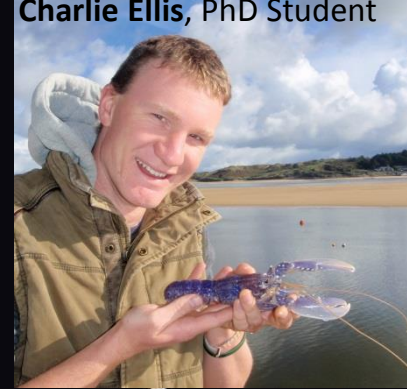


Paternity and population genetics of the lobster in Cornwall

- University of Exeter collaborating with the National Lobster Hatchery, Padstow
- Stock enhancement of the European lobster, *Homarus gammarus*





Overview

- Rationale
- Microsatellites

- Paternity
- Population structure
- Implications







- Coastal NE Atlantic range, including Mediterranean



- Extensive trap fishery
 - >75% of landings UK/Ireland
 - 370 Cornish vessels; mostly <10m
 - Large continental export value
 - Scandinavian stock collapse



- Genera relatively well studied, but mostly from *H. americanus* and still considerable understanding gaps



- Need knowledge of molecular, reproductive and population ecology to conserve species and fisheries

Hatchery stocking and genetics



- Release of hatchery-reared juveniles to supplement or restore natural fisheries
- Overcomes recruitment bottleneck of planktonic larval phase in captivity
- Undertaken in UK, Ireland, Norway, Germany, France, Spain and Italy
- National Lobster Hatchery in Cornwall released 60K juveniles in 2014

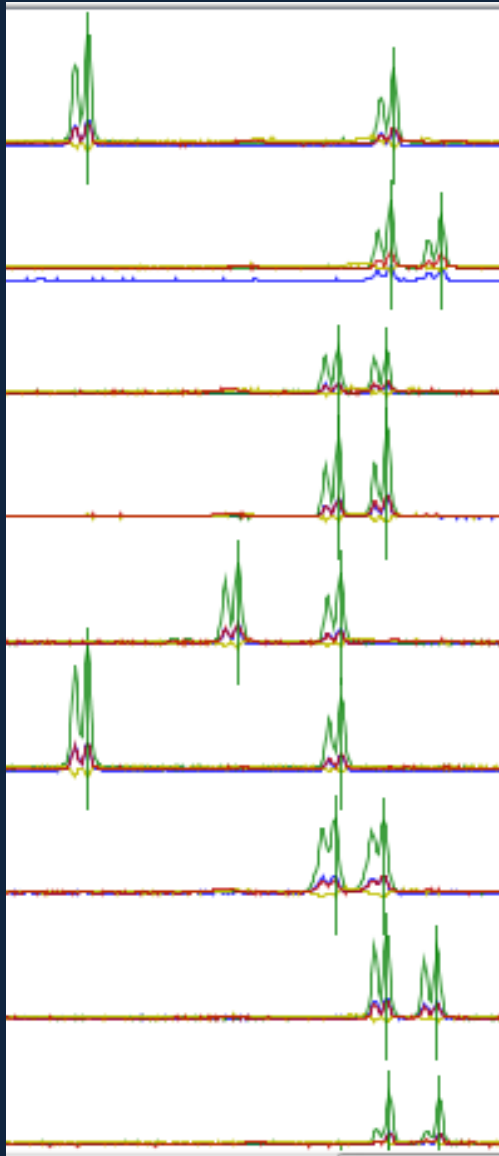
- Increased awareness of need for genetic management in stocking wild populations
- Negative impacts in other species (e.g. inbreeding depression, domestic selection, loss of diversity/adaptation/structure/fitness)
- Genetic tools > risk assessment / mitigation
> reference for genetic impact
> tagging via parentage
> ecology and management



Microsatellite DNA genotyping

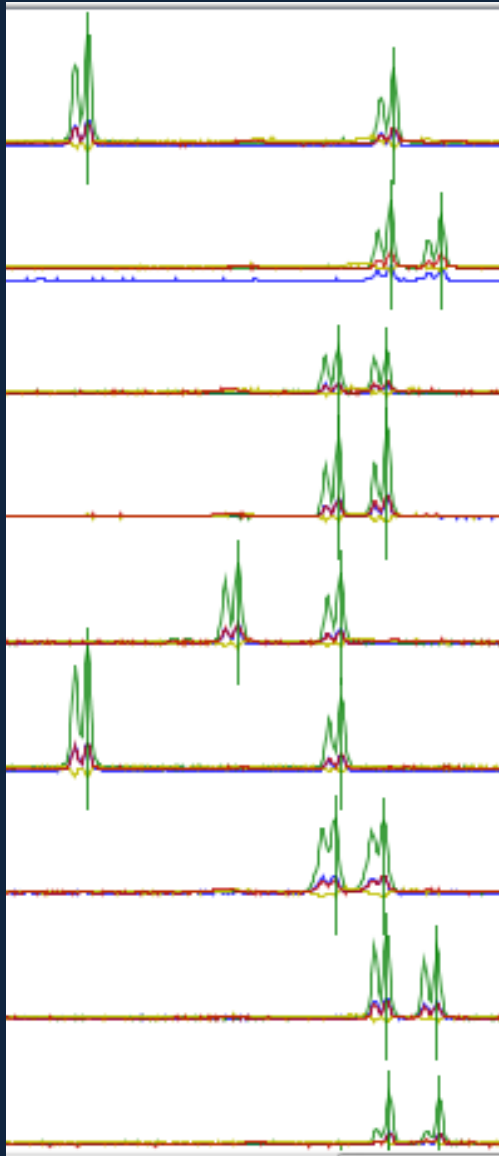
- Variable regions of DNA
- Bi-parental inheritance
- Sections vary in length
- Can be amplified with fluorescent tags to measure
- Powerful tool to discriminate individuals and populations when combined

Microsatellite DNA genotyping



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One microsatellite locus



Microsatellite DNA genotyping

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One microsatellite locus

Lobster 1

Lobster 2

Lobster 3

Lobster 4

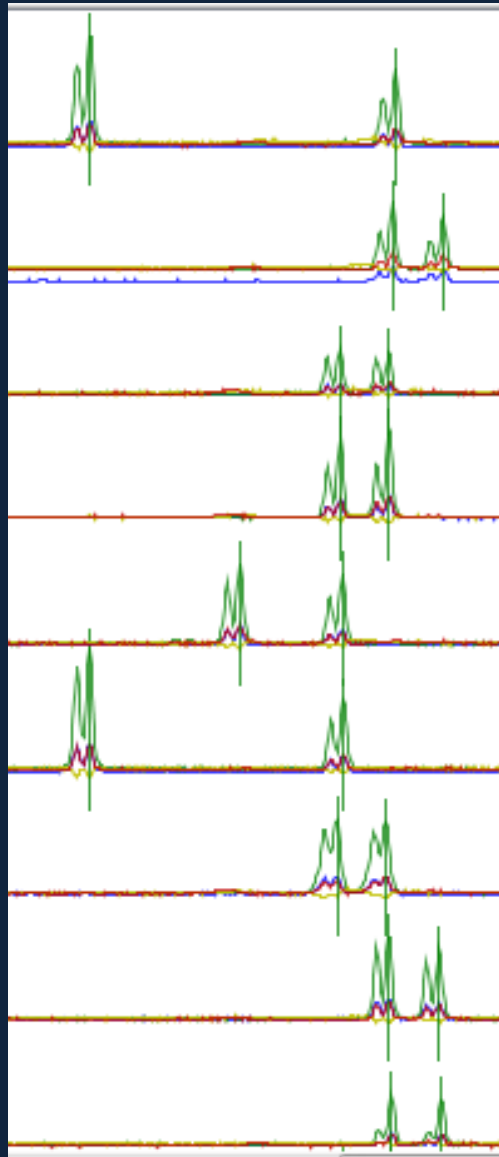
Lobster 5

Lobster 6

Lobster 7

Lobster 8

Lobster 9



Microsatellite DNA genotyping

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- Bi-parental inheritance
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One microsatellite locus

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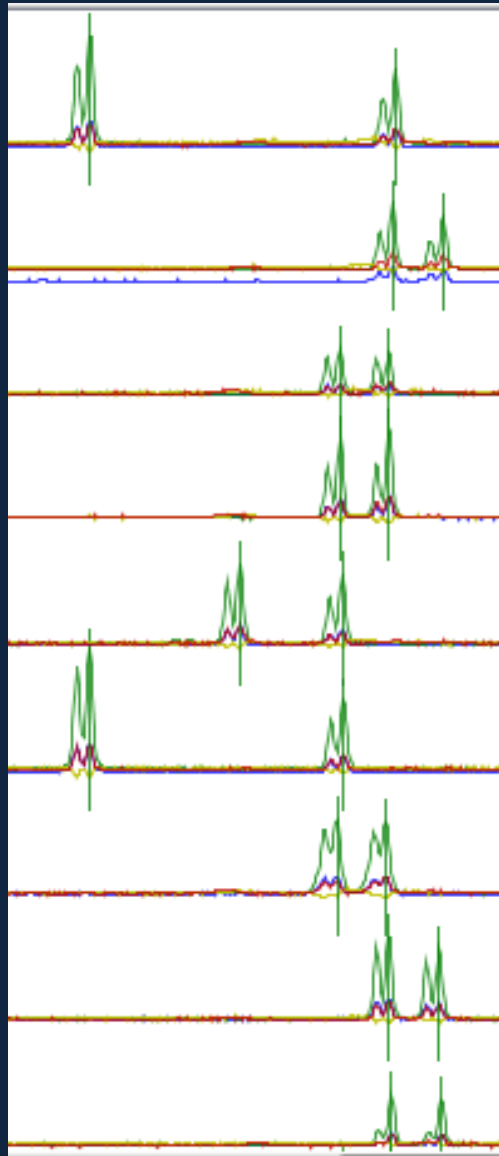
Lobster 5

Lobster 6

Lobster 7

Lobster 8

Lobster 9



Alleles

↑
1

↑
2

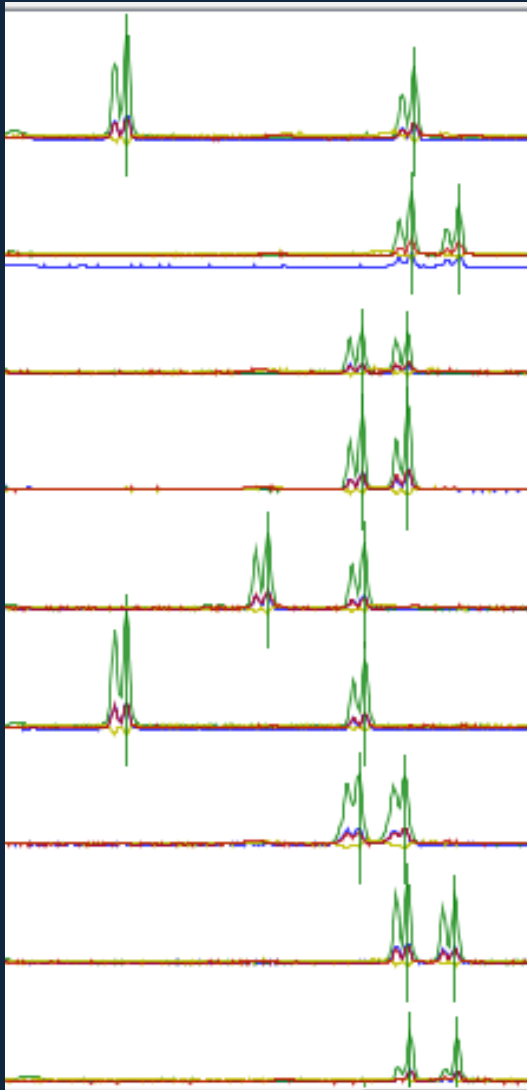
↑
3

↑
4

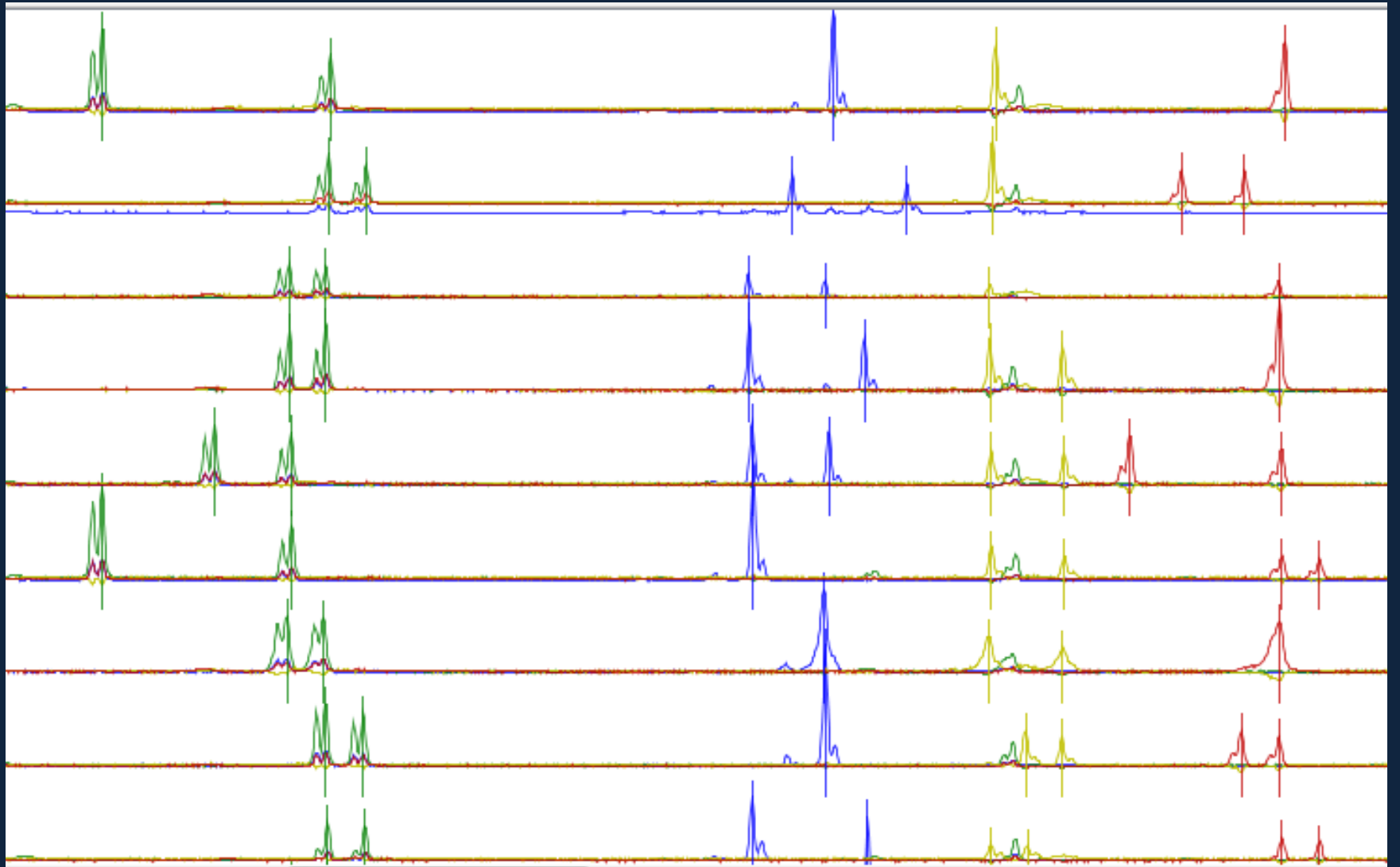
↑
5

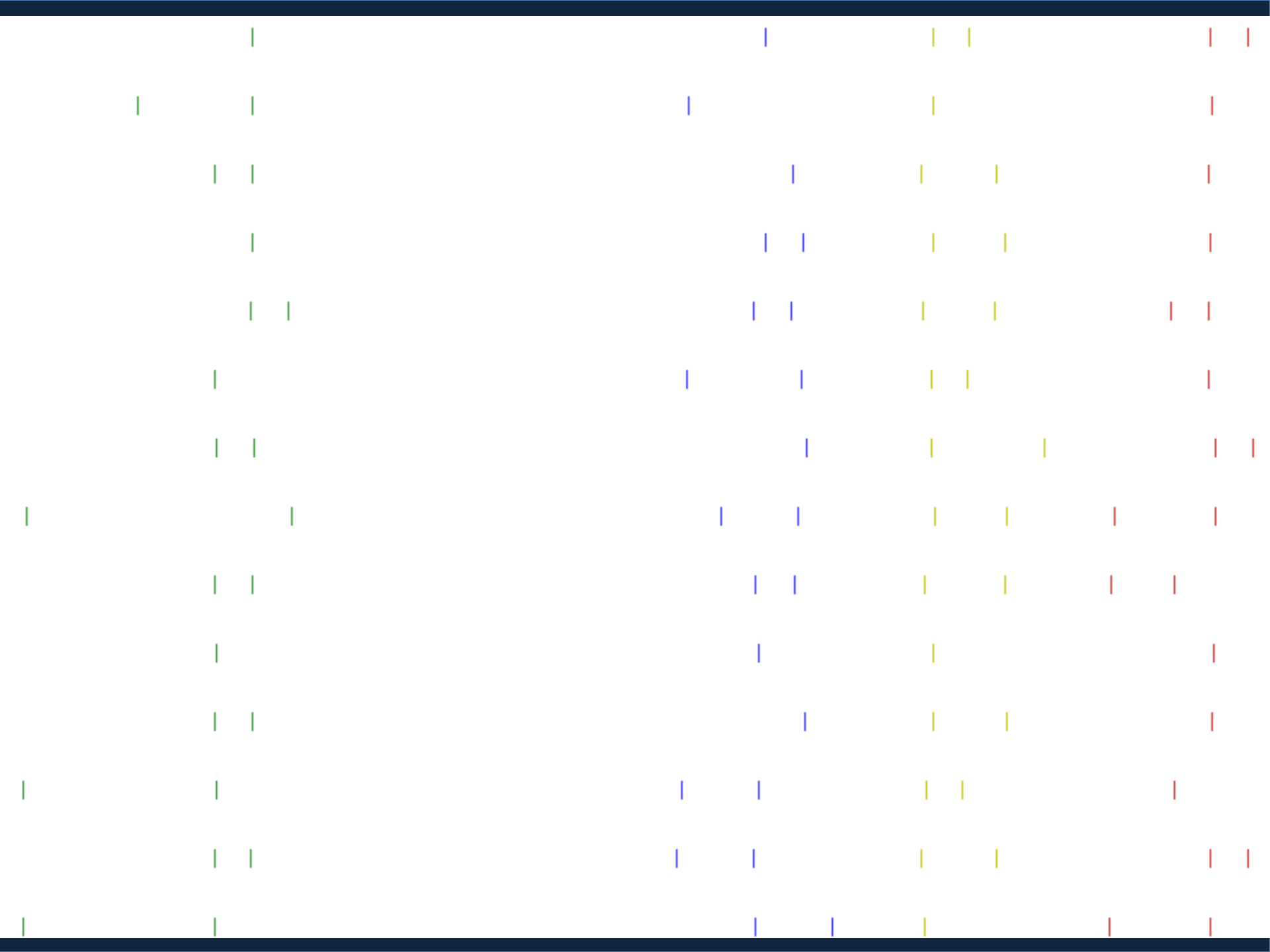
Microsatellite DNA genotyping

- Variable regions of DNA
- Bi-parental inheritance
- Sections vary in length
- Can be amplified with fluorescent tags to measure



- Genotype at one locus isn't very insightful
- More loci = greater resolution
- Powerful tool to discriminate individuals and populations





	A	B	C
2	Sample	Population	All Alleles
3	POP1	POP	
4	IoS1	Scilly Isles ,	156156 284284 212236 274278 240240 162186 258258 289289 295299 182186 239243 192216 239247 291293
5	IoS9	Scilly Isles ,	152156 284284 224232 274278 236248 186190 258258 277285 303303 174186 239275 188188 255267 285293
6	IoS17	Scilly Isles ,	152156 284284 212212 274274 232240 182186 258258 289289 303303 174186 243275 188192 259267 289297
7	IoS25	Scilly Isles ,	144164 284284 232236 274278 232244 182186 258266 289289 295299 174174 275275 188196 247263 289301
8	IoS10	Scilly Isles ,	144156 276284 212212 274274 232232 162182 258266 289293 303303 178186 239263 200204 251263 279301
9	IoS18	Scilly Isles ,	152152 276284 212232 274274 240240 182186 258266 289289 303307 178186 251275 188204 263267 293305
10	IoS26	Scilly Isles ,	156156 276276 212212 254274 240240 186190 262266 285289 299311 178186 235239 216216 239247 293297
11	IoS11	Scilly Isles ,	140164 284284 212232 274282 244244 182186 258258 289293 299303 174182 239239 192200 263267 287293
12	IoS19	Scilly Isles ,	156160 276280 212216 278278 240244 186186 258262 289289 295303 178186 239239 192200 251251 293299
13	IoS4	Scilly Isles ,	152152 276284 192212 274278 248248 186186 250258 289289 303303 178182 239275 188204 259267 285297
14	IoS12	Scilly Isles ,	152156 280284 212212 274278 236244 186186 258258 289289 295303 174174 275275 200200 243247 297301
15	IoS20	Scilly Isles ,	140156 276284 224232 274274 240240 182182 254258 277289 303303 182186 239279 200208 267267 297297
16	IoS13	Scilly Isles ,	156164 284284 192212 274278 240244 186186 258266 289289 295303 186186 239239 188216 243251 281297
17	IoS21	Scilly Isles ,	152152 276288 232236 274274 232244 174186 258270 289289 291299 178186 223235 188192 235243 297301
18	IoS6	Scilly Isles ,	152152 280284 212232 274282 240240 182186 250266 289289 295303 186186 223223 192192 247259 283305
19	IoS14	Scilly Isles ,	152156 276292 212216 274274 240248 182186 258258 285289 295303 178186 239239 188208 251263 289297
20	IoS22	Scilly Isles ,	144156 276284 212232 274274 232240 186186 258266 289289 283303 174186 239275 188200 263267 297305
21	IoS7	Scilly Isles ,	148156 284292 212212 254274 240240 182186 258258 289289 299303 166186 251275 200208 259267 283289
22	IoS15	Scilly Isles ,	152164 276284 212212 278278 232244 174182 266270 285289 303303 178182 239239 188192 255263 281295
23	IoS23	Scilly Isles ,	152156 284296 212212 262278 240240 186186 266266 289289 303303 178182 275275 188216 255275 297297
24	IoS8	Scilly Isles ,	156156 272284 212232 254274 236244 162186 258258 289289 299303 186186 239275 188212 243255 289293
25	IoS16	Scilly Isles ,	156160 276284 212212 254278 232248 182186 258266 289289 299307 178186 239275 184188 243263 285297
26	IoS24	Scilly Isles ,	140156 284284 212232 254274 232236 162186 258258 289289 303307 166178 239239 188216 247263 249249

= Multi-locus
genotypes

Dynamic paternity in lobsters



$$\text{♂} = 1$$

or

$$\text{♂} = >1$$



Molecular Ecology (2005) 14, 1517–1525

doi: 10.1111/j.1365-294X.2005.02498.x

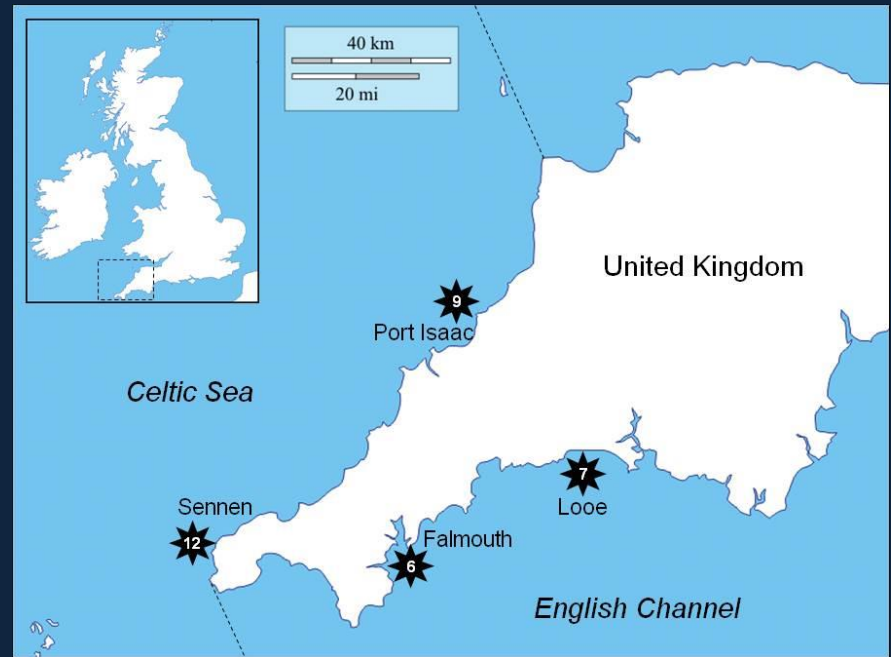
Geographic variation of multiple paternity in the American lobster, *Homarus americanus*

THIERRY GOSSELIN,*† BERNARD SAINTE-MARIE† and LOUIS BERNATCHEZ*

*Québec-Océan, Département de Biologie, Université Laval, Québec, QC, G1K 2P4, Canada, †Direction des invertébrés et de la biologie expérimentale, Institut Maurice-Lamontagne, Pêches et Océans Canada, 850 route de la mer, C. P. 1000, Mont-Joli, QC, G5H 3Z4, Canada

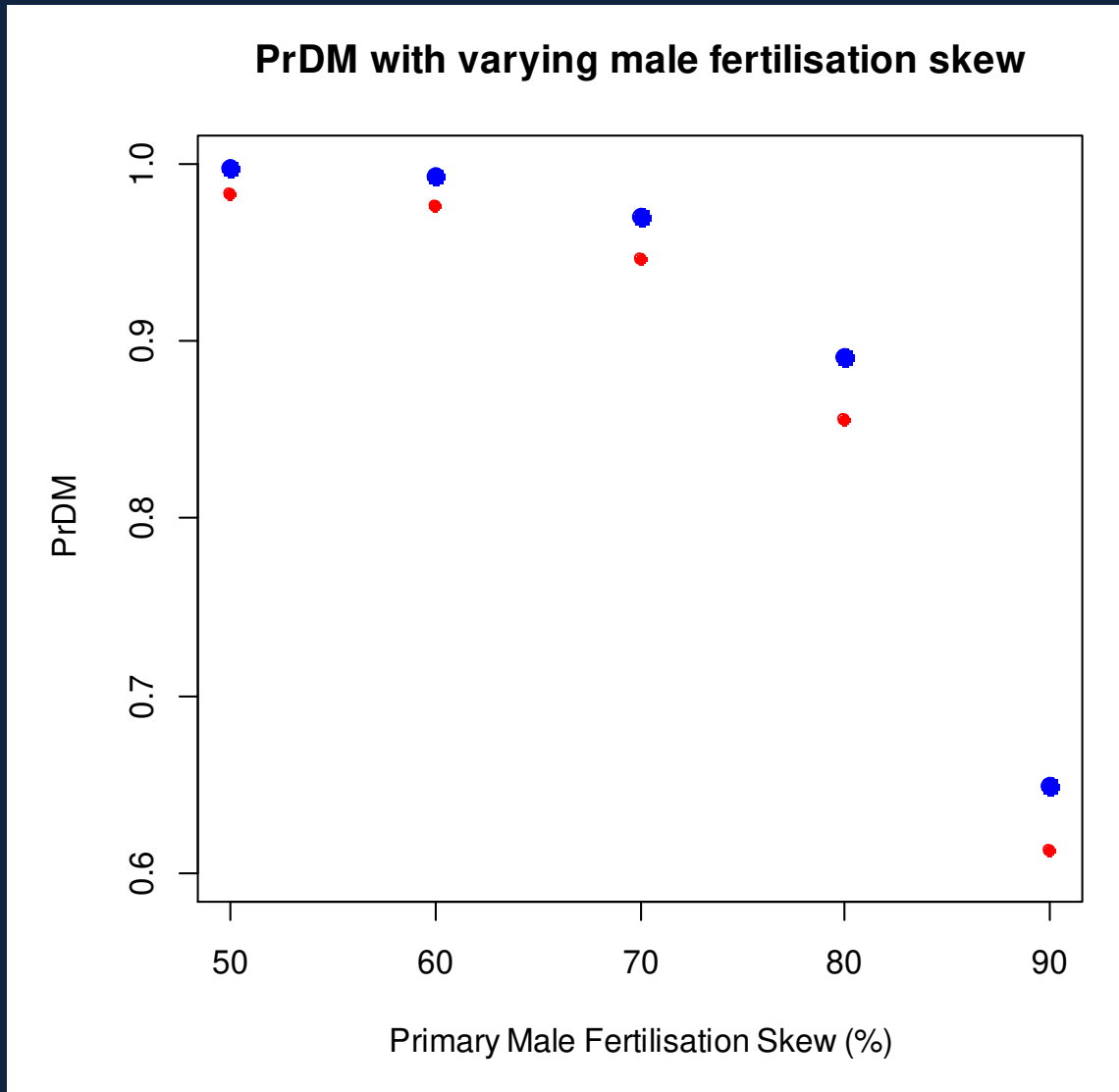
- Single paternity is regular in *H. americanus*, but multiple paternity detected and exhibits spatial variation in frequency
- Correlates to exploitation rate
- Does overfishing cause sperm limitation or breakdown in male hierarchy?
- Could paternity be a reference point?
- Can sex-biased management work?

Paternity assessment



- Genotyping maternal and progeny tissues to reconstruct paternal contribution
- 34 females across two sites on each coast
- 10 eggs per female, spatially sampled
- Genotyped at 13 microsatellite loci

PrDM and fertilisation skew



- PrDM diminished by skews of male fertilisation success
- PrDM
>0.99 when ♂ 50:50
>0.95 when ♂ 74:26
=0.65 when ♂ 90:10
- Even at highest skew (90:10) and least frequent incidence (11%) in *H. americanus*, expect three occurrences of multiple paternity (with one missed)
- Not a big reduction in PrDM with only 3 most informative loci

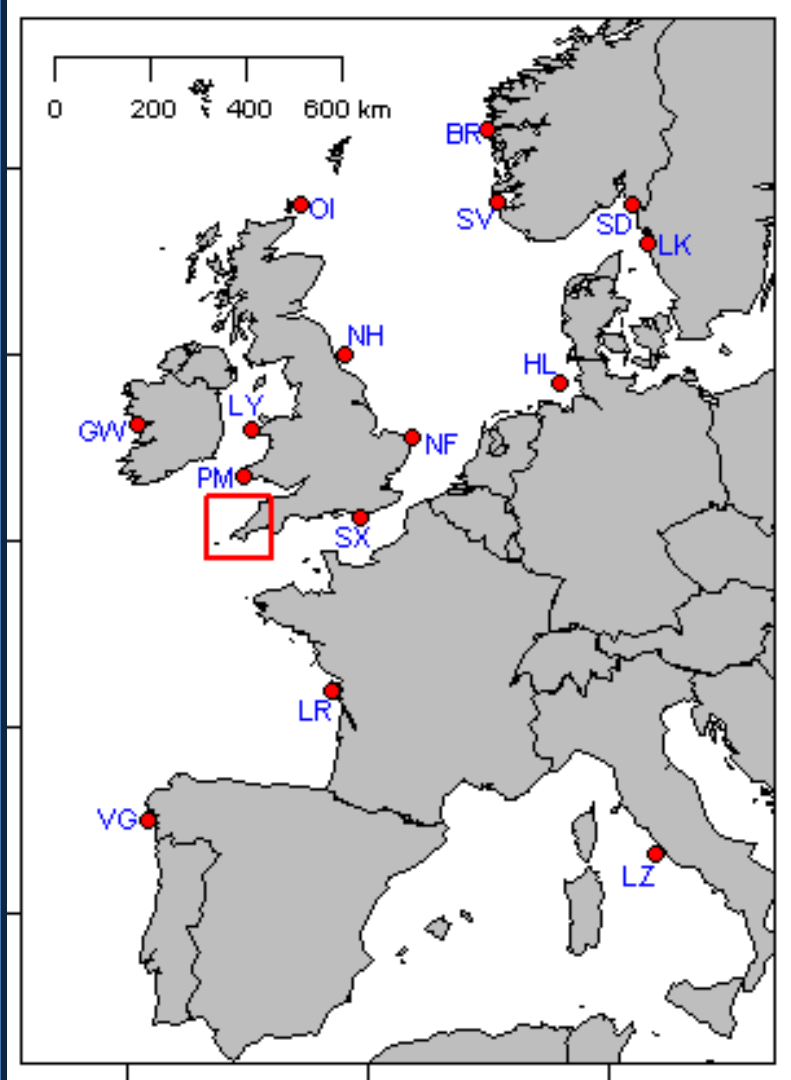
Single paternity in Cornish lobsters



- For all 34 females, one paternal reconstruction explained all progeny
- 34 different males
- Reconstruction method conservative, but heterozygosity of males matched females, suggesting single paternal contributions not underestimated
- Multiple paternity likely to be absent, or rare with fertilisation highly skewed in favour of a single male
- Mechanism preserving $\sigma=1$ is presumed to be monandrous mating, but post-copulatory processes too?
- Need to check other locations to test whether fishing pressure / demographic differences disrupt single paternity in *H. gammarus*

- Fine-scale – within Cornwall
- Broad-scale – across Europe
- Important application to spatial range of hatchery stocking

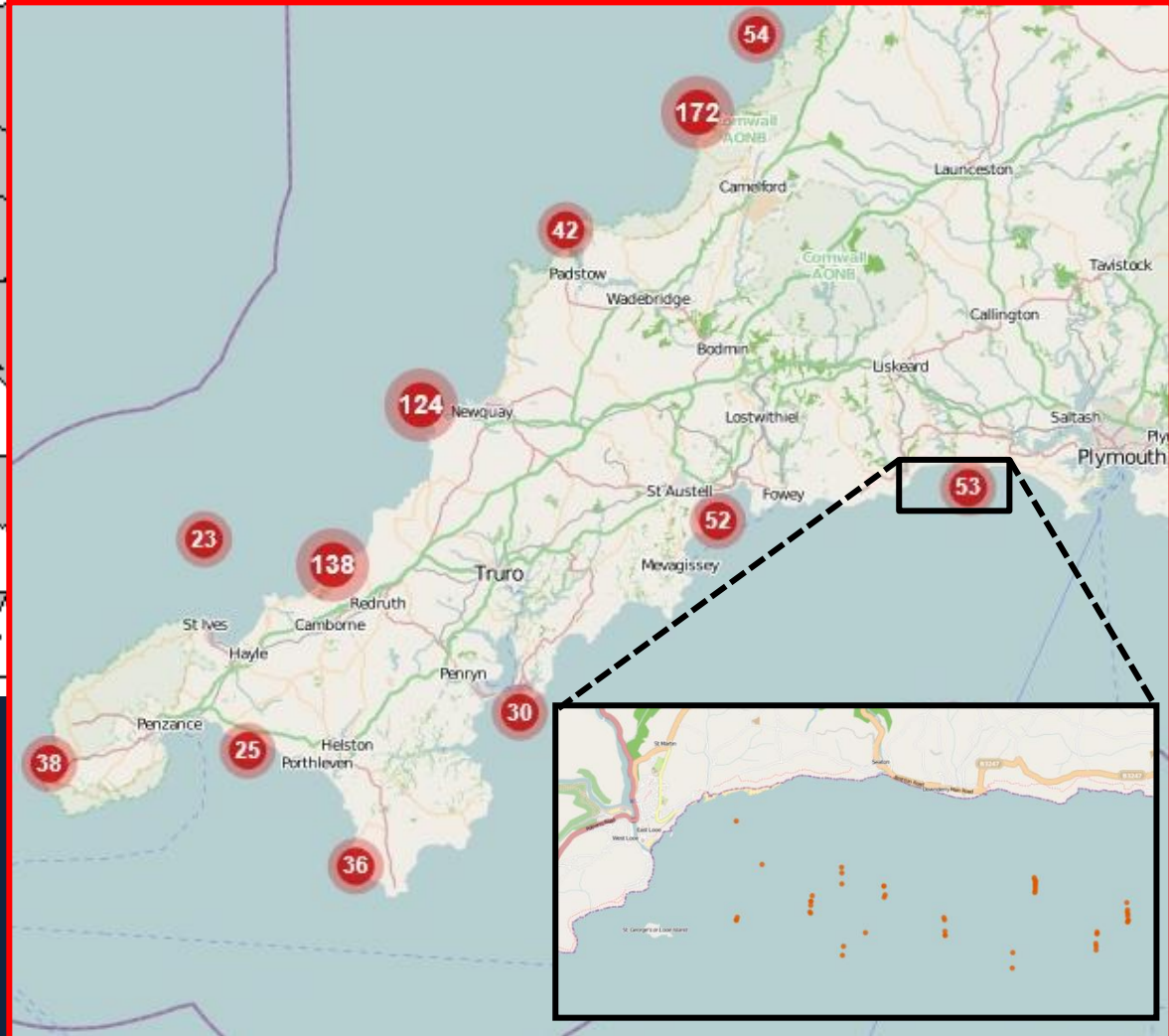
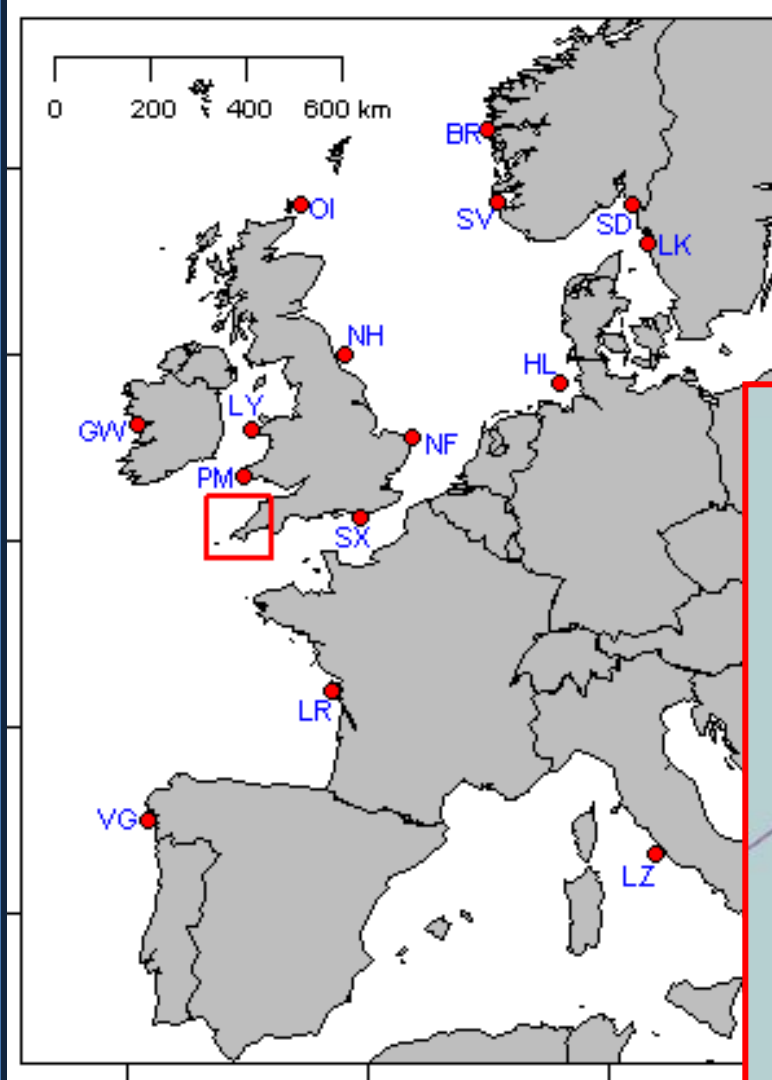
**Population
genetic structure**



- Fine-scale – within Cornwall
- Broad-scale – across Europe
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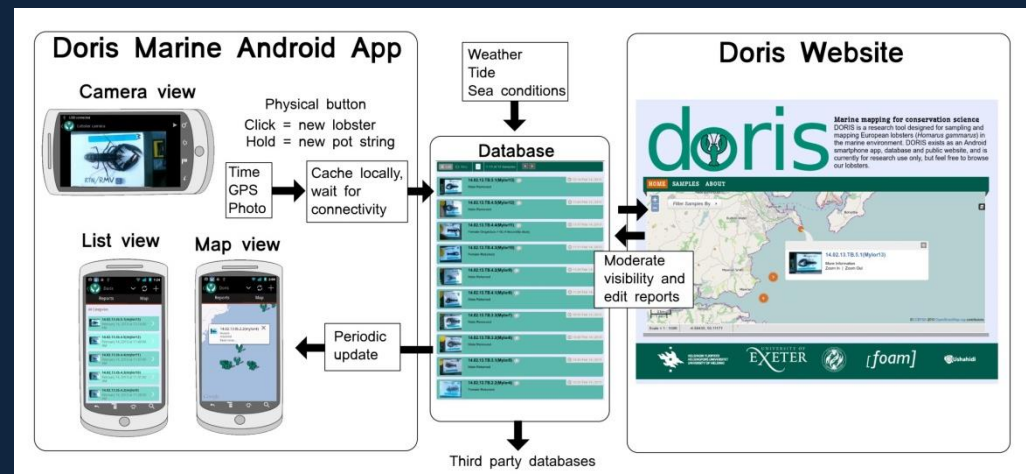


**Population
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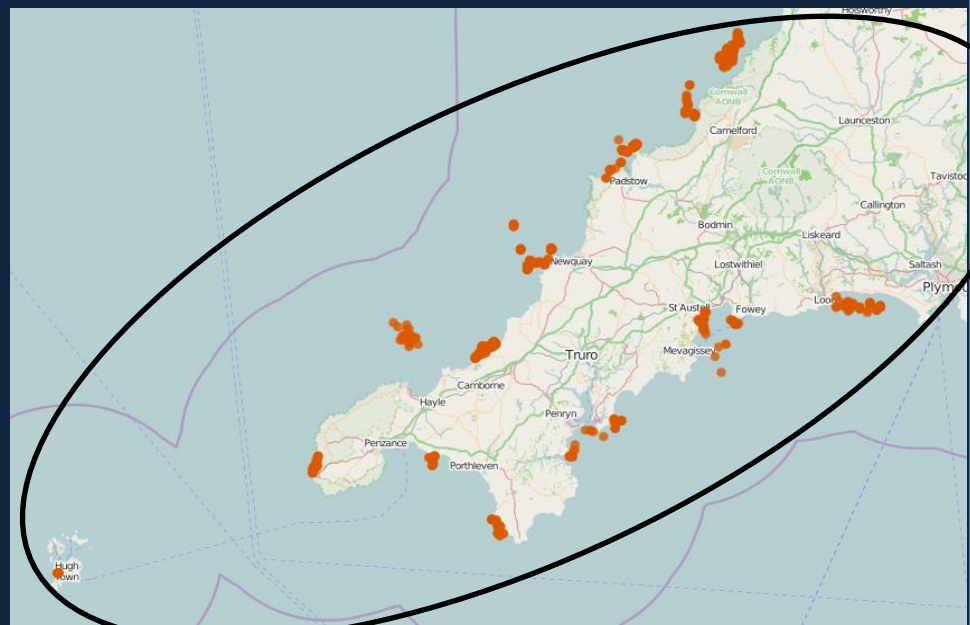
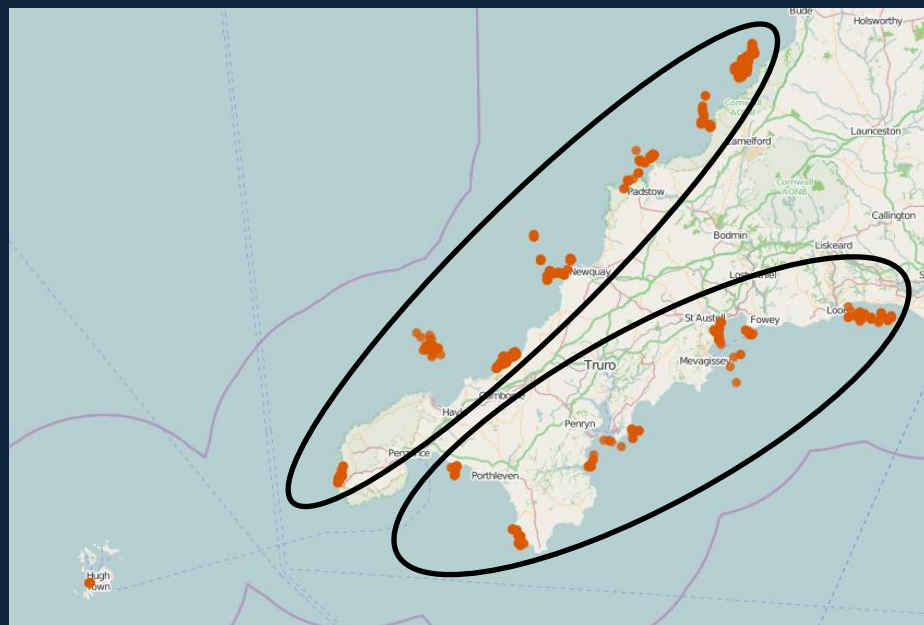
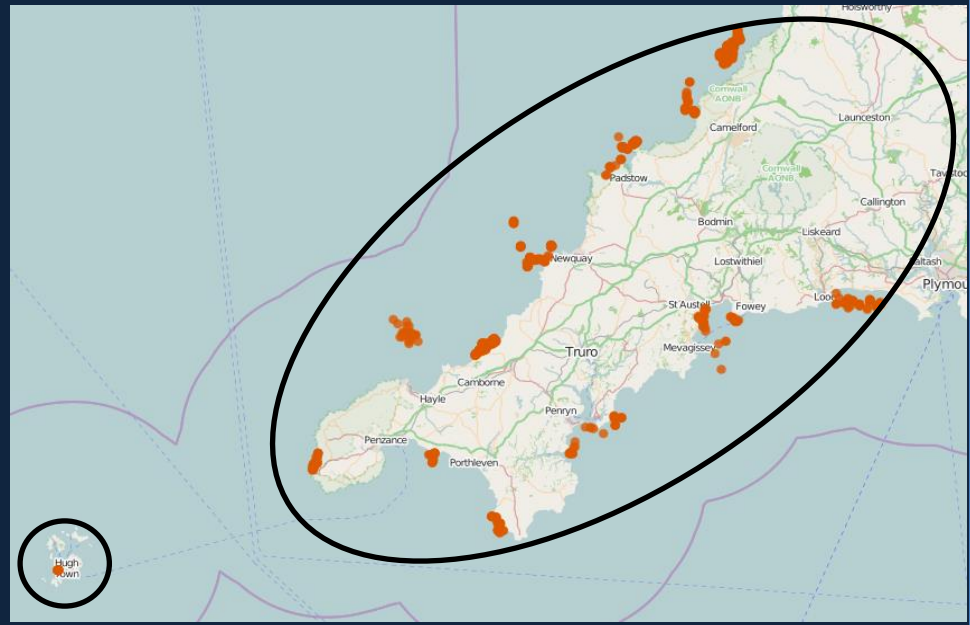
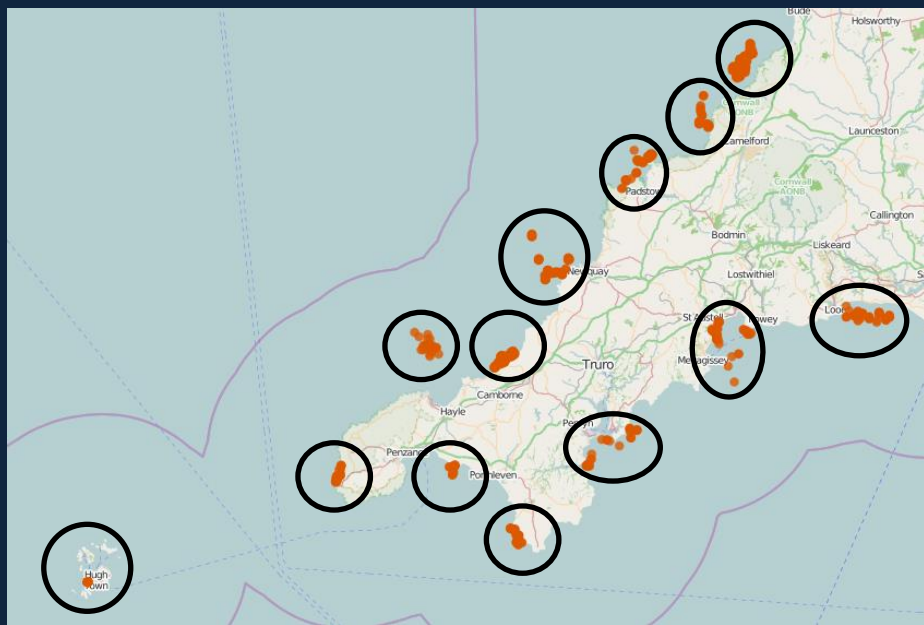
Population sampling

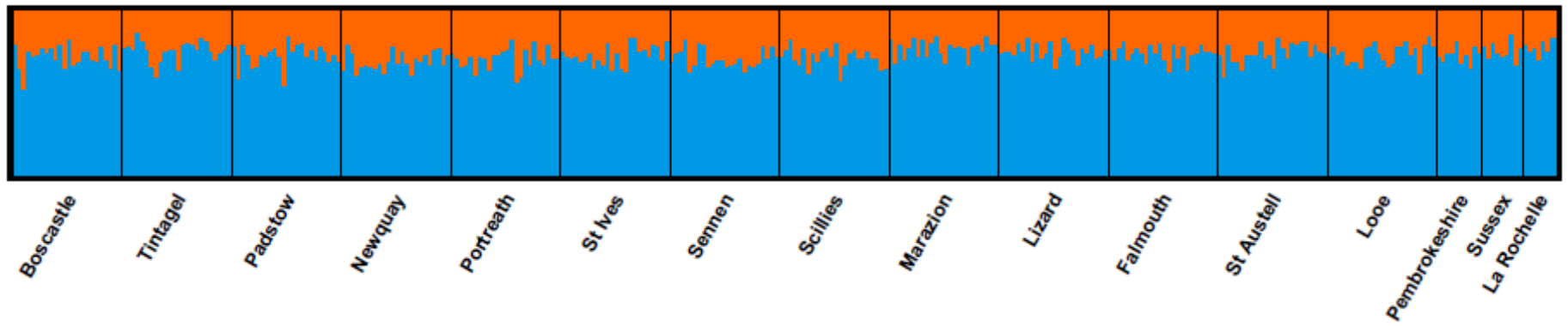


- Multi-locus genotypes (13 μ sats) for
 - 312 fine-resolution Cornish samples
 - 300 samples of European outgroups
- Unique alleles and differences in allele frequencies used to measure genetic diversity and estimate gene flow
- Search for population structure at local and continental scales

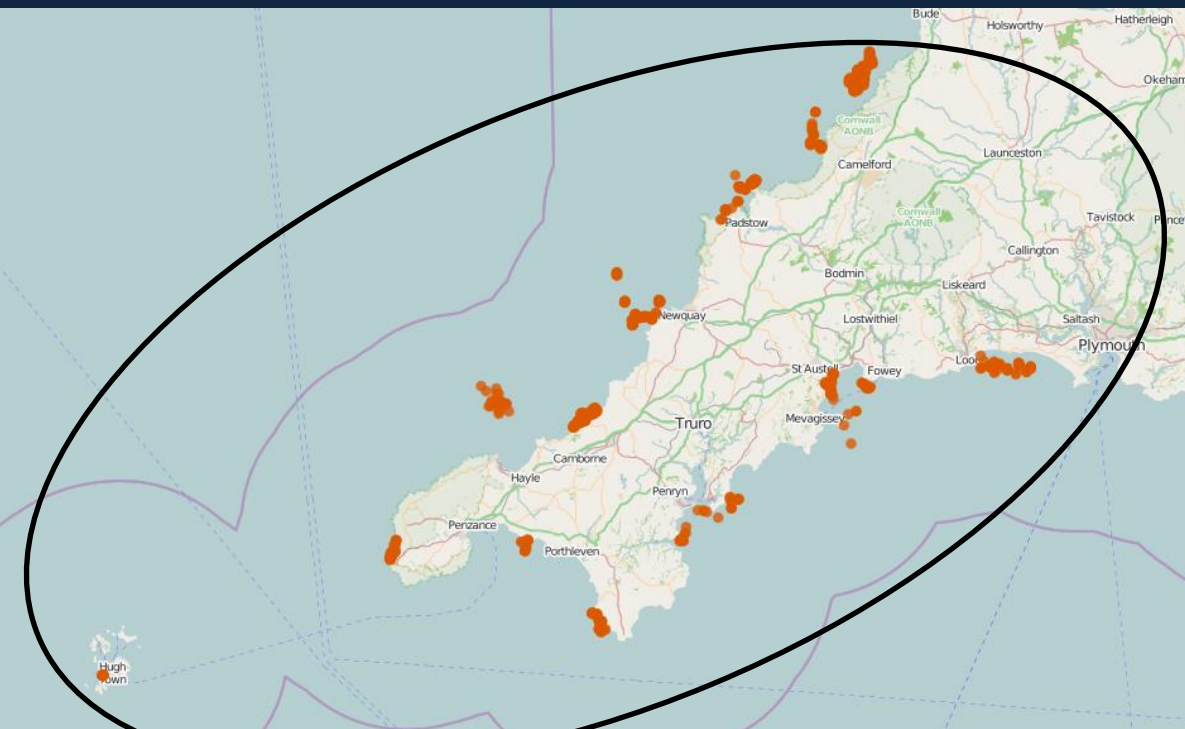


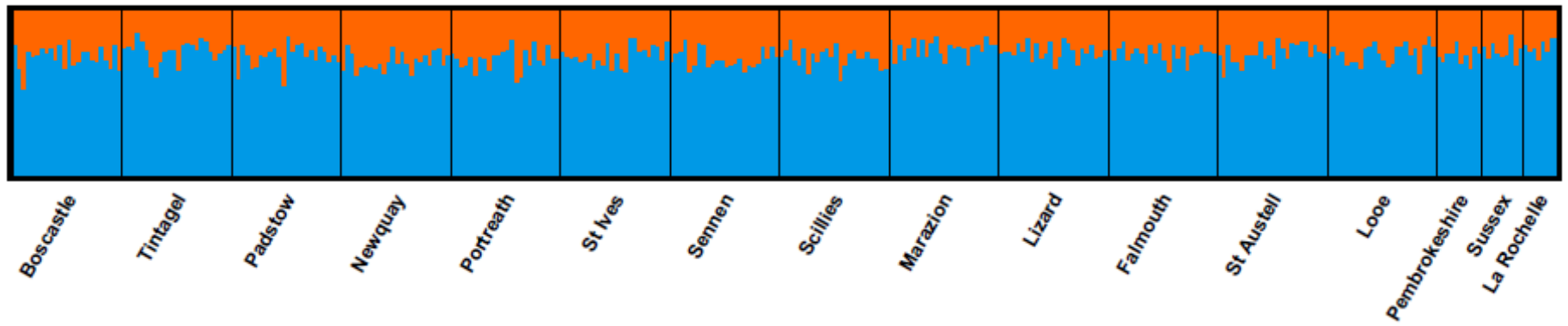
Fine-scale structure in Cornwall





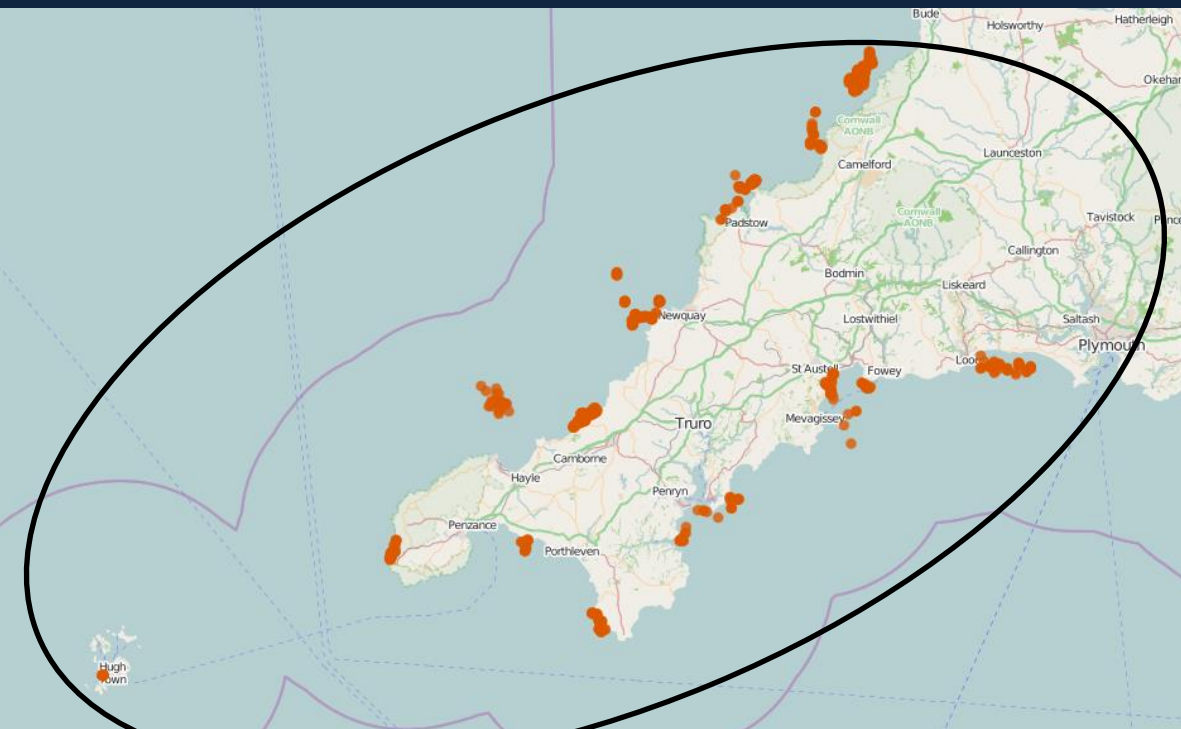
○ No evidence of regional sub-populations



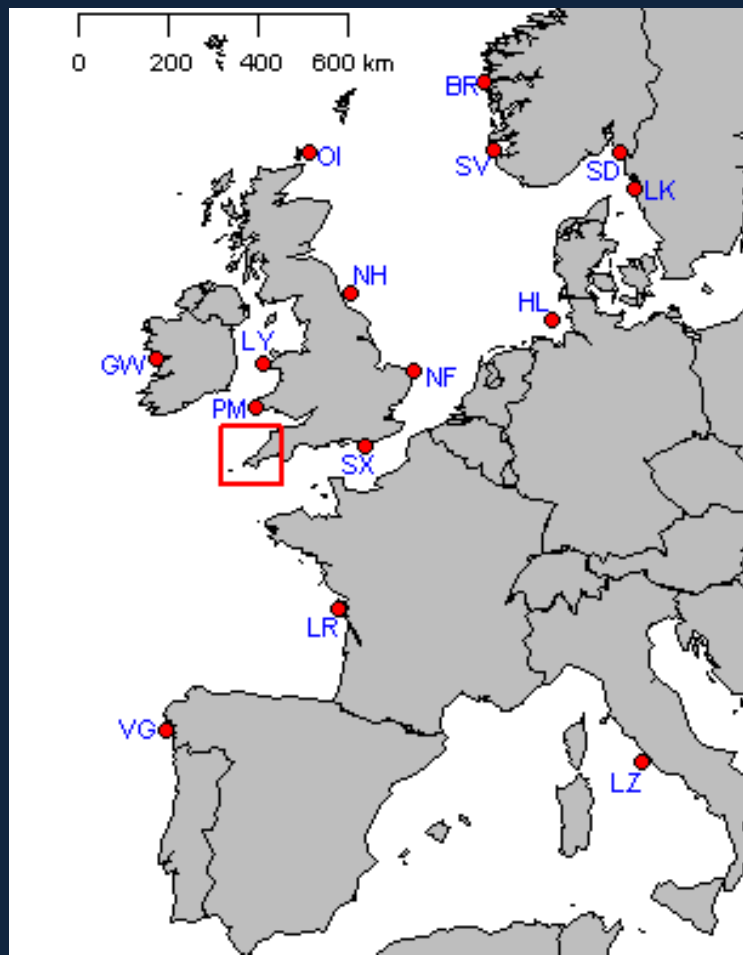


○ No evidence of regional sub-populations

- Overall F_{ST} low (0.0005)
low, but higher than similar area in Skagerrak
- No significant pairwise F_{ST} (0.012 to -0.007)
- No divergence via coalescent clustering
- Geographic vs genetic distance – not significant

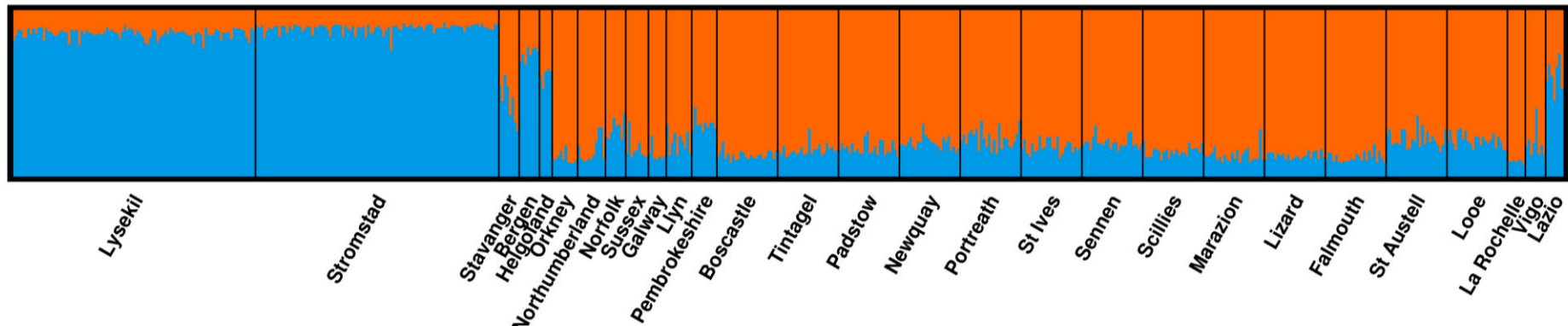
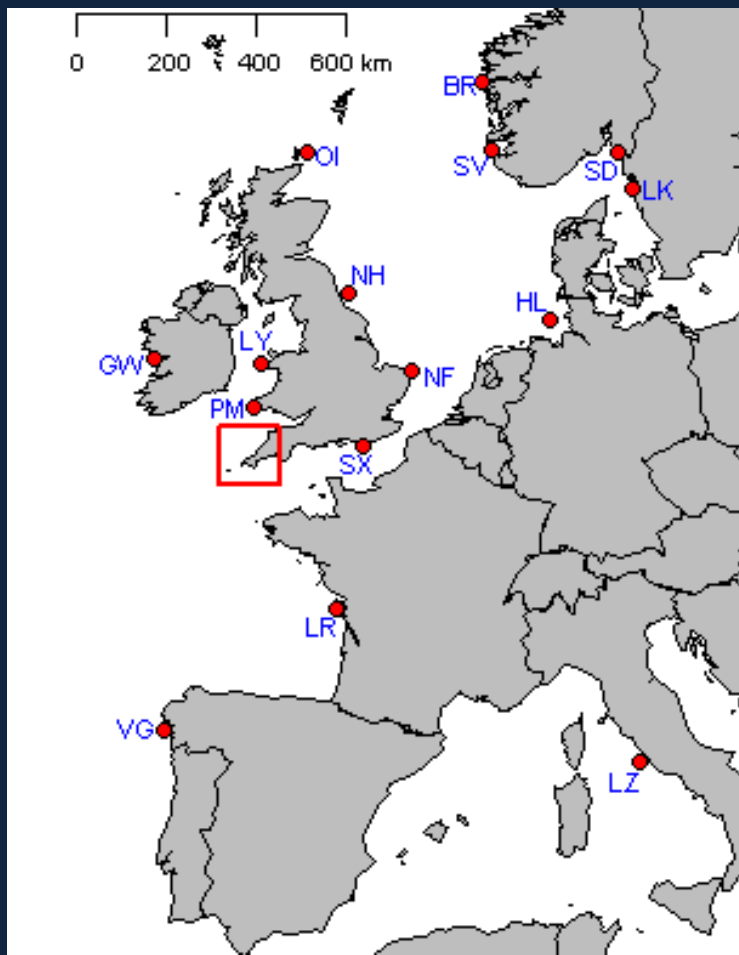


European scale structure



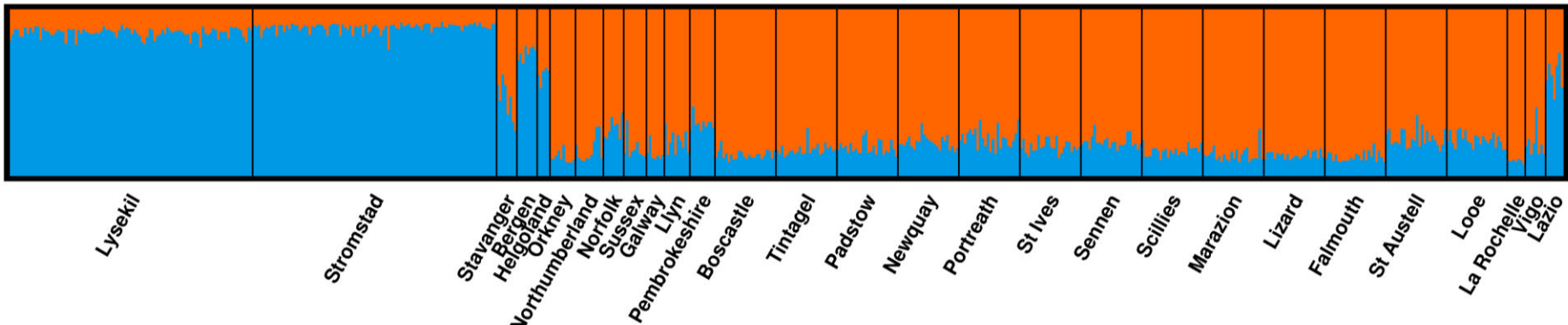
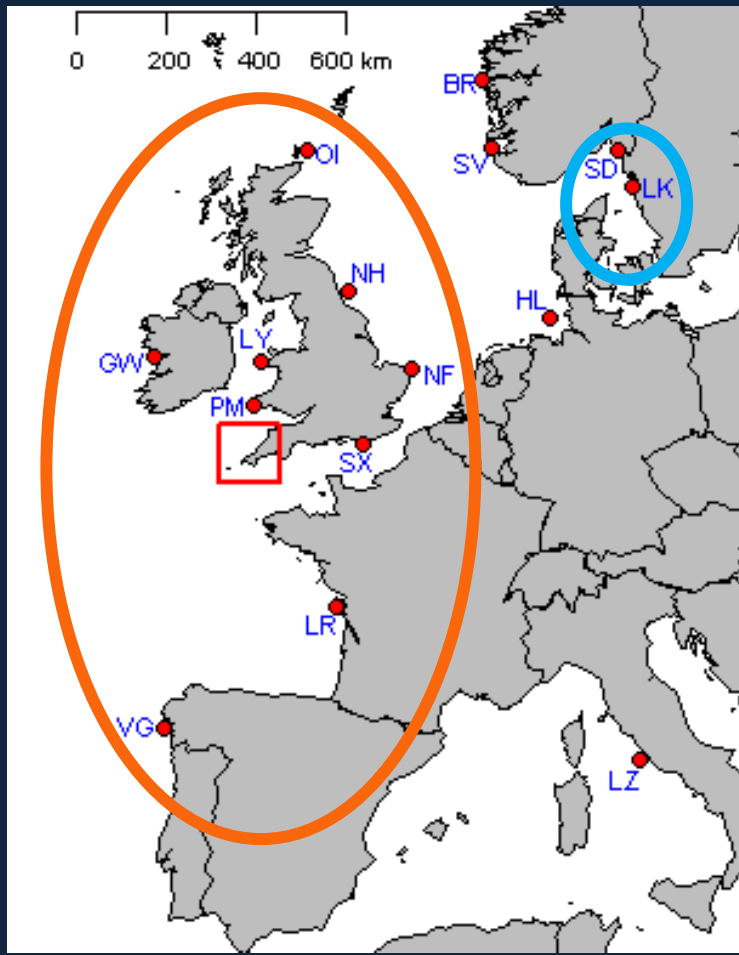
European scale structure

- Strong evidence of differentiation between two population clusters



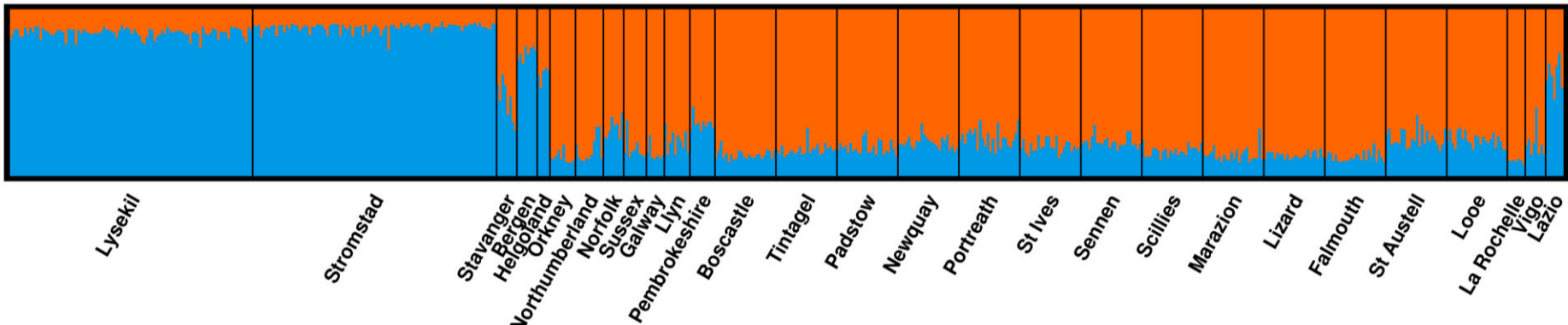
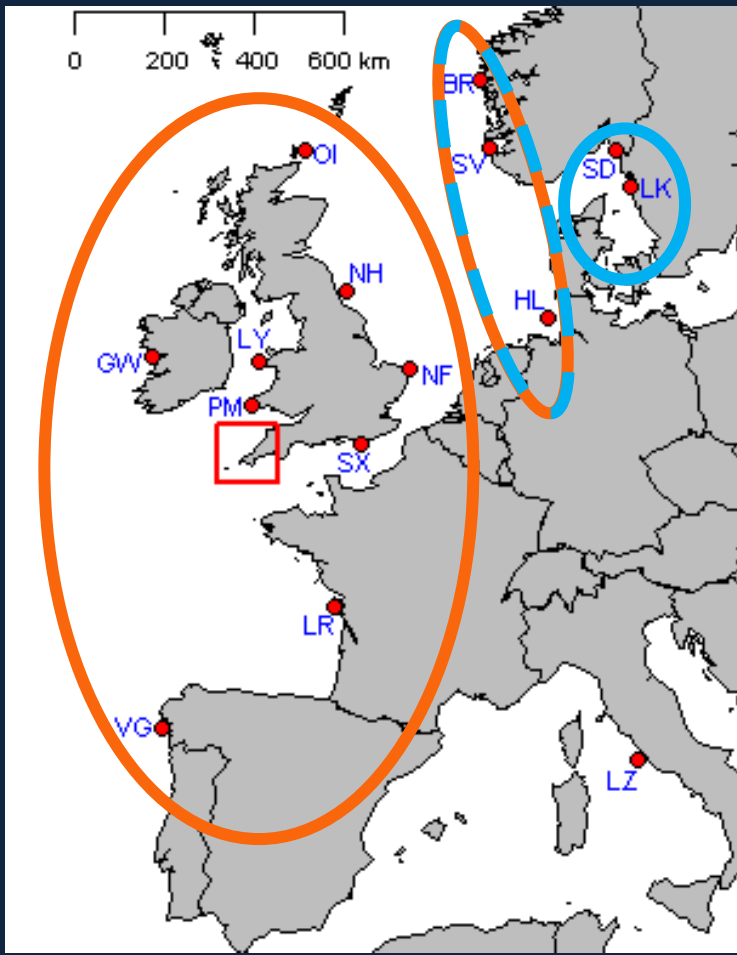
European scale structure

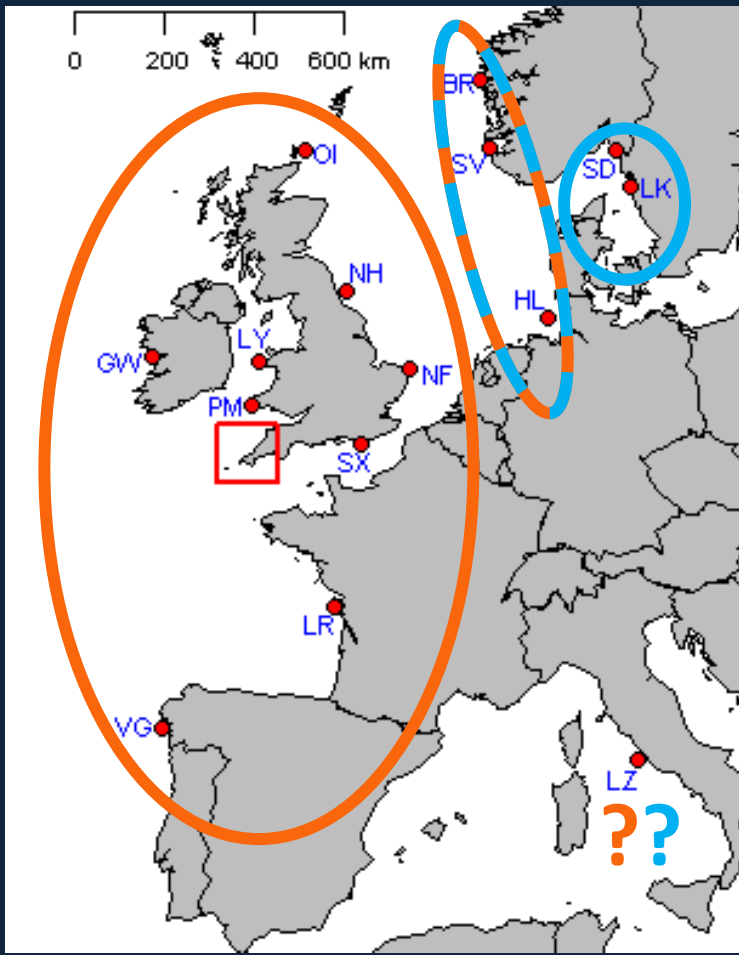
- Strong evidence of differentiation between two population clusters
 - > U.K. / Atlantic western Europe
 - > western Sweden



European scale structure

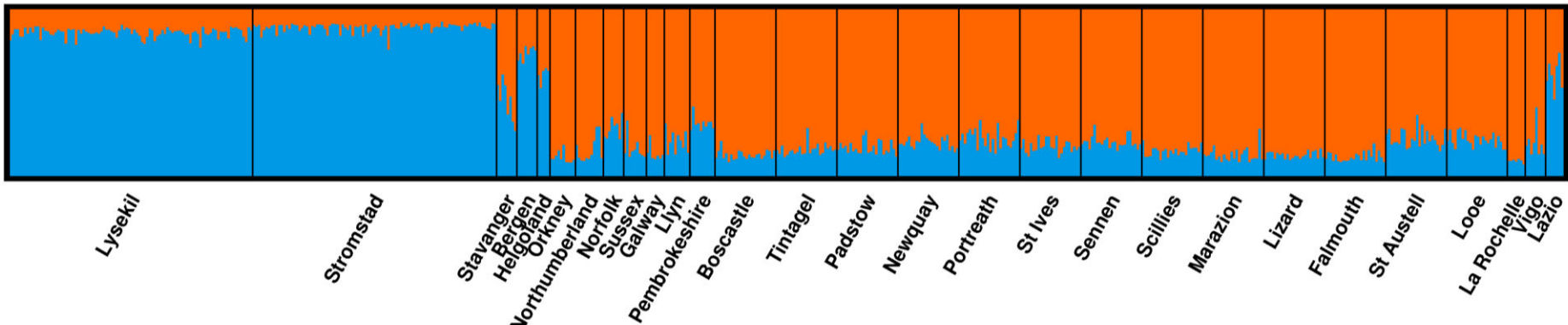
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- Mixing zone in eastern North Sea?





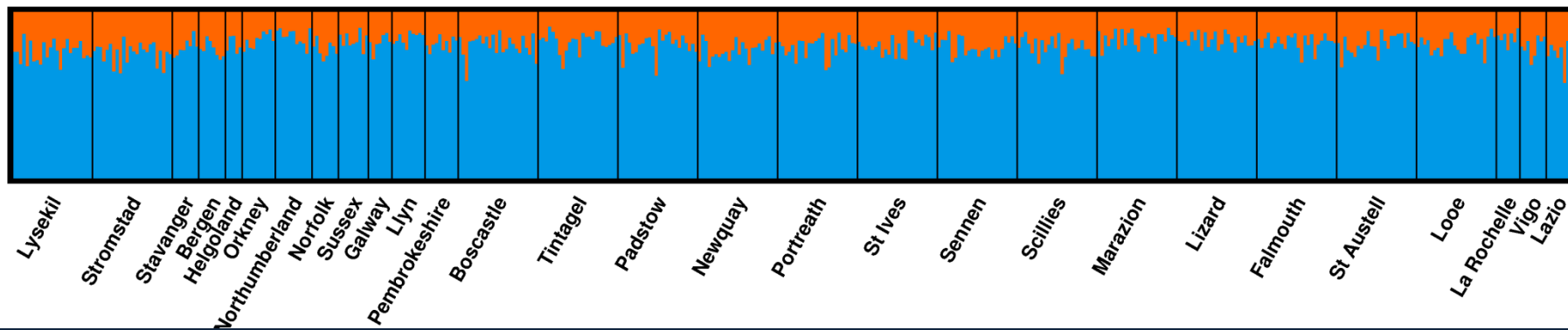
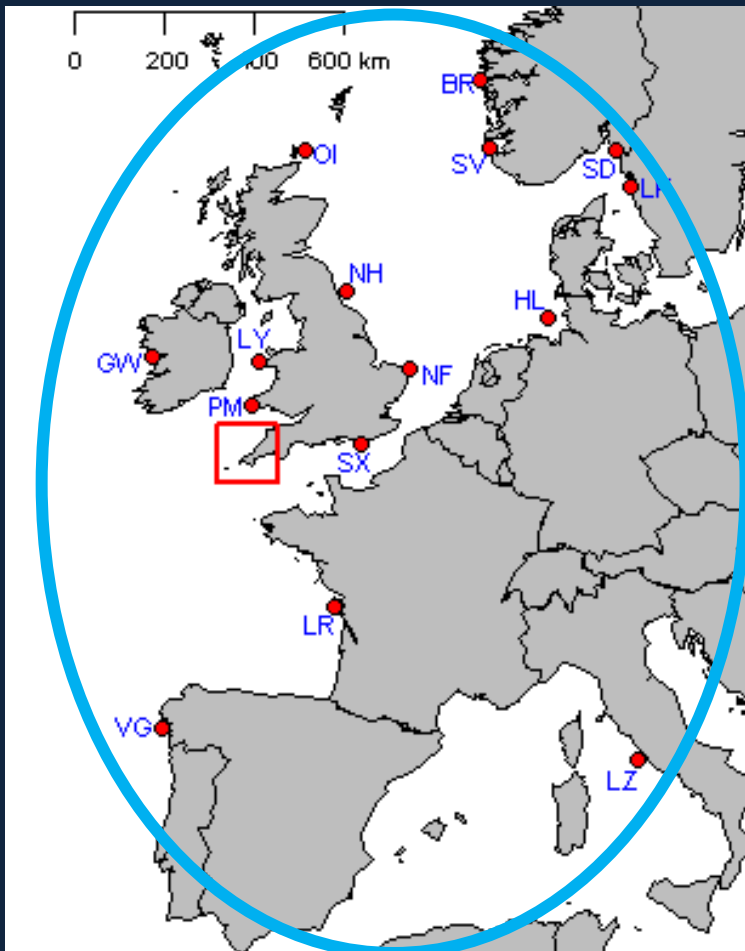
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- Italian alignment unexplained; more Mediterranean samples to be tested

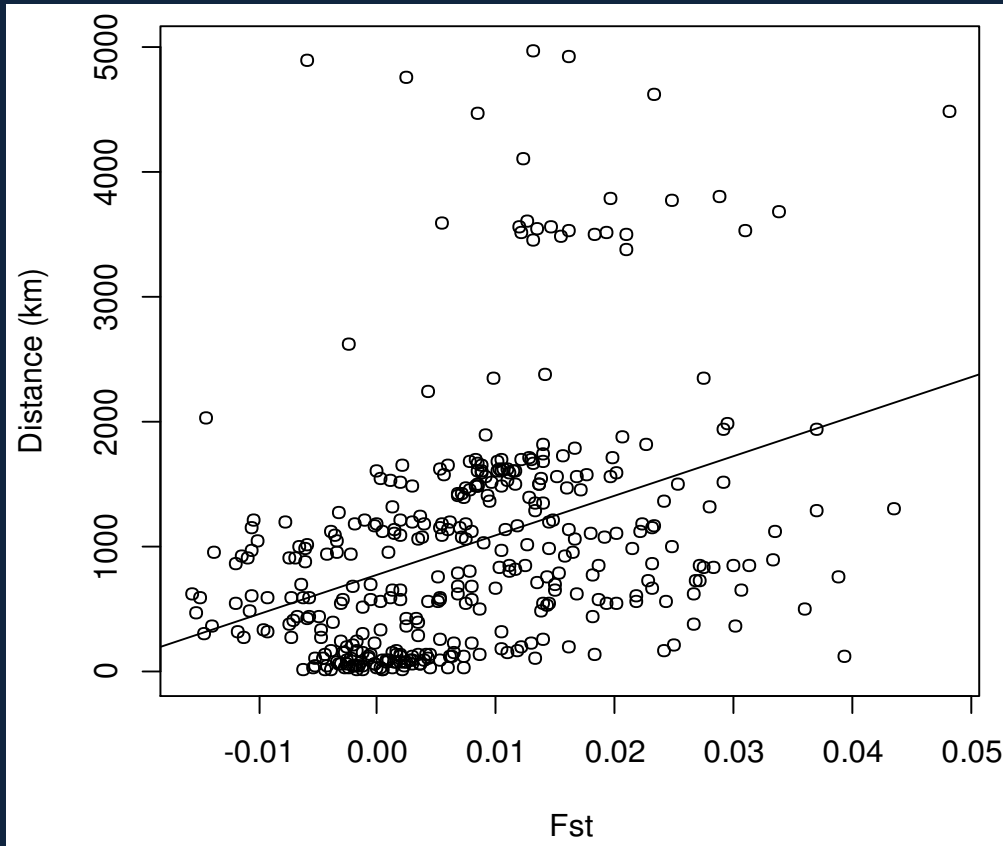


European scale structure

- Strong evidence of differentiation between two population clusters
 - > U.K. / Atlantic western Europe
 - > western Sweden
- Mixing zone in eastern North Sea?
- Italian alignment unexplained; more Mediterranean samples to be tested
- Signal lost when Swedish sample sizes reduced to low number of individuals



Connectivity, gene flow and diversity



- Genetic diversity high among all samples ($H_O = 0.60-0.72$)
- Divergence supported by significant overall F_{ST} (0.007), some pairwise F_{ST} values (0.048 to -0.016) and AMOVA
- Isolation by Distance highly significant across all geographic samples ($p = 0.0001$)
- Increased sampling may reveal further structure

- Some regional structuring and strong IBD
- No reduction in diversity in Scandinavia
- Stepping stone model of gene flow
- Larval dispersal limited in 2-4 week duration
- Direct connectivity of distant areas unlikely



Towards informed appraisal of stocking

- Robust frameworks exist for a 'Responsible Approach' to hatchery stocking
 - Blankenship & Leber (1995), Lorenzen *et al.* (2010)
 - Ensure that released hatchery fish can be identified
 - Use an empirical process for defining optimal release strategies
 - Define management plans with clear targets and measures of success
 - Use genetic resource management to maximize effectiveness of enhancement and avoid deleterious effects on wild populations
 - Assess and manage ecological impacts



ICES Journal of Marine Science



ICES Journal of Marine Science; doi:10.1093/icesjms/fsu196

European lobster stocking requires comprehensive impact assessment to determine fishery benefits

Charlie D. Ellis^{1,2*}, David J. Hodgson³, Carly L. Daniels², Dominic P. Boothroyd², R. Colin A. Bannister⁴, and Amber G. F. Griffiths¹

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²National Lobster Hatchery, South Quay, Padstow, Cornwall PL28 8BL, UK

³Centre for Ecology and Conservation, University of Exeter, Penryn Campus, Penryn, Cornwall TR10 9FE, UK

⁴Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, Suffolk NR33 0HT, UK

*Corresponding author: tel: +44 7940 316348; fax: +44 2326 254243; e-mail: cde204@exeter.ac.uk

Ellis, C. D., Hodgson, D. J., Daniels, C. L., Boothroyd, D. P., Bannister, R. C. A., and Griffiths, A. G. F. European lobster stocking requires comprehensive impact assessment to determine fishery benefits. – ICES Journal of Marine Science, doi: 10.1093/icesjms/fsu196.

Implications to N.L.H. stocking



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LOCAL PICTURE

- Unlikely to affect gene flow beyond the extent of natural dispersal
- Likely to limit negative genetic impacts to wild stock
- Important reference of genetic diversity



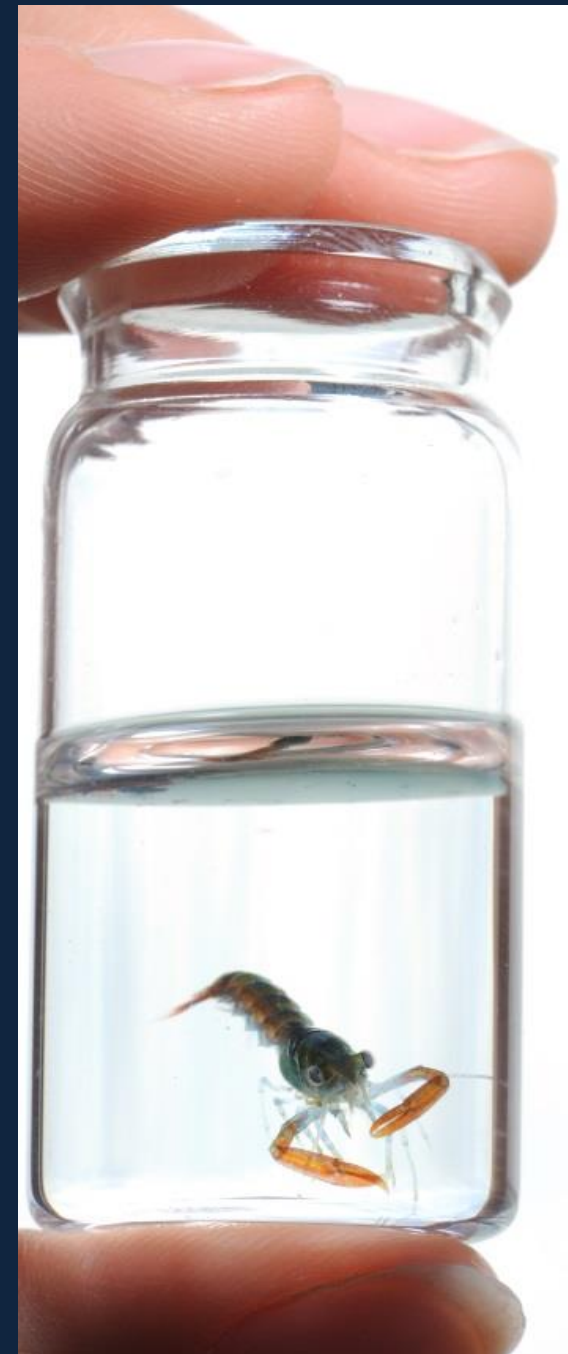
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FURTHER AFIELD

- Long-term enhancement of stocks may extend far beyond release areas
- Use of local broodstock to rear juveniles for release in distant areas inadvisable without empirical evidence of high natural genetic connectivity



Implications to N.L.H. stocking

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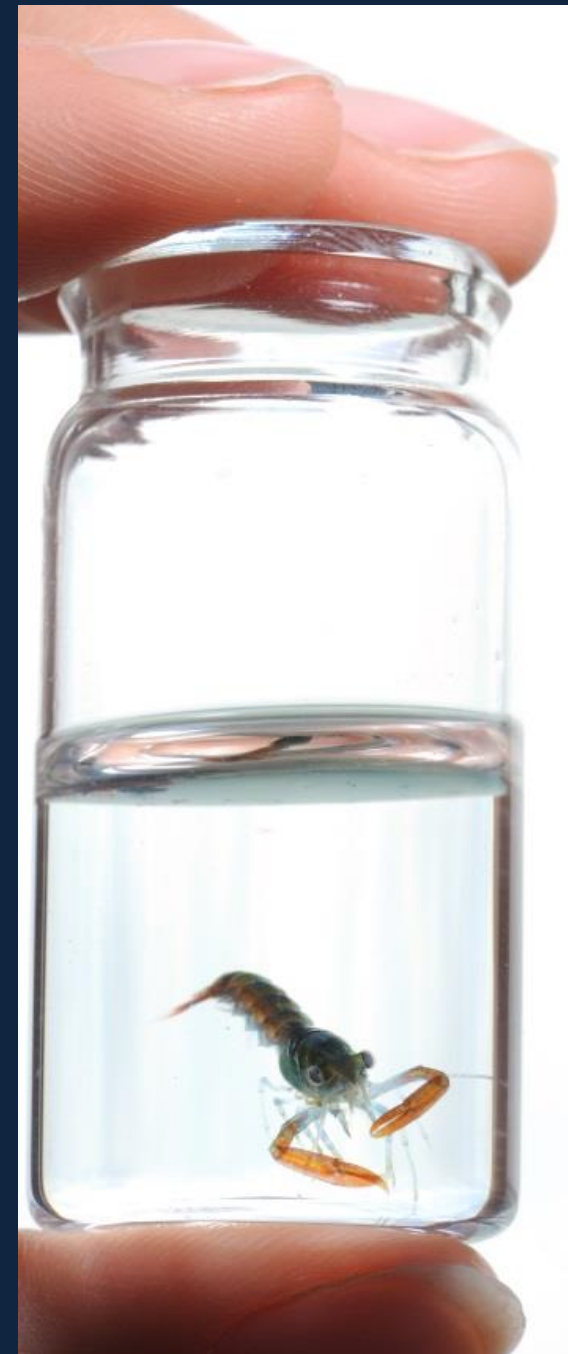
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DOWN THE LINE

- High genetic diversity and single paternity suggest parentage assignments can effectively identify hatchery lobsters in the wild – tissue archiving underway for future impact assessment
- Molecular tools available to assess unknown dynamics of the rearing environment





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