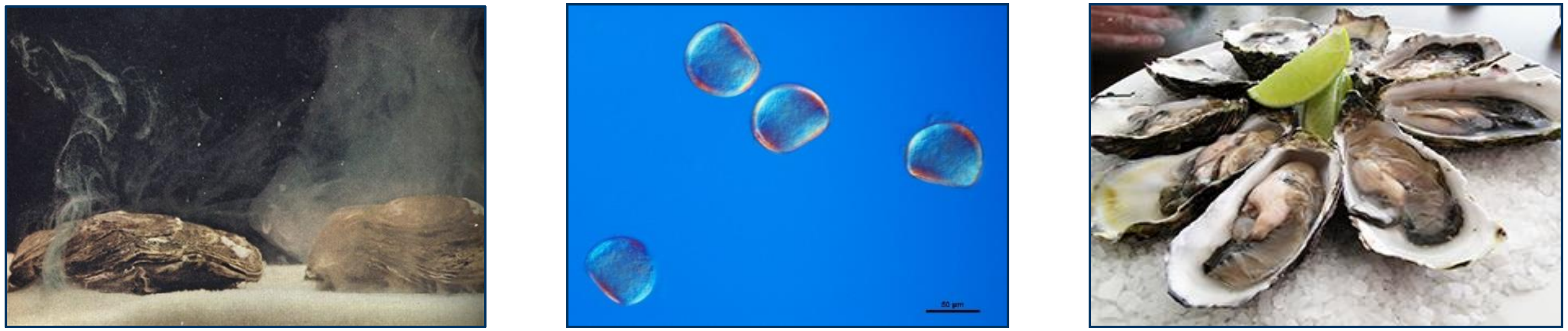
Genomic Approaches to Selective Breeding for Disease Resistance in Pacific Oysters (*Crassostrea Gigas*)



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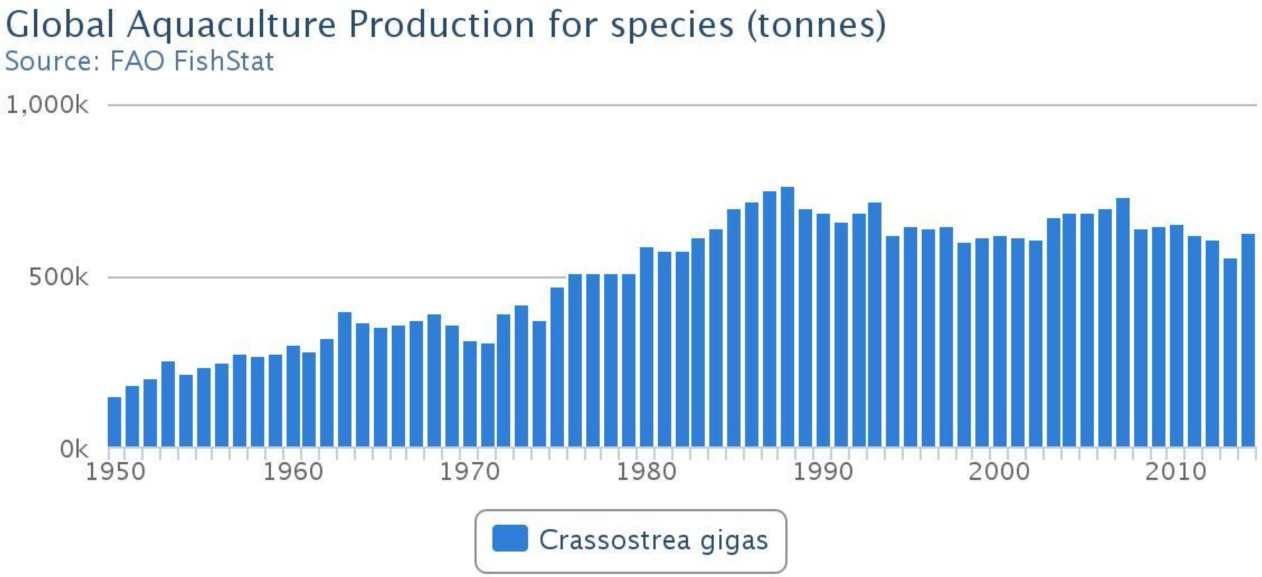
Vivaldi Workshop, Montpellier, 29th August 2018

Outline

i. Oyster aquaculture and disease
ii. Creating an oyster SNP chip
iii. Oyster Herpes Virus experiments
iv. Genetic basis of host resistance
v. Applications of genomic selection

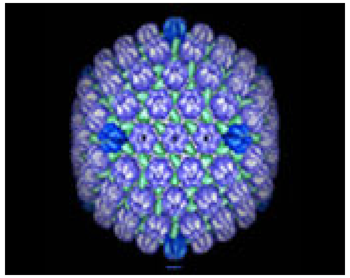
Background

- **Pacific oyster (*Crassostrea gigas*)**
  - **Species of global importance to aquaculture**



  - **Widely introduced to Europe and America: fast growing and robust**
  - **Substantial and expanding genomic toolbox**

Background



- **Pacific oyster aquaculture: threat of infectious disease**
  - **Mass mortality in juveniles due to Oyster Herpes Virus (OsHV-1)**
  - **Virulent form (micro variant) emerged in last 10 years**
  - **Biosecurity measures limited efficacy to prevent outbreaks**

A deadly herpes virus is threatening oysters around the world

September 11, 2017 1.40am BST

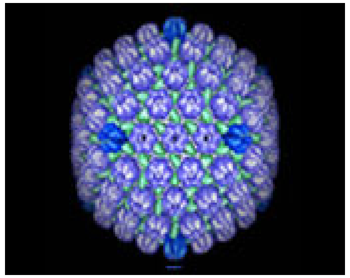
Colleen Burge counts oysters on an oyster aquaculture lease in California. Collin Closek, CC BY-ND

Oysters Can in Fact Get Herpes, And It's Killing Them in Droves

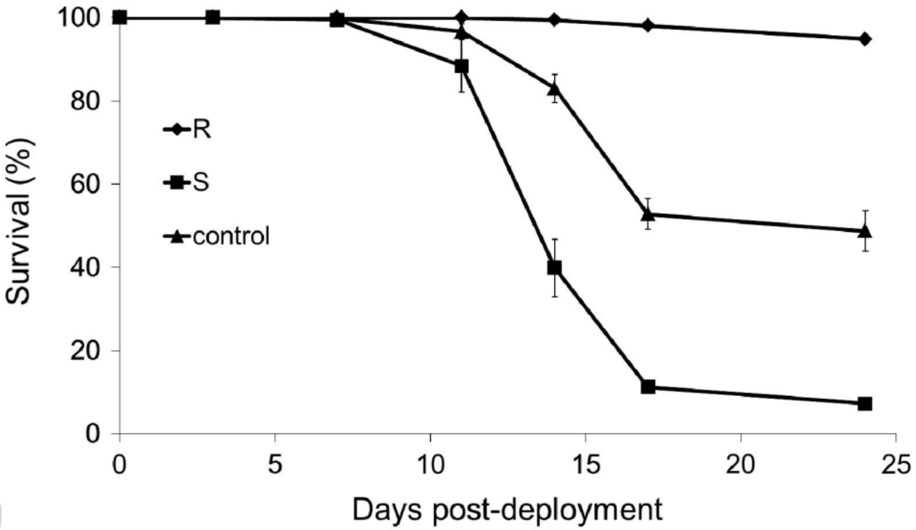
OutdoorHub Reporters
2 weeks ago

176
Shares

Background



- **Host resistance to OsHV has a large genetic component**
  - Heritability estimates in field & lab up to 0.6 (Degremont *et al.* 2015)
  - High genetic correlation between field and lab r_G~0.7 (Degremont *et al.* 2015)
- **Selective breeding has potential to produce resistant stocks**



From Degremont (2011):
Survival rate in a 'field' trial of samples from C. gigas lines derived from 4 generations of selection: Resistant (R), Susceptible (S) and Control lines

Background

- **Commercial-scale genetic improvement of *C. gigas***
  - **Reliance on hatchery stocks commonplace**
  - **Family-based programmes underway in France, Australia, N.Z….**
  - **Genomic tools not yet routinely applied**
- **Our aims:**
  - **i. To create a SNP array for efficient genome-wide genotyping of Pacific and European oysters**
  - **ii. To assess the genetic architecture of host resistance to OsHV-1**
  - **iii. To test and develop cost-effective genomic selection for oysters**

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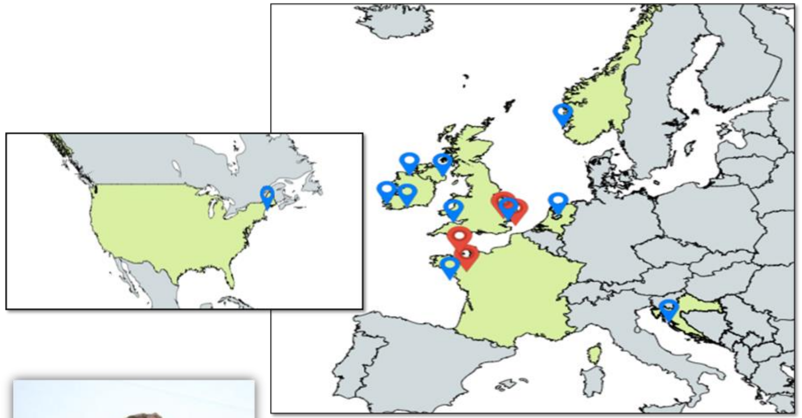
SNP Array

- **Population samples for sequencing and SNP discovery**



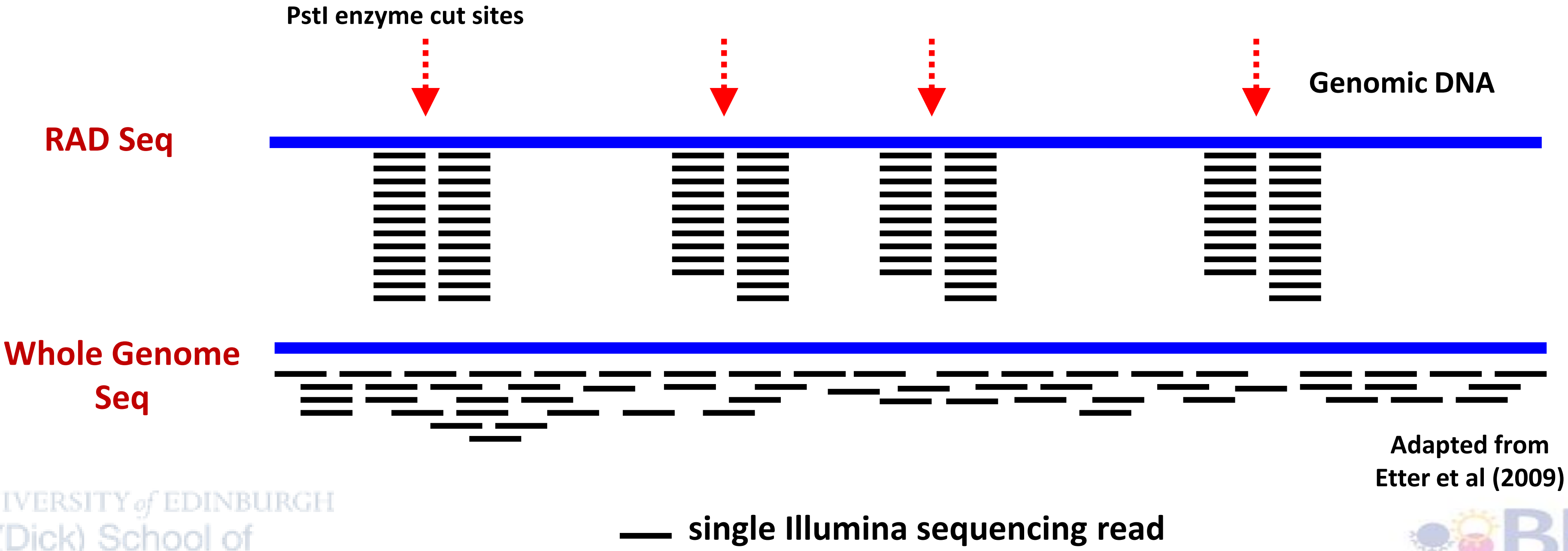
11 populations of *O.edulis*
- UK and Ireland
- Norway
- Netherlands
- Croatia
- USA

8 populations of *C. gigas*
- UK:
  - Hatcheries
- France
  - Hatcheries
  - Selection lines (Ifremer)

Species	Country	Site	Number
O_edulis	Croatia	Croatia	14
O_edulis	England	Mersea	15
O_edulis	France	Baie de Quiberon	15
O_edulis	Ireland	Rossmore (Cork)	15
O_edulis	Ireland	Tralee	13
O_edulis	Ireland	Lough Foyle	15
O_edulis	USA	Damariscotta, Maine	14
O_edulis	Netherlands	Lake Grevelingen	15
O_edulis	N. Ireland	Larne	14
O_edulis	Norway	Sveio	15
O_edulis	Swansea	Swansea Bay	15
C_gigas	France	Ifremer selection lines	16
C_gigas	France	France Turbot	29
C_gigas	France	Vendée Naissain	26
C_gigas	France	Novostrea	29
C_gigas	France	SATMAR	28
C_gigas	UK	Guernsey Sea Farms	47
C_gigas	UK	Sea Salter	13
C_gigas	UK	Maldon	15

SNP Array

- **Sequencing strategy for SNP discovery**
  - **Single libraries of pooled genomic DNA from each population**
  - ***C. gigas* → whole genome Illumina resequencing (reference genome), ~70x**
  - ***O. edulis* → RAD sequencing, *Pst*I enzyme (reference-free), ~100x**



Adapted from Etter et al (2009)

SNP Array

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- **Combined species SNP array created with ~50 K genetic markers**
  - **Candidate SNPs on array: ~35K for *C. gigas,* and ~15K for *O. edulis***
  - **Validated in several populations and families**

Informative C. gigas SNPs

	Sample *N*	MAF > 0	
		# SNPs	Average MAF
UK (combined)[a]	56	27,313	0.186
GSF + parents	38	26,549	0.19
Maldon	9	22,079	0.216
Sea Salter	9	22,821	0.214
Average within UK populations[b]		23,816	0.207
France (combined)[a]	52	26,891	0.182
Ifremer	13	23,010	0.203
Hatchery 1	10	21,479	0.217
Hatchery 2	10	20,141	0.221
Hatchery 3	10	21,730	0.215
Hatchery 4	9	22,052	0.214
Average within French populations[b]		21,682	0.214
All populations (combined)[a]	108	27,697	0.182

Informative O. edulis SNPs

	Sample *N*	MAF > 0	
		# SNPs	Average MAF
Croatia	9	8,474	0.234
Foyle_IRL	10	10,013	0.224
Grevelingen_NLD	10	9,946	0.224
Larne_NIRL	10	8,927	0.231
Mersea_UK	10	9,980	0.224
Quiberon_FR	10	9,973	0.226
Rossmore_IRL	10	9,846	0.228
Sveio_NOR	10	9,118	0.226
Swansea_UK	9	9,696	0.224
Tralee_IRL	10	9,980	0.219
Maine_USA	10	9,614	0.221
Average within population[a]		9,597	0.225
All populations (combined)[b]	108	11,151	0.210

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Gutierrez et al. 2017, G3, 8: 1273-80

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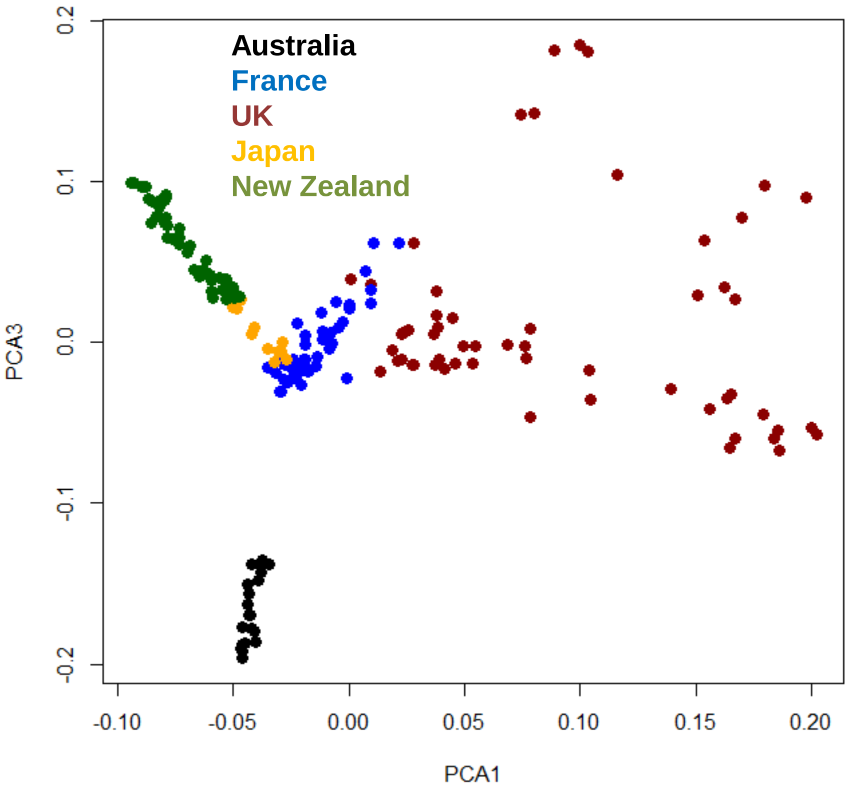
SNP Array

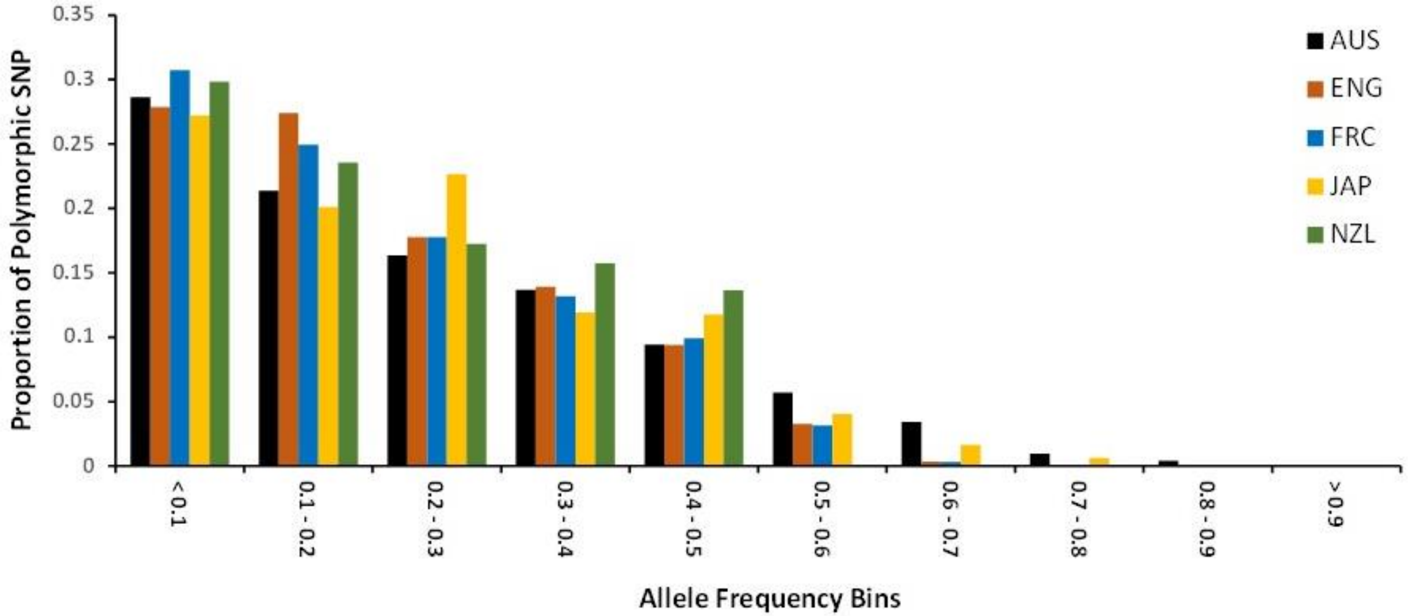
- **Combined species oyster SNP array created with ~50 K genetic markers**
  - **Freely available to purchase from ThermoFisher (Axiom technology) ~30 Euro**
  - **Being tested / used by researchers in several countries**

SNP Array

Courtesy of James Kijas, CSIRO

- **Study of genetic diversity of Pacific oyster samples**



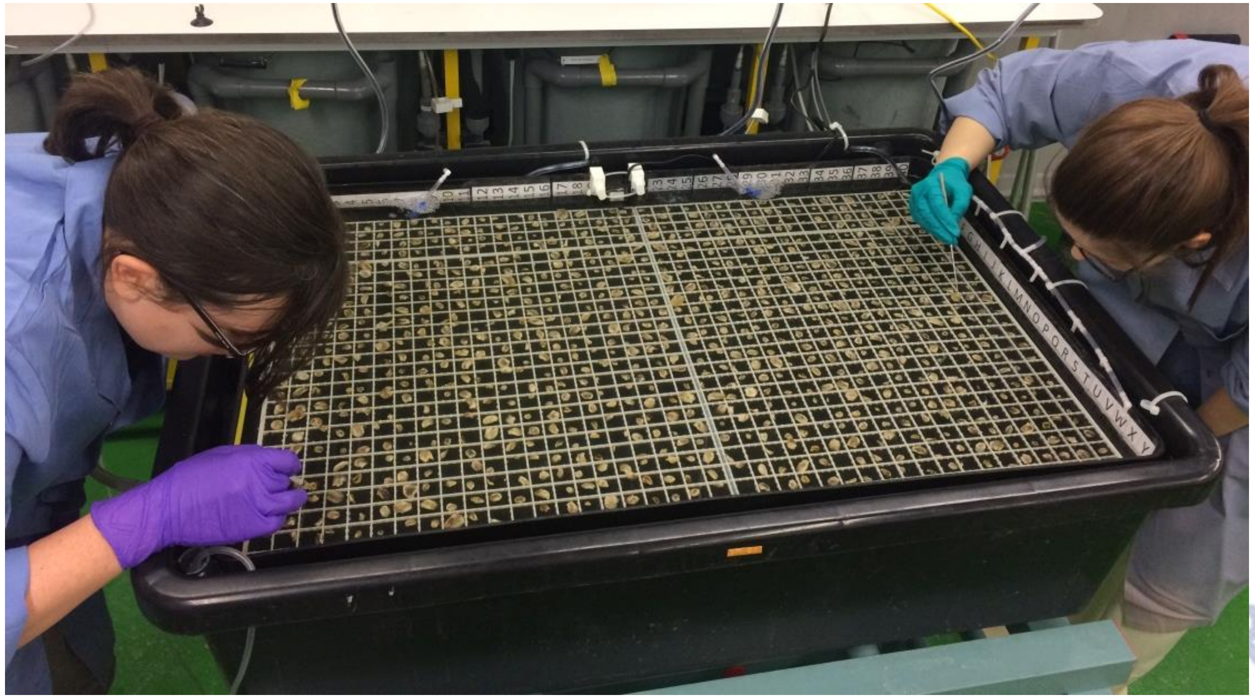


SNP array used to assess population structure

SNPs highly informative in oyster populations around the world

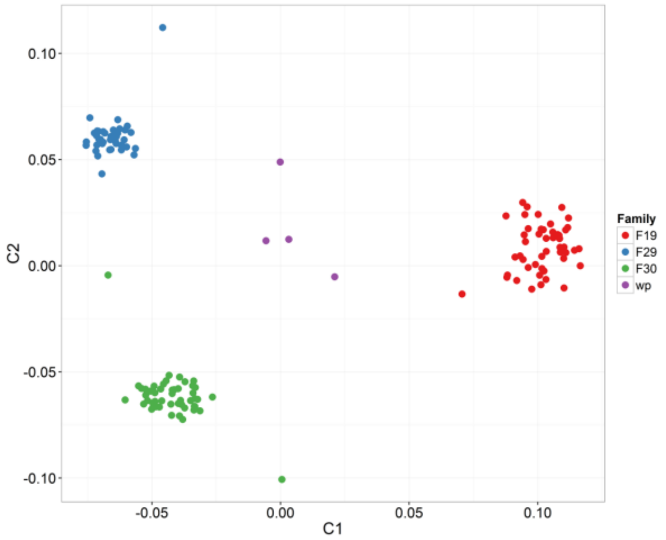
Disease Resistance

- Disease challenge study with Oyster Herpes Virus (OsHV-1)
  - Three large oyster families (each n = 60)
  - Oysters batch spawned from 28 parents (n = 820, family assignment by genotyping)
- Recently settled oyster spat (~2months) bath challenged
  - Single large tank
  - Grid structure
  - Flow-through system
  - Aeration and mixing

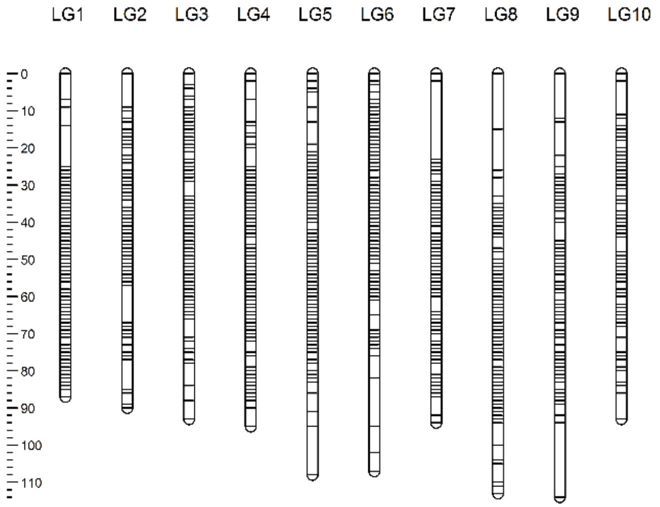


Disease Resistance

- **Samples of mortalities and survivors for genotyping**
  - **Overall mortality rate ~ 20 %**
  - **Genomic DNA extracted from all samples and parents & genotyped for array**
  - **Defining family structure and mapping the oyster genome**



SNP array genotypes can distinguish between families

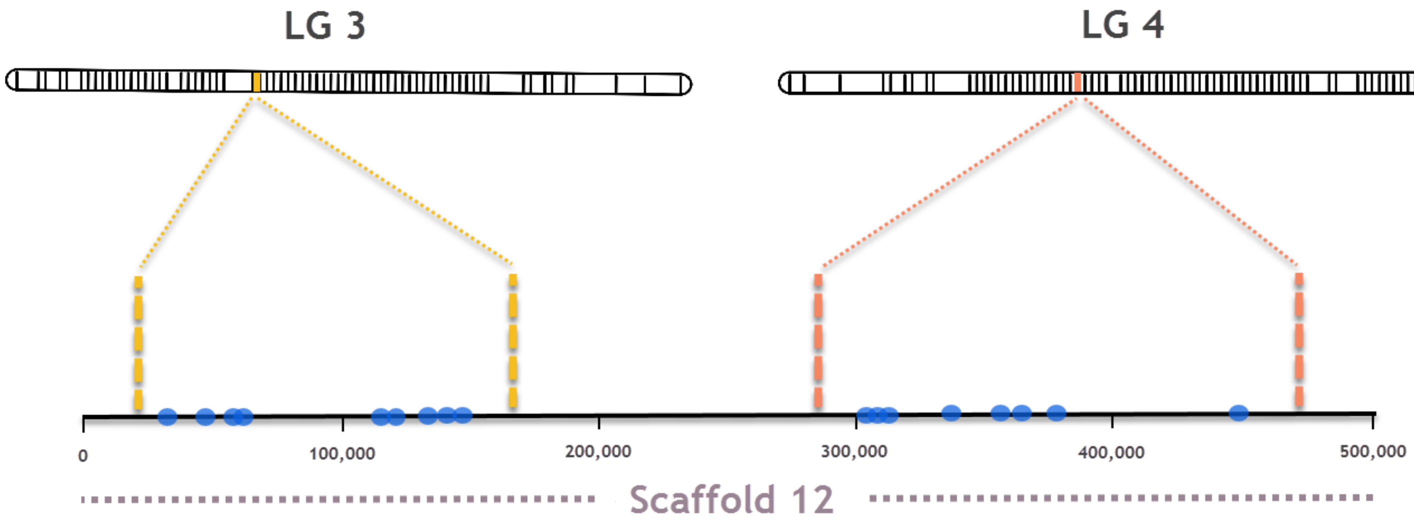


Family material used to build high density (~20K) linkage map of oyster genome

Disease Resistance

- **High density linkage map highlighted issues with *C. gigas* genome assembly**
  - **~40% of scaffolds mapped to >1 linkage group**
  - **Consistency with earlier linkage map of Hedgecock *et al.* (2015)**

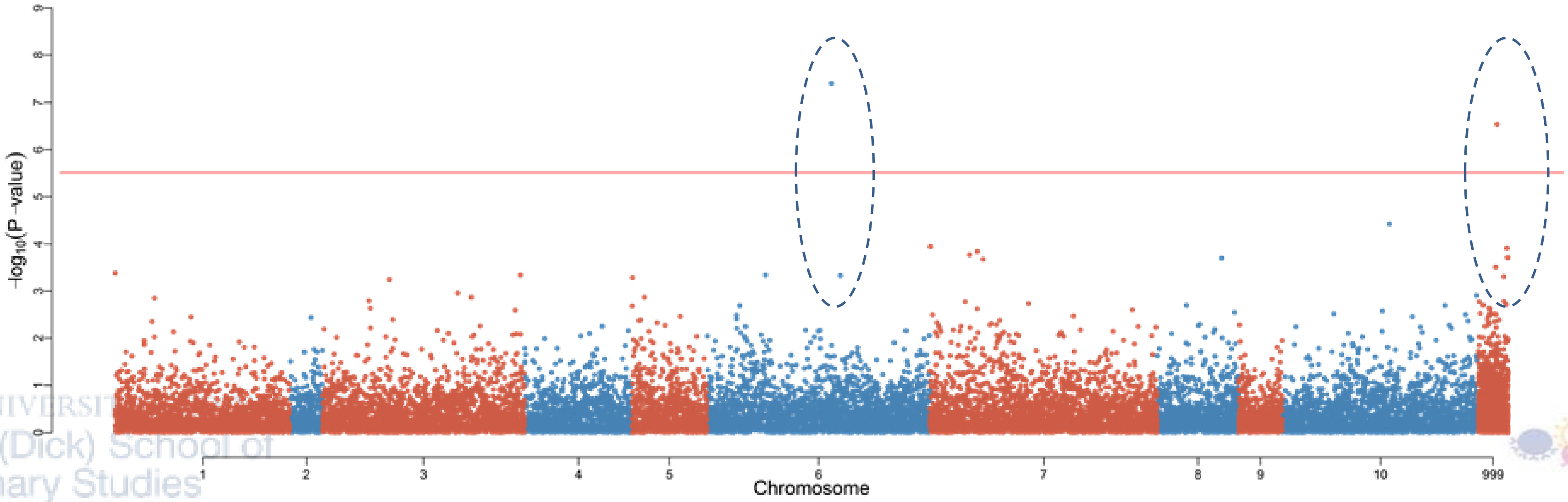
Example of reference genome scaffold mapping to two linkage groups



Disease Resistance

- **GWAS for OsHV resistance in oysters**
  - **Heritability of resistance (underlying liability scale) = 0.25**
  - **Individual loci (QTL) affecting resistance to herpes virus**
    - **Both survival and viral load affected by gene(s) on Chromosome 6**
    - **Candidate gene on Chr6: *RAN Binding Protein 9-like gene* (Interferon γ signalling)**

Genome-wide association study for OsHV survival



Disease Resistance

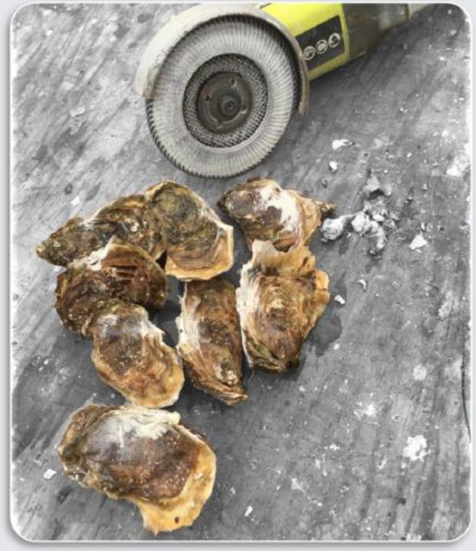
- **Application in UK oyster hatchery production**
  - **Sampling and testing broodstock oysters using targeted low density SNP panel**
  - **Avoidance of inbreeding and selection for resistance markers**

(i)

Oyster tagged

- Dorsal section of left valve ground away
- Each oyster tagged with unique identifier

Tissue biopsied



- Loose shell removed from anterior side of oyster.
- Tissue removed through the small gap between valves.
- Most oysters recover in 2-3 days. Some mortalities (<5%).

Disease Resistance

- **Application in UK oyster hatchery production**
  - **Sampling and testing broodstock oysters using targeted low density SNP panel**
  - **Avoidance of inbreeding and selection for resistance markers**

(ii)

SNP panel analysed

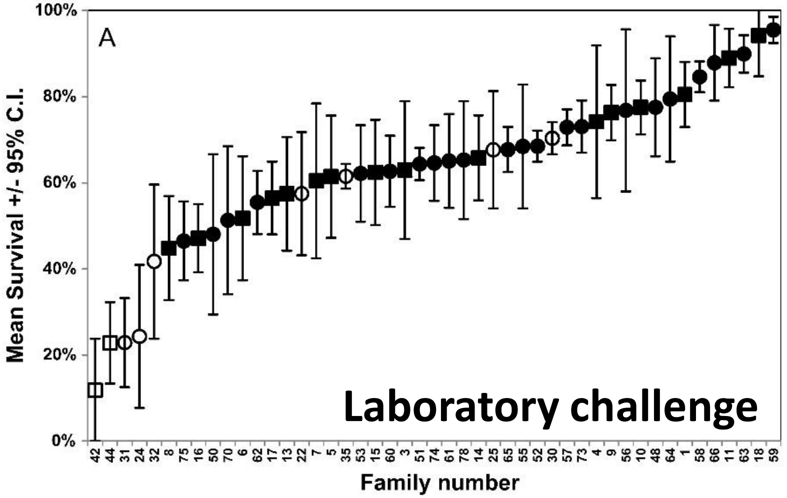
- 96 SNPs analysed through allele specific PCR.
- Data transferred to hatchery and to geneticists.

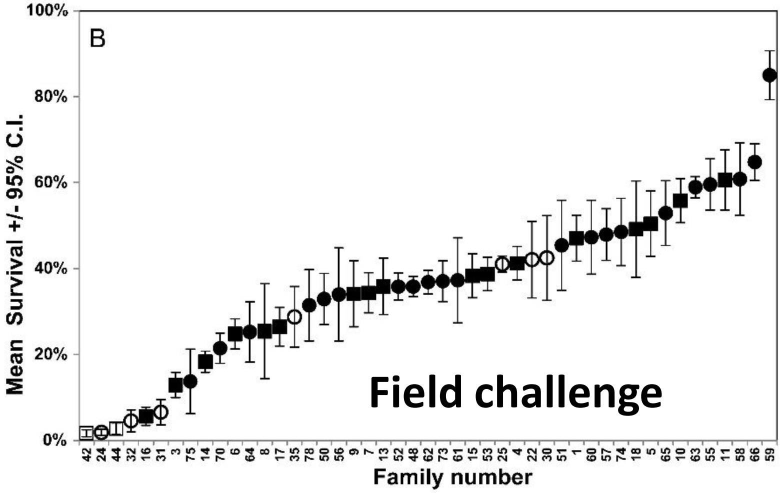
Breeding strategy designed

- Breeding patterns recommended.
- Commercial spat production continues.
- Next generation of broodstock produced.

Disease Resistance

- **OsHV resistance in an independent population**
  - 31 families OsHV challenged at Cawthron in 2015
  - Based on challenge model described in Camara et al. 2017, Aquaculture, 469:50-58 (validated in field)
  - Genotyped using SNP array (n~800): ~24K informative *C. gigas* SNPs
  - Animal model, probit link function (binary survival)
  - Heritability estimate for OsHV resistance in genotyped animals (using G Matrix) = 0.44 (0.05)
  - GWAS and genomic prediction analyses (ongoing)

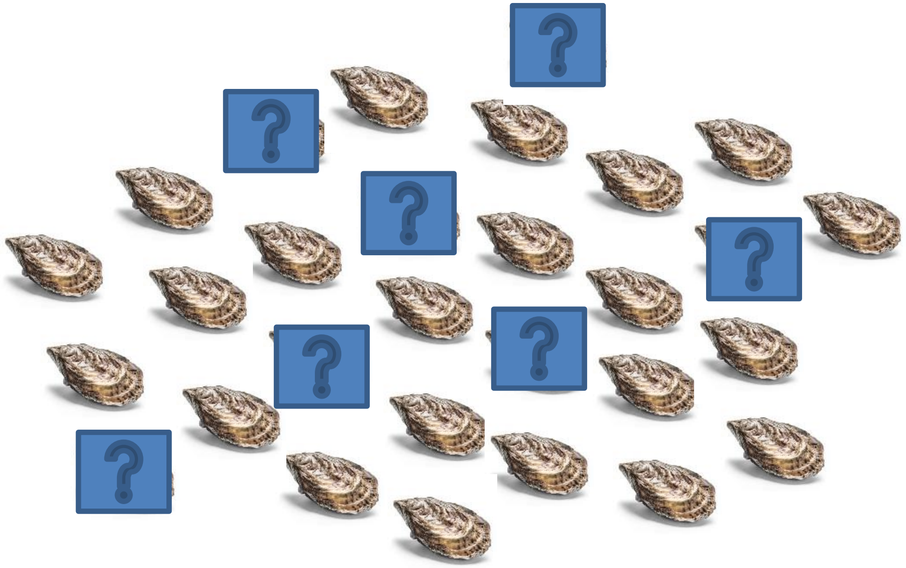




Genomic selection

- **Testing genomic prediction of herpesvirus resistance in oysters**
  - **Split population into training (80%) and validation (20%) sets**
  - **Mask the phenotypes of the validation sets**
  - **Use the genomic prediction model trained on the 80% to predict the 20% (GBLUP)**
    - **Repeat several times (5-fold cross validation), adjusted correlation (r) between estimated breeding value and trait value of masked individuals**

Sampled oysters with phenotypes and SNP array genotypes



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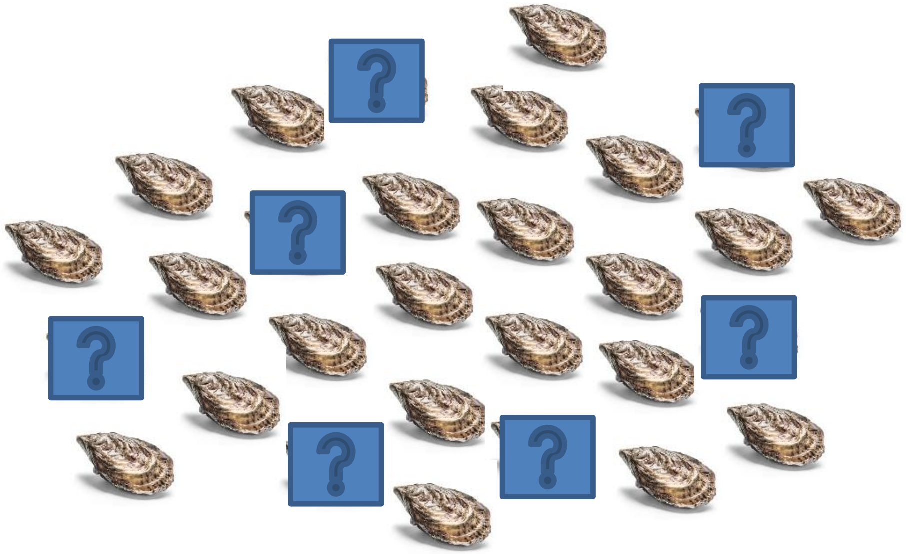

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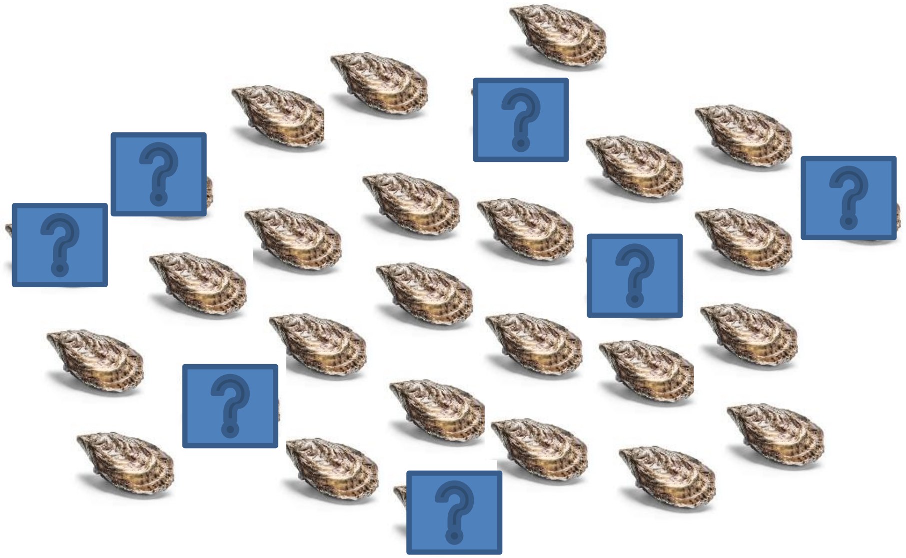
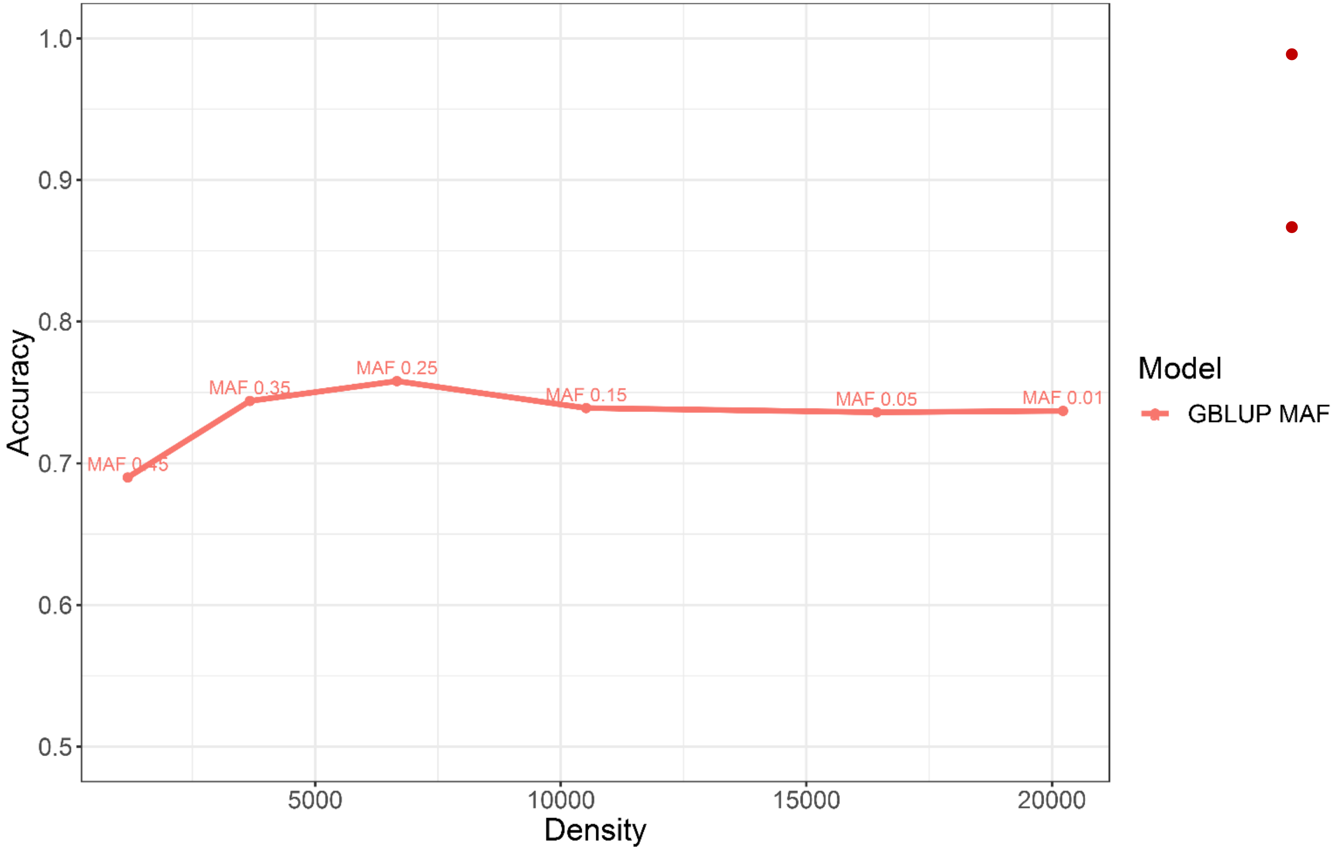


Genomic selection

- **Testing genomic selection for selective breeding in oysters**
  - **Genomic prediction (GBLUP) accuracies with varying SNP densities**
  - **Prediction accuracy substantially higher than with pedigree (pedigree h2 lower)**



- **Low SNP density (<1000 SNPs) = improved prediction accuracy**
- **Promising for cost-effective genomic prediction in oyster breeding**

Conclusions / Discussion

- **Combined species 50K SNP array for *C. gigas* and *O. edulis***
  - **Highly informative in *C. gigas* populations tested across the globe**
  - **Effective for detecting population and family structure**
- **Oyster Herpes Virus resistance is controlled by several QTL**
  - **Moderately heritable oligogenic trait**
  - **Promising markers / genomic regions for further study**
- **Genomic prediction effective with low marker density**
  - **Markedly higher prediction accuracy than pedigree, even with <1,000 SNPs**
  - **Potential for combined parentage / genomic prediction panel?**

Acknowledgements

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Natalie Lowe
Carolina Penaloza
Steve Bishop

Cefas

Mike Gubbins

edinburgh genomics

Karim Gharbi
Richard Talbot

Guernsey Sea Farms: Mark Dravers

CSIRO

James Kijas
Sean McWilliam
Curtis Lind
Peter Kube

Institute for East China Sea Research

Kiyoshi Soyano

Queen's University Belfast

Paulo Prodohl

Ifremer

Lionel Dégremont
Sylvie Lapegue
Jean-Baptiste Lamy
Pierre Boudry
Tristian Renault

SYSAAF: Pierrick Haffray

University of Southern California

Dennis Hedgecock

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