Potential effects of stock enhancement with hatchery reared seed on genetic diversity and effective population size.

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ABSTRACT
The present study was undertaken to investigate the genetic efficiency of enhancing populations of wild scallops using hatchery produced seed scallops. Scallops from four sites around Isle of Man (IOM) and from a French scallop hatchery were genotyped using 15 microsatellite markers. Heterozygosity was equivalent in the IOM and the hatchery scallops, whereas allelic richness was slightly lower in the hatchery sample. The effective population size ($N_e$) of the hatchery scallops was estimated at 32.4 (95% CI: 24.4 – 44.9). The confidence intervals for the estimates of $N_e$ for the IOM samples included infinity. When $N_e$ becomes large the genetic signal is weak compared to the sampling noise therefore, whilst we can be confident that the $N_e$ of IOM scallops is larger than that of the hatchery, the precise difference is uncertain. Simulations showed that with a census size larger than $10^8$, enhancement with small numbers of scallop seed should not affect the enhanced population’s $N_e$. However, with larger numbers of surviving seed or a smaller census size it is likely that the $N_e$ of the enhanced population could be decreased. Some benefit from enhancement of wild populations of scallops with hatchery seed is possible when the wild population has a very low effective population size and the number of seed scallops used is small. However this can rapidly change from an increase in the enhanced population’s $N_e$ to large decreases when larger numbers of seed scallops are used. In order to avoid a possible detrimental outcome of introducing hatchery scallop seed we suggest that effort is made to estimate both the wild population’s census size and effective population size to allow the prediction of the outcome of stock enhancement.
INTRODUCTION

Global scallop (Pectinid) capture production has risen from 500,000 tonnes in the 1980’s to 840,000 tonnes in 2010 (FAO 2012) and these scallop fisheries are highly valuable: The Canadian sea scallop had a landings value of CAN$93 million in 2008 (DFO 2011); the north east American sea scallop is worth approximately US$160 million annually (Beukers-Stewart and Beukers-Stewart 2009); Queensland scallop fisheries are worth approximately AUS$18 million annually (Beukers-Stewart and Beukers-Stewart 2009); Scallops were worth £54.5 million in the UK in 2010 and are the third most valuable fishery in the UK (Almond and Thomas 2011) and *Pecten maximus* accounts for over 65% of all fishery income in the Isle of Man (Beukers-Stewart et al. 2003).

Scallop stocks are highly variable both spatially and temporally with cyclical, irregular or spasmodic recruitment characterising many scallop species (Orensanz et al. 2006) with many occurrences of stock collapses being documented worldwide (Orensanz et al. 2006). This creates challenges for their sustainable management.

High fishing intensity since the start of the Isle of Man *Pecten maximus* fishery in 1937 has led to a decrease in the density of scallops found on commercial beds, with less than 3/100 m$^2$ now typical at the beginning of the open season and 1/100 m$^2$ at the end of the fishing season compared to a maximum estimate of 4/100 m$^2$ to 11/100 m$^2$ in the early 1980’s (Brand 2006). There also has been a reduction in the average age from greater than nine years old to less than five years old over an exploitation period of 15 years (Brand et al. 1991; Brand and Prudden 1997). This has meant that the king scallop fishery is now vulnerable to the variability and relative strength of each recruiting year class (Beukers-Stewart et al. 2003).
This uncertainty could be alleviated through stock enhancement and scallop ranching projects. Juvenile scallops can be produced in hatcheries and grown out on the seabed to supplement the natural exploitable population and thereby enhancing the spawning stock biomass.

However, there are concerns about the genetic consequences of the release of aquaculture stock into the natural environment (Utter 1998; Gaffney 2006; Roodt-Wilding 2007). Gaffney (2006) identified two main genetic concerns related to shellfish restocking programs: (i) changes in the effective population size ($N_e$) and (ii) the genetic composition in the enhanced population relative the original native wild population. Ryman & Laikre (1991) identified that if a transferred hatchery Salmonid population made up approximately 30% of the post enhancement Salmonid population then there would be a decrease in the overall $N_e$ of approximately 50%. However, even overfished shellfish populations rarely reach such low population sizes as these salmonids and Gaffney (2006) argued that an increase in $N_e$ is the more likely scenario in hatchery enhanced shellfish populations. A population with low $N_e$ will have a greater rate of loss of alleles and heterozygosity (Crow 1986) and such loss of genetic variability could lead reduced resilience.

There is mounting evidence that the genetic diversity of hatchery progeny may be lower than their wild counterparts (Hedgecock and Sly 1990; Liu et al. 2010). The introduction of progeny with low genetic diversity into wild salmonid populations has led to lowered overall genetic variability leading to concerns regarding the fitness of the resulting populations (Utter 1998). However, whereas the low population sizes in salmonid species led to a “swamping” of native populations with “hybrid swarms” (Utter 1998), the comparatively large population sizes of scallops may mean that such swamping is less likely to occur unless extremely large
numbers of hatchery derived seed are used. However, low genetic variability in Scandinavian populations of the European oyster (*Ostrea edulis*) was thought to be partially due to transplantation from one location to another as well as overfishing and disease (Johannesson *et al.* 1989). It is therefore important that seed stock has as high a genetic variability as possible to avoid adverse effects on the recipient wild population. The seed should also have genetic composition that is representative of the recipient population to avoid outbreeding depression and the breakdown of local genetic adaptation.

The present study quantified the genetic composition and diversity at microsatellite loci in wild scallops from the Isle of Man, Irish Sea as compared with that of hatchery progeny that were being considered for use in scallop restocking projects around the island, and thereby evaluates the efficiency of such an approach to enhance the local fishery.

**METHODS AND MATERIALS**

*NATIVE AND HATCHERY SCALLOP SAMPLING*

Sampling was carried out in 2009 onboard the RV Prince Madog using Newhaven dredges at each of four commercially fished sites around the Isle of Man (Figure 1). 50 scallops from each site were collected and approximately a 3mm$^2$ piece of mantle tissue from each scallop and stored in 90% ethanol. Each scallop was aged by counting the growth check rings on the flat valve of the shell following Mason (1957) who studied scallop growth off the south west coast of the IOM.

A sample of 50 scallops from a single spawning event was sourced from a hatchery. Wild scallop broodstock were spawned in March 2009 and the juveniles produced were grown on in sea cages until June 2010. From each of the scallops a sample of mantle tissue was then preserved in 90% ethanol. The hatchery use new wild broodstock for every spawning and the F1 generation is never used as broodstock, thereby minimising the occurrence of inbreeding.
The total number of individuals used for broodstock varies from year to year depending on the gamete production of the spawners and the survival of the eggs. However, the number of broodstock exceeds several hundred each year.

Figure 1. Sampling sites for wild *Pecten maximus* from the waters around the Isle of Man, Irish Sea. CHK= Chickens Rock, BRO = Bradda Offshore, TAR = Targets, LAX = Laxey

**DNA extraction and microsatellite genotyping**

For full genetic methodology see Hold 2012 (PhD thesis) but in brief DNA was extracted from each sample using CTAB and phenol-chloroform. 15 microsatellite markers were then genotyped for each sample.
Wild scallop census size estimation

Scallop abundance in the Isle of Man waters up to the 12 nautical mile limit were estimated using still photography methods adapted from Lambert et al. (2011). Scallop abundance was analysed for 50 photographs at 145 sites (Figure 2). The abundance at each station was assumed to be representative of an area of 25 km$^2$ (each station was 5 km apart). The total abundance for all 145 sites (3625 km$^2$) was then calculated.

Figure 2. Map showing the video sampling sites for the estimation of abundance of *Pecten maximus* in the waters off the Isle of Man. Grey line indicates the 12 nautical mile limit.
**Data analysis**

For detailed data analysis methods please see Hold 2012 (PhD thesis).

**Data quality**

Microchecker (Van Oosterhout *et al.* 2004) was used to check for genetic scoring errors and non-amplifying (null) alleles. Allele frequencies from each population were then tested for concordance with Hardy-Weinberg Equilibrium (HWE) in the software package Arlequin v 3.5 (Excoffier and Lischer, 2010). Linkage disequilibrium (LD) was tested for using Genepop.

**Genetic diversity**

Genetic diversity was assessed using a variety of summary statistics; the number of alleles, number of effective alleles and observed and expected heterozygosity using GenAlEx (Peakall and Smouse 2006). To test the difference in heterozygosity between IOM and the hatchery scallops, each individual scallop’s heterozygosity was estimated by assigning a value of 0 to homozygous loci and 1 for heterozygous loci for each individual at each locus in all populations. The score for each individual was then divided by the number of loci genotyped for that individual. Analysis of variance (ANOVA) was then used to investigate any significant differences in individual heterozygosity among populations.

For the comparison of allelic richness, only the age group of scallops with the greatest number of individuals was used for each IOM site (This varied between sites). This was due to the fact that the hatchery samples were all from a single cohort and it is possible that scallops show variable reproductive success with different spawning events. This means that the diversity of a single cohort may be less than the overall population; the sweepstake effect (Hedgecock 1994). Using a single age group for each site from the IOM meant that there was less bias in the comparison of allelic diversity. However, this approach resulted in unequal sample sizes, therefore allelic richness estimates were adjusted for unequal sample sizes.
which we performed using adjustment by rarefaction using the software HP-RARE v1.1 (Kalinowski 2005).

*Effective population size*

“The assumption that a gene is equally likely to come from any parent does not mean that each parent produces exactly the same number of progeny, for there will be random variability, but that each parent has the same *expected* number. Most actual populations depart from this ideal. Therefore we define the *effective population number*, \( N_e \), as the size of an idealised population that has the same probability of identity as the actual population being studied”

It is the magnitude of effective population size rather than census population size has implications for a population’s genetic diversity and rate of loss of rare alleles and propensity for inbreeding. When estimating the effective population size for just a single cohort the number estimated is called the effective number of breeders; essentially it is an estimation of the number of individuals it would require to be breeding to obtain the genetic composition or diversity seen in the samples.

The effective number of breeders (\( N_b \)) was estimated by using single cohorts and the linkage disequilibrium method implemented in the software package LDNe (Waples and Do 2008). As small sample size can bias the estimates of \( N_b \), especially for larger \( N_b \)s, and as no significant genetic population structure has been observed in IOM samples (Hold 2012) scallops of the same age group from all sites were combined into a single sample group. The effective number of breeders was then calculated for this group and compared to that of the hatchery samples.
Effect of Hatchery Seed on Wild Populations $N_e$

To estimate the effect of hatchery seed on the effective population size of the wild stock we used the method of Ryman and Laikre (1991);

\[ \frac{1}{N_e} = \frac{X^2}{N_h} + \frac{(1-X)^2}{N_w} \]

Equation 1

Where $N_e$ is the effective population size following enhancement, $X$ is the relative contribution to the offspring from hatchery progeny, $N_h$ is the effective population size of the hatchery progeny and $N_w$ is the effective population size of the wild population. Each individual scallop was assumed to have the same chance of breeding, therefore the relative contribution of hatchery stock to offspring is simply the number of seed (that survive) divided by the census size calculated from the photographic survey.

Population structure

The level of population genetic differentiation or structure was calculated between the IOM samples and the hatchery scallops by calculating Fst values and analysis of molecular variance.

RESULTS

Data quality

There was no genotyping errors detected by Microchecker. There was no evidence of LD for any pair of loci in any population with the exception of markers W12 and W5 in the hatchery sample after correction for multiple testing. There was no evidence of LD in any pairs of loci in all populations suggesting that loci were not closely linked and can be treated as independent variables. Twenty two out of the 75 locus/population combinations deviated from HWE, which occurred due to a deficiency of heterozygotes and all populations were significant for the global test over all loci for heterozygote deficiency ($P < 0.0001$). Two
markers deviated from HWE in all populations but none of the populations deviated from HWE at all loci. Microchecker highlighted the possible presence of null alleles in the population and loci combinations which were not in HWE, this can cause an excess of homozygotes and is the likely cause of the deviations from HWE.

Genetic diversity
The mean number of alleles per locus was lowest in the hatchery samples, and the mean effective number of alleles was slightly lower in the hatchery populations than the Isle of Man populations (Table 1). Average allelic richness varied between 4.14 in hatchery samples to 5.50 in the Laxey population after rarefaction (Table 1) However, single locus comparisons show that hatchery allelic richness is only lower than IOM samples at 8 out of the 15 loci. The mean heterozygosity was similar in all populations, including the hatchery, ranging from 0.35 in Laxey to 0.39 in Targets (Table 1), this heterozygosity was not significantly different between the IOM and hatchery samples (ANOVA: F_{4,234} = 1.78, P = 0.13).
Table 1. Locus characteristics for *Pecten maximus* samples from the Isle of Man and hatchery progeny. Na = number of alleles, Ne = number of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity, Nr = allelic richness adjusted by rarefaction.

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Census and Effective population size

The estimated census population size of IOM scallops was $3.2 \times 10^8$.

Estimates of the effective number of breeders ($N_b$) showed that the hatchery population had a low $N_b$ of 32.4 (95% CI: 24.4 – 44.9). The estimate for the IOM had confidence intervals which included infinity (394.6 to infinity). The point estimate was 1141.4.

Effect of Hatchery Seed on Wild Populations $N_e$

Due to the likely error associated with the estimation of the census population size and the inherent variability in population numbers on a temporal scale, three different census sizes were used to estimate the effect of seeding on the effective population size of the enhanced population; $10^7$, $10^8$ and $10^9$ (Figure 3). In the equation for estimating the effect of seed on enhanced the populations $N_e$, the effective population size of the hatchery scallops ($N_h$) was set to 30 (representative of our estimate of $N_b$ from the hatchery samples), but due the inaccuracy of the estimate of $N_b$ for the wild population, a range of values were used for the value of $N_w$ (500, 1000, 10000, 100000). The effect of enhancing a wild population with hatchery progeny depends on both the number of seed which survive to reproduce and both the wild $N_e$ and census size (Figure 3). With a moderate to large wild $N_e$ there is little benefit from enhancing with hatchery seed (up to 1% increase in wild $N_e$ in some scenarios) and these increases are generally only achieved with low numbers of hatchery seed (Figure 3).

The wild $N_e$ can drop by as much as 96% when large numbers of seed are added to wild populations that have a moderate to large $N_e$. Doubling the hatchery scallops $N_e$ only slightly improved the outcome of these seeding scenarios (Figure 3b). A wild population with a small $N_e$ can potentially benefit from enhancement with hatchery seed ($N_e = 60$) in some scenarios (Figure 4). In these scenarios, with a hatchery effective population size of 60 and a census size that is small to moderate, an increase in the wild populations $N_e$ is achieved with low numbers of hatchery seed, but higher numbers of seed scallops can lead to a rapid decline in
the wild $N_e$ (Figure 4). With larger census sizes and a small wild $N_e$ an increase in the wild populations $N_e$ can be achieved even with larger numbers of seed but as the census size increases further then this benefit plateaus out becoming neutral (Figure 4). Using a lower $N_h$ of 30 in these low $N_e$ scenarios leads to declines in the recipient populations $N_e$ with lower numbers of seed even with larger census sizes compared to the $N_h = 60$ scenarios (data not shown).

**Population structure**

Fst values among the IOM sample groups were very low and were not significantly different from zero suggesting that there is little genetic differentiation between scallops from different sites around the IOM. Between the IOM and hatchery populations there was low but statistically significant genetic structure between the hatchery sample and three of the four IOM sample groups (Table 2). AMOVA showed that 98.3% of the variance occurred within populations, 1.5% of the variance occurred between the hatchery samples and IOM samples and 0.2% was between the populations within the IOM group. Fisher’s exact test of population differentiation revealed a similar pattern: The hatchery sample was significantly different from all IOM samples, but sites within the IOM were not significantly different to each other (Table 2).
Table 2. Above diagonal: Fisher’s exact test of population differentiation in the scallop *Pecten maximus*. Below diagonal: Pairwise Fst values comparing *P. maximus* from four Isle of Man (IOM) Sites and hatchery progeny. Bold face type face = significant following correction of 0.05 alpha value for multiple testing using the false discovery rate.

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Figure 3. Predicted post enhancement effective population size following the introduction of hatchery seed. Enhanced with hatchery seed with an effective size of (a) 30 and (b) 60. The effect of different wild effective population size is shown in the different graphs: (i) 1000 (ii) 5000 (iii) 10,000 (iv) 100,000. The effect of variation in census size is shown by: solid line N=10^7, dashed line N=10^8 and dotted line N=10^9.
Figure 4. Predicted post enhancement effective population size following the introduction of hatchery seed (N_e = 60) to wild populations with small effective population sizes of (a) 30, (b) 100 and (c) 200. Wild population census sizes of: Solid line; 10^8, dotted line; 10^7, dashed line; 10^6, dash and dots; 10^5.
DISCUSSION

Levels of genetic diversity and population structure were quantified for four IOM and a hatchery sample. Heterozygosity was found to be similar in both the IOM and the hatchery sample, whereas allelic richness was slightly lower in the hatchery samples when compared to all 4 of the IOM sites (when averaged over all loci), but this was not the case for all of the single locus comparisons. It is not possible, with only a single hatchery replicate, to test if this decrease is significant. To compensate for the presence of only a single cohort in the hatchery samples they were compared to individuals from a single age group at each IOM site. Nevertheless, average allelic richness was still higher in all IOM samples compared to hatchery. However it is possible that more than one cohort is present in each IOM age group as it has been shown that there are two spawning peaks in the Isle of Man (Mason 1958). Ageing the scallops to either spring or autumn spawning is very difficult due to the lack of a visible first year growth check ring. This could increase the allelic richness in the Isle of Man samples as sweepstake reproductive success is often seen in broadcast spawning marine invertebrates and causes lower allelic richness in single cohorts compared to multiple cohorts (Hedgecock 1994; Flowers et al. 2002; Hedrick 2005). It is unclear whether other studies that compared wild and hatchery bivalves have used single cohorts or a total population sample. Decreased allelic richness with the heterozygosity maintained has been seen in other studies (e.g. Dillon & Manzi (1987)). A decrease in allelic richness means that some rare alleles, and therefore genetic diversity, have been lost in the hatchery samples. If this decrease is real and not an artefact of sampling more than a single cohort in the IOM, it could be detrimental to the wild recipient population. Utter (1998) described the negative effect of population enhancement using seed with compromised allelic richness as a “swamping” of the native genetic diversity. However, in the case of the scallop around the Isle of Man,
which supports a large annual fishery, it is unlikely that the census number would be low enough that this could occur without extremely large numbers of seed being used and surviving thereafter to reproduce.

There was low but significant genetic structure between the IOM and hatchery sample as supported by the Fisher’s exact test. The AMOVA analysis suggested that 1.5% of the variance occurred between the IOM group and the hatchery samples. This finding could lead to concern that the genetic composition of the native scallops might be compromised with the introduction of such seed scallops. Local populations will have evolved in response to the conditions in which they reside and transferring scallops that have evolved in different environments could affect their survival to maturity and the subsequent success of their direct or “hybrid” (hatchery x wild) offspring such that transferred scallops may have poor survival and therefore may not be economically viable. Alternatively, hatchery scallops may have preferential survival over native scallops and outcompete them. An example of ecological differences that are thought to be genetically mediated in *P. maximus* is the reproductive strategy. Scallops at some locations have single peak spawning events whilst others show bimodal or even extended “trickle” spawning. This is thought to be genetically mediated as, when transplanted among sites, the transplanted scallops maintained their native spawning schedule (Cochard & Devauchelle 1993, Mackie & Ansell 1993, Magnesen & Christophersen 2008). However, there is a history of movement of *P. maximus* around its range including transfers of scallops into IOM waters (Beaumont 2000), so contamination of the native genetic composition has probably occurred already. It should also be noted that the Irish Sea is subject to considerable marine traffic. Ballast water contains large quantities of plankton and the numbers of larvae transferred in ballast water may be greater than in transfer projects (Beaumont 2000).
The $N_e$ estimate we obtained for the hatchery sample was small with small CIs. However the estimate for IOM sample had extremely wide CIs, including infinity, so little weight should be given to the point estimates of $N_e$ for the IOM. $N_e$ estimation has difficulty in distinguishing between estimates of moderate and large size and has poor precision with large $N_e$s because the genetic signal is weak and the sampling noise is large (Waples and Do 2009).

It is likely that this was the case with the IOM sample, so although we can be confident that the $N_e$ of the wild population is larger than that of the hatchery scallops we cannot be precise about the magnitude of this difference. This high effective population size is to be expected for such an abundant marine species however, Hold 2012 found that a wild population in Falmouth Bay, western English Channel, had an extremely small $N_e$ of 24 (20 – 29 – 95% confidence intervals) suggesting that the protocols used by the hatchery are able to at least match the $N_e$s found in smaller wild populations.

By using a range of values for both wild $N_e$ and census size we were able to simulate the effect that transferring seed would have on the wild population in these different scenarios. The effective population size used for the hatchery seed was 30 (to represent that estimated for our hatchery seed) and 60 (to represent combining seed from multiple spawning events with different broodstock). Enhancing wild populations with seed scallop can have vastly differing outcomes depending on the census and effective size of the recipient population, ranging from an increase in the enhanced populations’ $N_e$ to a decrease. Thus it is important to estimate both of these parameters prior to transferring seed to ensure a positive outcome for the wild population with the enhancement with hatchery scallop seed. The effect of doubling the hatchery effective population size from 30 to 60 has only a very small effect on the outcome in these scenarios. However, increasing the effective population size of the hatchery seed, for example by using multiple spawning events with different broodstock, can be beneficial when enhancing a wild population with a very low effective population size and
using small numbers of seed. Using multiple spawning events with different broodstock should also increase the allelic richness of the seed scallops which was shown to be slightly lower in the hatchery scallops than the wild population. The importance of maintaining high Ne sizes relates to the negative relationship of Ne to rate of loss of alleles and heterozygosity (Crow 1986). Therefore populations with low Ne are at higher risk of losing genetic variability.

The simulations assume that the hatchery seed have an equal chance of contributing to the next generation compared to the wild scallops. Whilst this is a reasonable assumption it is not reasonable to assume that this occurs with random mating within the whole population. In broadcast spawning invertebrates, many studies have shown that fertilisation success decreases rapidly with distance (Levitan 1991; Levitan et al. 1992) and therefore scallops are much more likely to mate with their neighbours than scallops some distance away. A concern of scallop ranching would be that if a large number of hatchery seed were released into one area of the seabed that theses scallops would be more likely to mate with other hatchery seed than with the wild population, creating an F1 generation. This is also confounded by the fact that enhancement of wild populations would be more likely to occur in depleted and low density wild populations rather than an area of the seabed that has high densities of wild scallops.

This study has shown that there is no change in heterozygosity but that there is the possibility of lower allelic richness in the hatchery scallops compared to wild IOM populations. When combined with the weak, but significant genetic structure between the hatchery and IOM populations and a low hatchery Ne, these data indicate that it would be wise to take a precautionary approach to the transfer of hatchery scallops to enhance wild populations.
If stocking is to take place then we recommend the following precautions:

1. Efforts should be made to estimate the census population size and, if possible, the wild $N_e$ in order to more accurately estimate the outcome of the effect of transplanted seed on the effective population size.

2. With a census size larger than $10^8$, enhancement with small numbers of seed (100,000 surviving to reproduce) should not affect the enhanced populations’ effective population size.

3. Even with a moderate to large wild $N_e$ populations with a census size of less than $10^9$ could lead to a decrease in the enhanced populations $N_e$ with the introduction of large amounts of seed.

4. Ranching should not be used as an alternative for good management of the wild populations as, if the native population numbers decline to numbers lower than $10^8$ then there can be negative effects from enhancement even with small numbers of seed.

5. Increasing the hatchery scallops effective population size (for example by using multiple spawning events with different broodstock) should be considered in scenarios with very low wild effective population sizes.

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REFERENCES


