Progress Report: Genetic investigation into the spill over of scallop larvae from Port Erin closed area

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PROGRESS REPORT: GENETIC INVESTIGATION INTO THE SPILLOVER OF SCALLOP LARVAE FROM PORT ERIN CLOSED AREA.

INTRODUCTION

Marine Protected Areas (MPAs) can be implemented to achieve one of two goals. Firstly to conserve biodiversity and secondly as a fisheries management tool(Hastings and Botsford 2003). In the role of fisheries management, MPAs have the potential to enhance the sustainability of stocks, especially of sessile or sedentary species by protecting habitats where adults can achieve higher densities (Beukers-Stewart *et al.* 2006; Kaiser *et al.* 2007) with larger gonad sizes (Kaiser *et al.* 2007) leading to increased reproductive output. High densities of adults can also to lead to an increase in the reproductive success of sedentary broadcast spawners as close proximity to potential mates is essential for fertilisation (Levitan *et al.* 1992). The planktonic larval phase in many marine species can lead to eggs and larvae being able to "spill-over" to areas outside the reserve, thereby allowing the reserve to act as a source of recruits for the fishing grounds (Gell and Roberts 2003).

The success of a closed area for fisheries management will depend on the level and patterns of connectivity between it and the exploited grounds. Genetic data can provide information on connectivity as it relies on two contrasting factors; firstly, larval dispersal and adult migration will serve to connect populations (gene flow) and this will be shown by a homogenisation of genetic structure between populations; conversely lack of larval dispersal and adult migration will lead to random genetic drift and local adaptations causing genetic heterogeneity between populations and genetic structure will being evident. King scallops (*Pecten maximus*) are sedentary during their adult lives with their pelagic larvae spending approximately 25 days at sea (Le Pennec *et al.* 2003) therefore, there is potential for dispersal of larvae from an MPA to neighbouring exploited areas. There have been several studies identifying low levels of genetic structure in king scallop populations (Beaumont 1982; Heipel et al. 1998; Heipel et al. 2000; Kenchington et al. 2006). However these are on a large geographic scale and looking at connectivity on a finer scale, between an MPA and

neighbouring fishing grounds, it is less likely that any genetic differences will be found, thus making the use of genetic tools for studying connectivity in such situations less feasible.

Port Erin is a small (2 km^2) closed marine area in the Isle of Man (IOM). It has been closed to fishing since 1989 and densities of *P. maximus* are higher inside the closed area than outside (Beukers-Stewart *et al.* 2006), reproductive output is also higher inside the closed area (Beukers-Stewart *et al.* 2005). However, the question of whether the closed area contributes to recruitment in local fishing grounds has yet to be answered. In 2003 approximately 40,000 *P. maximus* were transferred into the Port Erin closed area from the Isle of Skye (IOS) as part of a stock enhancement program. If differentiation between the two populations exists then an opportunity to track this IOS genetic signature outside the boundaries of the closed area has been created which could allow identification of the contribution of Port Erin to the local fishing grounds.

This paper analyses the genetic structure of the IOS and IOM populations and reports on the feasibility of using genetic techniques to trace transplanted IOS progeny outside of the MPA.

METHODS

Sample Collection

P. Maximus samples were collected from the same grow out facility in the IOS that the transferred juveniles came from. *P. maximus* samples from the IOM of an age that prohibits them being the progeny of the transferred scallops (ie: 6 years or older at the beginning of 2009) were collected from the scallop processing plant in the Isle of Man and from the annual scallop survey onboard the research vessel Prince Madog. The left shell of each individual was kept to allow aging using annual growth rings (Mason 1957). Tissue was preserved in 90% ethanol.

DNA Extraction

Molluscs have high levels of muccopolysachharides which can contaminate DNA extractions therefore DNA was extracted using CTAB extraction buffer (Doyle and Doyle 1987). Samples were homogenised in 400ul of CTAB buffer (40ml of 2% CTAB buffer to 20ul of mercaptoethanol) and incubated overnight with Proteinase K (200ug) at 59 ^{o}C. Phenol-chloroform-isoamyl alcohol (24:24:1) extraction was followed by two Chloroform-Isoamyl alcohol washes (24:1). The DNA was precipitated using 2x the volume of ice cold absolute ethanol as the volume of DNA extract and 0.1x volume of sodium chloride (pH8, 5M). After

precipitation at -20 °C for 1 hour and centrifugation at max speed for 10 minutes a pellet was formed and the remaining ethanol drained off. The pellet was then washed in 70% ethanol and re-centrifuged for 5 minutes. The Ethanol was drained and the pellet allowed to air dry for 10 minutes to ensure all remaining ethanol was removed. The DNA was resuspended in 50ul of TE buffer (pH 8) and stored long term at -20 ^{o}C or at 4 ^{o}C for short term storage.

Microsatellite amplification and genotyping

Previous testing of published microsatellite markers for *P. maximus* (Watts *et al.* 2005) yielded usable PCR products in seven out of the nine markers(List15-004, List15-005, List15-008b, List15-011, List15-012, List15-013, List15-15). Three of these markers (List15-011, List15-013, List15-015) were too polymorphic for use at the population level (for example in 68 individuals 63 alleles were identified) which left only 4 markers (which we will call W4, W5, W8 and W12 to indicate that they are markers from Watts *et al* 2005). To increase power 12 new transcriptome derived microsatellite markers were developed (See Hold *et al* 2012), which we will label with a prefix of "P", giving a total of 16 microsatellite markers which were amplified using 4 multiplex PCR reactions. Each 12µl reaction contained 1µl of DNA (~10ng), 6µl of Qiagen Type IT multiplex PCR mix, 0.5µl of BSA (10mg/ml), 2.5µl of water and 2µl of Primer mix. The final primer concentrations and fluorescent tails of each primer are shown in Table 1. Three thermocycling profiles were used (Table 2). PCR products were resolved on an ABI 3030XL sequencer using the LIZ 600 size standard and Genemapper software was used to size alleles.

Table 1. Multiplex PCR primer concentrations and tails for *Pecten maximus* microsatellite markers.

Multiplex	Primer	PCR concentration	Fluorescent tail (PCR Concentration)	Thermocycle profile
A	P9 Forward	0.05	VIC (0.2)	1
	P9 Reverse	0.20		
	P19 Forward	0.08	PET (0.33)	
	P19 Reverse	0.33		
	P20 Forward	0.05	FAM (0.2)	
	P20 Reverse	0.20		
B	P11 Forward	0.05	PET (0.2)	2
	P11 Reverse	0.20		
	P23 Forward	0.05	NED (0.2)	
	P23 Reverse	0.20		
C	P59 Forward	0.05	FAM (0.2)	1
	P59 Reverse	0.20		
	P60 Forward	0.04	VIC (0.17)	
	P60 Reverse	0.17		
	P62 Forward	0.05	PET (0.2)	
	P62 Reverse	0.20		
	P68 Forward	0.04	PET (0.2)	
	P68 Reverse	0.17		
	P70 Forward	0.04	FAM (0.17)	
	P70 Reverse	0.17		
	P73 Forward	0.05	NED (0.2)	
	P73 Reverse	0.20		
	P75 Forward	0.04	FAM (0.17)	
	P75 Reverse	0.17		
D	W4 Forward	0.05	PET (0.22)	3
	W4 Reverse	0.20		
	W5 Forward	0.05	NED (0.2)	
	W5 Reverse	0.20		
	W8 Forward	0.05	VIC (0.2)	
	W8 Reverse	0.20		
	W12 Forward	0.05	FAM (0.2)	
	W12 Reverse	0.20		

Table 2. Three thermocycling profiles for *Pecten maximus* microsatellite multiplex PCR reactions.

PROFILE 1		
Temperature	**Time**	**Cycles**
95 °C	5 minutes	1
95 °C 64 °C; -1 °C per cycle 72 °C	30 s 90 s 30 s	6
95 °C 58 °C 72 °C	30 s 90 s 30 s	9
95 °C 50 °C 72 °C	30 s 90 s 30 s	15
72 °C	45 minutes	1

PROFILE 2		
Temperature	**Time**	**Cycles**
95 °C	5 minutes	1
95 °C 51 °c 72 °C	30 s 90 s 30 s	16
95 °C 50 °c 72 °C	30 s 90 s 30 s	19
72 °C	45 minutes	1

PROFILE 3		
Temperature	**Time**	**Cycles**
95 °C	5 minutes	1
95 °C 60 °C - 1°C cycle 72 °C	30 s 90 s 30 s	10
95 °C 50 °c 72 °C	30 s 90 s 30 s	13
72 °C	45 minutes	1

DATA ANALYSIS

Data Quality and loci characteristics

Microchecker (Van Oosterhout *et al.* 2004) was used to check for stuttering, large allele dropout and null alleles. Pairwise genotypic linkage disequilibrium (LD) was tested in Genepop v 4.0 using the log likelihood ratio statistic (G-test). Concordance with Hardy Weinberg Equilibrium (HWE) was tested using the exact test in Genepop v4.0. For all Genepop analyses burn-in for the Markov Chain Monte Carlo algorithm was set at 10,000 iterations and there were 100 batches each with 5000 iterations. Alpha values for multiple testing were corrected using the False Discovery Rate (FDR) of Benjamini & Hochberg (1995). Observed and expected heterozygosity was calculated using GenAlEx (Peakall and Smouse 2006).

Population differentiation

Population structure was tested using Weir & Cockerham's (1984) mulitallelic and multilocus adaptation of FST as implemented in Genepop v 4.0. FST corrected for null alleles was also calculated using the software FreeNA (Chapuis and Estoup 2007).

Assignment testing

The ability of the suite of microsatellite markers to assign individuals to either IOM or IOS populations was tested by self assigning the samples using GeneClass2 (Piry *et al.* 2004). Each individual was assigned to either IOS or IOM based on their genotypes and the percentage of individuals correctly assigned to their population of origin was calculated.

RESULTS

Data quality and loci characteristics

There was no evidence of stuttering or large allele dropout. Four markers showed evidence of null alleles in both populations (P9, P19, P20 and P68) There was no evidence of significant linkage disequilibrium between any loci pairs in either population. Four markers (P9,P19,P20 and P68) deviated significantly from HWE in both populations and a further four (P70, P75, W4, W8) deviated only in the IOM population. Observed and expected heterozygosity (Table 3) show an excess of homozygotes causing the deviation from HWE, possibly due to null alleles as suggested by Microchecker.

Population differentiation

Both calculated F_{ST} Values, FSTAT (-0.00011) and FreeNA (0.00002), were very low and not significantly different from zero suggesting no evidence differentiation between the IOM and IOS populations.

Assignment testing

Percentage correct assignment was low, only achieving 47.7% of individuals correctly assigned back to their source population (more were assigned to the wrong population than the correct one).

Table 3. Observed (Obs.Het.) and expected (Obs.Het.) heterozygosities and tests for Hardy-Weinberg Equilibrium for *Pecten maximus* microsatellites from the Isle of Man and Isle of Skye. P-values estimated using the exact test in Genepop v4.0. P-values in bold italics were significant after correction for multiple testing using the False Discovery Rate of Benjamini and Hotchberg (1995).

	Obs.Het.	Exp.Het.	P-value	Obs.Het.	Exp.Het.	P-value
Locus	**ISLE OF MAN**			**ISLE OF SKYE**		
P9	0.11765	0.22125	***0.01227***	0.25581	0.35267	***0.00448***
P11	0.29787	0.25623	0.57026	0.34694	0.40269	0.58243
P19	0.13333	0.24802	***0.00839***	0.02500	0.33196	***<0.00001***
P20	0.43750	0.85913	***<0.00001***	0.40476	0.79231	***<0.00001***
P23	0.28571	0.30952	0.39302	0.19048	0.21715	0.45344
P59	0.61765	0.67559	0.94868	0.64865	0.69900	0.20637
P60	0.35556	0.42172	0.48223	0.39623	0.45103	0.39209
P62	0.68182	0.65517	0.40772	0.63462	0.69044	0.07002
P68	0.50000	0.74363	***0.00096***	0.48649	0.83710	***0.00001***
P70	0.06977	0.15650	***0.00090***	0.09804	0.09416	1.00000
P73	0.08889	0.12884	0.17162	0.11538	0.18148	0.03323
P75	0.26471	0.59394	***0.00007***	0.38889	0.49257	0.10021
W4	0.02632	0.07754	***0.01326***	0.23913	0.21906	1.00000
W5	0.95000	0.88797	0.80008	0.89744	0.89677	0.80101
W8	0.15556	0.24694	***0.00217***	0.19298	0.24034	0.05718
W12	0.71429	0.85944	0.03286	0.67347	0.83589	0.06794

DISCUSSION

Microsatellite data suggests that there is no genetic population differentiation between the Isle of Man and Isle of Skye populations of *P. maximus*. This lack of structure will make tracing the offspring of the transplanted IOS seed unfeasible as demonstrated by the very low success in correctly assigning the parent samples back to their population of origin.

Homogenisation of the allele frequencies could be a consequence of current day connectivity between the two populations. Connectivity during the larval phase would be possible through the North Channel. The mean residual flow out of this passage towards the Malin shelf is estimated to be 0.02 – 0.03 m s^{-1} (Knight and Howarth 1999). This residual flow is primarily wind-driven with large winter storms accounting for much of this flow (Knight and Howarth 1999). During the summer months it would be expected that residual flow would be minimal due to decreased wind forcing. However, Mason (1958) felt that the primary spawning event in the IOM was in the autumn, which he observed in August and September. With the planktonic larval phase being approximately 3-4 weeks long this could mean that the larvae are in the water column when the outflow increases due to the onset of the autumnal storms. It has also been suggested that spawning may be happening approximately a month later than this in recent years (Chapter three) increasing the likelihood of the larvae encountering increased northerly residual flow. The connectivity required to homogenise allele frequencies only needs to equate to one or two individuals per generation (Crow and Kimura 1970) and therefore chance spawning events coinciding with an autumnal storm allowing flow through the North Channel would suffice.

Another possibility is that the 2 populations have limited contemporary gene flow but share a common ancestor in relatively recent history and have not yet had time to diverge. With parts of the British Isles being glaciated as recently as 10,000 years ago it is likely that the IOM and IOS population expanded from a common refugia population within this timescale. However, the lack of genetic structure taken together with predicted larval dispersal from particle tracking models (Neil and Kaiser 2008) suggests that gene flow is occurring through the North Channel, connecting scallop grounds within the Irish Sea and with the west coast of Scotland. The presence of connectivity between scallop grounds will allow scallop larvae spawned inside an MPA to potentially settle a distance away from their natal grounds. MPAs

allow increases in adult scallop density, size and reproductive output (Beukers-Stewart *et al.* 2005; Kaiser *et al.* 2007) and can therefore create a protected source of new scallop recruits for neighbouring and possibly more distant commercial fishing grounds. However, from this genetic data it is not possible to estimate the magnitude of this migration between populations of *P. maximus* or to estimate if this level of connectivity is on a scale which would influence population dynamics over an ecological timescale.