GENETIC CHARACTERISATION OF POPULATIONS AND ITS USE IN CONSERVATION DECISION MAKING IN FISH

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Summary
The conservation needs of fishes are special in many respects compared to those of terrestrial organisms. For example, aquatic organisms are the only major human food source that are primarily harvested from wild populations. This report aims to briefly outline some of the main applications for which molecular marker data are applied for conservation decision making in fish populations including: determining population genetic structure, estimation of effective population size and detection of population size changes.

Keywords
Fish, genetic diversity; population genetics; fishery management; microevolution

The conservation need of fishes are special in many respects compared to those of terrestrial organisms and in particular, compared to those domestic species considered in other presentations at this workshop. For example, aquatic organisms are the only major human food source that are primarily harvested from wild populations (Ryman et al. 1995). Therefore, methods for the development of population management guidelines often more closely follow those commonly used for wildlife compared to domestic animals and plants. This report aims to briefly outline some of the main applications for which molecular marker data are applied for preserving fish biodiversity.

Determining population genetic structure
One of the main applications of molecular markers related to the conservation of commercially exploited fishes is to aide in the development of guidelines enabling sustainable harvesting of populations (Carvalho and Pitcher 1994). An important first step towards this, is to characterise the genetic structure of the populations being harvested which assists in defining the biological units which should be considered when developing a management strategy: the so-called 'genetic stock concept' (e.g. Carvalho and Hauser 1994). While not directly aimed a conserving biodiversity, but rather aimed at maximising sustainable harvest levels, the implementation of management strategies based on molecular data can have indirect benefits for population biodiversity as the aim of such management plans is to avoid population crashes which would negatively affect harvesting, but this aim also benefits the maintenance of population genetic diversity. Studies investigating the population genetic structure of fishes have been conducted for a number of decades, firstly using protein coding allozyme loci (e.g. Utter et al. 1987) and then, starting in the mid-1980's, using mitochondrial DNA (mtDNA) polymorphisms (reviewed by Avise 2004). More recently, tandemly repeated microsatellite DNA markers have become the molecular marker of choice for determining intraspecific population genetic relationships. (e.g. Koskinen et al. 2002a). In general markers that are used today utilise the polymerase chain reaction (PCR) as it enable analysis of archive

material such as scales. For more details regarding the different marker types, readers are directed to e.g. Frankham et al. 2002; Avise 2004).

From a fisheries management perspective, the aim of determining the intra-specific population genetic structure is to determine the units between which limited gene-flow occurs: if such units are overfished, it is unlikely that population sizes will recover due to migration, and hence a collapse of the fishery may occur. While understanding population genetic structure is important from an applied perspective, the same knowledge is also the basis of any biologically sound conservation strategy. For example, similar genetic criteria to those described above are the basis of several definitions of the so-called 'Evolutionary Significant Unit' (ESU; reviewed by Fraser and Bernatchez 2001). For example, since 1978, U. S. the Endangered Species Act (ESA) affords protection to "*Any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature*" (Endangered Species Act, Sec. 3 (15). A distinct population segment (DPS) is not well described in the ESA, but some definitions have been developed. For example, the National Marine Fisheries Service (NMFS) developed the following criteria for salmonid populations to be considered a DPS:

(1) It must be substantially reproductively isolated from other con-specific population units; and;

(2) It must represent an important component in the evolutionary legacy of the species.

Thus, molecular marker information can assist in defining ESUs, which in turn can be used for developing conservation strategies of populations. A related technique which has been useful for delineating the population of origin of individuals in mixed stock fisheries is individual assignment, individuals are assigned to the population from which their multi-locus microsatellite genotype has the highest probability of occurring (see e.g. Conrnuet et al. 1999 and the program 'GeneClass', available at http://www.ensam.inra.fr/URLB). From a conservation perspective, assignment tests can be useful for identifying native born individuals from those that are hatchery reared, or for identifying individuals whose genetic makeup most closely resembles the original stock, based on e.g. archived scale data (see review in Hansen et al. 2001).

Understanding the general effects of different aquatic environments on fish genetic diversity and differentiation

Based on the wealth of population genetic research that has already been conducted in fishes, several studies summarising data based on allozymes (Gyllensten 1985; Ward et al. 1994) and microsatellites (DeWoody and Avise 2000) have been published which demonstrate some general trends in genetic structuring for species occupying different habitats during their life cycle. These studies have revealed marked differences in the level of genetic differentiation and genetic diversity between populations of marine and freshwater species, with marine species generally exhibiting lower levels of inter-population differentiation (Gyllensten 1985; Ward et al. 1994) and higher genetic diversity (Gyllensten 1985; Ward et al. 1994; DeWoody and Avise 2000). This general observation has generally been hypothesised to be a result of higher effective population sizes and/or higher inter-population migration rates in marine, compared to freshwater, environments and has implications for the conservation of genetic diversity. Lower effective population sizes and/or lower inter-population migration rates in the freshwater environment predicts that populations of freshwater species are expected to be more prone to extinction than marine species and thus should be of particular conservation concern. This does not however imply that marine populations are immune to such effects (see below).

Estimation of effective population size

Accurate estimates of effective population size (N_e) are central to the development of appropriate conservation strategies in any species as N_e predicts e.g. the rate of loss of neutral genetic variation, the fixation rate of deleterious and favourable alleles, and the rate of increase of inbreeding experienced by a population (Frankham et al. 2002). Importantly, the N_e of a population is often many times smaller than the census size (N) of the population, the N_e /N ratio averaging just 0.11 in a survey of vertebrate species (Frankham 1995). In fishes, N_e /N ratios may be expected to be even more extreme due to the high female fecundity of many species enabling large census size numbers to be obtained from minimal numbers of breeding individuals. For example, the winter chinook salmon run in the Sacramento River of California consists of around 2000 mature individuals, however the effective size of the population has been estimated to be only 85 (N_e /N = 0.04: Bartley et al. 1992).

While estimates of N_e can be gained using direct methods based on field data (estimates of sex ratio bias, offspring production, variation in family size etc.), obtaining such data can be very cumbersome in many wild populations, especially in aquatic species. Hence, indirect methods for N_e estimation based on molecular marker data have also been developed. From a practical viewpoint, these methods can be broken down into two categories: those that require data from a single population sample (single generation methods: e.g. Hill 1981; Pudovkin et al. 1996, Beaumont 1999, Luikart and Cornuet 1999) and those requiring samples from the same population collected at least one generation apart (temporal methods: Waples 1989; Anderson et al. 2000, Wang 2001; Berthier et al. 2002). The temporal methods generally utilise variation in temporal allele frequencies to estimate the level of genetic drift, and hence, the effective population size. This groups of methods tends to give more reliable results than the single generation methods. An important recent advance has been the development of methods which take into consideration the effects of migration on N_e estimation (Wang and Whitlock 2003; the MLNE program is available from http://www.zoo.cam.ac.uk/ioz/software.htm). The major limitation to use of these methods is that double the sampling effort is required. This can be a particularly large problem for late breeding species as collection of samples at least one generation apart can fall outside the timeframe of a funded study. However, due to the existence of historical scale samples in a number of commercially important species (which were originally collected for understanding the age structure of populations) from which sufficient DNA can be extracted, temporal methods for N_e estimation have been applied relatively frequently in fishes. In a high profile example, Hauser et al. (2002) demonstrated through microsatellite analyses of a time series of historical scale samples, that New Zealand snapper (*Pagrus auratus*) have undergone a decline in genetic diversity during their exploitation history. In addition, effective population sizes were estimated to be five orders of magnitude lower than estimated census sizes. This study provided one of the first indications that genetic problems can potentially exist in marine species for which individual numbers are in their millions.

Detection of population size changes

From a conservation perspective, detection of recent dramatic changes in population size (population bottlenecks) is another important aspect of any population monitoring programme (Frankham et al. 2002). Signals of past population bottlenecks can be detected using molecular genetic analyses. For example, one commonly applied method makes use of the assumption that populations which have experienced a recent reduction in N_e will show a reduction in both heterozygosity and allele number at polymorphic loci. However, the reduction in allele number is faster than the reduction in heterozygosity. Therefore, in a recently bottlenecked population, the observed heterozygosity is higher than the expected

equilibrium heterozygosity when calculated from the observed number of alleles, under the assumption of a constant-size population (Luikart and Cornuet 1997). Several statistical tests have been developed to determine whether a population exhibits a significant number of loci with heterozygosity excess, hence indicating the occurrence of a population bottleneck (Cornuet and Luikart 1996; implemented in the program 'Bottleneck', available at http://www.ensam.inra.fr/URLB). Additional tests for identifying reductions or expansions in population size based on DNA sequence data (Rogers and Hardpending 1992; Templeton 1998) or allele frequency data (e.g. Beaumont 1999) have also been developed. The choice of method depends on a number of factors including the samples available, the molecular marker data available and the time frame of any potential bottlenecks.
An important feature of all the molecular data applications described above is that temporal information i.e. data collected for the same populations over an extended time period, can be extremely valuable. Luckily, due to the existence of historical scale samples in a number of commercially important species (which were originally collected for understanding the age structure of populations) from which sufficient DNA can be extracted, the collection of such data is often more feasible in fishes than in other taxonomic groups, which has been utilised to good effect by fish conservation geneticists (e.g. Nielsen *et al.* 1997; Nielsen *et al.* 1999; Hansen et al. 2002, Hauser et al. 2002; Koskinen et al. 2002). In addition, regular monitoring of populations is important for enabling a distinction between normal population size fluctuations and those severe enough to warrant conservation measures (Laikre 1999).

Prioritising populations for conservation
Given that resources for preserving biodiversity are limited there is an important need for biologically sensible criteria which can be applied simply to prioritise fish genetic diversity conservation efforts. Currently, however, such methods are rarely applied (but see Allendorf et al. (1997) for an exception), but decisions are often made in a rather *ad hoc* manner or based on solely non-biological criteria such as monetary value. While such criteria should not necessarily be ignored, they should not completely replace biological criteria. Although methods for identifying populations harbouring a higher proportion of a species genetic diversity exist (e.g. Crozier et al. 1997, Caballero and Toro 2002; Reist-Marti et al. 2003, Simianer et al. 2003) such methods are yet to be applied in a fish biodiversity preservation context. One model outlining a method for population conservation prioritisation, based on experiences in brown trout is outlined in Laikre (1999). This model combines molecular genetic, phenotypic and socio-economic/cultural criteria to prioritise populations for conservation.

Conflicting needs of different sectors of society
As noted above, due to the commercial and cultural (e.g. recreational angling) importance of many fish populations, conservation guidelines of commercially and/or culturally important population potentially conflict with the needs of other interest groups such as commercial and recreational anglers. Therefore, a challenge when developing a conservation program for such populations is to find a balance with which all groups are satisfied. While considerable effort will undoubtedly be put into improving the analytical methodologies described above in the future, it should also be recognised that efforts aimed at decreasing the gaps between different interest groups (such as researchers, decision makers and end users) are likely to be equally important for fish biodiversity preservation and increased communication between these groups should be encouraged.

REFERENCE LIST

1. Allendorf FW, Bayles D, Bottom DL, Currens KP, Frissell CA, Hankin D, Lichatowich JA, Nehlsen W, Trotter PC, and Williams TH (1997) Prioritizing Pacific salmon stocks for conservation *Conservation Biology* 11:140-152
2. Anderson EC, Williamson EG, and Thompson EA (2000) Monte Carlo evaluation of the likelihood for Ne from temporally spaced samples *Genetics* 156, 2109-2118
3. Avise JC (2004) Molecular markers, natural history and evolution, 2nd edition. Chapman & Hall, New York, USA. 511 pp.
4. Bartley D, Bagley M, Gall G and Bentley B (1992) Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations *Conservation Biology* 6, 365-375
5. Beaumont M (1999) Detecting population expansion and decline using microsatellites *Genetics* 153, 2013–2029
6. Berthier P, Beaumont MA, Cornuet J-M, and Luikart G (2002) Likelihood-Based estimation of the effective population size using temporal changes in allele frequencies: A genealogical approach *Genetics* 160, 741-751
7. Caballero A and Toro MA (2002) Analysis of genetic diversity for the management of conserved subdivided populations *Conservation Genetics* 3: 289-299
8. Carvalho GR, Hauser L (1994) Molecular-Genetics And The Stock Concept In Fisheries *Reviews In Fish Biology And Fisheries* 4: 326-350
9. Carvalho GR and Pitcher TJ (1994) *Molecular Genetics in Fisheries*. Chapman & Hall, London, 131pp.
10. Cornuet JM and Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data *Genetics* 144:2001-2014
11. Cornuet J, Piry S, Luikart G, Estoup A and Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153: 1989--2000.
12. Crozier RH (1997) Preserving the information content of species: genetic diversity, phylogeny, and conservation worth. *Annual Reviews in Ecology and Systematics* 28: 243-268.
13. DeWoody JA and Avise JC (2000) Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology*, 56, 461-473.
14. Frankham R 1995 Effective population size / adult population size ratios in wildlife: a review *Genetical Research* 66: 95-107
15. Frankham R, Ballou JD and Briscoe DA (2002) *Introduction to conservation genetics* Cambridge University Press.
16. Fraser DJ and Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology* 10, 2741–2752
17. Gyllensten U (1985) The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. *Journal of Fish Biology*, 26, 691-699.
18. Hansen MM, Kenchington E and Nielsen EE (2001) Assigning individual fish to populations using microsatellite DNA markers *Fish & Fisheries* 2, 93–112
19. Hansen MM, Ruzzante DE, Nielsen EE, Bekkevold D, Mensberg KLD (2002) Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations *Molecular Ecology*, 11, 2523-2535
20. Hauser L, Adcock G, Smith PJ, Bernal Ramirirez JH and Carvalho GR (2002) Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (Pagrus auratus) *PNAS* 99: 11742-11747
21. Hill W G (1981) Estimation of effective population size from data on linkage disequilibrium *Genetical Research* 38: 209-216
22. Koskinen MT, Ranta E, Piironen J, Veselov A, Nilsson J and Primmer CR. (2002) Microsatellite data detect low levels of intrapopulation genetic diversity and resolve phylogeographic patterns in European grayling, *Thymallus thymallus*, Salmonidae. *Heredity* 88: 391-401
23. Koskinen MT, Piironen J, Sundell P and Primmer CR. (2002) Spatiotemporal evolutionary relationships and genetic assessment of stocking effects in grayling (*Thymallus thymallus*, Salmonidae) *Ecology Letters* 5: 193-205

24. Laikre L (ed) 1999 Conservation genetic management of brown trout (*Salmo trutta*) in Europe Report by the Concerted action on identification, management and exploitation of genetic resources in the brown trout (*Salmo trutta*) ("TROUTCONCERT"; EU FAIR CT97-3882) (ISBN 87-987732-0-8 1999) 91 pp
25. Luikart G and Cornuet JM (1997) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data *Conservation Biology* 12: 228-237
26. Luikart G and Cornuet J-M (1999) Estimating the effective number of breeders from heterozygote excess in progeny *Genetics* 151, 1211-1216
27. Nielsen EE, Hansen MM and Loeschcke V (1997) Analysis of microsatellite DNA from old scale samples of Atlantic salmon: a comparison of genetic composition over sixty years *Molecular Ecology* 6, 487– 492
28. Nielsen EE, Hansen MM and Loeschcke V (1999) Genetic variation in time and space: Microsatellite analysis of extinct and extant populations of Atlantic salmon *Evolution* 53, 261–268
29. Pudovkin AI, Zaykin DV, and Hedgecock D (1996) On the potential for estimating the effective number of breeders from heterozygote excess in progeny *Genetics* 144, 383-387
30. Reist-Marti SB, Simianer H Gibson J, Hanotte O and Rege JEO (2003) An approach to the optimal allocation of conservation funds to minimize losses of genetic diversity between livestock breeds *Conservation Biology* 17: 1299-1311
31. Rogers AR, Harpending HC (1992) Population growth makes waves in the distribution of pairwise genetic differences *Molecular Biology and Evolution*, 9, 552–569
32. Ryman N, Utter F, Laikre L (1995) Protection of intraspecific biodiversity of exploited fishes *Reviews in Fish Biology and Fisheries* 5: 417-446
33. Simianer H, Marti SB, Gibson J, Hanotte O and Rege JEO (2003) An approach to the optimal allocation of conservation funds to minimize losses of genetic diversity between livestock breeds *Ecological Economics* 45: 377-392
34. Templeton AR (1998) Nested clade analysis of phylogeographic data: Testing hypotheses about gene flow and population history *Molecular Ecology* 7, 381–398
35. Utter F, Aebersold P and Winans G (1987) Interpreting genetic variation detected by electrophoresis. *In* Ryman, N. and Utter, F. (eds.) Population Genetics and Fishery Management. Washington Sea Grant Program/University of Washington Press, Seattle, USA.
36. Wang J, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time *Genetics* 163, 429-446
37. Wang J (2001) A pseudo-likelihood method for estimating effective population size from temporally spaced samples *Genetical Research* 78, 243-257
38. Waples RS (1989) A generalised approach for estimating effective population size from temporal changes in allele frequency *Genetics* 121, 379-391
39. Ward RD, Woodwark M and Skibinski DOF (1994) A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology* 44: 213--232