OsHV-1µVar In Ireland

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The Emergence of OsHV-1µVar

- May-June 2008: 40-100% Mortality reported in all areas in France
- August: France report detection of OsHV-1 and Vibrio species
- August – September: 3 sites in Ireland report high mortality in seed
- OsHV-1 detected in Irish samples

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The Emergence of OsHV-1µVar

- April 2009 – 1st outbreaks of Mortality reported in the south of France
- May 2009 – Mortality spreads northwards through France
- 28th May – French Ministry decides to Ban exports
- 14th June – 1st reports of Mortality received in Ireland
- June-August - 15 Bays record abnormal levels of mortality. Variant strain of OsHV-1 detected

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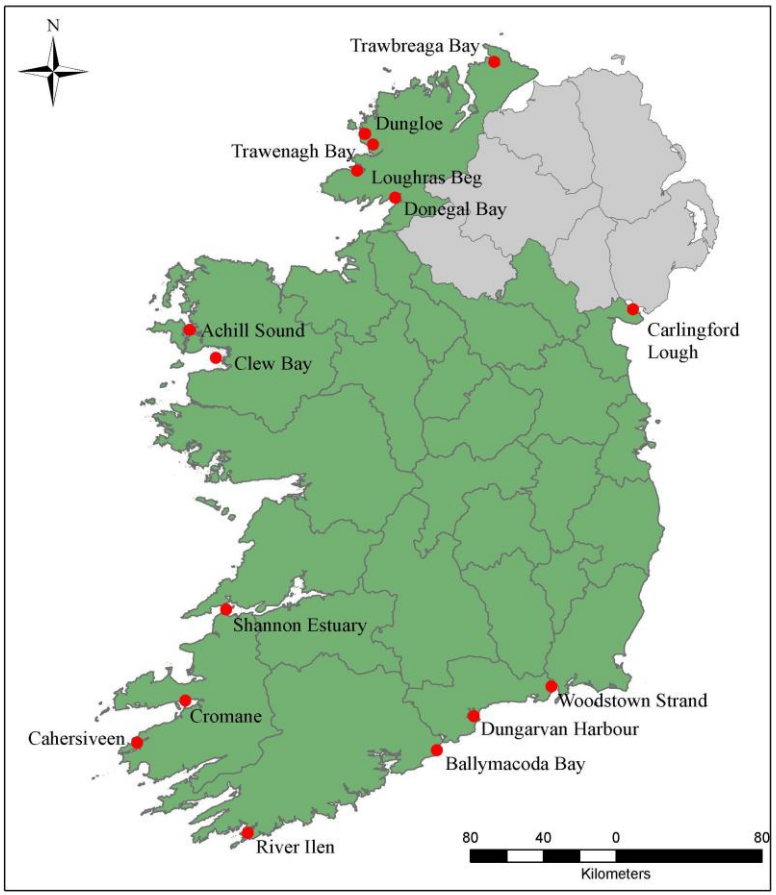
Establishment and spread within Ireland

- In 2008 Woodstown, Dungarvan & Cromane were infected
- In 2009 15 bays became infected
  - 9 cases: confined to 2009 French seed
  - 4 cases: Guernsey & English seed as well
  - 1 case: seed from England only
  - 1 case: oysters moved from an area that subsequently reported mortality





Establishment and spread within Ireland

- In 2009 it became clear that regulation was required to control the spread of OsHV-1
- Commission Regulation 2010/175 introduced in early 2010
  - Surveillance Programme introduced to test all areas considered to be free of OsHV-1µVar
  - Dictated methodology to test oysters (PCR Cf-Cr)
  - Trade Restrictions through introduction of containment areas

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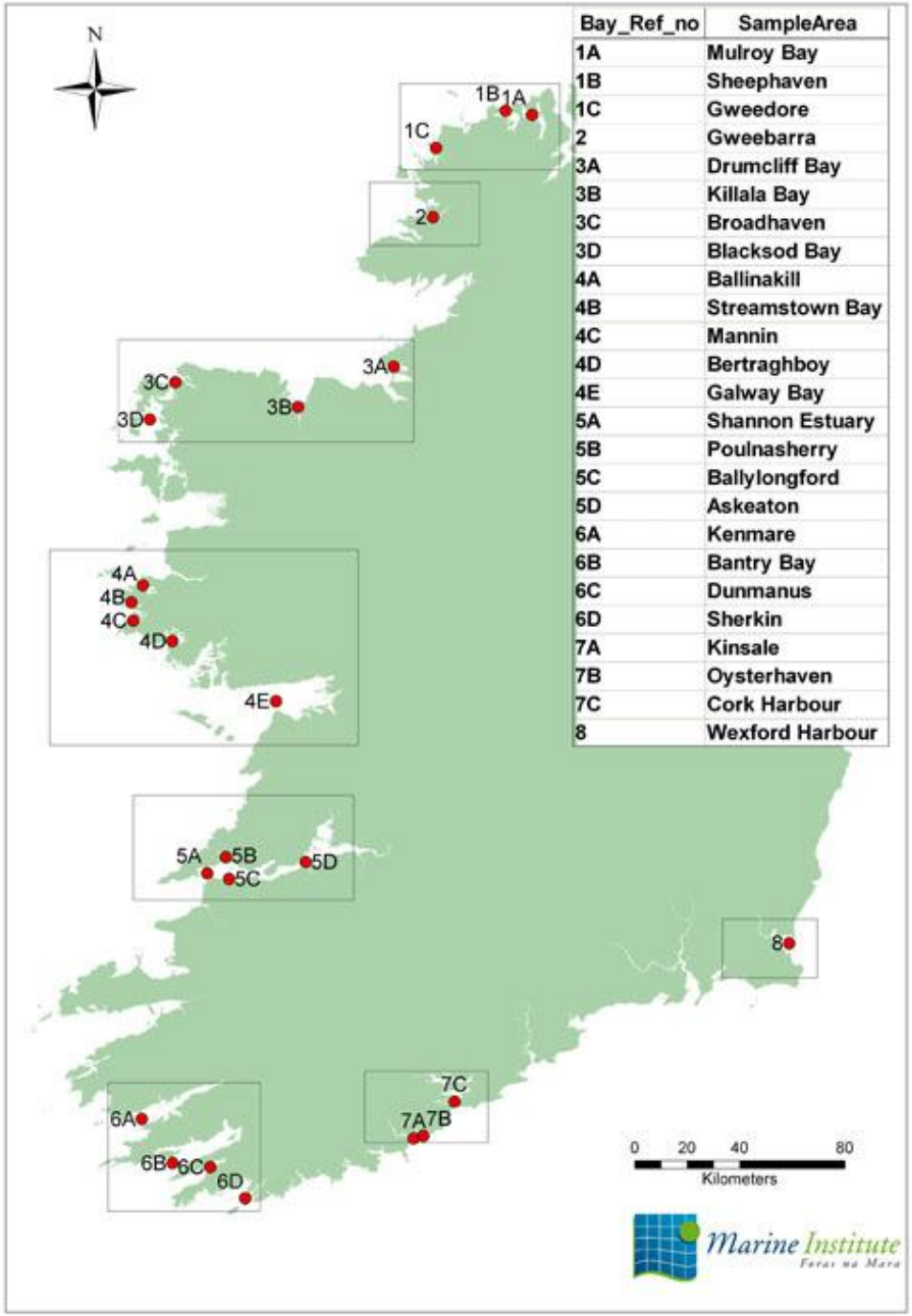
175 / 2010: Surveillance Programme

- 26 Bays considered to be free of OsHv-1µVar
- Testing of 150 oysters
- Most susceptible age classes tested
- 6 Sites found to be positive in 2010



Bay_Ref_no	SampleArea
1A	Mulroy Bay
1B	Sheephaven
1C	Gweedore
2	Gweebarra
3A	Drumcliff Bay
3B	Killala Bay
3C	Broadhaven
3D	Blacksod Bay
4A	Ballinakill
4B	Streamstown Bay
4C	Mannin
4D	Bertraghboy
4E	Galway Bay
5A	Shannon Estuary
5B	Poulnasherry
5C	Ballylongford
5D	Askeaton
6A	Kenmare
6B	Bantry Bay
6C	Dunmanus
6D	Sherkin
7A	Kinsale
7B	Oysterhaven
7C	Cork Harbour
8	Wexford Harbour



Legislation in 2011

- Commission Decision 2011/187/EC came into effect on 1st May 2011

The key features of this legislation are:

- The establishment of disease free compartments
- A targeted testing regime to ensure the health status of each compartment is known and can be protected.
- Restrictions on trade in *C.gigas* into surveillance areas - all movements of *C,gigas* into surveillance areas must originate from other surveillance areas.

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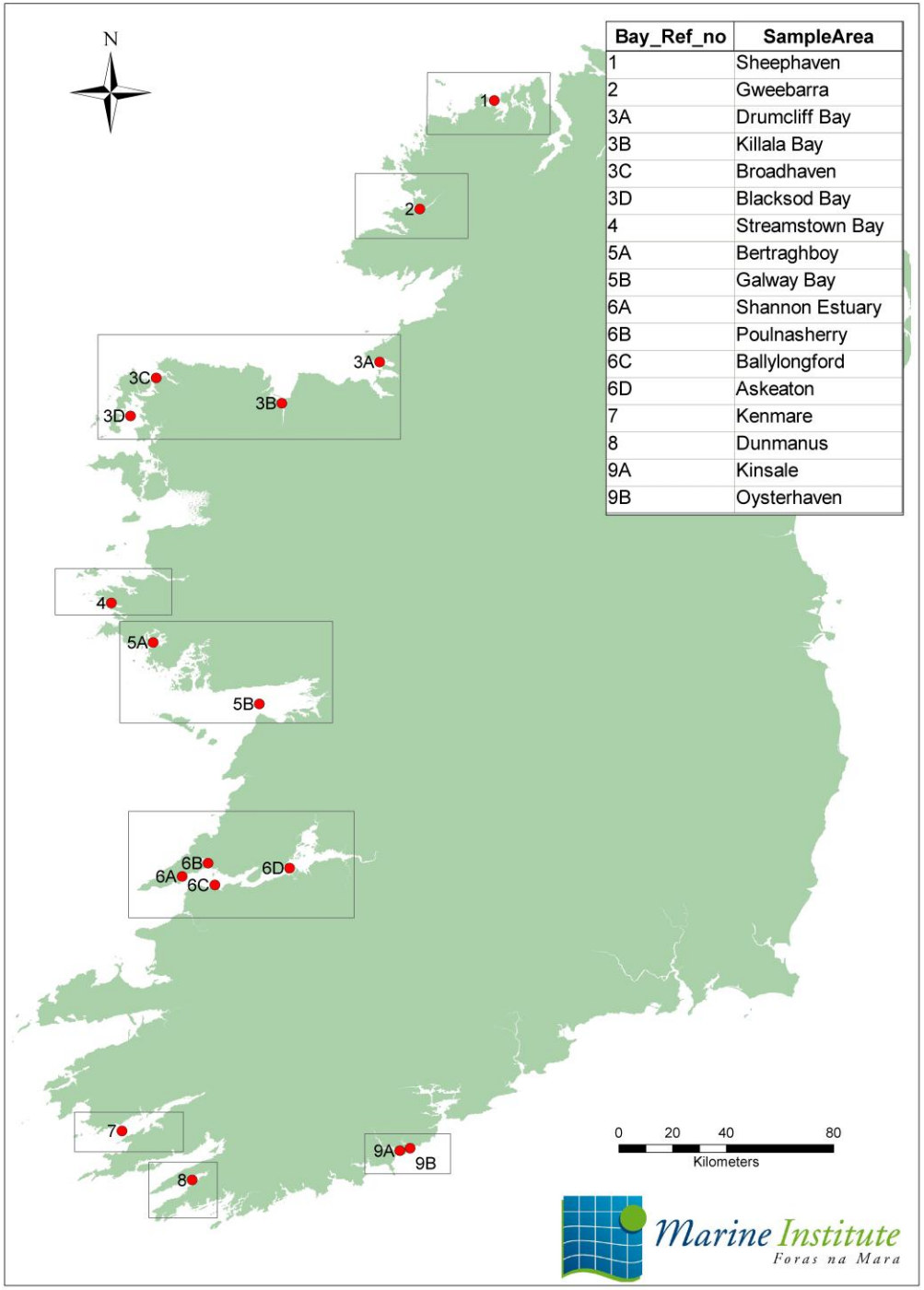
Surveillance and testing 2011

- 19 Bays included in 2011 surveillance programme
- OsHV-1 detected in surveillance sample from one site in Donegal
- 1/150 oysters found to be positive in a second site.



Bay_Ref_no	SampleArea
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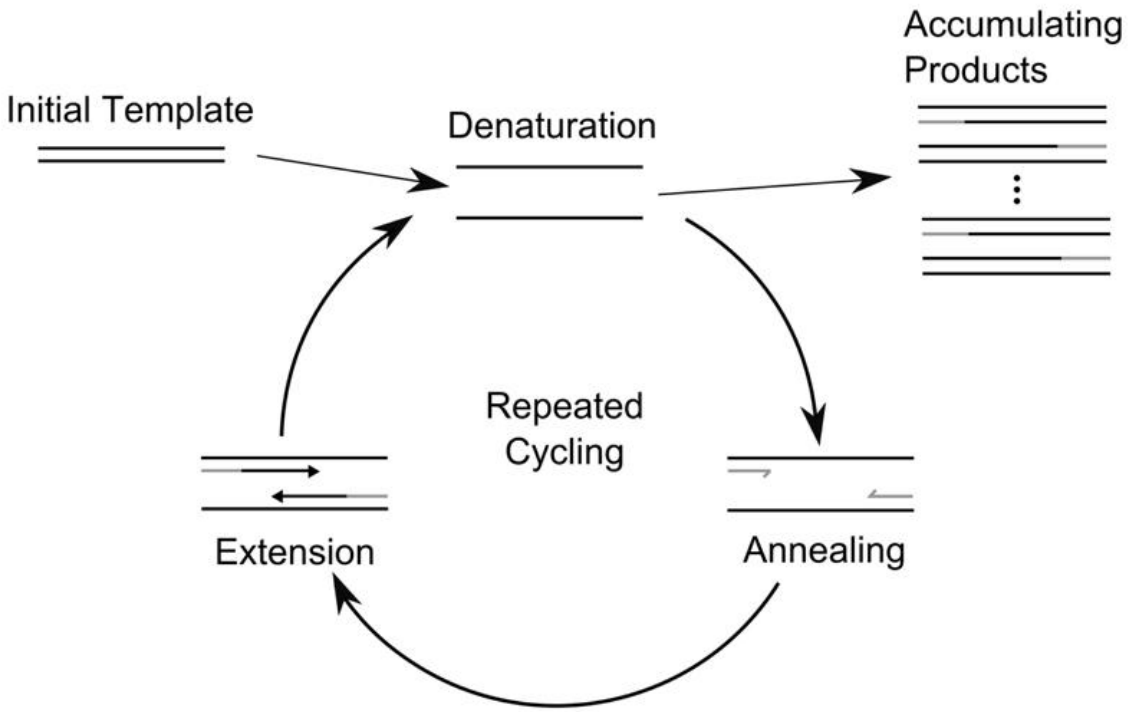
6C	Ballylongford
6D	Askeaton
7	Kenmare
8	Dunmanus
9A	Kinsale
9B	Oysterhaven



Testing Methodology - PCR

- specific amplification of a region of DNA of interest
- multiple copies of DNA can be visualized and sequenced
- method allows for specific identification of OsHV-1



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Testing Methodology - PCR

Why change from Conventional to Real Time?

- Conventional uses end point detection
- Real time detects whilst reaction is occurring
- Some of the problems with **End-Point Detection**:
  - Poor Precision,
  - Low sensitivity,
  - Low resolution,
  - Size-based discrimination only,
  - Results are not expressed as numbers

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Testing Methodology - PCR

Real time PCR

- 2 Different Chemistries:
  - SYBR Green, Taq Man
- SYBR Green - Uses SYBR Green I dye, a highly specific, double-stranded DNA binding dye, to detect PCR product as it accumulates during PCR cycles
- TaqMan - Uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR cycles.

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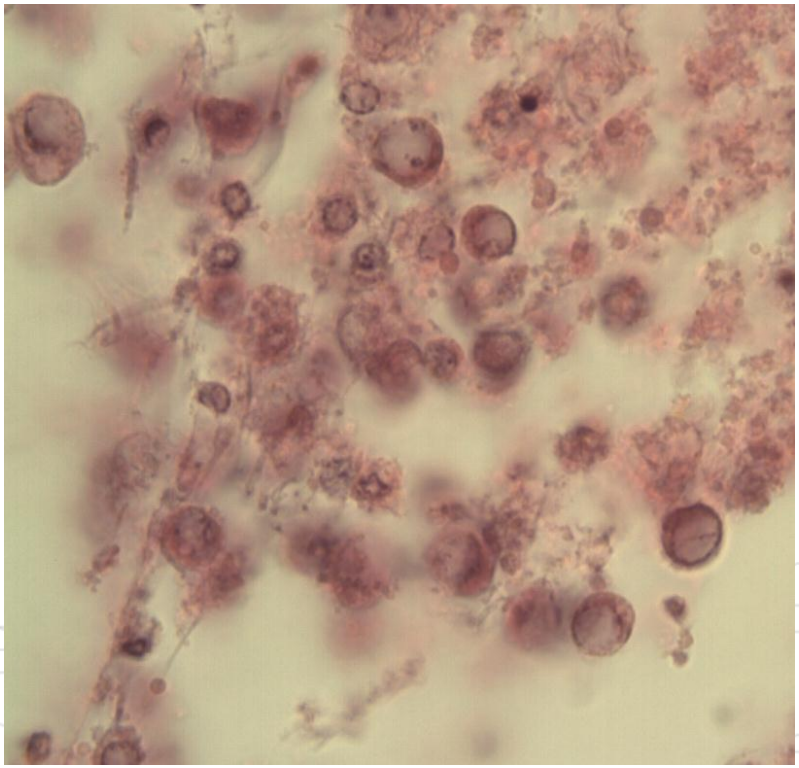
Testing Methodology - PCR

	TaqMan®-Based Detection	**SYBR®-Green Based Detection**
Specificity	Detects specific amplification products only	Detects all amplified double-stranded DNA, including non-specific reaction products
Advantages	Reduction in false positives & Background Possible to detect 2 different sequences in one No post PCR processing	Possible to monitor amplification of any DS DNA product No probes are required, which reduces your assay setup and running costs
Disadvantages	A different probe has to be synthesized for each unique target sequence	Possible false positives



Other Methodology

- Histology:
  - can see changes which could indicate the presence of a virus
  - Doesn't allow for specific identification of OsHV-1
- Electron Microscopy:
  - Visualisation of viral particles
  - Not specific for OsHV-1



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Evolution of Testing In Ireland

- Testing has evolved each year as our knowledge of the new variant has developed
- 2008: Conventional PCR with Primer C2-C4
- 2009: Conventional PCR with Primers C2-C4 & C2-C6
- 2010: 175/2010 states use of Conventional PCR with Primers Cf-Cr
- 2010: Ireland Used SyBr Green PCR with primers C9-C10 (Pepin et al, 2008) for screening with confirmatory testing using Conventional PCR with Primers Cf-Cr

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Evolution of Testing

- 2011: Ireland Used SYBR Green PCR (primers C9-C10) with confirmatory testing using a nested PCR with Primers C2-C6 followed by internal primers designed by David Stone (CEFAS)
- 2012: Testing of Taqman PCR (Martenot el al, 2010). Strain differentiation using nested PCR (as 2011)

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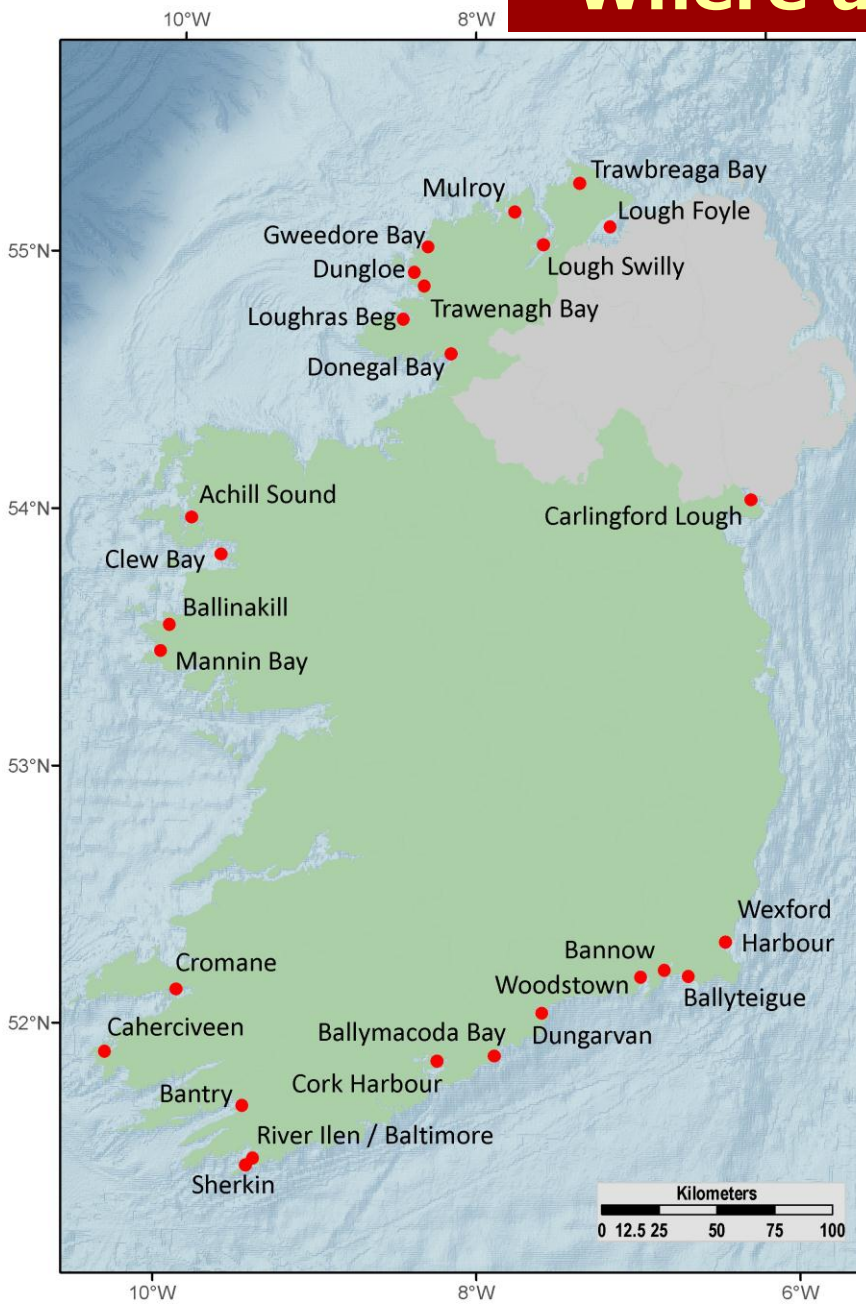
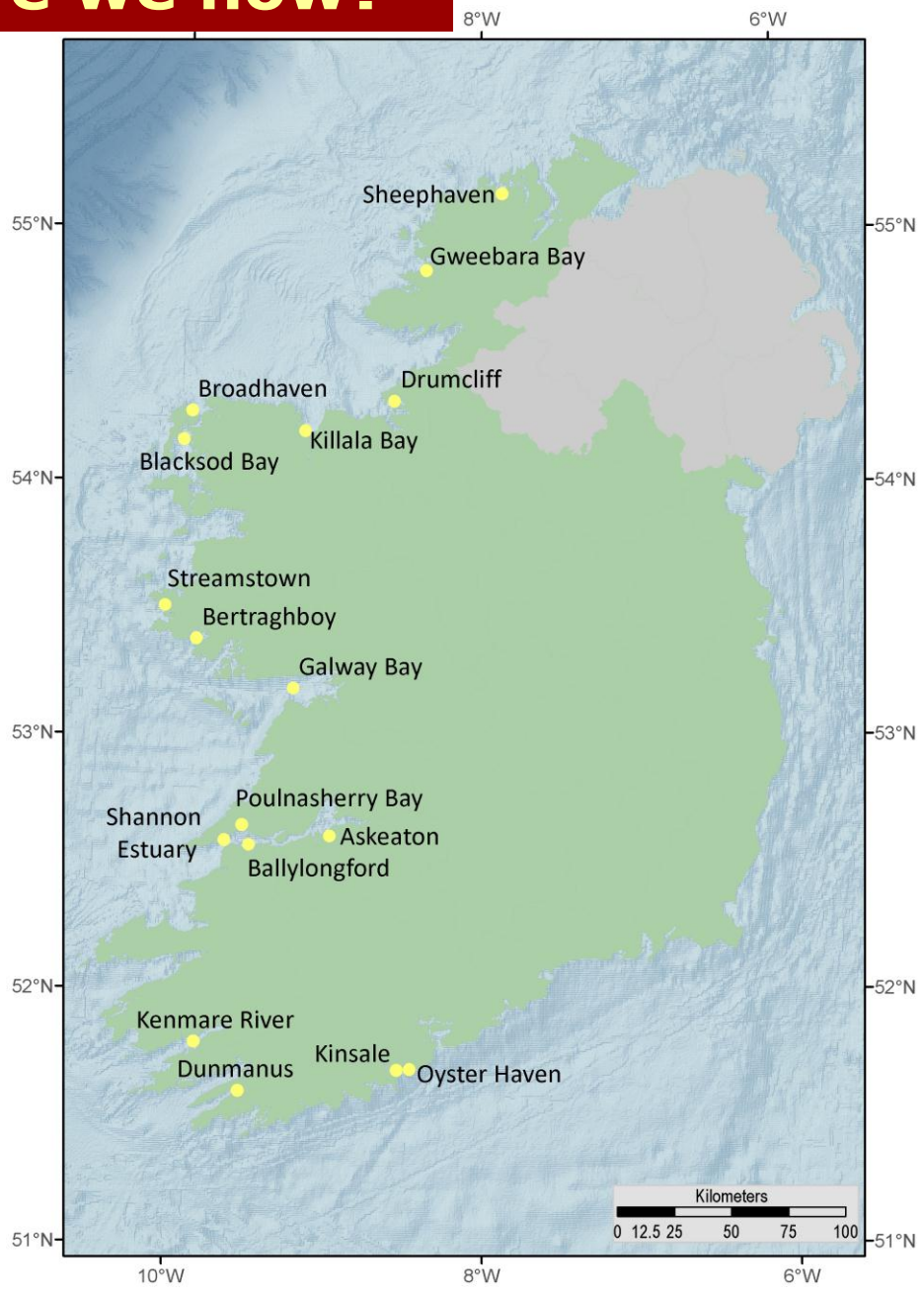
Where are we now?







Mitigation Measures

- Little is still understood about the best ways to mitigate against the effects of OsHV-1µVar
- Since 2009 MI has worked closely with industry to try to understand the factors that contribute to the mortalities
- In late 2009 a Questionnaire Study was undertaken in conjunction with CEFAS
- In 2010, MI became a partner in an FP7 funded project on OsHV-1µVar
- In 2011 an epidemiological study was launched

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2009 Study: Conclusions

- There was a strong association between introduction of stock from areas where the OsHV-1 was known to be present, relative to areas which remain free of the virus. This is consistent with spread of the virus through movements of stock from infected areas.
- 29% of triploid batches had mortality >20 % compared with 12% of diploid batches which is highly statistically significant
- The dates of onset by site in bays with more than 5 sites is consistent with spread of the virus between sites within the same bay

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2009 Study: Conclusions

- There was considerable variation in the level of reported mortality per batch. However, few clear associations with management factors could be identified.
- mortality due to OsHV1 is likely to be determined by
  - age of oysters when first infected,
  - condition of the oysters,
  - temperatures,
  - other environmental factors

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2009 Study: Conclusions

- maximum duration of the daily exposure to air (position on beach) and possibly handling are potential management factors which should be investigated in future studies
- In general no clear mitigation measures were apparent from the study

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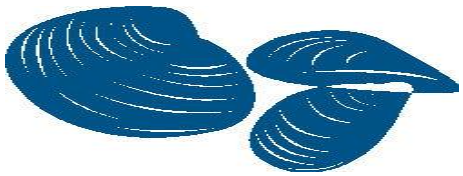
FP7 Project: Bivalife

Project Aims

- improve, validate and transfer existing methods for detection and identification of Pacific cupped oyster and mussel pathogens;
- search and characterise Pacific cupped oyster and mussel pathogens in relation to the presence or absence of mortality;
- assess the relationship between the presence of Pacific cupped oyster and mussel pathogens and environmental risk factors in the development of mortality or disease;



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FP7 Project: Bivalife

Project Aims

- investigate mechanisms allowing Pacific cupped oyster and mussel pathogens to survive outside the host;
- identify pathogen intrinsic virulence factors and effects on host defence mechanisms;
- develop methods and recommendations for pathogen control and eradication in Europe.

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FP7 Project: Bivalife

4 scientific work packages:

- Detection and identification of relevant pathogens and associated risk factors (WP2)
- Mechanisms allowing concerned pathogens to survive outside the host (WP3)
- Relevant pathogens: identification of intrinsic virulence factors and effects on host defence mechanisms (WP4)
- Pathogen control and eradication: development of methods, field tests and recommendations (WP5)

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FP7 Project: Bivalife

Our Role

- Detection and identification of OsHV-1µVar and Vibrio species and associated risk factors in 3 sites in Ireland
  - Sampling 5 times throughout the year
  - Testing for the relevant pathogens
  - Monitoring environmental parameters
- Samples collected will also be passed onto other partners for further analysis

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2011 Epidemiological Study

- No data analysed yet
- Preliminary results expected April
- Results will decide the course of the study in 2012

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Thank you for your attention…

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