Genetic profiling of Welsh whelk (Buccinum undatum) populations

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EXECUTIVE SUMMARY

- Population structuring of whelk populations investigated by mitochondrial DNA sequencing.
- Significant genetic differentiation among sites supports population structuring on small geographical scales.
- Genetic patterns also implicate environmental heterogeneity as a driver of reported phenotypic variation.
- Genetic variation among Welsh whelk seems to be considerably lower than other regions which may be attributed to small population sizes and/or overharvesting.
- Genetic stock structure must be included in management plans aimed at sustainable harvesting.
- Such plans must also endeavour to prevent further loss of genetic variation as this represents the ‘raw material’ for populations to respond to future climate change.

INTRODUCTION

Populations constitute interbreeding units with more or less autonomous dynamics and recruitment and are frequently defined as harvest stocks in relation to fisheries management (Carvalho & Hauser 1994). In terrestrial and freshwater environments, populations are often well delimited by conspicuous physical barriers, however, in the marine environment distinct populations are more difficult to detect and for many marine species it is unclear to what degree distinct populations exist at all, or whether they are organised into larger panmictic units (McQuinn 1997). This distinction is critical, in particular for exploited species, as different populations may possess different genetic, physiological, behavioural or other characteristics that may cause differences in life history traits such as fecundity and mortality rates and ultimately production and abundance (Gold & Richardson 1998). As recruitment and sustainability may be properties specific to individual populations failure to identify, and independently manage, distinct populations can lead to local overfishing and ultimately to severe declines or stock collapse (Hutchings 2000; Knutsen et al., 2003).

Genetic markers represent powerful tools for examining population structure that in the marine environment might otherwise be undetected due to difficulties in implementing standard ecological methods such as mark-recapture or behavioural observation (Shaklee & Bentzen 1998). Populations that are not linked by dispersal (i.e. exchanging genes/gene flow) are expected to accrue different gene frequencies. Therefore, by characterising the geographical distribution of genetic variation population units can be identified

Buccinum undatum, the common whelk, is a subtidal snail that is widely distributed in East Atlantic (Bay of Biscay to Norway) and West Atlantic (New Jersey to Newfoundland) waters wherein it is typically found at depths between 20 and 30 metres. The species exhibits limited potential for dispersal of gametes and larvae since fertilisation is internal, egg capsules are firmly adhered to sea bed substrates, and development is direct. In addition, adult movement is reported to be limited (Himmelmann 1988, Himmelmann & Hamel 1993) In the only mark recapture study performed to date, off Whitstable (UK), one individual (of 3099) was recaptured out of its original 8km2 release plot over a 3 yr period (Hancock 1963).

Based on the specie's limited dispersal capacity isolation of populations on local geographical scales would be expected. In such cases populations that are overharvested may not be replenished by recruits from other populations, at least not on the timescales of interest to fishery managers. Population genetic studies of the species have reported significant genetic differentiation over small geographical difference in English (Weetman et al. 2006), Irish (Mariani et al. 2012) and Icelandic (Palsson et al. 2014) waters indicative of parochial populations and fine scale recruitment independence. In addition localised depletions of fishery stocks have been reported (Morel & Bossy 2004) compatible with fine scale population differentiation and predictions of local population vulnerability.

The UK whelk fishery expanded considerably through the 1990s due to global trade and demands from the Far East (Fahy et al. 2000). More recently UK landings have increased from 12 900t (2009) to 20 000t (2013) with an estimated value of 13.7 million (MMO, 2014). As other fisheries decline or experience restrictions there is expected to be a displacement of effort into the whelk fishery which will increase pressure on stocks. As there is already suggestion that stocks may be overharvested (e.g. McIntyre et al. 2015), there is a growing need to implement genetic monitoring of stocks. This research reports the first genetic study of whelk in Welsh waters. Specific objectives were to (i) investigate the occurrence of population structuring among Welsh samples and (ii) assess levels of variability in comparison with other regions.

MATERIALS AND METHODS

Sample collection and DNA extraction

Samples of whelk were obtained from five sites (Fig.1), in all cases through liaisons with local fishers. A sample from Carmarthen was organised by Dr Leanne Llewellyn (Welsh Government Marine and Fisheries Division). Samples from the other four locations were obtained as part of collaboration with Bangor University (Prof Michael Kaiser) and represented a subset of samples included in the study by Haig et al., (2015). Individual tissue biopsies were removed and stored in absolute ethanol. Total DNA was then extracted from each individual using a standard CTAB-phenol/chlorofrom/isoamylalcohol method (Winnepenninckx et al. 1993).

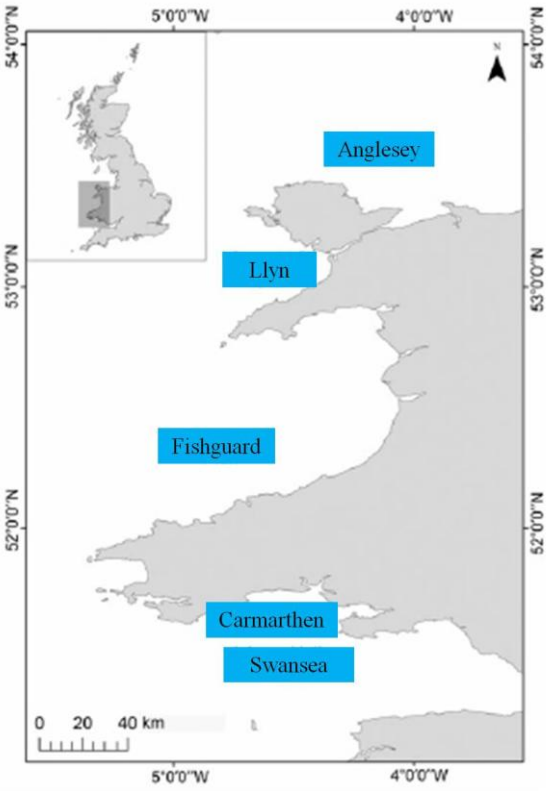

Figure 1. Map of approximate sample sites. Specific locations are not provided out of courtesy to local fishers

Genetic analysis

Individuals were genotyped by sequencing a portion of the mitochondrial DNA (mtDNA) genome. Specifically, a 530 base pair (bp) fragment of the Cytochromie Oxidase I gene (COI) was amplified by polymerase chain reaction (PCR) using the primers (FCOI and RCOI) described by Iguchi et al., (2007). MtDNA PCRs were carried out in a total volume of 50 µl, containing 5-50 ng of *B. undatum* DNA, 1mM each primer, 0.2 U of *Taq* DNA polymerase (Bioline, UK), 1 X the supplied PCR buffer, 2.0 mM $MgCl_2$ and 0.2 mM dNTPs, and involved an initial denaturation step (95 ^{o}C for 3 min) followed by 35 cycles of 30 s at 95 ^{o}C, 30 s at 54 ^{o}C and 60 s at 72 ^{o}C. PCR products were then purified using ExoSAP and

sequenced in both directions using ABI BigDye Terminator v3.1 (Applied Biosystems) and ABI 377 sequencer.

DNA sequences were manually checked using CHROMAS and aligned across individuals using BIOEDIT (Hall 1999). Levels of genetic variability were quantified as the number of haplotypes (i.e. different sequence types) and haplotype diversity (i.e. the probability (0 – 1) that any two randomly chosen sequences from a sample are different). Genetic differentiation among samples was quantified using F_{ST} (Wright 1951), the coefficient of inter-sample genetic variation, which ranges from 0 (identical gene frequencies) to 1 (samples fixed for different genetic variants). The significance of F_{ST} estimates were tested by permutation (following Goudet et al. 1996) whereby individuals/genotypes were shuffled among samples (10 000 iterations for each F_{ST} tested) and F_{ST} recalculated. The probability of the null hypothesis (i.e. no structure - $F_{ST} = 0$) was taken as the proportions of replicates that yielded a value of F_{ST} that was equal to, or higher than, the observed value.

RESULTS

Sequences were obtained for 50 individuals from each of the five samples. Following editing and trimming of sequence chromatograms a 515 bp segment could be aligned across all individuals. This revealed a total of 7 different sequence types (haplotypes) across all samples. Levels of variability differed across samples (Table 1; Fig. 2). This was most apparent for the Llyn sample, wherein all individuals were shared a single haplotype (Table 1; Fig.2).

Table 1. Genetic variation with samples as measured by number of haplotypes (*n*HAP) and haplotype diversity (*h*)

	*n*HAP	*h*
Anglesey	6	0.68
Llyn	1	0
Fishguard	4	0.56
Carmarthen	3	0.26
Swansea	3	0.41

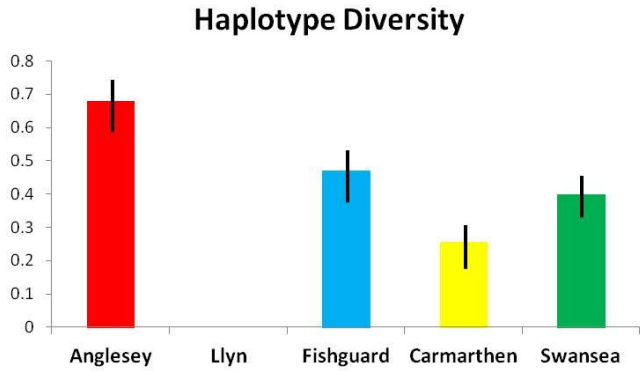

Figure 2. Illustration of variation in haplotype diversity among Welsh samples

Across all samples global F_{ST} was 0.01 and not significant ($P > 0.3$), however a number of comparisons between pairs of samples yielded signifcant F_{ST} results indicating statistically significant haplotype frequency differences (Table 2).

Table 2. Estimates of F_{ST} between pairs of samples. Statistically significant values are in bold and with an *.

	Anglesey	Llyn	Fishguard	Carmarthen	Swansea
Anglesey	-				
Llyn	**0.15***	-			
Fishguard	0.02	**0.05***	-		
Carmarthen	**0.07***	0	0	-	
Swansea	0.02	**0.1***	0	0.02	-

To place the genetic patterns within a wider geographical context data for the Welsh samples were pooled and compared with data obtained from a previously published study by Palsson et al., (2014) which focused on Icelandic samples. This comparison indicated a generally lower level of genetic variation among UK samples compared to northern samples and particularly low for the Welsh samples (Fig. 3).

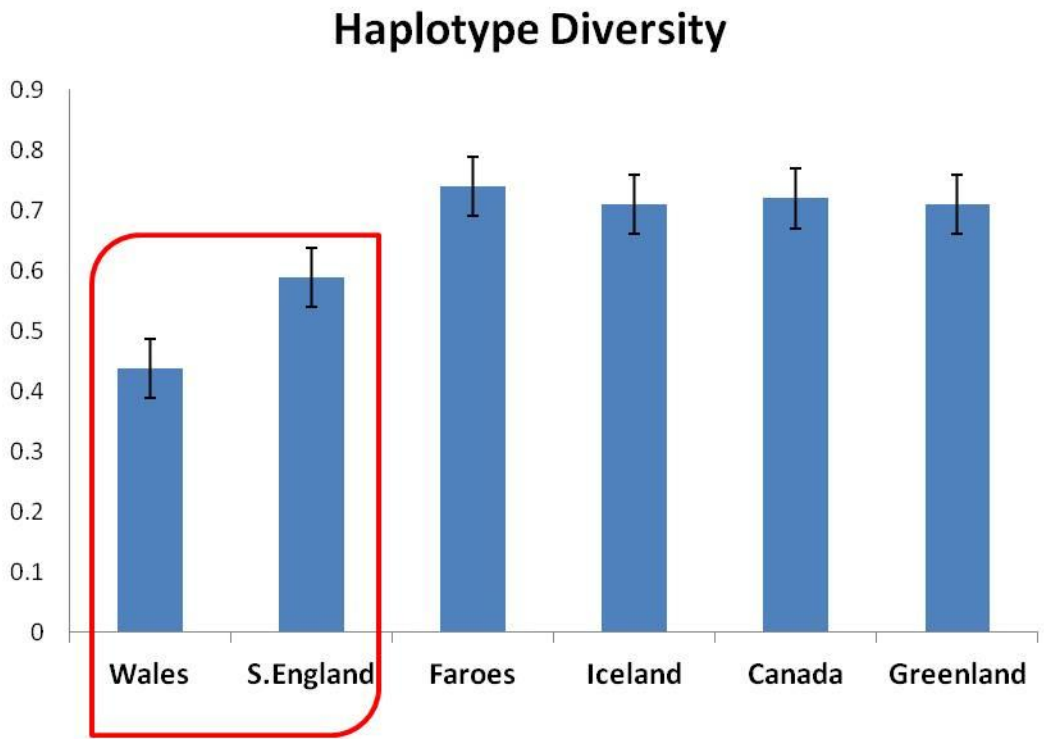

Figure 3. Comparison of levels of mtDNA variability, estimated using haplotype diversity , among regions.

DISCUSSION

Significant population genetic structure

Although the global test of differentiation was not significant, pairwise test of differentiation indicate significant genetic population structure among samples and reject the null hypothesis that Welsh whelk belong to a single homogenous genetic population. The strong differentiation between the Anglesey and Llyn samples indicates that there is significant population/stock independence at small geographic scales. This is consistent with *a priori* predictions based on life history of the species which lacks both a planktonic larval stage and mobile postlarval stage. It is also compatible with evidence of local population structuring obtained from population genetic studies in other regions. For example, Palsson et al., (2014) describe highly significant differentiation among Icelandic sites separated by as little as 20-30 km and sampled within a single bay. Mariani et al., (2012) also reported significant differentiation among samples from Irish waters separated by 30 km.

An important consideration in interpreting the results of this study is that the low levels of mtDNA genetic variation (i.e. haplotype number) will limit the power to detect population stricture. As such the estimates of genetic differentiation must be regarded as highly conservative estimate of population structure. In cases where no differentiation has been detected between sites, such as between Anglesey and Swansea, it cannot be ruled out that the sites may be effectively self-recruiting on timescales, and at rates, of interest to fishery managers and thus require local management. Analysis of more variable genetic markers such as microsatellites will be required to perform higher resolution population genetic analysis.

While the pairwise tests revealed greater differentiation among the northern samples, than among the southern samples, as well as differentiation between both groups, overall the pattern would be described as geographically patchy. For example, Llyn was differentiated from Fishguard and Swansea but not from the Carmarthen sample collected between these sites. A number of non-mutually exclusive factors may be contributing to such a pattern. Firsly, as mentioned previously the low level of genetic variability may limit the precision of estimates of genetic differentiation and more rapidly evolving markers such as microsatellites may be needed to more accurately quantify connectivity/isolation and thus resolve any subtle geographic pattern. Second, while other studies have reported correlations between genetic and geographical distances (isolation by distance – IBD) such IBD effects are only significant at larger geographic scales (e.g. > 250 km) and may thus not have been detectable at the scale of this study (Mariani et al. 2012; Palsson et al. 2014). Thirdly, the patterns may indicate that populations are not organised according to a ‘stepping stone model’ but rather represent locally isolated ‘islands’ (see Hellberg et al. 2002). Local isolation of small populations has been suggested to explain genetic structure among Scandinavian whelk (primarily from the Skaggerak) wherein populations separated by 1-2 km exhibited as much genetic differentiations as those separated by 20-200 km (Valentinsson 2002). Interesting both Weetman et al., (2006) and Mariani et al., (2012) suggest that samples collected from inshore areas may exhibit higher levels of isolation than samples collected from offshore areas, with offshore areas more connected to other offshore populations. As the exact locations of the sampled sites analysed here remain private out of courtesy to the fishers future studies would benefit from comparative analysis of offshore and inshore sites. This is especially important as inshore sites may represent important ‘asymmetric’ contributors to offshore populations and be particularly vulnerable to overexploitation (Weetman et al. 2006).

Independence of genetic and phenotypic variation

The patterns of genetic differentiation here differ from phenotypic patterns among the same samples reported by Haig et al., (2015). Haig et al., (2015) described the most pronounced differences in size at maturity between whelk sampled from Fishguard and Swansea, two sites that were not genetically differentiated here. Discordance between genetic and phenotypic variability has also been reported among Irish whelk (Mariani et al. 2012). Such discordance points to the influence of environmental heterogeneity on phenotypes with consequent phenotypic variation reflecting interplay between genetic adaptation and short term plasticity.

Regional genetic variability

Levels of genetic variability are influenced to varying degrees by historical and recurrent processes. Habitat changes associated with the Pleistocene glaciations have left profound imprints on the phylogeographic structure of N Atlantic marine taxa. A particularly common signature is one of lower genetic variability in northern populations compared to southern ones (Maggs et al. 2008) associated with the survival in, and expansion from, southern glacial refugia. The detection of lower levels of among UK samples, and in particular Welsh samples suggests (i) the existence of northern refugia (Palsson et al. 2014) and/or (ii) more recent loss of genetic variability. In this study the sample from Llyn exhibited a strikingly low level of mtDNA variability ($h = 0$). This may indicate that recruitment within this area is maintained by a small 'effective' breeding population. Such a reduced 'effective' population size could be due to fishing. Localised low levels of genetic variation have also been reported among Irish populations and linked to overharvesting (Mariani et al. 2012). Weetman et al., (2006) reported evidence of recent genetic bottlenecks among UK samples from the Solent area. In that study the authors suggest that these bottlenecks have been anthropogenically induced by tributly tin (TBT) pollution which can cause imposex in whelk.

Implication for management

The results of this study have implications for short term and loner term management and conservation of the species. The detection of population structure emphasises that the resource should not be managed as a single unit and future studies employing more sensitive genetic markers are needed to describe spatial and temporal patterns of population connectivity/isolation in the region. Sustainable management of whelk fisheries must take such structure into account to avoid overexploitation of local populations. Management must also endeavour to prevent any further loss of genetic diversity as this represents the raw material needed for species to respond to future climate change and environmental perturbation (Fogarty & Botsford 2007).

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