

Nakayama, A., & Numakunai, T. (2000). A novel life history strategy of the hydrozoan, *Hybocodon prolifer*. *Journal of the Marine Biological Association of the UK*, *80*(02), 363–364.

Neumann, R. (1979). Bacterial Induction of Settlement and Metamorphosis in the Planula Larvae of *Cassiopea andromeda* (Cnidaria: Scyphozoa, Rhizostomeae). *Marine Ecology Progress Series*, *1*, 21–28.

Nevalainen, T. J., Peuravuori, H. J., Quinn, R. J., Llewellyn, L. E., Benzie, J. A. H., Fenner, P. J., & Winkel, K. D. (2004). Phospholipase A2 in cnidaria. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*, *139*(4), 731–5.

Nichols, D., & Barker, M. (1984). A comparative study of reproductive and nutritional periodicities in two populations of *Asterias rubens* (Echinodermata: Asteroidea) from the English Channel. *Journal of the Marine Biological Association of the United Kingdom*, *64*, 471–484.

Nickell, T., Davidson, K., Fox, C., Miller, P., & Hays, G. (2010). Crown Marine Estate Research Report, 1–70.

NOAA's National Ocean Service: Diagram of nematocyst cell. (2008). http://oceanservice.noaa.gov/education/kits/corals/media/supp_coral01b.html

Nomura, T., Yoshimizu, M., & Kimura, T. (1992). An epidemiological study of furunculosis in salmon propagation. In *Salmonid diseases* (p. 187). Hokkaido University Press.

O.S. (2015). Bing Maps: Ordnance Survey. Retrieved November 15, 2015, from www.bing.com/mapspreview

O'Hara, C. M., Brenner, F. W., & Miller, J. M. (2000). Classification, Identification, and Clinical Significance of *Proteus*, *Providencia*, and *Morganella*. *Clinical Microbiology Reviews*, *13*(4), 534–546.

OIE, 2009. Chapter 1.1.3 Methods for disinfection of aquacultural establishments. In *Manual of Diagnostic Tests for Aquatic Animals*. OIE World Health, pp. 31–42.

Ortman, B. D., Bucklin, A., Pages, F., & Youngbluth, M. (2010). DNA Barcoding the Medusozoa using mtCOI. *Deep-Sea Research II*, *57*, 2148–2156.

Orton, J. H., SOuthward, A. J., & Dodd, J. M. (1956). Studies on the biology of the limpets II. The breeding of *Patella vulgata* L. in Britain. *Journal of the Marine Biological Association of the United Kingdom*, *35*, 149–176.

Osborn, A. M., Moore, E. R., & Timmis, K. N. (2000). An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. *Environmental Microbiology*, *2*(1), 39–50.

Östman, C. (2000). A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. *Scientia Marina*, *64*, 31–46.

Panteleeva, N. (1999). *Obelia longissima* (Pallas, 1766) and *Obelia geniculata* (L., 1758) (Hydrozoa, Thecaphora, Campanulariidae) in the Barents Sea. Morphology, distribution, ecology and special life history features. *Zoosystematica Rossica Supplement*, *1*, 51–65.

Pazos, F., Santos, Y., Macias, A. R., Nunez, S., & Toranzo, A. E. (1996). Evaluation of media for the successful culture of *Flexibacter maritimus*. *Journal of Fish Diseases*, *19*(2), 193–197.

Powell, M., Carson, J., & van Gelderen, R. (2004). Experimental induction of gill disease in Atlantic salmon *Salmo salar* smolts with *Tenacibaculum maritimum*. *Diseases of Aquatic Organisms*, *61*(3), 179–85.

Powell, M. D., Harris, J. O., & Carson, J. (2005). Effects of gill abrasion and experimental infection with *Tenacibaculum maritimum* on the respiratory physiology of Atlantic salmon *Salmo salar* affected by amoebic gill disease. *Diseases of Aquatic Organisms*, *63*(2-3), 169–174.

Primo, A. L., Marques, S. C., Falcão, J., Crespo, D., Pardal, M. A., & Azeiteiro, U. M. (2012). Environmental forcing on jellyfish communities in a small temperate estuary. *Marine Environmental Research*, *79*, 152–9.

Purcell, J., & Arai, M. (2001). Interactions of pelagic cnidarians and ctenophores with fish: a review. *Hydrobiologia*.

Purcell, J. E. (1984). The functions of nematocysts in prey capture by epipelagic siophonophores (Coelenterata, Hydrrozoa). *Biol. Bull.*, *166*(5495), 310–327.

Purcell, J. E. (2012). Jellyfish and ctenophore blooms coincide with human proliferations and environmental perturbations. *Annual Review of Marine Science*, *4*, 209–35.

Purcell, J. E., Uye, S. I., & Lo, W. T. (2007). Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review. *Marine Ecology Progress Series, 350,* 153–174.

Rajendhran, J., & Gunasekaran, P. (2011). Microbial phylogeny and diversity: small subunit ribosomal RNA sequence analysis and beyond. *Microbiological Research*, *166*(2), 99–110.

Richardson, A. J., Bakun, A., Hays, G. C., & Gibbons, M. J. (2009). The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in Ecology & Evolution*, *24*(6), 312–22.

Ringø, E., Sperstad, S., Kraugerud, O. F., & Krogdahl, Å. (2008). Use of 16S rRNA gene sequencing analysis to characterize culturable intestinal bacteria in Atlantic salmon (*Salmo salar*) fed diets with cellulose or non-starch polysaccharides from soy. *Aquaculture Research*, *39*(10), 1087–1100.

Rivas, A. J., Balado, M., Lemos, M. L., & Osorio, C. R. (2011). The *Photobacterium damselae* subsp. *damselae* hemolysins damselysin and HlyA are encoded within a new virulence plasmid. *Infection and Immunity*, *79*(11), 4617–27.

Romalde, J. L. (2002). *Photobacterium damselae* subsp. piscicida: an integrated view of a bacterial fish pathogen. *International Microbiology*, *5*(1), 3–9.

Roosen-Runge. (1970). Life cycle of the hydromedusa *Phialidium gregarium* (A. Aggasiz, 1862) in the laboratory. *Biology Bulletin of the Woods Hole Oceanographic Institute, 166,* 206–215.

Rossi, S., Gili, J. M., & Hugues, R. G. (2000). The effects of exposure to wave action on the distribution and morphology of the epiphytic hydrozoans *Clava multicornis* and *Dynamena pumila*. *Scientia Marina*, *64*(S1), 135–140.

Russell, F. (1953). *The medusae of the British Isles Vol. T: Anthomedusae, Leptomedusae, Limnomedusae, Trachymedusae and Narcomedusae. Journal of the Marine Biological Association of the United Kingdom*. Cambridge University Press.

Sasaki, R., & Sheperd, S. A. (1995). Larval dispersal and recruitment of *Haliotis discus hannai* and *Tegula* spp. on Miyagi Coasts, Japan. *Marine and Freshwater Research*, *46*, 519–529.

Schmid, V. (1979). The use of an anthomedusa in establishing an in vitro regeneration system. *Annual Society For Biological Development.*, 35–38.

Scottish Salmon Farming Economic Report June 2015. (2015). Scottish Salmon Producers Organization.

Seaton, D. D. (1989). Fish kills by planktonic organisms. *Aquaculture Information Series*.

Sen, K. (2005). Development of a rapid identification method for *Aeromonas* species by multiplex-PCR. *Canadian Journal of Microbiology*, *51*(11), 957–66.

The Highland Council Planning and Development Service. (2002). *Loch Bracadale Aquaculture framework Plan*. 1-33

Shanks, A. L. (1998). Apparent oceanographic triggers to the spawning of the limpet *Lottia digitalis* (Rathke). *Journal of Experimental Marine Biology and Ecology*, *222*(1), 31–41.

Shanks, A. L. (2009). Pelagic larval duration and dispersal distance revisited. *The Biological Bulletin*, *216*(3), 373–85.

Shucksmith, R., Irvine, H., & McEvoy, L. (in preparation.) *Seasonal changes in the abundance and community composition of gelatinous zooplankton in northern Scotland*.

Skerry, B. (2011). Hard corals carpets a shallow seafloor on Kingman Reef. National Geographic.

Slobodov, S., & Marfenin, N. (2004). Reproduction of the colonial hydroid *Obelia geniculata* (L., 1758)(Cnidaria, Hydrozoa) in the White Sea. *Hydrobiologia*, (1999), 383–388.

Sommer, C. (1992). Larval biology and dispersal of *Eudendrium racemosum*. *Scientia Marina*, *56*, 205–211.

SSPO. (2014). *SCOTTISH SALMON FARMING ANNUAL REPORT 2014*. Retrieved from www.sspo.org.uk

Stebbing, A. R. D. (1981a). Hormesis—stimulation of colony growth in *Campanularia flexuosa* (hydrozoa) by copper, cadmium and other toxicants. *Aquatic Toxicology*, *1*(3-4), 227–238.

Stebbing, A. R. D. (1981b). The effects of reduced salinity on colonial growth and membership in a hydroid. *Journal of Experimental Marine Biology and Ecology*, *55*, 233–241.

Stebbing, a. R. D. (2002). Tolerance and hormesis - Increased resistance to copper in hydroids linked to hormesis. *Marine Environmental Research*, *54*(3-5), 805–809.

Steinum, T., Sjåstad, K., Falk, K., Kvellestad, A., & Colquhoun, D. J. (2009). An RT PCR-DGGE survey of gill-associated bacteria in Norwegian seawater-reared Atlantic salmon suffering proliferative gill inflammation. *Aquaculture*, *293*(3-4), 172–179.

Stepanjants, S. (1998). Obelia (Cnidaria, Medusozoa, Hydrozoa): phenomenon, aspects of investigations, perspectives for utilization. *Oceanography and Marine Biology: An Annual Review*, *36*, 179–215.

Stewart, E. J. (2012). Growing unculturable bacteria. *Journal of Bacteriology*, *194*(16), 4151–60.

Stibor, H., & Tokle, N. (2003). Feeding and asexual reproduction of the jellyfish *Sarsia gemmifera* in response to resource enrichment. *Oecologia*, *135*(2), 202–8.

Szmant, A. M. (1986). Reproductive ecology of Caribbean reef corals. *Coral Reefs*, *5*(1), 43–53.

Tamkun, A., & Hessinger, D. (1981). Isolation and partial characterization of a haemolytic and toxic protein form the nenatozyst venom of the Potuguese man-of- war, *Physalia physalis*, *667*, 87–98.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*, 512–526.

Tan, L., Qu, Y., Zhou, J., Li, A., & Gou, M. (2009). Identification and Characteristics of A Novel Salt-Tolerant Exiguobacterium sp. for Azo Dyes Decolorization. *Applied Biochemistry and Biotechnology*, *159*(3), 728–738.

Taq PCR Master Mix Kit - QIAGEN. https://www.qiagen.com/gb/shop/pcr/end-point-pcr-enzymes-and-kits/regular-pcr/taq-pcr-master-mix-kit/#productdetails

Thiel, M., & Gutow, L. (2005). Oceanography and Marine Biology: An Annual Review. *Oceanography and Marine Biology: An Annual Review*, *42*, 181–264.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, *25*(24), 4876–82.

Thuesen, E. V., & Kogure, K. (1989). Bacterial Production of Tetrodotoxin in Four Species of *Chaetognatha*. *Biol. Bull.*, *176*(2), 191–194.

Todorova, S. G., & Costello, A. M. (2006). Design of *Shewanella*-specific 16S rRNA primers and application to analysis of *Shewanella* in a minerotrophic wetland. *Environmental Microbiology*, *8*(3), 426–32.

Torres-Ramos, M., & Aguilar, M. (2003). Recent Advances in Cnidarian Neurotoxin Research. *Comments on Toxicology*, *9*(2), 161–174.

Tørud, B., & Håstein, T. (2008). Skin lesions in fish: causes and solutions. *Acta Veterinaria Scandinavica*, *50*(Suppl 1), S7.

UKHO. (1991a). UK Hydrographic Office, Shetland Isles North-East Sheet 3282. UK Hydrographic Office Admiralty Charts and Publications.

UKHO. (1991b). UK Hydrographic Office, Shetland Isles North-West Sheet 3281. Taunton: UK Hydrographic Office Admiralty Charts and Publications.

UKHO. (2001). UK Hydrographic Offic, Shetland Isles South Sheet 3283. UK Hydrographic Office Admiralty Charts and Publications.

Underwood, A. H., & Seymour, J. E. (2007). Venom ontogeny, diet and morphology in *Carukia barnesi*, a species of Australian box jellyfish that causes Irukandji syndrome. *Toxicon : Official Journal of the International Society on Toxinology*, *49*(8), 1073–82.

Valdenegro-Vega, V. A., Crosbie, P., Bridle, A., Leef, M., Wilson, R., & Nowak, B. F. (2014). Differentially expressed proteins in gill and skin mucus of Atlantic salmon (*Salmo salar*) affected by amoebic gill disease. *Fish & Shellfish Immunology*, *40*(1), 69–77.

Vartoukian, S. R., Palmer, R. M., & Wade, W. G. (2010). Strategies for culture of "unculturable" bacteria. *FEMS Microbiology Letters*, *309*(1), 1–7.

Vengatesen, T., Lau, S., Mandy, T., Zhang, W., & Qian, P. (2010). Monitoring bacterial biodiversity in surface sediment using terminal restriction fragment length polymorphism analysis (T-RFLP): application to coastal environment. Nagasaki.

Visca, P., Seifert, H., & Towner, K. J. (2011a). *Acinetobacter* infection--an emerging threat to human health. *IUBMB Life*, *63*(12), 1048–54.

Vynne, N. G., Månsson, M., Nielsen, K. F., & Gram, L. (2011). Bioactivity, chemical profiling, and 16S rRNA-based phylogeny of *Pseudoalteromonas* strains collected on a global research cruise. *Marine Biotechnology (New York, N.Y.)*, *13*(6), 1062–73.

Wade, W. (2002). Unculturable bacteria--the uncharacterized organisms that cause oral infections. *Journal of the Royal Society of Medicine*, *95*(2), 81–3.

Wallace, C. (2015). Personal communication with Marine Harvest Ltd. re: treatment and mortality.

Wang, N., & Li, C. (2015). The accumulated effect of temperature on the *Aurelia* sp.1 strobilation process. In *China-Korea Joint Workshop on Jellyfish Research* (p. 20).

Wang, S., & Feng, S. (2015). Searching for the field population of blooming scyphozoan jellyfish along the China coast. In *China-Korea Joint Workshop on Jellyfish Research* (p. 13).

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews. Genetics*, *10*(1), 57–63.

Weisburg, W., Barns, S., Pelletier, D., & Lane, D. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, *173*, 797–703.

Werner, B. (1958). Die Verbreitung und das jahreszeitliche Auftrete der Anthomedusae *Rathkea octopunctata* M. Sars. sowie die Temperaturabhangigkeit, ihrer Entwicklung und und Fortpfanzang. *Helgolander Wiss. Meeresunters*, *6*, 137–170.

Werner, B. (1961). Morphology un Lebensgeschichte, sowie Temperaturabhangigkeit dur Verbreitung und das jahreszeitlichen Auftretens von *Bougainvillia superciliaris* (L. Agassiz) (Athecatae-Anthomedusae). *Helgolander Wiss. Meeresunters*, *7*, 206–237.

Werner, B. (1962). Verbreitung und jahreszeitliches Auftreten von *Rathkea octopunctata* (M.Sars) und *Bougainvillia superciliaris* (L. Agassiz) (Athecatae-Anthomedusae). *Ein Beitrag Zur Kausalen Marinen Tiergeographie. Kieler Meerresforsch*, *18*, 55–62.

Widmer, C. (2004). The hydroid and early medusa stages of *Mitrocoma cellularia* (Hydrozoa, Mitrocomidae). *Marine Biology*, *145*(2).

Widmer, C. (2015). PhD Thesis: *Influences of temperature and salinity on asexual reproduction and development of scyphozoan jellyfish from the British Isles*. University of St Andrews.

Woese, C. R., Kandler, O., & Wheelis, M. (1990). Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 4576–4579.

Woo, P. C., Cheung, E. Y., Leung, K., & Yuen, K. (2001). Identification by 16S ribosomal RNA gene sequencing of an Enterobacteriaceae species with ambiguous biochemical profile from a renal transplant recipient. *Diagnostic Microbiology and Infectious Disease*, *39*(2), 85–93.

Yoon, J.-H., Kim, I.-G., & Oh, T.-K. (2007). *Acinetobacter marinus* sp. nov. and *Acinetobacter seohaensis* sp. nov., isolated from sea water of the Yellow Sea in Korea. *Journal of Microbiology and Biotechnology*, *17*(11), 1743–50.

Zaiontz, C. (2015). Real Statistics Using Excel. Milan, Italy: Charles Zaiontz. www.real-statistics.com

Zhao, D. H., Sun, J. J., Liu, L., Zhao, H. H., Wang, H. F., Liang, L. Q., Li, G. F. (2009). Characterization of Two Phenotypes of *Photobacterium damselae* subsp. *damselae* Isolated from Diseased Juvenile *Trachinotus ovatus* Reared in Cage Mariculture. *Journal of the World Aquaculture Society*, *40*(2), 281–289.

Zhong, S., Young, J., Zheng, Y., Chen, Y., Liu, B., Shao, Y., Xiang, J., Zhangjun, F.,Giovannoni, J. (2011). High-Throughput Illumina strand-specific RNA sequencing library preparation. In R. Sever (Ed.), *Cold Spring Harbour Protocols* (3rd ed., pp. 940–949). Cold Spring Harbor Laboratory Press.

Zibordi, G., Berthon, J.-F., Mélin, F., D'Alimonte, D., & Kaitala, S. (2009). Validation of satellite ocean color primary products at optically complex coastal sites: Northern Adriatic Sea, Northern Baltic Proper and Gulf of Finland. *Remote Sensing of Environment*, *113*(12), 2574–2591.