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Report of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM)

24–27 March 2006

Newport, Ireland

International Council for the Exploration of the Sea
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Executive summary

The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met at the Marine Institute, Newport Ireland April 24–27, 2006. The meeting was well attended; with in total 18 representatives present from 10 countries (nine national delegates and nine experts appointed by the Chair).

Five terms of reference were on the agenda for 2006. The first issue addressed was that of genetically based domestication in fish and shellfish by unintentional (natural) selection in the hatchery environment. To a large extent these genetic changes are related to the relaxation of selection pressures on traits important in the wild (e.g. reproduction, migration, predator avoidance). There is ample evidence for domestication in fish, with carp and salmon as the most prominent case studies. For shellfish evidence of domestication is also apparent, in particular in relation to selection of brood-stock. The group recommends recognising both intentional and unintentional domestication in aquaculture as powerful evolutionary forces to improve production in aquaculture, but also makes a cautionary note in association with release or escape of domesticated hatchery fish and their potential impact on wild populations.

GSI (Genetic Stock Identification) now allows determination of the structuring of fish stocks into breeding populations and the estimation of proportions of contributing populations in mixed aggregations with high precision. To further exploit the immense potential of GSI in fisheries management it is necessary to improve the integration, accessibility and management of existing and future genetic information. Also, it is of paramount importance to preserve and catalogue irreplaceable archival biological material (e.g. historical scales and otoliths), which can be used "to go back in time" and serve as reference or baseline material to reveal genetic changes in populations. The group recommends the establishment of genetic "meta-databases" managed by organisations such as ICES responsible for coordinating and improving the biological data platform on which management of fisheries is based.

The group discussed the subject of local adaptation in marine fish populations. Failing to recognize locally adapted populations in fisheries management and conservation biology will result in erroneous estimation of short term demographic processes as well as long term population dynamics and response to harvesting and global change. The group produced a very thorough review aimed at the non-specialist explaining what local adaptation is, how to detect local adaptation and in addition provided a number of case studies. The group recommends that current knowledge of genetic basis of adaptive heterogeneity should be incorporated into fisheries management and more research should be diverted towards demonstrating local adaptation in the wild, identifying the environmental drivers responsible for local adaptation and in turn elucidating the genetic basis of important adaptive traits.

The TOR proposed in 2005 "Assess, through a case study of anadromous salmonids, the potential of genetic and spatial data analysis methods for resolving spatial boundaries of finfish and shellfish populations.." was postponed to 2007 since Professor Tom Cross who was to lead for this TOR was unable to attend the meeting.

Finally, the WG assessed the genetic effects of the introgression of farmed Atlantic salmon on wild salmon populations, following a request from NASCO (North Atlantic Salmon Conservation Organisation). Recently there have been a number of important new studies, which have been adding information to our general good understanding of the effects of the interactions between farmed and wild salmon. Accordingly, the group summarized and discussed this new information, particularly its potential to improve management of wild fisheries. A number of the group members were involved in a very recent review of the impact of farm escapes (Ferguson *et al.*, 2006) consequently (with the kind permission of the authors) some of this text was used to provide a part of the advice here.

1 Introduction

The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met at the Marine Institute, Newport Ireland, from 24 to 27 April 2006 to address its Terms of Reference (ToRs) for 2006 (Annex 1). The ToRs were decided by Council Resolution adopted at the ICES Statutory meeting held in Copenhagen, Denmark in 2005. Einar Eeg Nielsen (Denmark) chaired the meeting, which opened at 09:00 on Tuesday, 24 April and closed at 12:00, Friday, 27 April 2006.

1.1 Attendance

Eighteen persons from ten countries attended the meeting (Annex 2). Nine were official members (or substitutes) for their countries and nine were appointed experts by the Chair for 2006. The latter were registered with ICES prior to the meeting.

1.2 Venue

The meeting was held at the Marine Institute, Newport, Ireland. The WG expressed its appreciation to the local host Phil McGinnity and the rest of the staff at the institute for their kind hospitality. The meeting venue was ideal with accommodation available in Newport and "shuttle bus service" provided by our local hosts which took us to the institute just outside town in the morning and evenings.

1.3 Meeting Format

WGAGFM has an established framework for completing its ToRs. Prior to the meeting, small *ad hoc* working groups, under the leadership of one person, are established to prepare position papers related to specific issues in the Terms of Reference. The leader of the ToR is responsible for presenting the position paper in plenary at the meeting and chairing the discussion. Thereafter, volunteers undertake the task of editing and updating position papers according to points raised in the plenary discussions. The ToR leader is responsible for preparing the final report text from their sessions. Prior to the meeting an agenda is circulated to all members. For 2006 special "open sessions" were introduced allowing individual members to present data, software, management problems etc. to the rest of the group in an informal setting.

The 2006 WGAGFM meeting proceeded under the following direction: E. E. Nielsen chaired the business and general scientific session as well as the open sessions, T. Johansen chaired ToR a), E. Verspoor chaired ToR b), J. Mork chaired ToR c), E.E. Nielsen chaired ToR d) and P. McGinnity chaired ToR e).

2 Terms of Reference for 2006

2.1 Assess selected case studies and report on the current knowledge of the genetic basis of domestication processes in farmed fish and shellfish

This text was based on a working paper prepared by Torild Johansen, Martha O´Sullivan, Jochen Trautner and Pierre Boudry, adopted by WGAGFM at Newport, Ireland, 2006.

2.1.1 Introduction

Domestication has to be distinguished from simple taming of wild animals. The domestication process involves genetic changes in a group of breeding individuals caused by intentional or unintentional selection. According to Hale (1969) domestication is the breeding (by choice of

the reproducers and isolation from wild counterparts), care (shelter, food, protection against predators) and feeding of animals and is more or less controlled by humans. Therefore simply rearing of animals in an adequate environment (as for oyster or mussel) is not considered domestication (Mignon-Grasteau *et al.*, 2005).

There are three main processes involved in the evolution of animals during domestication: genetic drift, inbreeding and selection (Ollivier, 1981). The first two are depressive processes resulting from the limiting size of the population and leading to random variations in the gene frequency. Selection can be divided into artificial selection and natural selection.

Artificial selection involves human selection of favourable traits and is the main aim of domesticating animals. In the past selective breeding programs have altered the genetic makeup of various aquaculture strains e.g. carp, salmon, rainbow trout, seabass, seabream, tilapia and catfish. The efficiency of artificial selection has improved since quantitative genetics and, more recently, marker assisted selection has been used to create selective breeding programs (Liu and Cordes, 2004). However, compared to other animal groups for example poultry the selective process in most fish/shellfish species is still in its infancy and there is still large scope for further optimisation of certain traits beneficial to the fish farmer.

Natural selection in captivity is more or less unintentional selection resulting in the modification of traits in a given and foreseeable direction. It mostly consists of a relaxation of selection pressure existent in the wild which applies to traits that are important in nature but not in captivity such as food finding, seasonal reproduction, external colour, or predator avoidance. Domestic animals can thus be more variable in these traits then their wild counterparts. But natural selection in captivity also eliminates animals unable to reproduce in captivity, and favours animals, which can wean a high proportion of young in the environment provided by humans. However it is difficult to describe and quantify the potentially very high level of unintentional selection that occurs during the domestication process. To make this process more understandable a fictitious example is given: In a typical aquaculture situation the first selection occurs when choosing the fish intended to be kept in captivity (se e.g. Dahle *et al.*, 2006). Depending on the method (net, trap etc.) used the fast and “clever” ones may be selected against (simply because they are more difficult to catch) and also of those caught the smaller individuals may pass through the net and so too be underrepresented in the catch. These fish are then transported to a stressful aquaculture environment where the “less stress tolerant” individuals will not survive. Individuals will next undergo pressure of weaning to artificial feeding, mature and/or reproduce and only those adapting to the situation will survive and reproduce under aquaculture conditions. So without having actually performed an intentional selection up to this point many selective factors have already altered the gene pool of the animals used in the aquaculture situation compared to the wild population. To investigate the extent of domestication within a species wild and domestic stocks can be compared genetically, phenotypically or behaviourally, under the same environmental conditions. Longitudinal analysis of wild animals kept in captivity allows for genetic, phenotypic and behavioural changes over time in wild populations to be studied (Price, 2002). The purpose of such studies is to quantify the rate of evolution over time.

2.1.2 Case studies in fish/shellfish

The **domestication of carp** can be traced back 2000 years to the Romans with the original broodstock originating from the Danube River system (for review see Balon, 2004). The common carp were produced in pond systems including spawning and growing ponds. Unintentional artificial selection has been taking place between the 12th and mid-14th century, and deep bodied and variously scaled or scaleless domesticated forms appeared in nearly every pond system. Some colour aberrations appeared in the 1950s in Japan, which, as koi, became the most expensive of fish. Accordingly, in the domesticated forms of the species morphology and colour has changed dramatically compared to the wild type.

By comparison the **domestication of salmonid fish** is in its infancy having only begun about 40 years ago, a time span equivalent to as few as eight generations. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) production in aquaculture is based mainly on a few strains originating from a few wild ancestor populations. Breeding programs alter the genetic makeup of farmed strains e.g. in broodstocks of rainbow trout egg hatchability, fry survival, feed conversion efficiency, growth, and fecundity are highly affected by levels of inbreeding (reviewed in Kincaid, 1983). These genetically altered strains usually perform much better in aquaculture than their wild counterparts do (Gjøen and Bentsen, 1997). However when released into the wild these 'domesticated' strains may display a lower fitness than the wild fish (McGinnity *et al.,* 2003). In many cases behavioural differences are responsible for these differences in fitness for example, the avoidance of predators (Berejikan 1995, Fleming *et al.,* 2000; McGinnity *et al.,* 2003). Some of these negatively altered traits are traits that developed unintentionally by relaxed natural selection under aquaculture conditions. Lucas *et al.* (2004) investigated behavioural differences between clonal lines and crosses of rainbow trout and found that the domestication history of the source line had a major influence on behavioural traits like mean swimming level, hiding, foraging, startle response, and aggression level. The progeny of populations reared in captivity for over 100 years exhibited reduced predator avoidance behaviour patterns and an increase in aggression compared to progeny from more recently domesticated populations. Johnsson and Abrahams (1991) and Berejikan (1995) both demonstrated that wild steelhead trout were less susceptible to predation than farmed trout (death rate 9% and 17% wild and farmed, respectively, (Berejikan, 1995). This could be due to greater risk taking by domesticated animals due to the naivety of the farmed strain with respect to the presence of predators i.e. more likely to take more risks in the presence of natural predators than their wild counterparts are. Domesticated masu salmon for example feed closer to the surface, where they are more susceptible to predation (Reinhardt 2001). They also show a shorter latency to feeding after the introduction of chemical alarm signals, a mixture of masu salmon body homogenate and water from a recirculating tank containing piscivorous predators (Yamamoto and Reinhardt, 2003).

Unintentional selection can be investigated by comparing the breeding history of wild and domesticated fish. Kallio-Nyberg and Koljonen (1997) for example examined the breeding history of wild, hatchery-reared and released Baltic Atlantic salmon. By releasing 2-year-old tagged smolts into a river they compared the progeny of mating groups with differing parental breeding histories and parental sea-age. They found that the sea growth rate of progeny was not independent of the parental traits, breeding history or sea-age with the progeny of ranched parents growing more rapidly in the sea than the progeny of the wild parents. The sea-age at maturity was also found to be dependent on the parental traits with 52% of the progeny of ranched parents returning as grilse while from wild parents it was just 37% ($p=0.026$). They also found that the progeny of ranched females were larger after two sea-winter growth periods and were captured in a different area than the progeny of the ranched two-sea-winter females. This study illustrates that selection occurs in the aquaculture/ ranching environment for characteristics which are not intentionally being selected for by the farmer. Despite recognition that unintentional selection does occur and that unselected domestic characteristics can be found in many farmed species it will be challenging to identify the genetic basis for these traits. It is known that molecular genetic information will contribute to a better understanding of the history of domestication (see Bruford *et al.,* 2003 for a review,). However, though such work has begun (Roberge *et al.,* 2006; Wright *et al.,* 2006). Wright and co workers (2006) have recently identified QTLs for anti-predator behaviour in zebrafish. Roberge *et al*. (2006) found parallel changes in gene expression in domesticated Atlantic salmon from Canada and Norway compared to wild fish independent of their geographical origin. Such work is still in its infancy and need further attention with respect to fish species.

2.1.3 Shellfish

Unlike for fish (Busack and Currens, 1995; Roberge *et al*., 2006), little is known for shellfish species about intentional and unintentional consequences of domestication. Inbreeding depression has been studied in several bivalve species by recording the performance in progenies of sib families or selfing hermaphrodites (with expected inbreeding coefficients $0.25 < F < 0.5$), or in the framework of a breeding program, where more limited inbreeding ($F = 0.0625$) also lead to significant inbreeding depression of yield and individual growth rate in two-year old oysters (Evans *et al.*, 2004).

In many cultured bivalve species, the larval phase is most likely to be affected by artificial rearing conditions, and therefore a potential domestication process, since the later stages are usually grown under more natural conditions (i.e. tidal and costal areas). However, most studies about selection in bivalves have focused on juvenile and adult development stages (for oysters, see Sheridan, 1997), but relatively few give consideration to the larval stage (Ernande *et al.*, 2003). Inbreeding depression was observed at the larval stage in *Ostrea edulis* (Bierne *et al.*, 1998), *Crassostrea virginica* (Longwell and Stiles, 1973), *Pecten maximus* (Beaumont and Budd, 1994), *Argopecten circularis* (Ibarra *et al.*, 1995), and *Crassostrea gigas* (Hedgecock *et al.*, 1995; Launey and Hedgecock, 2001). Launey and Hedgecock (2001) have clearly demonstrated the high load of deleterious mutations carried by *C. gigas*.

Unintentional selection during hatchery and nursery stages have recently been investigated in the Pacific oyster *Crassostrea gigas* by Taris (2005), using a mixed-family approach and microsatellite-based family assignment (Taris *et al.*, 2005). Two main factors have been studied: (1) the effect of culling (i.e. discarding of slow growing larvae) and (2) the effect of high temperature (in *C. gigas* hatcheries, water temperature during larval rearing is usually around 26–28 °C, which is much higher than in the wild, to speed up this phase). Culling, by selective sieving of the smallest larvae, is an advantageous practice at a phenotypic scale as it reduced variance in larval size, variance of developmental rate and time to settlement. However, culling represents a substantial risk for diversity loss, because it increases the variance of reproductive success among parental oysters (Taris *et al.*, in press). Taris (2005) also compared larval growth, survival and genetic composition in populations reared at 20 and 26°C. Significant genotype x environment interactions were observed for growth and survival. Interestingly, a higher temperature exerted a positive influence on the expression of genetic variability for larval growth. Consequently, Taris conclude that a temperature of 26°C coupled with culling, to common practice in oyster hatcheries, is likely to amplify the selection pressure for fast growing larvae.

To test this hypothesis, they compared larval developmental traits in the progeny of a hatchery broodstock closed for seven generations, with the progeny of wild oysters and the two possible hybrids. These results showed that selection of fast growing larvae can counteract presumed inbreeding depression, due to higher mean relatedness among hatchery broodstock than in the wild. Genetic effects of intensive rearing conditions at larval stage, notably those related to culling and temperature, are significant and should be taken into account in oyster hatchery practices, especially in terms of genetic diversity management.

In the Pacific white shrimp (*Litopenaeus vannamei*), Arena *et al.*, (2003) studied the effect of artificial selection to increase body weight on the adaptation ability of shrimp to use dietary carbohydrates as source of energy. They observed a reduction in amylase activity related to domestication in two selected populations showing reduced genetic variability for amylase genes. Results suggest that the efficiency with which shrimp transform energy into biomass was reduced by artificial selection.

In summary domestication is an evolutionary process resulting in a ‘farmed population’ where many physiological, morphological and behavioural traits differ from those present in their wild counterparts. These differences are the result of both intentional and unintentional

selection pressures and result in individuals whose performance in captivity is changed positively i.e. from an aquaculture point of view (e.g. faster growth, disease resistance, age at maturation) but exhibit a lower performance than the wild stocks. This could result in organisms less adaptable to unexpected environmental changes. This is important to have in mind in the process of domesticating new species among others e.g in the growing interest for farming of cod-fishes. Our knowledge of the genetic basis of domestication in aquatic organisms is limited and to further understand the processes involved it is important that experiments demonstrating the changes and the genetic basis of those changes are carried out in order to optimise breeding programs.

2.1.4 Recommendations

- To promote studies on unintentional natural selection to understand the process of domestication and more experiments demonstrating the change and the genetic basis of such traits
- To genetically monitor hatching of fish/shellfish to be used for sea-ranching, aquaculture based fisheries or restocking purposes and to carefully estimate unintentional selection occurring during captivity.
- To be aware of the unintentional selection going on in the hatcheries, which can/may have implications/potential effect on the wild population or induce further domestication.

To engage in more research regarding gene expression analysis for further understanding the nature of the genetic changes associated with domestication.

2.1.5 References

Arena, L., Cuzon, G., Pascual, C., Gaxiola, G., Soyez, C., Van Wormhoudt, A., Rosas, C. 2003. Physiological and genetic variations in domesticated and wild populations of Litopenaeus vannamei fed with different carbohydrate levels. J Shellfish Res., 22(1): 269–279.

Balon, E.K. 2004. About the oldest domesticates among fishes. Journal of Fish Biology 65(Sup. 1): 1–27.

Beaumont, A.R., Budd, M.D. 1983. Effects of self-fertilization and other factors on the early development of the scallop Pecten maximus. Mar. Biol., 76: 285–289.

Berejikan, B.A. 1995. The effect of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhyncus mykiss*) to avoid benthic predators. Can. J. Fish. Aquat. Science., 52: 2476–2482.

Bierne, N., Launey, S., Naciri-Graven, Y., and Bonhomme, F. 1998. Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. Genetics, 148: 1893–1906.

Busack, C.A., and Currens, K.P. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. American Fisheries Society Symposium, 15: 71–80.

Dahle, G. Jørstad, K.E., Rusaas, H.E., and Otterå, H. 2006. Genetic characteristic of Broodstock collected from four Norwegian coastal cod (Gadus morhua) populations. ICES Journal of Marine Science, 63: 209–215.

Ernande, B., Clobert, J., McCombie, H., and Boudry, P. 2003. Genetic polymorphism and trade-offs in the early life-history strategy of the Pacific oyster, *Crassostrea gigas* (Thunberg, 1795): A quantitative genetics study. J. Evolution. Biol., 16: 399–141.

Evans, F., Matson, S., Brake, J., and Langdon, C. 2004. The effects of inbreeding on performance traits of adult Pacific oysters (*Crassostrea gigas*). Aquaculture, 230: 89–98.

Fleming, I.A., Hindar, K., Mjolnerod, I.B. *et al.* 2000 Lifetime success and interactions of farm salmon invading a native population. Proceedings of the Royal Society of London. Series B, Biological Sciences, 267, 1517–1523.

Gjøen, H.M., Bentsen, H.B. 1997 Past, present, and future of genetic improvement in salmon aquaculture. ICES Journal of Marine Science, 54: 1009–1014.

Hale, E.B. 1969. Domestication and the evolution of behaviour. In: Hafez, E.S.E. (Ed.), The Behaviour of Domestic Animals. Bailliere, Tindall and Cassell, London, pp. 22–42

Hedgecock, D., McGoldrick, D.J., Bayne, B.L. 1995. Hybrid vigor in pacific oysters: an experimental approach using crosses among inbred lines. Aquaculture, 137: 285–298.

Ibarra, A., Cruz, P., and Romero, B.A. 1995. Effects of inbreeding on growth and survival of self-fertilized catarina scallop larvae, *Argopecten circularis*. Aquaculture, 134: 37–47.

Johnsson, J.I., and Abrahams, M.V. 1991. Interdreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. Aquaculture 12: 73–77

Kallio-Nyberg and Koljonen 1997. The genetic consequences of hatchery-rearing on life-history traits of the Atlantic salmon (*Salmo salar* L.): a comparative analyse of sea-ranched salmon with wild and reared parents. Aquacuulture, 153: 207–224.

Launey, S., and Hedgecock, D. 2001. High genetic load in the Pacific oyster *Crassostrea gigas*. Genetics 159: 255–262.

Liu, Z.J., and Cordes, J.F. 2004 DNA marker technologies and their applications in aquaculture genetics Aquaculture, 238(1–4): 1–37.

Longwell, A.C., and Stiles, S.S. 1973. Gamete cross incompatibility and inbreeding in the commercial American oyster, Crassostrea virginica Gmelin. Cytologia, 38: 521–533.

Lucas, M.D., Drew, R.E., Wheeler, P.A., Verrell, P.A., and Thorgaard, G.H. 2004. Behavioral differences among rainbow trout clonal lines. Behav. Genet., 34(3): 355–65.

McGinnity, P., Prodohl, P., and Ferguson, K. *et al.* 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, Salmo salar, as a result of interactions with escaped farm salmon. Proceedings of the Royal Society of London. Series B, Biological Sciences, 270: 2443–2450.

Mignon-Grasteau, S., Boissy, A., Bouix, J., Faure, Jean-Michel, Fisher, A. D., Hinch, G.N., Jensen, P., Le Neindre, P., Mormede, P., Prunet, P., Vandeputte, M., and Beaumont,C., 2005. Genetics of adaptation and domestication in livestock. Livestock Production Science, 93: 3–14.

Ollivier, L. 1981. Eléments de génétique quantitative. Masson Paris

Price, E.O. 2002. Ed. by E.O. Price. *In* Animal Domestication and Behaviour. CABI Publishing, Wallingford, UK, pp 1–9.

Reinhardt, U.G. 2001. Selection of surface feeding in farmed and Sea-ranched masu juveniles. Trans. Am. Fish. Soc. 130: 155–158.

Roberge, C., Einum, S., Guderley, H., and Bernatchez, L. 2006. Rapid parallel evolutionary changes of gene transcription profiles in farmed Atlantic salmon. Mol. Ecol., 15: 9–20.

Roselund, G., and Skretting, M. 2006. Worldwide status and perspectives on Gadoid culture. ICES Journal of Marine Science, 63: 194–197.

Sheridan, A.K., 1997. Genetic improvement of oyster production - a critique. Aquaculture, 153: 165–179.

Taris, N., Baron, S., Sharbel, T.F., Sauvage, C., and Boudry, P. 2005. A combined microsatellite multiplexing and boiling DNA extraction method for high throughput parentage analyses in the Pacific oyster (*Crassostrea gigas*). Aquac. Res. 36: 1-3.

Taris, N., Ernande, B., Boudry, P. In press. Phenotypic and genetic consequences of size selection at the larval stage in the Pacific oyster (*Crassostrea gigas*).Journal of Experimental Marine Biology and Ecology (in press).

Withler, R.E., Supernault, K.J., and Miller, K.M. 2005. Genetic variation within and among domesticated Atlantic salmon broodstocks in British Columbia, Canada. Anim Genet., 36(1): 43–50.

Wright, D., Nakamichi, R., Krause, J., and Butlin, R.K. 2006. QTL Analysis of Behavioral and Morphological Differentiation Between Wild and Laboratory Zebrafish (*Danio rerio*). Behav Genet, 12: 1–14.

Yamamoto, T., and Reinhardt, U.G. 2003. Dominance and predator avoidance in domesticated and wild masu salmon *Oncorhyncus masou*. Fish. Sci., 69: 88–94.

2.2 Identify and provide recommendations on the technical and organisational requirements for establishing practical, functional and integrated international databases and supporting repositories for genetic stock identification (ToR b)

This text was based on a working paper prepared by E. Verspoor, R. Hanel O. Vasins and A. Was, adopted by WGAGFM at Newport, Ireland, 2006.

2.2.1 Background and Issue

Understanding of biodiversity (i.e. genetic diversity) within currently recognised Linnean fish species, and how it is impacted by factors such as fisheries exploitation and global climate change, is critical for the development of effective management programmes for the conservation and restoration of fish stocks. Analysis of the distribution of genetic variation, in both space and time, can deliver valuable insights in support of fisheries management in relation to:

- the structuring of fish stocks into genetic populations, the fundamental biological units underpinning fisheries recruitment; this understanding of intraspecific biodiversity is important for the support of biodiversity conservation and restoration under national and international legislation such as the Rio Convention, and the EU Water Framework and Habitats Directives
- the proportional contribution of different genetic populations, or regional groups of populations to fish aggregations and fisheries catches, to help manage exploitation of individual populations within sustainable levels
- the impacts of exploitation on fisheries, to avoid detrimental changes and guide stock restoration initiatives
- the impacts of global climate change on fisheries, to understand how these affect fish abundance and distribution and, thereby, fisheries catches

The potential of genetic studies to contribute to understanding of population structuring and intraspecific biodiversity, and to mixed stock analysis, is well illustrated by work on salmonids fishes, particularly in the northeast Pacific Ocean (e.g. Beacham *et al.*, 1999; Shaklee *et al.*, 1999). Potential also exists to apply molecular genetic analyses to help understand the impacts of fisheries exploitation and global climatic change but this application is more recent and currently less advanced.

Methods for the direct analysis of DNA variation are now widely available and there is an increasing amount of genetic information available. As a result for many species, we are now starting to be able to resolve population structuring and assess population change. However, the growth in DNA data can be expected to be substantial as new and increasingly rapid and cost-effective methods of genetic screening are developed. This should lead to increasing insights in the coming years.

The contribution of genetic studies is dependent on the quality and amount of genetic information available. This depends on individual studies with respect to the type and number of the molecular markers screened and the statistical analyses carried out. However it is also

dependent on 1) the availability of both contemporary and historical samples, and 2) the integration and utilisation of genetic data across studies. The extensive geographical range of most species, the complexity of species' genomes, and the limitations on resources for genetic screening mean that gaining a clear overview of the population genetic character of a species poses a major challenge and most studies only provide a limited geographical and genomic insight. However, this insight can be substantially increased where it is possible to link genetic studies, something which can increase the extent and quality of geographical and temporal analyses (e.g. Verspoor *et al.*, 2005), provided common sets of genetic loci are screened.

Linkage of contemporary work to historical information from the past studies or availability to DNA in archival materials is critical for assessing the genetic impacts of global climate change, habitat change (e.g. as caused by fish farming activities), and fisheries. If not, such assessments are impossible or can only be carried out once suitable temporal time series of data or materials have been accumulated, something which will be both costly and require decades to achieve. This can be avoided in many cases if contemporary data sets are linked to historical data, or by exploiting DNA in historical soft tissue, scale and otolith collections.

For this reason, archival material is an important resource and needs to be protected. Yet historical material in many cases is at serious risk of being lost. Its importance is not always appreciated by its custodians and a lack of awareness of its existence means it may not be considered in the design of scientific studies. The risk of loss is serious as collections of useful material are often maintained within organisations only through the care and attention of individuals and once they leave or retire are at risk of being lost or neglected.

2.2.2 Proposed action

Genetic understanding in the future could be substantially increased by:

- Improving the integration and accessibility of existing and future genetic information collected by studies carried out at different times and by different research groups.
- Developing more effective measures for preserving, cataloguing and accessing archival biological material containing DNA e.g. scales, ootoliths and museum specimens for future genetic analyses

The institutional development of two types of linked data bases for fish species covered by the ICES remit would be valuable for making sure that historical information and material which exists is available and accessible for genetic work. These are meta-data and primary data bases which are linked as follows.

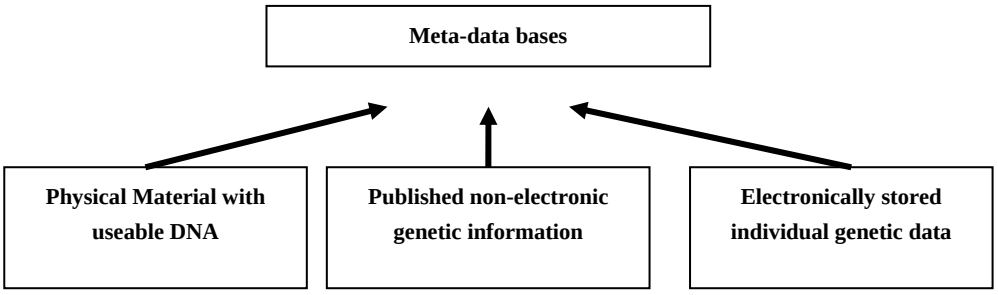

Figure 2.2.2.1.

Meta-data bases are catalogues or lists of what information is available, its quality and extent, and where it can be found, but not the primary information itself. Three types of meta-data bases which do not currently exist would help to achieve the stated aims:

- Lists of existing sources/locations of primary genetic data
- Catalogue of repositories of archival material useful for DNA analysis
- Directory of Researchers and research relating to the genetics of a species

Meta-databases should be accessible to interested parties and information sources be catalogued taxonomically as well as geographically with existing meta-databases like FISHBASE (Froese and Pauly, 2005) serving as useful templates. This suggests that they are most useful if they are centrally managed by an organisation such as ICES which has a vested interest in increasing research productivity in the field of fisheries genetics.

Primary data bases represent the actual genetic information or material. This can encompass:

- collections of genetic data which include either genetic information on samples e.g. allele frequencies for individual loci, or individual haplotype data, information on geographical origin of a sample or individual, date of collection, collector, researcher and linked data sets e.g. on size, age, state of maturation etc.
- Published non-electronic data sets e.g. papers, reports –
- Repositories of Biological Material e.g. soft tissues, scales, otoliths

Species specific genetic information should be collated into integrated trans-national and, where possible, range-wide electronically stored data banks. Valuable published text based data, found in historical papers and reports, should be scanned and centrally available in electronic form. Repositories of biological material should be promoted, maintained, and co-ordinated at a national level. This includes developing standard policies and operating practises for collecting and storing existing and new samples so their present and future usefulness is maximised. Optimal methods and approaches need to be reviewed. It is easier to sample much more primary material than it possible to analyze (using the newest and the most powerful analyzers) but at the same time if sufficient suitable material is not available studies of historical change are not possible.

The existence of meta and primary data bases on the genetics of fish species will also ensure that available information and resources can be rapidly and effectively accessed so that existing information is taken into account in new research programmes and is available to inform the development of fisheries management policy and decisions. The activity in creation of databases has to be a basic platform for international research cooperation with the main goal being to increase the scientific activity on the field of fish genetics.

2.2.3 Implementation

The development, maintenance and holding of meta and primary data bases needs to be organised and supported at the institutional level and a framework for doing so needs to be put in place. This requires careful consideration as it will determine data base utility and effectiveness, now and in the future. It will also influence the extent to which future research efforts of different laboratories work to integrate data on a given species across studies. If properly implemented, it should encourage integration and their continued development, increasing their value for addressing scientific questions beyond the sum of the results of the individual studies. Placed in the right institutional framework, the two types of data bases will help ensure that in the design of new research, past and on-going work can be easily and fully taken into account and, to the extent possible, integrated into existing information increasing the information value of new work. This will be valuable at both the individual and institutional level.

Meta data bases should be developed and implemented in a centralized way, as has been done for FishTrace, Fishbase and Genbank, by an organisation such as ICES with a vested interest in promoting and using genetics research of fish species. For primary data bases, standardised methods for data collection will need to be developed and the data bases will need to be able to be freely accessed by all potential research workers, both now and in the future. This can be done in a centralised or dispersed way, each of which has its own merits, with a combination of approaches likely to be most productive. Central to either approach will be establishing and maintaining collaborative networks of researchers investigating each species of interest. These will help to keep researchers up-to-date, ensuring that analyses are not needlessly repeated by different research groups, increasing research efficiency, and provide the forum for standardisation of methods and integration of data sets. Primary data bases should, where possible, be linked to other biological data on samples.

ICES is ideally placed to host and maintain GIS based genetics meta-data bases on the species covered by its remit; these could be linked to existing GIS based species data bases. This could be achieved by nominating someone to lead in their development and be responsible for the promotion and coordination of primary data bases and national and trans-national sampling and data mining programs.

One of the most important tasks of such an individual would be to oversee the cataloguing of existing historical material of potential use for genetic investigations (dried, frozen or ethanol-preserved organic material of any kind). This should be done at the national level and driven by national fisheries boards, and central, or centrally administered, archives established and maintained. Guidelines for the preservation and use of material should be developed at the international level. This will be especially important where there is a limited amount of material to ensure that existing historical resources are most effectively and productively used. With an eye on the future, archiving of new geographically, temporally and taxonomically representative samples of DNA or tissue should be encouraged.

Funding for the establishment of both meta- and primary data bases should be sought from trans-national funding bodies such as the EU and should be considered a funding priority for programmes such as FP7. Funding should be encouraged for 2 or 3 pilot projects on species for which existing genetics research programmes are underway as a way of developing optimal approaches which can be used across all fish species of interest.

The funding should be directed at supporting the set up of meta data bases and research networks for individual species to establish primary data bases and co-ordinate on-going and future research activities. Primary data bases should be lodged in libraries and with relevant national and international organisations such as national fisheries boards and ICES. The organization structure needs to be as simple and cheap as possible, and structured to provide flexibility for future development. In this respect, there should not be one central depository. Funding also needs to be made available for research into the optimal ways of storing DNA and DNA containing materials, both from a degradation and cost perspective e.g. the relative merits of ethanol versus dry filter paper based systems. This is also best done at a trans-national level, as is the development of “best practice” guidelines for sample cataloguing and use. Where internationally important collections exist, international funding to support their protection may be needed in some cases and should be considered.

2.2.4 Recommendations

- WGAGFM should define standardised structural and operational requirements for species specific meta and primary genetic data bases.
- National and EU funding programs should provide financial support for the formation and maintenance of research networks to develop and appropriately archive primary genetic data bases, and exploit these data bases to answer fundamental questions on GSI in marine fishes.

- ICES should take the lead in the development and maintenance of a meta-data base related to genetics in fisheries and mariculture linked to existing fisheries assessment data bases.
- National fisheries institutions within each member country should catalogue and centrally list all existing historical collections of biological material from fish species within the ICES remit which have a potential use for genetic investigations.
- National fisheries institutions should encourage the creation of high level central national "DNA Banks" of archived historical biological material, and extend these with contemporary geographically, temporally and taxonomically representative collections to facilitate past and future time series investigations. (EU data collection regulation – integrated).
- WGAGFM should undertake a review of methods for DNA archiving and storage has as well as clear, practicable guidelines for DNA sampling in fisheries surveys.
- ICES should encourage the formulation of a policy on the utilization of scarce historical and future collections suitable for DNA analysis to ensure their optimal exploitation for trans-national research goals

2.2.5 References

Beacham, T.D., Pollard, S., and Le, K.D. 1999. Population structure and stock identification of steelhead in Southern British Columbia, Washington, and the Columbia River based on microsatellite DNA variation. Transactions of the American Fisheries Society, 128: 1068–1084.

Froese, R., and Pauly, D. (Eds.) 2005. FishBase. World Wide Web electronic publication. www.fishbase.org

Shaklee, J.B., Beacham, T.D., Seeb, L., and White, B.A. 1999. Managing fisheries using genetic data: case studies from four species of Pacific salmon. Fisheries Research, 43: 45–78.

Verspoor, E., Beardmore , J.A., Consuegra, S., Garcia de Leaniz, C. Hindar, K. Jordan, W. C. Koljonen, M-L. Mahkrov, A. A, Paava, T. Sánchez, J.A. Skaala, O. Titov, S., and Cross T.F. 2005. Population Structure in the Atlantic Salmon: Insights From 40 Years of Research into Genetic Protein Variation. Journal of Fish Biology, 67(Supplement A): 3–54.

2.3 Synthesize the evidence and methods for detecting local (genetic) adaptation in marine fishes (ToR c)

This text was based on a working paper prepared by J. Mork, D. Bekkevold, H. Knutsen, R. Wenne C. André, E. Gosling, S. Mariani and B. Hernande, adopted by WGAGFM at Westport, Ireland in 2006.

2.3.1 Abstract

This report gives the results of the discussions in the working group. Starting with an overview of topics, definitions, and the genetic framework and evolutionary mechanisms involved in local adaptation, the report gives an overview of areas where the knowledge of such adaptation is important in practical management of fisheries as well as aquaculture activities. The need for knowledge on local adaptation for the conservation of genetic resources is also treated. It is realised that the marine environment itself represent a special challenge for the detection and description of local adaptation, and a set of proven useful approaches is described along with a set of case studies in different species and stocks. The deliberations of the working group on these topics are condensed in a set of recommendations for further research and for incorporation into current management practices.

2.3.2 What is local adaptation?

At the population level, local adaptation is the change in a gene pool that occurs as a response to selective forces set up by local environmental factors. The genetic change works through fitness differences between individuals/families in the population. Evolution that is due to stochastic changes in the gene pool (random genetic drift) can occur, but is not part of a local adaptation. Usually, individuals have some capacity to deal with environmental stress through phenotypic plasticity (humans shiver when cold, sweat when warm), but such general and temporary phenomena are not regarded as adaptive in the evolutionary sense.

The concepts of relative fitness and selection

The genetic setup of individuals is determined by genes on the chromosomes. The place on a chromosome where a specific gene is located is called a *locus* (latin for "place"; in plural *loci*). In diploid species (like humans) individuals receive one chromosome from each of the parents, and hence one gene from each parent for a specific trait, forming its genotype for that trait. The genes from each parent can be identical (in homozygotes), or different (in heterozygotes).

The fitness coefficient (w) is a relative measure ($0<w<1$); it tells how efficiently an individual genotype passes on its genes to the next generation, compared to other, different genotypes for the same trait. For the abnormal gene for the sickle cell haemoglobin (HbS) in humans), the fitness coefficient is only a fraction of that of the normal haemoglobin. In double dose (i.e. the S type haemoglobin gene both from mother and father), the homozygote HbSS is lethal (babies die early in life du to sickling of the red cells when de-oxygenized). For multilocus genotypes the relative fitnesses are determined by the joint contribution from the involved single loci. Based on the differences in relative fitness of individuals, some reproduce better than others. The favoured (best) individuals is said to be selected for, and the rest is selected against. Thus fitness and selection are related concepts, and the selection coefficient (s) is defined as one minus the fitness coefficient ($s=1-w$).

The concept of genetic variability (allelic vs by recombination)

Without individual genetic variation the concepts defined above would be meaningless, as would also the concept of evolution. Thus, genetic polymorphisms are crucial in evolution. The genetic polymorphisms arise by mutations in existing genes. (The human AB0 blood groups is an example of a genetic polymorphism). Mutations accumulate through evolution so that most species today are polymorphic at a large part of their loci (i.e. their genes).

In population genetics, the frequencies of the various mutational forms of a gene are called alleles. The frequencies of the various alleles are, under certain assumptions set by the Hardy-Weinberg law, constant over generations in a population, and the proportion of the different genotypes in diploids are determined by the allele frequencies. Different populations can have different allele frequencies, which then can be utilized as stable population characteristics.

A very useful working definition in population genetics is that "…the smallest unit of evolution is any change in allele frequencies in populations".

It is axiomatic in evolution that genetic variability (polymorphisms) is favourable in order to cope with and survive environmental changes. This applies to taxa at all levels. The type of genetic variability that has accumulated by mutations during evolution is called allelic variability; it is the arsenal of different genes that a population or species possess to meet unpredictable scenarios of environmental change. In addition, however, there is a type of genetic variability that stems from the sexual reproduction process itself. The process of crossing-over between chromosomes from mother and father creates new combinations of genotype for evolution to work with. It is important to be aware that this recombinatoric

variability is not creating new genes – it is simply a re-shuffling of existing ones. In an adaptational process, however, this type of variability is probably very important.

Qualitative *vs* quantitative genetic traits

Some inhereted characters are coded for by genes at single loci. Individual genetic variability caused by allelic genes at single loci is called qualitative. They are typically either-or traits like blood type, where the genetic setup for an individual can be determined accurately. Traits coded for by the joint effect of genes at many loci (multi-locus traits) shows quantitative genetic variation, like e.g. growth rates, body length, and IQ. It is usually not possible to assess how many genes are involved in forming such traits, and how large the effect of each locus is on the trait. It is this type of variation that forms the basis for quantitative genetics and selective breeding. Probably also, it plays an important role in local genetic adaptations.

2.3.3 Factors affecting local adaptation in nature

Evolutionary forces

Local genetic adaptation is part of evolution. Above, evolution was defined as "...any change in allele frequencies in populations". Hence populations can be viewed as the most significant evolutionary units and, followingly, the evolutionary forces are those forces which can change allele frequencies in the populations. In population genetics theory these forces are grouped in four important categories:

1) Mutations;
2) Random genetic drift;
3) Gene flow;
4) Selection.

Mutations at protein-coding loci are rare, and usually shortlived in an evolutionary sense because most of them have unfavourable (sub-lethal or lethal) effects. However, favourable mutations may survive and accumulate in populations, species and higher taxa. They constitute the genetic variability that is the raw material for evolution, including local adaptations. Some DNA markers, like microsatellites, show substantially (millions of times) higher mutation rates than protein-coding loci. These markers are found widespread in the genome, but their actual function has not been very clear (at least among fisheries geneticists). If selectively neutral, they may hence be much more sensitive tools for detecting low levels of differentiation caused by genetic drift. (More on the question of microsatellite neutrality below).

Random genetic drift is the change in population allele frequencies that occur between generations due to chance. The change is a "sampling error" which arise because, among other factors, not all individuals in a population take part in reproduction each generation, and not all parents give the same number of equally fit offspring to the next generation. The magnitude of random genetic drift is highly dependent on population size; it is large in small populations and small in large populations. In infinitely large populations it is nil.

Gene flow is the process where different groups of individuals exchange genes by immigration/emigration. Gene flow is a very powerful evolutionary force which hamper or prevent genetic differentiation between groups at all loci simultaneously. If there are no limitations to gene flow, groups are regarded as panmictic, and no lasting differentiation (including local adaptations) can develope.

Selection was by Darwin seen as the driving evolutionary force. Based on fitness differences between individuals which at least to some degree are genetically determined, natural selection will change the gene pool in response to environmental factors and act to increase

the mean fitness of the local population. The efficiency of selection depends a.o. on the amount of genetic variability present in the population, and the selection intensity. ("Fisher's fundamental law of natural selection" states that the rate of evolutionary change is directly proportional to the level of genetic variation).

From the population point of view there are thus three genetically differentiating forces (mutations, random genetic drift and selection) and one homogenizing force (gene flow). Thus the question of if and how much genetic diffentiation (including local adaptation) occurs boils down to a question of how reproductively isolated the putative populations are from each other. It is generally acknowledged that factors like a more homogeneous environment, extensive pelagic phases of eggs and larvae, large populaitons sizes and the lack of strong restrictions to gene flow explain why marine fishes show lower levels of genetic differentiation than anadromous fish, which in turn are less differentiated than limnic fish species (Gyllensten, 1985; Ward *et al.,* 1994; DeWoody and Avise, 2000).

The potential for genetic change by local adaptations

As treated introductory the amount of genetic change that can occur and persist in local populations depends on the concerted impact of virtually all the evolutionary forces; mutations, genetic drift, gene flow and selection coefficients:

For multilocus (quantitative) traits information from natural populations is scarce, but some anecdotic information exist for Atlantic salmon rivers. For example, in a salmon river (Figga) in the inner part of the Trondheimsfjord, Norway, a small river is only accessible for salmon on spawning run during a short period early in the spring (snow melting and the spring flood). Salmon which resturn later will not be able to ascend and to reproduce in this river. Indeed, the salmon in river Figga return almost one month earlier in the spring than salmon from the other Trondheimsfjord salmon rivers (like Orkla, Gaula, Nidelva, Stjørdalselva, Verdalselva). This may seem to represent a case of local adaptation (return time) upheld by very strong natural selection (high selection coefficients).

The speed at which a local adaptation for quantitative traits can proceed depends also on the heritability of the traits. Although heritabilities for "production traits" like growth, age at maturation, disease resistence have been estimated for captive, production stocks in salmon and other mariculture species, comparatively little information exists as for fitness-related traits in natural populations. "Heritability" is a quite imprecise measure which, in reality, is valid only for the (captive) population in which it was measured. However, if we for the argument assume that heritability in nature is similar to that in many selectively bred stocks, traits like growth rate and age at maturity can theoretically change quite rapidly under favourable conditions with respect to population size, gene flow and selective pressure.

For example, slaughtering weight for highly bred Atlantic salmon was doubled during 15 years of selective breeding in Norway, and age at maturation was at the same time reduced. Jonas Jonasson (Iceland) has found that heritability for survival (return) for Icelandic salmon may be substantial (0.15–0.20). In livestock, selective breeding for milk production in Norwegian NRF cattle more than tripled the annual output per cow.

Thus, in domesticated animals it is not uncommon that selective breeding can move the mean values of traits several standard distances (SD). (It is also noted that behavioural traits is inherited in the same way as somatic traits like growth, etc.) To the degree that the situations are comparable in captivity and in the wild, local adaptations in fish may hence appear to have a considerable potential.

However, this rests on several strict assumptions about gene flow, initial level of genetic variability, selection coefficients and selection intensity (cf above), and also on the nature of the adaptations that typically occur in natural populations. In nature, the environment is not

stabilised and optimized like in livestock production. It appears likely that this situation favours selecton for genetic variability more than for genetic unidirectionality (which forms the basis for genetic gain in livestock production). If, for example, single- or multilocus heterozygotes have a fitness advantage in a particular natural habitat, local adaptation will not proceed very far because the prospect for a substantial genetic change is limited. It is an axiom in breeding genetics that the heritability of fitness-related multilocus traits is low.

The so-called "**breeders equiation**" depicts the important variables which determine the change in a trait due to selection (natural or artificial). In the following example, the change in average mean length of individuals per generation due to a selective removal (i.e. they are not allowed to reproduce) of the upper 20% of a group is estimated according to the formula:

$$\Delta L = h^2 * I * SD_p$$

where h^2= heritability of trait, I = selection intensity (difference in mean values between population mean and selected group, measured in SDs), and SD_p = phenotypic standard deviation for the trait in the natural population (note: $I*SD_p = S$; the selection differential). As an example; if heritability of body growth is 0.26, and one by selective gear removes the largest 20 % of the individuals at some age at which mean length = 35.0 cm (SD_p=2.5), the mean length at same age in the next generation is determined by the remaining 80% and is hence reduced to [35 + (0.26*(-.35)*2.5)] ~ 34.8 cm.

Mating patterns, life history variation, behaviour

Marine organisms exhibit an extensive range of behaviours and life-history variations both between and within species. Within species, such differences constitute mechanisms preventing or slowing gene flow among local populations. A rapidly increasing number of studies report non-random mating patterns in marine organisms, with local populations differing with respect to, for instance spawning time, mating behaviour and success of different reproductive tactics. Such traits may reflect specific, heritable adaptations to local environments, although they also may constitute plastic and/or socially incurred responses to prevailing conditions (see Section 1.3.1). Whether one or the other, knowledge of such traits is, however, an important prerequisite for predicting population response to human activities and environmental change, as they are tightly linked with both population demographics and levels of connectivity through gene flow.

Human activities (selective fishery, overfishing, escapees, transfers)

Selective fishery

Fishing gear which catch fish on basis of individual phenotypic characteristics (e.g. body size (growth, age) or behaviour) can, to the degree that the phenotype reflects the genotype, make changes to gene pools in at least two ways: Firstly; if a fishery targets populations at a stage when they occur in the same areas (e.g. Pacific salmon on feeding migrations), legally set total quotae and mesh size regulations may unproportionally decimate the numerically small populations and hence affect their evolutionary potential including local adaptivity. Secondly; the continuous removal of the largest individuals by e.g. active and passive net gear may have effects on growth rates and age at maturity. Such human activities represent an extra evolutionary force which interfers with the natural gene pools and their local adaptation processes.

By-catch

Even with modern, high-tech fisheries technology some unwanted bycatch is unavoidable. For example, in catching herring with highly efficient purse seines one will also get species which follows herring shoals and predate on them, like cod and saithe. The mesh sizes of herring

purse seines are smaller than net gear regulations dictate for the gadoids, hence they catch under-measure cod and saithe. Regulations aimed at reducing such bycatch are not always efficient, and probably a good deal of unfortunate killing of untargeted species occurs. For decimated, threathened and endangered species this is particularly unfortunate and may have serious consequences for their evolutionary potential.

Overfishing

Today, most of the large marine resource fish stocks show clear signs of overexploitation (reference to FAO web site). While only in a few, if any, cases the overfishing is expected to result in extinction of species, the reduction in stock sizes can result in change of behaviour (e.g. Atlantic herring after the crash in the 1960ies), geographic distribution (large abundance usually increases the distribution range and vice versa), and niche competetiveness (the decline of gadoid species (cod, haddock and hake) in the West Atlantic since 1990 have given opportunities for skates, dogfish and lobster which has increased in abundance (National Marine Fisheries Service, USA).

Escapees from aquaculture

Fish species under domestification are notoriously undergoing a change in genetic variability and composition. Along with planned selection regimes for higher performance in productivity traits including e.g. growth rates age at maturity, there will always be a passive selection for e.g. sedative behaviour and other adaptations to a life in captivity). However, the traits which are favourable in captivity may be completely opposite in the wild. Hence, escapees from aquaculture plants which introgress into wild populations are expected to reduce the mean fitnesses and local adaptations in the wild stocks. In some places, e.g. in Norwegian salmon rivers, the genetic impact from escaped farmed fish already appears massive. As the domestification process goes on and the genetic setup of the farmed fish deviates further from that of wild stocks, the potential magnitude of the genetic impact is likely to increase.

Transfers of marine finfish and shellfish

Up to some 50 years ago, the deliberate transfer of marine fishes to new ecosystems for production and recreation (trophy fish) purposes was practiced many places and was not much questioned. Today, the dangers for local ecosystems, species and populations with such practices are generally acknowledged. In general the ecological outcomes of species transfers are highly unpredictable, and when effects are observed they are usually negative for the native populations, species and ecosystems. WGAGFM has treated some aspects of the transfer problem in detail in previous reports, and assisted the specific ICES Working Group for dealing with these questions; WGITMO (Working Group on the Introduction and Transfer of Marine Organisms) in preparing the *"ICES Code of Practice for the Introduction and Transfer of Marine Organisms"*.

2.3.4 Relevance of local adaptation to fisheries management

Local adaptation is one of the most significant components of intra-specific biodiversity. The relevance of local adaptation to fisheries management can be divided into two main issues that differ with respect to temporal scale. First, local adaptations and population structure affect short-term demographics through their effects on local recruitment patterns, and second, local adaptations and genetic heterogeneity affect long-term population dynamics, both with respect to connectivity among stocks/populations and their resilience and response to environmental change and harvesting. Whereas the application of genetic methods to determine stock/population structure is slowly beginning to gain practical use (e.g. Nielsen *et al.*, 2001; ICES, 2005), the second consideration of effects of local adaptation and maintenance of

biodiversity on long-term sustainable fisheries management has yet to be implemented into management strategies.

Population heterogeneity: phenotypic plasticity vs. genetic heterogeneity

Fisheries management is traditionally based on targeting stock components, defined by their geographic distribution and/or temporal stability in expression of traits such as mean age-at-spawning, migration behaviour, spawning time, parasite fauna and meristic traits, such as numbers of vertebrae. The underlying assumption is that stock components sharing such traits represent demographic and reproductive entities, whose (short-term) dynamics are distinct from those of other stocks, and therefore can be managed as comprehensive entities. However, the fact that morphological differences need not reflect reproductive isolation, and that a rapidly increasing number of genetic studies in marine and anadromous fishes and shellfish provide evidence for stock/population structure on a level of sub-components (that in many cases differ little with respect to known morphological traits), call for a reassessment of the validity of assumptions for management on a basis of classical stock concepts. When examining whether population differences reflect adaptive diversification, it is important to distinguish between differences that reflect underlying population structure and genetic heterogeneity that should be incorporated into management strategies, and plastic responses to different environments. Phenotypic plasticity describes the capacity of individuals to express different phenotypic traits in response to cues from the biotic and/or abiotic environment. With phenotypic plasticity, two individuals may appear very different, e.g. with respect to growth and reproductive behaviour, even though they stem from a reproductively coherent and genetically uniform background. Although expression of phenotypic plasticity may in itself be underlying local selective pressures, such phenotypic differences do thus not reflect that different types each are specifically adapted to a local environment. An example of such phenotypic plasticity was indicated for Atlantic herring, where sympatric components with different spawning times have been observed in several stocks (reviewed by McQuinn, 1997). Bekkevold *et al.,* (in review) recently combined genetic analyses and otolith based determination of hatching- and spawning season to show that in a western Baltic location, herring spawned in spring (which there is the predominant spawning season) may switch spawning time and become winter spawners with different life-history and migratory behaviour. Although this has not been specifically evaluated, the development and presence of winter spawning components is expected to be a recurrent, transient phenomenon and management initiatives specifically targeting such sub-components would thus not be expected to yield large effect. The study, however, also showed that sympatric winter- and spring spawning components in other locations may represent demographically and genetically divergent components, which should be incorporated into management strategies.

Local adaptations may occur not just among patchily distributed stocks, but also in species with continuous distributions (e.g. Conover and Present, 1990). Moreover, lack of detectable differentiation in morphology, life-history and neutral marker allele frequencies need not reflect panmixia and lack of local adaptations among individuals across spatial components. This has for instance been shown in studies demonstrating counter-gradient variation. Counter-gradient variation describes a mechanism where stabilising selection reduces phenotypic differentiation among genetically differentiated sub-populations across environmental gradients (e.g. in response to latitudinal temperature variation). A recent example of such variation comes from cod in the Northwest Atlantic. Marcil *et al.,* (2006) compared juvenile morphology in wild and common-garden reared cod from both Nova Scotia and Newfoundland and found that whereas wild fish from the two populations did not exhibit differences with respect to body shape, common-garden-reared fish exhibited large shape variation between populations. This indicates that the two populations harbour genetic differences related to adaptations to local temperature regimes and/or larval environments. The two cod populations examined in the latter study are managed separately, but the

implications are that several stocks currently managed together, can be predicted to exhibit spatially explicit adaptive divergence. Salvanes *et al.,* (2005) e.g. also found indications of counter-gradient variation in feeding behaviour between Atlantic cod from two Norwegian coastal locations. Billerbeck *et al.,* (2000) demonstrated counter-gradient adaptive variation in another marine fish, the Atlantic silverside.

Effect of stock structure for precision in stock assessment parameters

Knowledge on local adaptation in marine fish and shell-fish populations is crucial in order to predict if depleted or extinct populations can be effectively replaced by re-colonisation from other populations. Extinction of individual populations obliterates locally adapted gene pools and can hence be expected to be detrimental to total recruitment. In the extreme, extinct locally adapted populations may be unlikely to be replaced by immigrants from neighbouring populations on an historic time-scale, and thus have serious repercussions both to stocks locally, and to total recruitment and fisheries yield. Evidence for extinctions of stocks due to fishing was summarised by Dulvy *et al.* (2003). They compiled 133 datasets for population or species extinctions in marine organisms and found that in 80% of cases, extinctions could be attributed to a single threat, for which exploitation was the key factor in 55% and habitat degradation/destruction was the main cause in 37% of the cases. However, due to the difficulty with obtaining information about local adaptations in already extinct stocks, no study has yet attempted to directly estimate how obliterating local stocks affects stock assessment precision. Individual herring stocks have been reported to change spawning, feeding and wintering habitat in response to fishing (reviewed in McQuinn, 1997). Although it is unknown if these changes are associated with loss or break-up of local adaptations and seemingly do not constitute biological extinctions, the losses from the perspective of local fishery communities, that in several cases lost access to harvesting the stocks, are nonetheless obvious. However, even if exploitation does not lead to complete extinction, but merely to significant reductions of local populations, adaptive variation may be lost due to genetic drift. Management units need not reflect biologically differentiated populations, and failure to recognise local stock structure is likely to lead to suboptimal management strategies and overexploitation that can have immediate repercussions to fisheries yield. Recent examples illustrating the application of genetic marker-based methods for assessing stock structure and stock-based fisheries exploitation in Atlantic herring are found in the seminal papers by Bekkevold *et al.* (2005), Mariani *et al.* (2005), Ruzzante *et al.* (in press) and Bekkevold *et al.* (in prep.). In the two former studies genetic marker analysis was applied to determine population structure for all major herring stocks in the Northeast Atlantic. Based on population specific allele frequencies estimated in these studies the latter studies then applied a genetic mixed-stock analysis approach to determine relative contributions of these population components to fisheries targeted mixed-stock aggregations in the North Sea and the Skagerrak. They showed that stocks previously assumed to contribute little to the herring fishery made up substantial proportions in the mixed aggregations, and hence that stock assessment failing to incorporate variance in contributions from individual components is expected to perform sub-optimally. Although none of these studies directly demonstrated that individual populations are locally adapted, the implications of temporally stable genetic differentiation among populations indicate recruitment to be local (for an example of genetic determination of local recruitment within subpopulations of Atlantic cod, see Nielsen *et al.*, 2005) and hence predict large demographic effects of misdirected management leading to overexploitation of individual components, as has occurred in several other herring stocks (e.g. McQuinn, 1997).

Predicting response to harvesting and environmental change

Knowledge about local adaptations is crucial to predictions about the response of organisms and populations to perturbations, be they caused by harvesting or environmental change.

Classical fisheries theory e.g. assumes both that removal of individuals by harvesting leads to increased fitness in non-harvested individuals because of the reduction in intra-specific competition, and that fisheries-induced changes to fitness are selectively neutral (Grift *et al.*, 2003). In dire contrast to this, an increasing number of studies report evidence for fisheries-induced 'local adaptation' in response to size-selective harvesting (e.g. Conover and Munch, 2002; Grift *et al.*, 2003; Barot *et al.*, 2004; Ernande *et al.*, 2004; Olsen *et al.*, 2004, 2005). The rationale is that size selective fishing induces selection against fast growth and for maturation at an early age, and this corresponds with observations that average age and size at maturation have decreased following intensive fisheries exploitation in several species and populations, and both novel methods for describing growth and maturation patterns in natural populations and experimental studies have demonstrated a heritable basis for these changes (Conover and Munch, 2002; Barot *et al.*, 2004; Ernande *et al.*, 2004; Olsen *et al.*, 2004; Walsh *et al.*, 2006). Early maturation at small size is correlated with several life-history traits, such as reduced egg size, larval growth, food consumption rates and survival (e.g. Walsh *et al.*, 2006) and the implication is that fisheries induced selection both directly lowers fisheries yield (Conover and Munch, 2002) and impedes recovery of over-exploited populations (Hutchings, 2005; Walsh *et al.*, 2006). Fisheries impact and the magnitude of selective responses are likely to differ greatly among species and populations (Ernande *et al.*, 2004) and both population history and local adaptations are likely to play important rôles in how the proximate response falls out in terms of impact on recruitment and yield, as well as capacity to rebuild following exploitation.

Response to environmental change, such as in connection with global climate change, also depends on local adaptation. In the case of temperature-related counter-gradient selection, the effect on fisheries of a rapid temperature increase is e.g. likely to depend on whether local populations are capable of adjusting, as replacement by individuals originating from and adapted to warmer waters may be prevented or slowed by divergent selection on other non-temperature related characters. Studies in Atlantic and Baltic cod have e.g. indicated a heritable component to egg buoyancy, with cod from the brackish Baltic Sea producing eggs that are buoyant at lower salinities than eggs produced by cod from the saline North Sea (Nissling and Westin, 1997). In this case, potential replacement of Baltic populations with cod immigrating from a high salinity environment would be highly constrained by such immigrants' suboptimal reproductive success in a brackish spawning environment.

Effects of local adaptations on stock enhancement approaches and in aquaculture

Enhancement of depleted stocks by stocking hatchery-reared brood has been used for both conservation and exploitation (e.g. recreational- and commercial fisheries interest) purposes in, for instance, anadromous salmonids and gadoids. Material used for stocking has commonly been based on non-local individuals that often are maintained in a hatchery environment through several generations, and thus have been subjected to more or less severe domestication selection. Domestication selection and loss of genetic variation has been demonstrated in hatchery brood stock in several species. Stocking such individuals into wild populations is expected to lead to interbreeding between wild and domesticated individuals. This leads to introduction of genotypes that are maladapted in local populations, to potential out-breeding depression through break-up of co-adapted gene complexes, and to decreases in the total genetic variation maintained within local populations (e.g. Lynch and O'Hely, 2001). Decrease of fitness in local populations through introgression with non-native domesticated strains has for example been demonstrated in Atlantic salmon (McGinnity *et al.*, 2003). Although introgressed populations in theory should be able to regain fitness through natural selection against mal-adapted genetic input, the process may be long and lead to stochastic variation in populations that are otherwise depressed by small population sizes. Whereas some supplementation initiatives have seemingly boosted local populations that were reduced to very low levels (reviewed in Tallmon *et al.*, 2004), numerous studies have shown stocking

with non-local fish to yield little effect (e.g. Svåsand *et al.*, 2000; Hansen, 2002; Waples *et al.*, 2004). As a consequence of genetic marker-based demonstrations that stocking with domesticated brown trout strains in Denmark largely has failed to boost dwindling wild populations (Hansen, 2002), Danish management strategies have now changed to ensure that stocking programmes use local, non-domesticated broodstock whenever possible (Hansen *et al.*, 2006).

In aquaculture adaptive variation and local adaptation also play an important role, as the genetic make-up for sought after traits such as high growth rates, delay of maturation and parasite resistance is likely to differ greatly among brood-stocks founded with individuals of different population origin. In contrast to salmonid breeding programmes for aquaculture, several of which have progressed over almost a century, most mariculture attempts with fully marine species are recent and underway. Especially here, combinations of ecological and genetic studies as well as direct genome-based examination of wild source populations with respect to heritable variation for traits of interest are expected to yield important insight into how aquaculture yield can be maximised (e.g. Goetz *et al.*, 2006).

A related issue concerns the effects of escapees from fish farms into wild populations, where concerns are along those related to actively stocking domesticated, non-natal fish into wild populations (see above). Reviews by Naylor *et al.* (2005) and Bekkevold *et al.* (2006) have recently addressed this issue. Although, a number of measures have been adopted by fish-farmers to reduce incidences of escapes it has for instance been estimated that up to 80% of Atlantic salmon entering rivers to spawn are escapees from sea-farms. Such huge numbers inevitably have effects on local populations in terms of e.g. competition for resources, parasite pressures and through hybridization between wild and captive genomes. Consequences to wild population viability are, however, difficult to predict, as they will depend on a suite of parameters, out of which expression of local adaptations presents an important factor. Moreover, large-scale escapes from sea-farms into wild populations are likely to be a recurrent phenomenon, leading to cumulative fitness depression and lack of re-adaptation in (numerically smaller) wild populations.

Adaptive variation as a component of biodiversity

Adaptive variation is expected to lead to resilience in harvested species (and in general) and theoretical models have demonstrated the importance of connectivity among networks of populations for persistence and strength of local populations. The metapopulation has been an important conceptual framework in management of many terrestrial and anadromous species, but has been less applied in marine organisms (e.g. Smedbol, *et al.*, 2001; Kritzer and Sale, 2004). Hillborn *et al.*, (2003) used sockeye salmon catch data from more than a century to examine changes in contributions to fisheries of individual populations within a common stock complex. They found that individual population components with divergent life-history traits (believed to reflect adaptations to local spawning rivers) contributed varying proportions to fisheries over time and that the populations' relative abundance varied with climatic changes. This shows that maintaining biodiversity confers resilience to environmental change, and has direct on yield to fisheries.

The establishment of Marine Protected Areas (MPA's) is expected to become an important tool in marine conservation and management, and may become an important instrument for preservation of adaptive variation in harvested species. Depending on their scale and focal species, the expectation is that establishment of marine protected areas, whether they constitute complete no-take zones or merely areas where fishing activities are reduced, will provide resilience against management failure, such as quotas that do not take population structure into consideration and hence induce risk of extinction of locally adapted components, or loss of gene complexes maximising potential for recovery from over-harvesting. Empirical studies suggest that populations of exploited species protected within

MPA's have higher densities, live longer, have larger mean body sizes, and that reproductive output and recruitment is larger (reviewed by Coté *et al.*, 2001). Expectations from theoretical models further are that no-take MPA's can protect and buffer against fisheries-induced selection for early maturation (Baskett *et al.*, 2005).

2.3.5 Specific challenges in detecting and describing local adaptation in the marine environment

Collection of relevant data in single locus studies

The study of natural selection and local adaptation is inherently plagued by the fact that many different scenarios could have caused the observed end effect in terms of allelic- or genotypic frequency changes. For analytical purposes it is important to have as much as, and as exact as possible, information on each individual in each sample. Sometimes this may, workwise, conflict with the equally important requirement of large sample sizes. Many laboratories have developed lists of individual measurements that are quick to perform but relevant and important for subsequent data analyses. Typically for a field situation, sampling data would include vessel, catch date, location, gear, depth and weather conditions, and individual measurements would include species, body length and weight, sex, and gonadic stage. Otloliths or scales would be collected for age reading, and tissue samples like blood, muscle, hearth, liver, gills, eye or skin (fin-clip) for subsequent protein or DNA genetic analyses. Categorical data like sex and age are particularly useful for testing hypotheses on Hardy-Weinberg equilibria. Measures like body length, weight and condition factor allows for sensitive tests on the relative performance of genotypes. Such tests can be very relevant, based on the argument that any difference in genotypic fitness would probably be detectable in growth parametres before it eventually leads to differential mortalities. All catch data and biological and genetic information pertaining to the samples should be kept and maintained in easily accessible databases (or spredsheets like Excel).

Scale and boundary problems – marine landscapes

Many fish species show a set of basic life history traits which are common to all members of the species regardless of their population affinity. For some marine fishes such traits can for example be spawning and birth at a specific time of the year which is timed to temperature and habitat productivity, developmental rates which results in a certain pelagic period of the spawning products, a subsequent searching behaviour for suitable settling habitats (demersal species), a tendency of stationarity and hiding during the vulnerable young stages, and a hormone-induced tendency of countercurrent migration at the time of sexual maturation. Successful and persisting local populations are often found in ecosystems/habitats which can support the individuals in all these life stages. There are strong elements of scale and physical environment in this support, and it may not be surprising that the most striking intra-specific differences in life history traits are observed between largely self-containing systems on the ecosystem-scale. Current examples of such large-scale systems can be the Baltic Sea, the North Sea, and the Barents Sea. These systems are geologically quite distinct, biologically old enough, and environmentally stable over long enough periods that natural selection can make its genetic effect in a directional manner; i.e. shaping local genetic adaptations. On this large scale, the systems are also relatively isolated from each other, meaning that the gene flow represents a lesser restriction to local genetic differentiation.

A relative reproductive isolation is not always dependent on geographical distance or topographical hindrances (marine landscapes) though; a cold or arid ocean current, and some times large depths can be very effective in preventing gene flow between groups for both pelagic and demersal fish species.

Within such large-scale landscape systems there are smaller-scale habitats where a combination of species-specific biology and environmental factors may favour a relative reproctive isolation which may allow for some local adaptation. For example some oceanic islands and fjords may be shielded from immigrants and may have retention mechanisms for emigrants. Over time these factors may result in a certain level of genetic uniqueness for the local inhabitants which at least in part may be adaptive. On the other hand most marine hydrograhical systems are relatively open, i.e. they allow for gene flow at levels which prevent substantial local adaptation. This openness, together with the comparatively large populations of marine fishes have been put forward as the explanation for the generally lower levels of genetic differentiation (being that by drift or local adaptation) in marine compared to anadromous and limnic fish species (Gyllensten, 1985; Ward *et al.,* 1994; deWoody *et al.,* 2000).

Gene flow and large population sizes

Genetic differentiation by random genetic drift is hampered both by large effective population sizes and by gene flow. Local adaptation, however, is favoured rather than hampered by large population sizes. This is so because in very small populations the effect of random genetic drift can be so strong that it overrides and practically excludes the directional genetic change in an adaptation process. Gene flow always reduces local genetic differentiation, no matter whether it is caused by random drift or by natural selection.

Thus the obtainable level of local adaptation, both for single-locus and quantitative traits, will depend on the relative strengths of the local selective forces and the level of gene flow into the target population from other populations with different selection regimes.

It is worth noting that the number of migrants might be high enough to prevent neutral differentiation, whereas migration is too low to counter-balance local selective coefficients and may permit adaptive differentiation; in addition, unless population size is extremely small, the rate of approach to equilibrium is likely to be higher for loci under selection than for those that are simply drifting (Reznick, 1997). Since exploited marine fish populations have generally relatively large population sizes and non-negligible migration rates, low or nil differentiation may be detected at neutral marker loci, while local adaptation at selective traits might still take place.

Utility of time series

Given the comparatively modest local adaptations allowed for by the gene flow levels for fish species in the marine environment, they may be difficult to detect. So far little or no genetic data exist on local adaptations for multilocus traits in marine species in the wild. This may not be surprising since their detection and assessment usually requires quite demanding logistics for experimental studies which rarely have been available.

For single-locus traits, cases of natural selection are more easily detected. This is both because the trait is directly linked to a specific, observable gene, and because the changes in allele frequencies caused by selection are faster for single locus- than for multi-locus traits. If the selection coefficients acting on genotypes at a single locus are strong, changes may be observable both within generations and between succeeding generations (cf case studies in cod below). However, whether the selection forces result in lasting local adaptations depends on the stability of the selection coefficient over generations, and hence on the stability of the environmental factors that set them up. Quite often, it is difficult to choose suitable populations/locations for realistic studies of selection coefficients, because any observed allele frequency changes can result from genetic drift and immigration as well as from selection.

Currently, the best way to overcome these difficulties is to study one population over several years or generations, i.e. a time-series. The study population should be selfsustaining by local

recruitment and relatively isolated reproductively in order to minimize gene flow by immigration, and large enough to minimize effects of genetic drift. These are factors that can only be assessed by a thorough knowledge of the biology of the population in question. For some, but far from all species/populations, sufficient knowledge exists for choosing a suitable model population for the study of natural selection and local adaptation for single locus traits.

Local recruitment studied by time series

The Flødevigen time series (e.g. Chan *et al.*, 2003) has demonstrated the utility of time series to study a range of variables pertaining to self-sustainability and local recruitment of fish populations. The series, which spans 85 successive years and is still running, have provided insight into the effect of variables like:

- The effects of climate fluctuations on recruitment (Ottersen and Stenseth, 2001), mediated via other species (Hjermann *et al.*, 2004, 2005);
- Density dependence within cohorts, as well as density-dependent effects between cohorts such as cannibalism (Bjørnstad *et al.*, 1999; Stenseth *et al.*, 1999; Chan *et al.*, 2003);
- The abundance of other species can affect recruitment via cannibalism (Hjermann *et al.*, 2004, 2005);
- Spatial variation in recruitment among (sub-) stocks (Fromentin *et al.*, 1997; Stenseth *et al.*, 2006);
- Abundance and timing (match-mismatch) of Calanus affecting the recruitment of North Sea cod (Durant *et al.*, 2005);
- Non-fish time series analysis to assess the importance of intrinsic and extrinsic factors (Lekve *et al.*, 2002, 2003);
- Direct studies of recruitment (Knutsen *et al.*, 2006 in review).

This richness of the different types of questions that can be enlightened by time series data illustrates the importance of maintaining such series, as a source of information that can not be obtained by point studies in time.

Utility of tagging-recapture experiments (migrations)

An important aspect of local adaptations of populations is their migratory behaviour, since this has a bearing on both reproduction/recruitment and gene flow by immigration/emigration. Very few (but some are known, though) behavioural variants are caused by single-locus polymorphisms. More complex behaviour traits may have a genetic component and be heritable like any other quantitative trait and with similar heritabilities. Behaviour is probably a very potent factor for shaping and maintaining local adaptations, as illustrated by the importance of the homing behaviour in anadromous salmonids like the Atlantic salmon. Most of our current knowledge on the migration dynamics of marine fish species is the result of tagging-recapture experiments, and so is much of our knowledge on their general life histories and biology. In those fish species where biology and life histories are most intimately known (e.g. cod, herring, salmon), hundreds of thousands of specimens have been tagged and tens of thousands recaptured.

Hence, whenever tagging and recapture experiments are feasible they should be performed, on immatures as well as maturing/mature specimens. The results are crucial to create a platform for more detailed genetic studies, not least on processes like local genetic adaptations.

The utility of tagging-recapture experiments can be increased substantially by combining the tagging procedure with the collection of tissues samples (biopsy) for genetic characterization of the individuals. Particularly in cases where samples are drawn from temprarily mixed populations, subsequent recaptures of tagged and genotyped individuals may reveal the

various components of the mixture in their "pure" forms when analyzed according to time and place for recapture.

2.3.6 Methods for detecting local adaptations in marine finfish/shellfish

Theoretical considerations and field observations

The meaning of life for marine fishes is essentially to stay alive long enough for reproduction. This requires the ability to get sufficient food, to avoid being eaten by predators, and to be able to compete succesfully with peers for a successful courting of the opposite sex. These factors determine an individual's relative fitness. By default, evolution selects for increased mean fitness both at the population and the species level. The genetic structure of a species that we observe today gives an indication of evolutionary processes of the past. Sometimes one can superimpose a time frame on processes. For example, local populations of marine fishes in the North Atlantic must have established themselves since the last glaciation, or ~10000 years.

Evolution can be defined as changes in allele frequencies of populations. Such changes are results of the action of the three evolutionary forces mutations, random genetic drift, and natural selection. The action of the fourth evolutionary force, gene flow, is to flatten out population differences in allele frequencies.

The main abiotic environmental variables in the marine environment are temperature, salinity, pressure (depth) and oxygenation. The function of gene products is often sensitive to variation in specific factors (e.g. haemoglobins and water temperature in cod). Therefore, at polymorphic loci different genotypes can have different performance, survival and hence fitness values depending on environmental factors.

If the environmental factor is sufficiently stable over many generations, and the target population is sufficiently isolated, local selection forces can shape the local gene pool to be different from others of the species. Sometimes, when the environmental factor forms a gradient (e.g. latitudinal temperature gradient in the Atlantic), populations along the gradient can show a corresponding cline in allele frequencies at certain polyorphic loci. Such clines have been observed in many cases (cf HbI and *Pan*I in the section Empirical results below). Although allele frequency clines can form by other scenarios as well (e.g. distance-dependent distribution of individuals between separate populations), their existence should alert the observer that a case of natural selection may be at hand.

Due to traits of their general biology, different species will have different tendencies and abilities to form reproductively isolated and locally adapted populations. The conditions for gene flow in the distribution range of the species is the main variable, and these conditions vary extensively between species. Look at two species with a similar geographic distribution in the east Atlantic, the dog whelk and the Atlantic cod: Obviously, the general biology of the dog whelk does not facilitate panmixia over large geographic distances; it is small, moves very slowly and can hence not travel very far, there is no pelagic period for its eggs and larvae, it forms only small local populations, and it produces relatively few offspring. The Atlantic cod, on the other hand, is a good swimmer which is fully capable of traversing the Atlantic as an adult. It is also a very fertile species (millions of eggs per female) whose eggs and larvae are pelagic for extended periods of time and represent a very potential gene flow. Studies have shown, just as would be expected from these traits of their general biology, that the genetic differentiation of the dog whelk is much higher than for the Atlantic cod over comparable geographic distances. How much of this variability is adaptational is not known, but certainly the potential for local adaptations is larger in the dog whelk than in the Atlantic

cod. Numerous examples of this type of link between general biology and level of genetic structuring have been reported.

Hence, by looking at the relevant traits of a species biology, quite reliable predictions about the degree of genetic structuring and local adaptation characterizing it can be obtained.

Measures of differentiation

Population differentiation can be estimated with a number of different parameters, such as F_{ST}, the proportion of genetic variation that exists among populations, and different measures of genetic distance (D).

Hitherto, fish stock genetics has mainly dealt with identifying reproductively isolated populations, providing information on stock boundaries important for population biology and informed management. The evolutionary force shaping stock structure is commonly genetic drift detected by presumed neutral genetic markers. When identified, selected genes are usually omitted in genetic stock identification (GSI) studies because selected loci are not optimal for inferring demographic processes or for estimating migration rates and population substructure, because selection bias parameter estimation. On the other hand, comparing the structures indicated by neutral and selected markers could help identifying the genetic basis for local adaptation, adaptive differentiation and, ultimately, speciation (Luikart *et al.*, 2003; Vasemägi and Primmer, 2005).

Recent advances with whole genome sequencing, e.g. in Salmon and Atlantic cod, will allow a rapid developments of many more markers and also new marker types, e.g. SNP's. Screening of a large number of loci simultaneously will allow the detection of outlier loci that can be either under selection or linked to genes under selection. In addition to the discovery of adaptive markers a large number of neutral genes are expected to be identified which can be used to increase the power in GSI studies.

A battery of both coding and non coding genes will allow better distinction between short and medium term conservation needs such as GSI, important for fisheries management, and longer term evolutionary conservation units (ESUs) (Frazer and Bernatchez, 2001).

Common garden experiments

Significant differences among populations as detected by neutral molecular genetic markers can give us valuable insights as to the degree of demographic independence among these units (Palumbi, 2003), and provide sufficient grounds for the separate management of stocks (Waples, 1998), at least over timescales of interest to fisheries management. However, from an evolutionary perspective, it is the genetic diversity for ecologically-relevant traits within local units that has a greater importance for the long-term success of a population (McKay and Latta, 2002).

Phenotypic variation

Phenotypic variation is often the result of the adaptation to a given environment. To some extent such variation is the result of developmental trajectories during the lifetime of an individual determined by environmental variation, whereas in other cases it reflects local genetic adaptation (of a number of genes regulating ecologically-relevant traits).

Common garden approaches

An important task in studying natural populations is to disentangle the phenotypic plasticity (see below) induced by the environment from the variation at phenotypic traits that has a direct genetic basis.

A direct approach to look into this is to rear specimens from different locations (for which phenotypic differences have been detected in the wild) in "common garden experiments", where all individuals are assayed in the same environmental conditions. In this way, the component of phenotypic variance dependent on environmental differences is removed, and any measurable quantitative variance (Q_{ST}) between populations will reflect genetic adaptive variance (McKay and Latta, 2002). If Q_{ST} turns out to be zero, all phenotypic variation observed in the wild is due to plasticity, and local adaptation cannot be demonstrated. On the contrary, if $Q_{ST} > 0$, there must be a genetically adaptive variation between samples from the two locations studied.

The advantage of common garden experiments is that the Q_{ST} values obtained can be compared with the variance measured at neutral genetic markers (F_{ST}), in order to determine whether quantitative genetic variation is of the same order of magnitude as neutral variation ($Q_{ST} = F_{ST}$), in which case, differences between locations largely depend on migration/drift equilibria, mutation and population size, and the quantitative traits are considered neutral (non adaptive) (Lande, 1992). On the other hand, if Q_{ST} is considerably higher or lower than F_{ST}, we can respectively ascribe this to either divergent or stabilizing selection. The former finding ($Q_{ST} > F_{ST}$) generally signifies that the two populations are adapted to different habitats.

Common garden experiments can be conducted across a range of different levels or rigour, from the simple, short-term rearing of samples from different locations under common conditions, to the generation of the F1 and the exact estimation of heritability of the traits of interest, up to the identification of particular regions of the genome associated with the quantitative traits (QTL mapping). The latter approach can be successful if a genomic linkage map and controlled pedigree information is available for the studied species (Slate, 2005), however, the recent development of linkage maps for sea bass (*Dicentrarchus labrax*) and gilt-head sea bream (*Sparus aurata*) shows that our potential to identify candidate genes involved in local adaptation is greatly improving.

Life-history traits are likely to remain the main focus of studies employing the common garden approach: they are relatively easy to collect, long term data series are available for stocks of most commercial species, they are phenoypically plastic, but they also are under polygenic control and may show relatively high heritability. Moreover, they are of great practical importance for stock maintenance and fisheries yields.

The reaction norm methodology

As previously noticed (see Section 1.3.1), when dealing with phenotypic variation one important challenge is to distinguish between variation due to phenotypic plastic and adaptive genetic differentiation, or rather separate out their relative contribution. Although both processes can indeed lead to observable phenotypic differences between populations (or in case of counter-gradient variation, cancel each other out, so that locally adapted populations look phenotypically homogeneous; see Section 1.3.1), their biological and management implications are rather different. In terms of biology, phenotypic plasticity represents a short-term adaptive response to environmental variability whereas adaptive genetic differentiation (or local adaptation) is a medium/long-term adaptation. Regarding management, phenotypic differences between populations based on local adaptation would justify considering these populations as separate management units whereas phenotypic plasticity would not.

Although common garden experiments (see Section 2.2.2) provide a way to assess local adaptation against phenotypic plasticity, as any experiments in controlled environment they lay themselves open to criticism in terms of ecological relevance because of the discrepancy between controlled and natural environment. Therefore, any corroborating evidence in the wild provides a complementary assessment of the relative strength of these two components of strong ecological relevance.

In principle, it is possible to disentangle phenotypic plasticity and genetic differentiation by estimating the reaction norm of the trait of interest for the different populations considered. A reaction norm is defined as the systematic profile of phenotypes a genotype produces across a given range of environments (Schmalhausen, 1949). A trait's reaction norm is thus a genotype's property characterizing its phenotypic plasticity. As such, reaction norms are ideal tools to disentangle phenotypic plasticity and genetic differentiation: differences in mean reaction norms between populations assess genetic differentiation (or local adaptation) whereas mere plastic adaptations leave reaction norms unchanged across populations (see Figure 2.3.6.1).

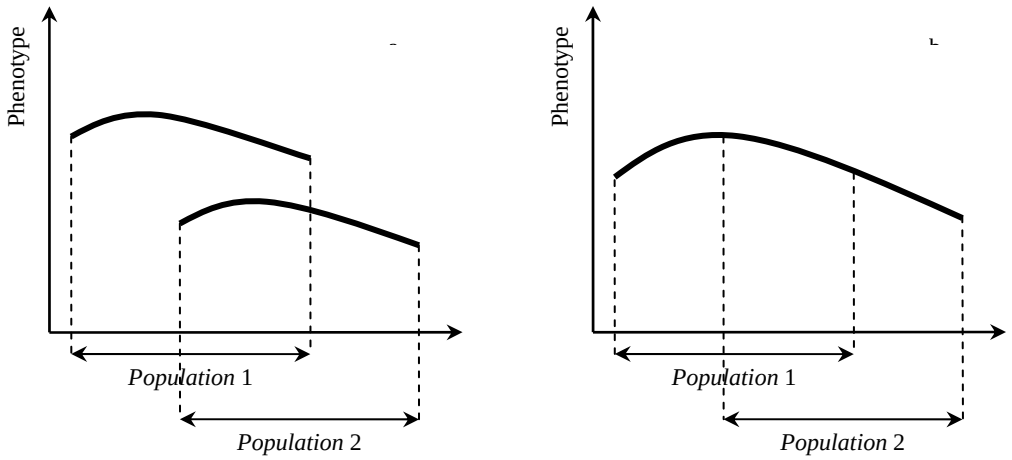

Figure 2.3.6.1: Reaction norm analysis principle. a. Genetic differentiation between two populations; b. Plastic response to the environment across two populations.

Although the reaction norm principle is theoretically applicable to any trait, in practice, accessing reaction norms requires being able to measure environmental variation affecting the life history trait considered, which is far from easy in the field. One important exception is maturation. In case of maturation, growth (or size-at-age) is considered as a *proxy* (supposedly) accounting for most environmental variation affecting the maturation process (Stearns and Crandall, 1984; Stearns and Koella, 1986). Reaction norms for age and size at maturation describe the age and size dependence of an organism's maturation process and were first introduced by Stearns and Koella (1986). Maturation is not, however, fully determined by age and size alone. The residual effects not captured by age and size introduce an unavoidable probabilistic element to the description of the maturation process. Taking this probabilistic nature of maturation into account is crucial if maturation reaction norms are to be estimated from data. This was made possible by the development of probabilistic maturation reaction norm (PMRN) analysis (Heino *et al.*, 2002a,b; Barot *et al.*, 2004a). The probabilistic reaction norm for age and size at maturation (Heino *et al.*, 2002b) is defined as the probability that an immature individual, depending on its age and size, matures during a given time interval. A probabilistic reaction norm is thus specified by determining these probabilities for all relevant ages and sizes (see Figure 2.3.6.2).

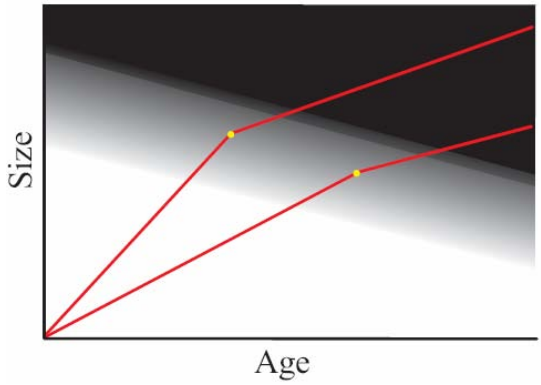

Figure 2.3.6.2: Probabilistic reaction norms for age and size at maturation describe how the probability of an organism to mature during a given time interval depends on its age and size. Shades of grey illustrate how this probability may vary with age and size. Two growth trajectories are shown in red, one for a slow-growing fish and one for a fast-growing fish. Yellow dots indicate the age and size at which these fish mature.

The traditional way of describing maturation in fisheries science is based on so-called maturity ogives. These depict the proportions of mature individuals in a population as functions of age and/or size. However, it is crucial to realize that maturity ogives characterize only the maturity status of a stock, and not the maturation process itself. This is because the maturity status of a population is determined not only by the maturation process itself but also by growth and survival. When analyzing trends in maturity ogives, changes in the maturation process are thus not distinguishable from changes in the rates of growth and/or mortality. All three factors are affected by fishing, and it is therefore very difficult, if not impossible to use maturity ogives for building an understanding of how specifically exploitation impacts a stock. Maturation reaction norms, by contrast, largely overcome these difficulties: by describing the maturation process itself (in terms of the effects of age and size) they strip away the confounding effects of varying growth and survival. This property of maturation reaction norms facilitates addressing important research problems, including disentangling phenotypically plastic and genetic changes in maturation.

2.3.7 Case Studies: Evidence for local adaptation in marine fish and shellfish

The killifish *Fundulus heteroclitus* (Powers *et al.*)

Selectively maintained genetic differentiation has only been demonstrated in a handful of cases in the marine environment. A well-known example is the lactate dehydrogenase (*Ldh-b*) cline in killifish (*Fundulus heteroclitus*) along the temperature gradient on the US east coast (Powers and Schulte, 1998). Laboratory experiments show that populations from Newfoundland are superior at low temperatures compared to populations from Florida. The performance difference is associated with allelic variation in *Ldh-b* locus. Expression of *Ldh-b* in heart and liver is about two fold higher in northern populations as would be expected for thermal conspensation (Schulte *et al.*, 2000).

Atlantic silverside (*Menidia menidia*) and life history traits

The application of common garden approaches in fisheries science is limited to those species that can be successfully reared in captivity and/or have short generation time (which ensures timely gathering of data). Evidence so far obtained has proven insightful at three levels: a) for detecting signal of local adaptation in samples of natural populations (Salvanes *et al*., 2004), b) for modelling adaptive processes that may be widespread across species, including those

that are impossible to rear (Conover and Munch, 2002), and c) for identifying the most suitable stock/strains for aquaculture enhancement.

The Atlantic silverside (*Menidia menidia*) has long represented a pivotal species for such breeding analyses, due to its relatively short generation time and its ease of captive rearing. More than two decades of research on this species have consistently shown that variation in life-history traits (in particular size-selective mortality and growth rate) is strongly linked to environmental gradients (Conover *et al.,* 2005). Evidence from these studies could be extrapolated to many other commercial species under the same type of pressure (Conover and Munch, 2002), and, alongside integrative field-based observations, might help developing better management strategies.

Breeding studies of both Canadian (Marcil *et al.*, 2006) and Norwegian (Salvanes *et al.*, 2005) cod (*Gadus morhua*) populations have demonstrated geographical and latitudinal counter-gradient variation in both morphological and life-history traits, showing that a large extent of phenotypic variation may be undetected in nature due to stabilizing selection (the success of specific genotype×environment combinations that produce the same optimal phenotype in different habitats).

Common garden studies of Atlantic salmon (*Salmo salar*), whose degrees of population structure and local adaptation are of course not comparable to those of most marine species, are still very explanatory to illustrate the power of these approaches in detecting population differences at adaptive phenotypic traits (e.g. migratory behaviour, McGinnity *et al*., 2004).

Cod and the *HbI** polymorphism

The haemoglobin locus *HbI** in cod is polymorphic with two common and several rare alleles. Allele frequencies were shown by Knud Sick to vary considerably between locations throughout the distribution range, on small as well as large geographic scales (Sick 1961; 1965a,b). A number of subsequent studies supported Sick's findings (Frydenberg *et al.*, 1965; 1967, 1969; Møller, 1966, 1968; Jamieson and Jonsson, 1971; Jamieson and Otterlind, 1971; Jamieson and Thompson, 1972; Karpov, 1979; Karpov *et al.,* 1984; Wilkins, 1971). In general, one of the two common alleles (here called the ***S*** allele) was predominant in cold waters of the north and the other (here called the ***F*** allele) in warmer waters in the southern part of the distribution range.

Evidence from field studies

In the east Atlantic, the *F* frequency forms a negative cline from south to north (approx. from. 0.80 in the North Sea to <0.10 at Swalbard; cf references above). Karpov and Novikov (1980) attributed this geographic cline to natural selection caused by temperature-dependent oxygen affinities for the genotypes.

Already in the sixties, Frydenberg *et al.* (1969) reported temporal instability of allele frequencies at at *HbI**, which could be caused by selection. This was supported by Mork and Sundnes (1985). Further support of a selection model is found in reports on genotypic differences in performance for several fitness-related traits (growth, age at maturity, within-season gonad maturation, haematocrit) in natural cod populations (Mork *et al.*, 1983, 1984).

On large geographic scales, the allele frequencies do not seem to have changed substantially in absolute values over the 3–4 decades that have passed since the first reports by Sick (1965a,b). On smaller geographic scales, however, both temporal (e.g. Mork and Sundnes, 1985), and spatial allele frequency heterogeneity is commonly observed (Mork and Sundnes, 1985; J. Mork, unpublished data from Trondheimsfjord cod). Hence there is ample evidence from field studies that the geographic variability observed at cod *HbI** actually reflects local adaptations to the environment.

Evidence from laboratory studies

Oxygen affinity

Karpov and Novikov (1980) reported clear effects of haemoglobin genotype on oxygen affinity in cod, in a way that appeared logical in relation to the distribution of the two common alleles in nature (cf above). Thus, the HbI(22) phenotype was superior at low temperatures, the HbI(11) phenotype at high temperature, with the heterozygotic HbI(12) was somewhat intermediate. Brix *et al.,* (1998) confirmed the differential genotypic effect, but found that the Hb-I(2/2) phenotype had a higher oxygen affinity Hb-I(1/1) at all temperatures except at 20° C. Both studies seem to give a logical explanation to why the Hb-I(2/2) phenotype is dominating in northern areas. The highest affinity, however, was found for a rare heterozygote involving the 2b allele (i.e. Hb-I(2/2b). This is most interesting because other studies (cf references in Brix *et al.,* (1998) have shown this genotype to be most abundant in coastal and warmer waters along the coast of Norway.

Growth

Nævdal *et al.,* (1992) studied growth rates of artificially produced cod fry at three different temperatures (6, 10, and 14°C). At approx. 70 days of age, samples from the three temperature regimes were genotyped for haemoglobin and size measured. Individuals with genotype *Hb-I(2/2)* showed the highest mean growth rate regardless of environmental temperature, and fish with genotype *Hb-I(1/1)* grew on average slowest at the two higher temperatures. By and large, these results were supported by results in Imsland *et al.* (2004) who tested cod *HbI** genotypic growth at 7, 10, 13, and 16°C. They found that the *Hb-I(2/2)* individuals grew best at the two higher temperatures while the *Hb-I(1/1)* individuals were best at 7°C. Brix *et al.*, (2004) and Pörtner *et al,* (2001) found that all genotypes showed best growth at temperatures around 10–12°C. Hence, results from laboratory experiments have shown that *HbI** genotype indeed has an effect on growth (a fitness-related trait).

Behavior

Salvanes and Hart (2000) reported that small cod with the *HbI*2/2* genotype have a higher motivation to feed and are better competitors for feed than individuals with the other haemoglobin genotypes. This suggests the existence of a link between *HbI** genotype and growth (cf above) through feeding behavior, at least under laboratory conditions..

Further support of this idea may be extracted from the experiments of Petersen and Steffensen (2003), which showed that the three common cod *HbI** genotypes had substantially different temperature preferences at normoxia and moderate hypoxia. Their study included only the two homozygotes, which showed the following preferred temperatures (ºC): *FF*: 15.4 (± 1.1), and *SS:* 8.2 (± 1.5).

The relevance of these temperature preferences is not in their relation to the large scale latitudinal or longitudinal temperature regimes in the cod's distribution range, but rather relative to the local temperature range available for cod to dwell in. Usually, there will be both a vertical (depth) and a horizontal temperature gradient in natural cod habitats. To the degree that cod in nature actually have the possibility to maximize their well-feeling by choosing a certain local temperature regime, the differential behaviour and preferences of the genotypes may theoretically affect both growth and other fitness-related traits.

Conclusion

Compared to most other protein marker loci employed in cod, the *HbI** system in cod shows substantial genetic differentiation throughout the distribution range. There is considerable evidence from field observations that natural selection acts on genotypes at this locus, and the clear correlation between latitudinal and allele frequency clines, especially in the East

Atlantic, suggest that the genetic differentiation at least in part is adaptive. This is supported by reports on associations between genotype and biological traits like gonadal maturation, growth, and haematological measures in natural populations. Laboratory experiments showing growth survival and behavioural differences between genotypes have provided strong evidence that *HbI** genotype affects fitness-related traits in ways which are temperature-dependent.

The question arises as to what potential effects the genotypic performance differences may have on the distribution of genotypes in natural environments, where temperature typically varies according to latitude, longitude, depth and season, and ultimately how this can affect our sampling from populations. Apparently, the relation between *HbI** genotypic performance in the laboratory, and the geographic distribution and fitness of these genotypes in nature is a more complex matter than just an oxygen affinity and temperature effect.

Obviously, the cod *HbI** system provides an exciting object for laboratory studies of genotype/environment interaction. On the other hand, the existence of selection and local adaptation inevitably reduces the utility of *HbI** genotype- and allele frequencies in studies of genetic population structure (e.g. for use in management), and even more so in studies of long term evolutionary processes.

In practice, selection which changes genotypic proportions within one generation can be difficult to detect with the Hardy-Weinberg goodness-of-fit test. Common sample sizes in population genetic studies are typically ~100 or less. In cases of balanced selection the heterozygote excess must be very pronounced to be statistically significant in such samples sizes, and under directional selection (i.e. with heterozygote survival intermediate between the homozygotes), the deviation from HW genotypic proportions will usually be too small to be detectable.

Cod and the *Pan*I polymorphism

The genetic differentition at the nuclear-encoded RFLP locus *Pan*I (the locus was first named *SypI*) in Atlantic cod was first described by Pogson *et al.* (1995) in a distribution-wide study of population structure. Several applications followed, on cod from Icelandic waters, the northeast Arctic cod, and coastal cod in northern Norway (Fevolden and Pogson, 1995, 1997; Jonsdottir *et al.*, 1999; Pogson and Fevolden, 2003).

Fevolden and Pogson (1995, 1997) and Pogson and Fevolden (1998) presented field observations suggesting that natural selection was acting on *Pan*I genotypes. This was later substantiated by studies at the molecular level (Pogson, 2001). Pogson and Fevolden (2003) used nucleotide sequence variation in the $PanI^{A}$ allele group to further explore the genetic differentiation at PanI between the Arctic and coastal cod complex of northern Norway. Karlsson and Mork (2003) examined the temporal stability of *Pan*I genotypic and allelic composition among groups in a time series spanning 26 cohorts from spawning locations of a cod stock in Trondheimsfjord, Norway. Recently, Case *et al.* (2005) reviewed and analyzed the macro- and micro-geographic variation in allele frequencies in East Atlantic and Baltic Seas cod relative to environmental factors such as temperature, salinity and depth.

Pogson and Fevolden (2003) pointed out that details of the dynamics of *Pan*I polymorphism in populations of cod remained elusive. This is still true. However, the literature on *Pan*I and its relationship to environmental factors has been steadily expanding, and some features of this relationship have now been treated in considerable detail.

Evidence from field studies

No other genetic marker in cod has shown such a remarkably high level of differentiation between groups of cod as *Pan*I. Though in general the differentiation seems to follow an

isolation by distance model, exceptions in form of abrupt changes in allele frequencies over geographically short distances are observed in northern Norwegian (Fevolden and Pogson 1995, 1997; Pogson and Fevolden, 2003; Sarvas and Fevolden, 2005) and Icelandic waters (Jonsdottir *et al.*, 1999).

The fact that *Pan*I behaves so different from other loci used in cod population structure studies is in itself an indication that some factor in addition to genetic drift and gene flow is affecting the genetic differentiation at this locus. This has been corroborated by many studies.

Already Fevolden and Pogson (1995, 1997) and Pogson and Fevolden (1998) reported genotypic growth differences suggesting that natural selection may act on *Pan*I genotypes. A latitudinal cline in *Pan*I allele frequencies that exist along the coast of Norway gives considerable support to this (e.g. Sarvas and Fevolden, 2005). Indeed, at the molecular level Pogson (2001) and Pogson and Fevolden (2003) found clear evidence for strong natural selection forces in the ratio of nonsynonymous to synonymous substitutions at *Pan*I. Pogson and Fevolden (2003) utilized nucleotide sequence variation in the *Pan*I^A-allele group to explore further the large genetic differentiation at *Pan*I among the Arctic and coastal cod complexes in northern Norwegian waters.

A different approach was taken by Karlsson and Mork (2003), who focused *Pan*I data from a time series of 26 succeeding cohorts from the spawning grounds of a local population of cod in Trondheimsfjorden, Norway. In addition to a microgeographic *Pan*I heterogeneity (6 km distance, 40 m depth difference), they reported significant differences in *Pan*I allele frequencies between cohorts as well as between males and females. In females, a significant excess of heterozygotes were also observed, as well as associations between *Pan*I genotype and phenotypic traits. Extensive hydrographical data were available for the time series, but no obvious correlation between annual temperature and *Pan*I genetic characteristics were detected.

Case *et al.* (2005a) reviewed earlier studies of *Pan*I, and extended the geographical coverage by analyses of *Pan*I in cod from the Baltic Sea and the North Sea. They found strong correlations between *Pan*I allele frequencies and key environmental factors like temperature, salinity and depth (pressure) in juvenile specimens (note that these factors are generally strongly intercorrelated in the marine environment).

Evidence from laboratory studies

The accumulated filed evidence that natural selection acts on *Pan*I genotypes referred to above clearly calls for laboratory studies of the performance of *Pan*I genotypes under controlled conditions with respect to genotype, temperature, pressure and salinity.

However, so far only one such study has been reported. Case *et al.* (2005b) cleverly utilized opportunistic data from a mesocosm experiment for a check of P*an*I genotypic growth and survival. The study, which was restricted to the AB and BB genotypes, detected no deviation from Mendelian ratios among single crossing offpsring, but gave strong evidence of genotypic differences in growth parametres like RNA/DNA ratios, dry weight, and body length in some but not all parallells. Being performed as a common garden experiment at ambient water temperature, the setup did not offer adequate opportunities for testing the effect of temperature, pressure or salinity on genotypic performance.

Conclusion

The pronounced geographic variability of *Pan*I genotypic and allelic frequencies has been thoroughly documented. Also, there is substantial evidence for non-neutrality of the *Pan*I polymorphism in natural environments. The information from laboratorial studies is so far too scarce to enable the identification of which evironmental factors are most important for the

function of proteins coded for by PanI. As pointed out by Case *et al.* (2005a), there are key variables in the marine environment which vary geographically, and which are known to affect ectoterm (e.g. fish) behaviour and performance; e.g. depth, temperature, salinity, and oxygen saturation. Using partial Mantel tests keeping one factor constant at a time, they found significant effects of all three factors in juvenile cod. However, they acknowledge that these factors are notoriously inter-correlated in the marine environment, making it very difficult to disentangle their individual effect in samples with unknown prehistory. Evidence for positive selection at *Pan*I has also been reported for another gadoid; the walleye pollock (Canino and Bentzen, 2004). In humans, the function of integral mebrane proteins of the synaptic vesicles (like pantophysin) is known to be very sensitive to changes in temperature (Prosser and Nelson, 1981). Temperature is maybe the most important environmental variable in determining distributional limits for marine ectoterms, and it appears reasonable that the observed *Pan*I differentiation, at least on large spatial and temporal scales, can be regarded as adaptations. The observed year to year fluctuation of allele frequencies in local natural populations (Karlsson and Mork, 2003) may, from an adaptational point of view, more reflect normally occuring local environmental variability without any predictable genetic endpoints for the gene pool. The sexual heterogeneity in allele frequencies reported by Karlsson and Mork (2003) could reflect differences in behaviour (e.g. habitat preferences) between males and females.

Undoubtedly, the *Pan*I system in cod yields exciting possibilities for studies on interactions between genome and milieu. Probably, however, further progress in this field must rely on laboratory experiments with control of important environmental factors.

Meanwhile, the rather tight correlation between allele frequency and latitude in the East Atlantic as well as an association between allele frequency and depth (Sarvas and Fevolden, 2005), suggest that local environmental factors may take part in shaping local *Pan*I genetic characteristics, and hence give rise to local adaptations.

Cod and the *LDH-3** and *PGI-1** polymorphisms

A broad panel of cod tissue enzymes were assayed for polymorphisms by Mork *et al.* (1982), reporting intermediate levels of genetic variability and a set of isozyme loci suitable for population genetic studies. In a subsequent distribution-wide study (Mork *et al.*, 1985), two loci stood out with the largest geographic differentiation in allele frequencies; *LDH-3** and *PGI-1**. The most pronounced differences were found between the West and East Atlantic, and between the Atlantic and the Baltic Sea at these two loci. At *PGI-1**, privat alleles were found in the geographically most extreme locations (the West Atlantic and the Baltic Sea). At *LDH-3**, a private allele was found in the central part of the distribution range; i.e. on Norwegian coastal locations and in the Barents Sea.

*LDH-3** (lactate dehydrogenase)

Evidence from field studies

The polymorphism at the tissue enzyme locus *LDH-3** (EC 1.1.1.27) was first reported by Odense *et al.* (1969). Two common alleles dominate in samples from throughout the distribution range (Mork *et al.*, 1985). It has been ackowledged for a long time that genotypes at this locus may be affected by natural selection in cod; Jamieson (1975) observed statistically significant heterozygote excesses in samples from the North American Banks. Heterozygote excess appears to be a common phenomenon at this locus (Mork and Giæver 1999 and references therein). Also, temporal instability of allele frequencies within a single population has been demonstrated (Mork and Giæver, 1999), and sexual differences in allele frequencies in spawning populations (Reisegg, 1983). (The latter phenomenon will actually

produce an excess of heterozygotes under panmixia, but not of the magnitude reported in the studies cited above).

Evidence from laboratory studies

Mork and Sundnes (1985) reported differential survival rates of cod *LDH-3** genotypes in an aquarium experiment with 0-group Norwegian coastal cod; the heterozygotes were superior. In a controlled crossing experiment with *LDH-3** heterozygotes, there was no significant differences in survival, but the heterozygotes showed a significantly higher mean length than both homozygotes at 72 days (Mork, 1997).

Conclusion

The physiological role of LDH is to take care of lactic acid that builds up during anaerobic muscle activity. There is substantial evidence from field and laboratory studies that cod *LDH-3** is affected by natural selection (i.e. genotypic fitness coefficients differ). Evidently, this is an example of a balanced polymorphism maintained, at the least, by an intra-population superiority of the heterozygote. LDH, like haemoglobin (see above), is a very important protein in the individual's interaction with the environment, and it may not be surprising that genotypes differ in performances relevant to fitness (catching prey, avoiding predators etc). The globally most common allele (*100*) is most frequent in the Baltic Sea (low salinity and winter temperatures), but any consistent latitudinal or longitudinal clines in allele frequencies are not apparent in the distribution range of the cod. The marked overdominance scenario at cod *LDH-3** makes it very attractive for laboratory studies of selection forces but so far, evidence of correlations between allelic or genotypic setup and specific environment factors has not been demonstrated. In cases of heterozygote advantage, direct links between genotypic fitness and environmental factors may be more difficult to detect than in directional selection. Also, it is always possible that heterozygote advantage is due to generally better catalytic properties at the molecular level, and hence that allele frequencies show little or no correlation with varying physical environment factors. Thus, there is currently no firm evidence that allele or genotypic frequencies at this locus reflect local adaptations.

PGI-1* (phosphoglucoisomerase)

The PGI (GPI; EC 5.3.1.9) is an important glucolytic enzyme catalyzing the interconversion of G6P and F6P. In ectotherm organisms experiencing contrasting environmental temperatures, its genetically determined kinetic and structural properties diverge in ways that correlate remarkably well the enzymes' respective thermal environments (Hall, 1985). Certainly, there are no shortages of thermal gradients in the marine environment, but no apparent cline of allele frequencies at ***PGI-1**** have been reported in cod. On the other hand, the presence of a private allele at a frequency of 0.031 in the cold (in winter) and brackish Baltic Sea might have some bearing to environment (but then, the Baltic cod also appears to be the reproductively most isolated cod stock studied so far).

Evidence from field studies

Dando (1974) first described the polymorphisms for cod PGI (E.C. 5.3.1.9). Allele frequencies at this locus have been utilised for studies on population structure throughout the species range (Cross and Payne, 1978; Jørstad, 1984; Mork *et al.*, 1985; Mork and Giæver, 1999). The two most common alleles are present in populations throughout the species range. No apparent latitudinal or longitudinal clines have been reported, but private alleles exist in noticeable frequencies in the West Atlantic, the East Atlantic, and the Baltic Sea cod (Mork *et al.*, 1985).

Evidence from laboratory studies

The experiment by Mork and Sundnes (1985b) revealed statistically significant differences in general survival between *PGI-1** genotypes, with the heterozygote clearly superior. Also, among whole-sibs produced by artificial crossing, the heterozygote showed significantly better growth than either of the homozygotes (Mork, 1997). However, direct links between genotypic fitness traits and any specific environmental factor have not been erected.

Conclusion

Laboratory studies (above) indicate fitness advantages of the heterozygote at this locus. However, heterozygote superiority does not necessarily lead to local adaptations because a better catalytic capacity of the heterozygous molecule may be a general pheomenon in the species. While such overdominance selection certainly will hamper the use of this locus in studies of genetic population structure and differentiation, its evolutionary effect would be more to maintain the polymorphisms than to contribute to local environmental adaptations. It is noted that both in the survival experiment by Mork and Sundnes (1985b), and the full-sib growth experiment of Mork (1997) reported above, the double heterozygote at *LDH-3** and *PGI-1**** was by far the superior genotype. This highlights the fact that individual genetic setups contribute to the average fitness of a population. Although so far not reported for any commercially important fish species, it is fully possible that some local environments are so demanding that a selection for high heterozygosity may be regarded as local adaptation process.

Fisheries-induced evolution in herring, cod and other species studied through reaction norms

In commercially exploited fish stocks, fishing is the major cause of mortality. Since all fish species were genetically adapted to the environmental conditions experienced prior to intensive exploitation, the current, drastically altered conditions cannot possibly leave their life-history patterns unaffected. In other words, fishing not only decreases the abundance of fish in exploited fish stocks, but also changes their genetic composition (Law and Grey, 1989; Stokes *et al.*, 1993; Palumbi, 2001; Conover 2002; Ashley *et al.*, 2003) thus leading to genetic adaptation of stocks to the local fishing regime, in other words local adaptation to anthropogenic pressure.

However, although selective pressures imposed by fishing has been recognized (Stokes *et al.*, 1993; Law, 2000; Heino and Godo, 2002; Stokes and Law, 2002), no substantive basis that fishing actually induced life history evolution that could affect yield of the resource was available until very recently. One major breakthrough in this area was made possible by the development of probabilistic maturation reaction norm (PMRN) analysis (Heino *et al.*, 2002a,b; Barot *et al.*, 2004a), which allowed demonstrating that most of the documented changes in the maturation properties of exploited fish populations are indeed evolutionary responses, and not mere effects of phenotypic plasticity (see Section 2.2.3).

Although *estimating* the PMRNs is a rather complex task, the principle for using them to detect ongoing evolutionary changes in the maturation process is rather simple as shown in Figure 2.3.7.1.

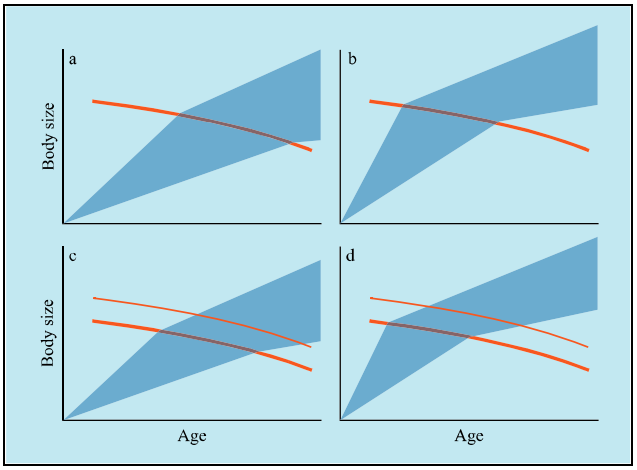

Figure 2.3.7.1: Probabilistic reaction norms for age and size at maturation. Thick orange curves shows reaction norm midpoints, that is, combinations of age and size at which the probability of maturing is 50%. In each of the four scenarios shown, maturation is likely to occur in the vicinity of this curve. Growth trajectories (spread across the dark blue area) determine which part of the reaction norm will be observed (a–d). An increase in growth rates (from a to b) leads to earlier maturation along a different part of the reaction norm, but will not change the observed position of the reaction norm itself (b). By contrast, a genetic change (from a to c or d) toward maturation at younger ages and smaller sizes results in a shift of the reaction norm: the observed midpoints (thick orange curves) therefore differ from the initial ones (thin orange curves). Importantly, the corresponding shift in the position of the reaction norm can be detected both in the absence (c) and in the presence (d) of concomitant environmental change in growth rates. Adapted from Olsen *et al.,* 2004.

Basically, any constant temporal trend in PMRNs is a signal for ongoing evolutionary/genetic changes affecting the maturation process in the considered population.

To date, probabilistic maturation reaction norms have been estimated for eleven marine and one freshwater stocks of exploited fish (see Table 2.3.7.1).

Table 2.3.7.1: Empirical case studies of fisheries-induced evolution in maturation reaction norms.

Stock	Period with data	Reference
Northeast Arctic cod	1932–1998	Heino *et al.*, 2002b
Georges Bank cod	1970–1998	Barot *et al.*, 2004b
Gulf of Maine cod	1970–1998	Barot *et al.*, 2004b
Northern cod	(1977)–1981–2002	Olsen *et al.*, 2004, 2005
Grand Bank cod	1971–2002	Olsen *et al.*, 2004, 2005
St. Pierre Bank cod	1972–2002	Olsen *et al.*, 2004, 2005
North Sea plaice	1955–1995	Grift *et al.*, 2003
Newfoundland plaice	1973–1999	Barot *et al.*, 2005
Grand Bank plaice	1969–2000	Barot *et al.*, 2005
St. Pierre Bank plaice	1972–1999	Barot *et al.*, 2005
Atlantic herring	1930–1992	Engelhard and Heino 2004
Norwegian grayling	20th century	Haugen and Vøllestad *in press*

All but one of the investigated stocks exhibited clear temporal trends in their maturation reaction norms. These always occurred towards increased maturation probabilities at younger ages and smaller sizes, in accordance with predictions from life-history theory. In the Canadian cod stocks, there are tentative signs for a reversal of this trend, resulting from the moratorium declared on directed offshore cod fishing in 1992.The one exception in an otherwise overwhelmingly clear pattern of evolutionary changes is the Atlantic herring stock, where maturity changes have been predominantly plastic.

Evidence of local adaptations in marine shellfish

The genetic characteristics of natural populations can change over time and space. The inference usually is that such changes occur as a response to some environmental selective factor, acting either directly on the gene locus itself or on an associated locus or loci. In most cases, however, the correlation between gene frequency and selective factor is purely inferential, i.e. there has been no attempt to pinpoint the selective agent nor demonstrate cause-effect relationships between selective agent and observed allele frequency.

One exception is the well-documented case involving leucine aminopeptidase (LAP) (Hilbish and Koehn 1985), an enzyme involved in the degradation of intracellular protein to free amino acids. Mussels osmoregulate by changing the concentration of intracellular amino acids in response to changing environmental salinity. Along the east coast of the USA the frequency of the Lap^{94} allele is ~ 0.55 in mussels in oceanic waters but declines to 0.15 in estuarine populations. Genotypes with the Lap^{94}, under field and laboratory conditions, excrete amino acids at rates nearly twice those of alternate genotypes, as a mechanism for relieving osmotic stress when salinity rises. However, this same activity leads to depletion in nitrogen resources and subsequent high mortality of Lap^{94} genotypes, generating a balancing selection that favours less active *Lap* alleles. Outside of N. America, however, the frequency of Lap^{94} is not correlated with environmental salinity (Bulnheim and Gosling, 1988; Varvio *et al.*, 1988; Väinölä and Hvilsom, 1991).

Still on the topic of adaptation to changing salinity, Ridgway and Naevdal (2004) reported significant correlation between allele frequencies at two polymorphic loci, PGM and GPI, and the salinity regime experienced by *Mytilus* in and around Bergen, Norway. Mussels from full salinity waters differed significantly from those collected at brackish water sites, and the differences were so large that the authors suggested that the Norwegian coast with very variable salinity may well be a mixing zone for the brackish water *M. trossulus*, and the more salt-dependent *M. edulis*.

Hummel *et al.* (1998) sampling the Baltic clam, *Macoma balthica*, over its total distribution range, from N. Russia to France (17 sites), found that clams in the Arctic Pechora Sea, Russia were significantly genetically differentiated (7 allozyme loci; genetic identity 0.79) from clams at all other sites, which they interpreted as genetic adaptation to Arctic conditions.

Additional indirect evidence for selection comes from a number of studies on the effects of various pollutants on allele and genotype frequencies. Beaumont and Toro (1996) found that mortalities in mussels (*Mytilus edulis*), exposed to 100 ppb copper, were genotype-dependent: heterozygotes at four allozyme loci survived longer and homozygotes succumbed sooner, which supported finding of an earlier, similar study on the same species (Hawkins *et al.*, 1989a). Survivorship was also associated with low protein turnover times, which have been shown to have great significance for energy metabolism and fitness (Hawkins *et al.*, 1989b). The most heterozygous individuals have the lowest protein turnover times and routine metabolic maintenance costs, and hence the greatest fitness.

Bivalves, the primary vectors of paralytic shellfish poisoning (PSP) in humans, show marked inter-species variation in their capacity to accumulate PSP toxins. Bricelj *et al.* (2005) have identified a molecular basis for inter-population variation in PSP resistance within the clam, *Mya arenaria*, that is consistent with genetic adaptation to PSPs. Clams from areas exposed to PSPs (from red tides) are more resistant to PSTs and accumulate toxins at a greater rate than sensitive clams from unexposed areas. Resistance has been shown to be genetically determined and thus PSPs act as potent natural selection agents, leading to greater toxin resistance in clams and increased risk of PSP in humans. An interesting case is that of the butter clam, *Saxidomus giganteus*, which sequesters the diet-derived dinoflagellate saxitoxin (STX), a highly potent neurotoxin, in its siphons as a defence against siphon-nipping fish (Kvitek, 1991). Resistance to STX is innate, rather than acquired with increased exposure,

since no difference in STX sensitivity was observed between clams from sites with different histories of toxin contamination (Kvitek and Beitler, 1991).

Oysters, *Crassostrea virginica*, from two populations, one that had been exposed to harmful algal blooms (HABs) and the other from a 'clean' site, were exposed to *Prorocentrum minimum* a dinoflagellate that causes blooms (Hegaret and Wikfors, 2005). After exposure, there was a 35% increase in haemocyte respiratory burst in oysters that had prior exposure to HABs (mortality 13%), with no effect on the oysters from the clean site (mortality 38%). Increased respiratory burst may represent an adaptation to annual blooms whereby surviving oysters protect themselves against tissue damage from ingested *P. minimum*. Whether this adaptation is genetically determined remains to be seen.

A quantitative, as opposed to a single locus, approach may be used to investigate genetic adaptation. Phenotypic plasticity can be an adaptive response to spatial and/or temporal varying environments. Ernande *et al.* (2004) investigated the quantitative genetics of plasticity in resource allocation between survival (S), growth (G) and reproductive effort (RE) in the oyster, *Crassostrea gigas* when food abundance varied spatially. Resource allocation shifted from S to G and RE as food abundance increased, and this plastic response was genetically polymorphic. Polymorphim was mostly due to strong variability in RE plasticity, whereas genetic variability in RE mean was moderate. In contrast, there was substantial heritability for S mean, whereas it was weak for S plasticity. There was no genetic variability for either G mean or plasticity. These results illustrate how *C. gigas* may deal with and adapt to spatial variation in food abundance, a critical environmental factor for sedentary organisms.

2.3.8 Recommendations

- Current knowledge of adaptive heterogeneity and scientific principles for genetic conservation should be incorporated into management strategies and practices for wild as well as captive stocks (Section 1.3).
- Proven approaches (Section 2.1) for identifying, describing, assessing, and managing genetic resources represented by local adaptations should be employed along with the development and use of new scientific methodology. New approaches include:
    - behaviour and life history analysis (Sections 1.2.3 and 2.1.5);
    - quantitative genetics analysis of captive populations (Section 2.2.2);
    - simulation modelling;
    - time series analyses (Section 2.1.4);
    - QTL analyses and genomics.
- Integrated approaches linking topological, hydrographical, biological, behavioural, phenotypic and genetic data should be employed in the research on local adaptations, with the following approaches being particularly useful in such studies:
    - Comparison of information from neutral and selected genetic markers;
    - Linking of ecological and biological data with genetic samples *(*Section 2.1);
    - Design of sampling schemes integrating marine landscapes, current systems, commercial landings data, and environmental information.

2.3.9 References

Ashley, M.V., Willson, M.F., Pergams, O.R.W., O'Dowd, D.J., Gende, S.M., and Brown, J.S. 2003. Evolutionarily enlightened management. Biological Conservation, 111: 115–123.

Barot, S., Heino, M., O'Brien, L., Dieckmann, U. 2004 Long-term trend in the maturation reaction norm of two cod stocks. Ecological Applications, 14(4): 1257–1271.

Barot, S., Heino, M., Morgan, M. J., and Dieckmann, U. 2005. Maturation of Newfoundland American plaice (*Hippoglossoides platessoides*): long-term trends in maturation reaction norms despite low fishing mortality? ICES Journal of Marine Science, 62: 56–64.

Barot, S., Heino, M., O'Brien, L., and Dieckmann, U. 2004a. Estimating reaction norms for age and size at maturation when age at first reproduction is unknown. Evolutionary Ecology Research, 6: 659–678.

Barot, S., Heino, M., O'Brien, L., and Dieckmann, U. 2004a. Estimating reaction norms for age and size at maturation when age at first reproduction is unknown. Evolutionary Ecology Research, 6: 659–678.

Barot, S., Heino, M., O'Brien, L., and Dieckmann, U. 2004b. Long-term trend in the maturation reaction norm of two cod stocks. Ecological Applications, 14: 1257–1271.

Baskett, M.L., Levin, S.A., Gaines, S.D., and Dushoff, J. 2005. Marine reserve design and the evolution of size at maturation in harvested fish. Ecological Applications, 15(3): 882–901.

Beaumont, A.R., and Toro, J.E. 1996. Allozyme genetics of *Mytilus edulis* subjected to copper and nutritive stress. J. Mar. Biol. Ass. UK, 76: 1061–71.

Bekkevold, D., Clausen, L.A.W., Mariani, S., André, C., Christensen, T.B., Mosegaard, H. Divergent origins of sympatric herring populationcomponents determined using genetic mixture analysis. Mar. Ecol. Prog. Ser. *In review*.

Bekkevold, D., Mariani, S., André, C., Hatfield, E.M.C., Clausen, L.A.W., Dahlgren, T.G., Hutchinson, W.F., Torstensen, E., Ryman, N., Ruzzante, D.E. (in prep.) Genetic mixture analysis for weakly differentiated populations: effects of baseline composition and mixture sample size on mixed-stock analysis in Atlantic herring. Manuscript in preparation.

Bekkevold, D., Hansen, M.M. and Nielsen, E.E. 2006 Genetic impact of gadoid culture on wild fish populations: predictions, lessons from salmonids and possibilities for minimising adverse effects. ICES Journal of Marine Science, 63: 198–208.

Billerbeck, J.M., Schultz, E.T., Conover, D.O. 2000. Adaptive variation in energy acquisition and allocation among latitudinal populations of the Atlantic silverside. Oecologia, 122(2): 210–219.

Bjornstad, O.N., Fromentin, J.M., Stenseth, N.C., Gjosaeter, J. 1999 A new test for density-dependent survival: the case of coastal cod populations. Ecology, 80(4):1278–1288

Bricelj, V.M., Connell, L., Konoki, J., MacQuarrie, S.P., Scheuer, T. *et al.* 2005 Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature, 434: 763–767.

Brix, O., Thorkildsen, S., Colosimo, A. 2004. Temperature acclimation modulates the oxygen binding properties of the Atlantic cod (Gadus morhua L.) genotypes HbI*1/1 , HbI*1/2, and HbI*2/2 by changing the concentrations of their major hemoglobin components (results from growth studies at different temperatures). Comparative Biochemistry and Physiology, 138A: 241–251.

Brix, O., Forås, E. and Strand, I. 1998. Genetic variation and functional properties of Atlantic cod hemoglobins: Introducing a modified tonometric method for studying fragile hemoglobins. Comp. Biochem. Physiol., 119A: 575–583.

Bulnheim, H.P., and Gosling, E.M. 1988. Population genetic structure of mussels from the Baltic Sea. Helgoländer. Wiss. Meeresunters, 42: 113–129.

Canino, M.F., and Bentzen, P. 2004. Evidence for positive selection at the Pantophysin (*Pan*I) locus in walleye pollock (*Theragra chalcogramma*) Mol. Biol. Evol., 21: 1391–1400.

Chan, K.S., Stenseth, N.C., Kittilsen, M.O., Gjøsæter, J., Lekve, K., Smith, T., Tveite, S., and Danielssen, D. 2003. Assessing the effectiveness of releasing cod larvae for stock

improvement: using long-term monitoring beach-seine data to resolve a century-long controversy. Ecological Application, 13: 3–22.

Chan, K.-S., Stenseth, N. C., Lekve, K., and Gjøsæter, J. 2003a. Modelling the population dynamical effects of pulse disturbances: the algae bloom along the Norwegian Skagerrak coast in 1988. Ecol. Monogr., 73: 151–171.

Conover, D.O., Arnott, S.A., Walsh, M.R., and Munch, S.B. 2005. Darwinian fishery science: lessons from the Atlantic silverside (*Menidia menidia*). Can. J. Fish. Aquat. Sci., 62: 730–737.

Conover, D.O., and Munch, S.B. 2002. Sustaining fisheries yields over evolutionary time scales. Science, 297: 94–96.

Conover, D.O., and Munch, S.B. 2002. Sustaining fisheries yields over evolutionary time scales. Science, 297(5578): 94–96.

Conover, D.O. Present, T.M.C. 1990. Oecologia, 83: 316.

Conover, D.O. 2002. Darwinian fishery science. Marine Ecology Progress Series, 208: 303–307.

Coté, I.M., Mosqueira, I., and Reynolds, J.D. 2001. Effects of marine reserve characteristics on the protection of fish populations: a meta-analysis. Journal of Fish Biology, 59: 178–189.

Dahlgren, T.G., Hutchinson, W.F., Hatfield, E.M.C., Torstensen, E., Brigham, J., Simmonds, E.J., Laikre, L., Larsson, L.C., Stet, R.J.M., Ryman, N., and Carvalho, G.R. 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. Proc R Soc Lond Ser B *In press online*.

Dando, P.R. 1974. Distribution of multiple glucosephospate isomerases in teleostean fishes. Comp. Biochem. Physiol., 47B: 663–679.

DeWoody, J.A. and Avise, J.C. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J. Fish. Biol., 56: 461–473.

DeWoody, J.A., and Avise, J.C. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J. Fish. Biol. 56: 461–473.

Dieckmann, U. 2004. Maturation trends indicative of rapid evolution preceeded the collapse of Northen cod. Nature, 428: 932–935.

Durant, J.M., Hjermann, D.O., Anker-Nilssen, T., Beaugrand, G., Mysterud, A., Pettorelli, N., Stenseth, N.C. 2005. Timing and abundance as key mechanisms affecting trophic interactions in variable environments. Ecology Letters, 8: 952–958

Engelhard, G. H., and Heino, M. 2004. Maturity changes in Norwegian spring-spawning herring “Clupea Harengus”: compensatory or evolutionary responses? Marine Ecology Progress Series, 272: 245–256.

Ernande, B., Boudry, P., Clobert, J., and Haure, J. 2004. Plasticity in resource allocation based life history traits in the Pacific oyster, *Crassostrea gigas*. 1. Spatial variation in food abundance. J. Evol. Biol., 17: 342–56.

Ernande, B., Dieckmann, U., Heino, M. 2004. Adaptive changes in harvested populations: plasticity and evolution of age and size at maturation. Proceedings of the Royal Society of London Series B-Biological Sciences, 271(1537): 415–423.

Fevolden, S.E., and Pogson, G.H. 1995. Differences in nuclear DNA RFLPs between the Norwegian coastal and the North‐east Arctic population of Atlantic cod. *In* Ecology of Fjords and Coastal Waters (Skjoldal, H. R., Hopkins, C., Erikstad, K. E. and Leinaas, H. P., eds), pp. 403–415. Amsterdam: Elsevier Science BV

Fevolden, S.E., and Pogson, G.H. 1997. Genetic divergence at the synaptophysin (*Syp* I) locus among Norwegian coastal and north‐east Arctic populations of Atlantic cod. Journal of Fish Biology, 51, 895–908.

Frazer, D.J., and Bernatchez, L. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecula Ecology, 10: 2741–2752.

Fromentin, J.M., Stenseth, N.C., Gjøsæter, J., Bjornstad, O.N., Falck, W., and Johannessen, T. 1997. Spatial patterns of the temporal dynamics of three gadoid species along the Norwegian Skagerrak coast. Marine Ecology-Progress Series, 155: 209–222

Frydenberg, O., Møller, D., and Sick, K. 1965. Haemoglobin polymorphism in Norwegian cod populations. Hereditas, 53: 252–271.

Frydenberg, O., Tønnes-Nielsen, J., and Sick, K. 1967. The population dynamics of the haemoglobin polymorphism in cod. Cienc. Cultur., 19: 111–117.

Frydenberg, O., Tønnes-Nielsen, J. and Simonsen, V. 1969. The maintenance of the haemoglobin polymorphism of the cod. Jap. J. Genet., 44(1): 160–165.

Goetz, F.W., McCauley, L., Goetz, G.W., and Norberg, B. 2006 Using global genome approaches to address problems in cod mariculture. ICES Journal of Marine Science, 63: 393–399.

Grift, R.E., Rijnsdorp, A.D., Barot, S., Heino, M., Dieckmann, U. 2003. Fisheries-induced trends in reaction norms for maturation in North Sea plaice. Marine Ecology-Progress Series, 257: 247–257.

Grift, R. E., Rijnsdorp, A. D., Barot, S., Heino, M., and Dieckmann, U. 2003. Fisheries-induced trends in reaction norms for maturation in the North Sea plaice. Marine Ecology Progress Series, 257: 247–257.

Gyllensten, U. 1985. The genetic structure of fish: Differences in intraspecific distribution of biochemical variation between marine, anadromous and freshwater species. J. Fish. Biol., 26: 691–699.

Gyllensten, U. 1985. The genetic structure of fish: Differences in intraspecific distribution of biochemical variation between marine, anadromous and freshwater species. J. Fish. Biol., 26: 691–699.

Hall, J.G. 1985. The Adaptationof Enzymes to Temperature: Catalytic Characterization of Glucosephosphate Isomerase Homologues Isolated from Mytilus edulis and Isognomon alatus, Bivalve Molluscs Inhabiting Different Thermal Environments. Mol. Biol. Evol., 2(3): 251–269.

Hansen, M.M. 2002. Estimating the long-term effects of stocking domesticated trout into wild brown trout (Salmo trutta) populations: An approach using microsatellite DNA analysis of historical and contemporary samples. Molecular Ecology, 11: 1003–1015.

Hansen, M.M., Bekkevold, D., Jensen, L.F., Mensberg, K.-L.D. and Nielsen, E.E. 2006. Genetic restoration of a stocked brown trout (*Salmo trutta*) population using microsatellite DNA analysis of historical and contemporary samples. Journal of Applied Ecology, in press.

Haugen, T. O., and Vøllestad, L. A. in press. Case study on grayling. In Fisheries-induced adaptive change. Ed. by U. Dieckmann, O. R. Godø, M. Heino, and J. Mork. Cambridge University Press, Cambridge.

Hawkins, A.J.S., Bayne, B.L., Day, A.J., Rusin, J., and Worrall, C.M. 1989b. Genotype-dependent interrelations between energy metabolism, protein metabolism and fitness. In: Reproduction, genetics and distribution of marine organisms, J. Ryland and PA Tyler (eds), Olsen and Olsen, Fredensborg, Denmark, pp. 283–292.

Hawkins, A.J.S., Rusin, J., Bayne, B.L., and Day, A.J. 1989a. The metabolic/physiological basis of genotype-dependent mortality during copper exposure in *Mytilus edulis*. Mar. Environ. Res., 28: 253–257.

Hegaret, H., and Wikfors, G.H. 2005. Effects of natural and field-simulated blooms of dinoflagellate *Prorocentrum minimum* upon haemocytes of eastern oysters, *Crassostrea virginica*, from two different populations. Harmful Algae, 4: 201–209.

Heino, M., and Godø, O. 2002. Fisheries-induced selection pressures in the context of sustainable fisheries. Bulletin of Marine Sciences, 70: 639–656.

Heino, M., Dieckmann, U., and Godø, O. 2002*a*. Estimating reaction norms for age and size at maturation with reconstructed immature size distributions: a new technique illustrated by application to Northeast Arctic cod. ICES Journal of Marine Science, 59: 562–575.

Heino, M., Dieckmann, U., and Godø, O. R. 2002b. Estimating reaction norms for age and size at maturation with reconstructed immature size distributions: a new technique illustrated by application to Northeast Arctic cod. ICES Journal of Marine Science, 59: 562–575.

Hilbish, T.J., and Koehn, R.K. 1985. Dominance in physiological phenotypes and fitness at an enzyme locus. Science, 229: 52–54.

Hjermann, D.O., Eikeset, A.M., Ottersen, G., and Stenseth, N.C. 2005. Climatic effects filtered through the food web affect the dynamics of Arcto-Norwegian cod. ICES CM 2005/AA:22

Hjermann, D.O., Stenseth, N.C., and Ottersen, G. 2004. Indirect climatic forcing of the Barents Sea capelin: a cohort effect. MEPS, 273: 229–238

Hummel, H., Bogaards, R., Bek, T., Polishchuk, L., Sokolov, K., Aiard-Triquet, C. *et al.* 1998. Growth in the bivalve *Macoma balthica* from its northern to its southern distribution limit: a discontinuity in North Europe because of genetic adaptations in Arctic populations? Comp. Biochem. Physiol.A: Mol Integr Physiol, 120: 133–141.

Hutchings, J.A. 2005 Life history consequences of overexploitation to population recovery in Northwest Atlantic cod (Gadus morhua). Can. J. Fish Aqat. Sci., 62(4): 824–832.

ICES. 2005. Report of the Herring Assessment Working Group for theArea South of 62ºN), 8–17 March 2005, ICES Headquarters. ICES CM 2005/ACFM:16. 599 pp.

Imsland, A.K., Foss, A., Nævdal, G., Johansen, T., Folkvord, A., Stefansson, S.O. and Jonassen, T.M. 2004. Variations in growth in haemoglobin genotypes in Atlantic cod. Fish Physiology and Biochemistry, 30: 47–55.

Jamieson, A. and Jónsson, J. 1971. The Greenland component of spawning cod at Iceland. Rapp. P.-v. Réun. Cons. int. Explor. Mer, 161: 65–74.

Jamieson, A. and Otterlind, G. 1971. The use of cod blood protein polymorphisms in the Belt Sea, the Sound and the Baltic Sea. Rapp. P.-v. Réun. Cons. int. Explor. Mer, 161: 55–59.

Jamieson, A., and Thompson, D. 1972. Blood proteins in North Sea cod (*Gadus morhua*). In XIIth European Conference on Animal Blood groups Biochemical Polymorphism, Budapest, pp. 585–591. The Hague: Dr W. Junk N.V.

Jamieson, A. 1975. Protein types of Atlantic cod on the North American Banks, p. 491–515. In: C.L. Markert (ed.) Isozymes IV. Genetics and Evolution. Acad. Press, New York.

Jónsdóttir, O.D.B., Daníelsdóttir, A. K. and Nævdal, G. 2001. Genetic differentiation among Atlantic cod (*Gadus morhua* L.) in Icelandic waters: temporal stability. ICES Journal of Marine Science, 58: 114–122.

Jónsdóttir, O.D.B., Imsland, A.K., Daníelsdóttir, A.K., Thorsteinsson, V. and Nævdal, G. 1999. Genetic differentiation among Atlantic cod in south and south·east Icelandic

waters: synaptophysin (*Syp* I) and haemoglobin (*HbI*) variation. Journal of Fish Biology, 54: 1259–1274.

Jørstad, K., and Nævdal, G. 1989. Genetic variation and population structure of cod, Gadus morhua L., in some fjords in northern Norway. J. Fish. Biol., 35: 245–252.

Jørstad, K.E., and Naevdal, G. 1994. Studies of associations between genotypes and growth rate in juvenile cod. ICES Marine Science Symposia, 198: 671–675.

Julliard, R., Stenseth, N.C., Gjøsæter, J., Lekve, K., Fromentin, J.M., and Danielssen, D.S. 2000. Natural mortality and fishing in a coastal cod population: A release –recapture experiment. Ecological Applications, 11: 540–558.

Karlsson, S., and Mork, J. 2003. Selection‐induced variation at the pantophysin locus (*Pan* I) in a Norwegian fjord population of cod (*Gadus morhua* L.). Molecular Ecology, 12: 3265–3274

Karpov, L.K., and Novikov, G.G. 1980. The haemoglobin alloforms in cod (*Gadus morhua* L.), their functional characteristics and distribution in the populations. (Translated from Russian.) Voprosy ichtiologi, 20: 823–827.

Karpov, A.K. 1979. Seasonal and geographic variability of White Sea cod as indicated by biochemical markers. *In* Ekologicheskaya Fiziologiya i Biokhemiya Ryb (Ecological Physiology and Biochemistry of Fishes) *1, Astrakhan*. Sturgeon Fisheries Research Center Publication.

Karpov, A.K., Osinov, A.G., and Novikov, G.G. 1984. Protein polymorphism in Atlantic cod *Gadus morhua* (Gadidae) of the White Sea. J. Ichtyol., 24: 71–79.

Knutsen H., Olsen, E.M., Ciannelli, L., Espeland, S., Knutsen, J.A., Simonsen J.H., Skreslet, S., Stenseth, N.Chr. 2006. Egg distribution, bottom topography and small-scale population structure in a coastal marine system. *In review*

Knutsen, H., André, C., Jorde, P.E., , Skogen, M.D., Thuróczy, E. and Stenseth N.C.S, 2004, Transport of North Sea cod larva into the Skagerrak coastal populations Proc. R. Soc. Lond., BB 271, 1337–1344.

Kritzer, J.P., and Sale, P. F. 2004. Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. Fish and Fisheries, 5(2): 131–140.

Kvitek, R.G. 1991. Paralytic shellfish toxins sequestered by bivalves as a defense against siphon-nipping fish. Mar. Biol., 111: 369–74.

Kvitek, R.G., and Beitler, M.K. 1991. Relative insensitivity of butter clam neurons to saxitoxin: a pre-adaptation for sequestering paralytic shellfish poisoning toxins as a chemical defence. Mar. Ecol. Prog. Ser., 69: 47–54.

Lande R. 1992. Neutral theory of quantitative genetic variance in an island model with local extinction and recolonization. Evolution, 46: 381–389.

Law, R. 2000. Fishing, selection, and phenotypic evolution. ICES Journal of Marine Science, 57: 659–668.

Law, R., and Grey, D. R. 1989. Evolution of yields from population with age-specific cropping. Evolutionary Ecology, 3: 343–359.

Lekve, K., Ottersen, G., Stenseth, N.C., and Gjosaeter, J. 2002. Length dynamics in juvenile coastal Skagerrak cods: effects of biotic and abiotic processes. Ecology, 83: 1676–1688

Lekve, K., Stenseth, N.C., Johansen, R., Lingjaerde, O.C., and Gjosaeter, J. 2003. Richness-dependent and climatic factors as regulating processes of coastal fish species richness. Ecology Letters, 6: 428–439.

Luikart, G., England, P.R., Tallman, D., Jordan, S., Taberlet, P. 2003. The power and promise of population genomics: from genotyping to genome typing. Nature Genetics, 4: 981–993

Lynch, M., O'Hely, M. 2001. Supplementation and the genetic fitness of natural populations. Conservation Genetics, 2: 363–378.

Marcil, J., Swain, D.P., Hutchings, J.A. 2006. Counter-gradient variation in body shape between two populations of Atlantic cod (*Gadus morhua*). Proc. Roy. Soc. Lond., *B* 273: 217–223.

Mariani, S., Hutchinson, W.F., Hatfield, E.M.C., Ruzzante, D.E., Simmonds, E.J., Dahlgren, T.G., Andre, C., Brigham, J., Torstensen, E., Carvalho, G.R. 2005. North Sea herring population structure revealed by microsatellite analysis. Mar Ecol Prog Ser., 303: 245–257.

McGinnity, P., Prodohl, P., Maoileidigh, N.O., Hynes, R., Cotter, D., Baker, N., O'Hea, B., Ferguson, A. 2004. Differential lifetime success and performance of native and non-native Atlantic salmon examined under communal natural conditions. J. Fish Biol., 65(Suppl. A): 173–187.

McKay, J.K., and Latta, R.G. 2002. Adaptive population divergence: markers, QTL and traits. Trends Ecol. Evol., 17: 285–291.

McQuinn, I.H. 1997. Metapopulations and the Atlantic herring. Rev Fish Biol Fish, 7:

Møller, D. 1966. Genetic differences between cod groups in the Lofoten area. Nature, Lond., 212: 824.

Møller, D. 1968. Genetic diversity in spawning cod along the Norwegian coast. Hereditas, 60: 1–32.

Mork, J., and Giæver, M. 1999. Genetic structure of cod along the coast of Norway: Results from isozyme studies. Sarsia, 84: 157–168.

Mork, J., and Giæver, M. 1999. Genetic structure of cod along the coast of Norway: Results from isozyme studies. Sarsia, 84: 157–168.

Mork, J., and Sundnes, G. 1985. O-group cod in captivity: Differential survival of certain genotypes. Helgolander Meeresunters., 39: 63–70.

Mork, J. 1997. Cod – choose your parents with care [Poster]. Annual meeting of Norske Havforskeres Forening (The Norwegian Oceanographers Association). Abstract in: Norske Havforskeres Forening. Årsmelding 1997. Available also at: http://www.nt.ntnu.no/users/vmbijmor/Projects/SELECTIO/poster1.html)

Mork, J., Giskeødegård, R. and Sundnes, G. 1984. Haemoglobin polymorphism in Gadus morhua: Genotypic differences in maturing age and within-season gonad maturation. Helgoländer Meeresuntersuch. 36: 313–322.

Mork, J., Giskeødegård, R. and Sundnes, G. 1984. The haemoglobin polymorphism in Atlantic cod (Gadus morhua L.): Genotypic differences in somatic growth and in maturing age in natural populations. Flødevigen rapportser., 1: 721–732.

Mork, J., Reuterwall, C., Ryman, N., and Ståhl, G. 1982. Genetic variation in Atlantic cod (*Gadus morhua* L.): A quantitative estimate from a Norwegian coastal population. Hereditas, 96: 55–61.

Mork, J., Reuterwall, C., Ryman, N., and Ståhl, G. 1982. Genetic variation in Atlantic cod (*Gadus morhua* L.): A quantitative estimate from a Norwegian coastal population. Hereditas, 96: 55–61.

Mork, J., Ryman, N., Ståhl, G., Utter, F., and G. Sundnes, 1985. Genetic variation in Atlantic cod (*Gadus morhua* L.) throughout its range. – Can. J. Fish. Aquat. Sci., 42: 1580–1587.

Mork, J., Ryman, N., Ståhl, G., Utter, F., and G. Sundnes, 1985. Genetic variation in Atlantic cod (*Gadus morhua* L.) throughout its range. – Can. J. Fish. Aquat. Sci., 42: 1580–1587.

Nævdal, G., and Jørstad, K.E. 1984. Importance of genetic variation in the propagation of cod. Ed. by E. Dahl, D.S. Danielsen, E. Moksness and P. Solemdal. *In* The propagation of cod *Gadus morhua* L. Flødevigen rapportser, 1:733–743.

Nævdal, G., Folkvord, A., Otterlei, E., and Thorkildsen, S. 1992. Growth rate related to genotype of 0-group cod at three environmental temperatures. Sarsia, 77: 71–73.

Naylor, R., Hindar, K., Fleming, I.A., Goldburg, R., Williams S., Volpe, J., Whoriskey, F., Eagle, J., Kelso, D., Mangel, M. 2005. Fugitive salmon: Assessing the risks of escaped fish from net-pen aquaculture. Bioscience, 55: 427–437.

Nielsen, E.E., Grønkjær, P., Meldrup, D. and Paulsen, H. 2005. Retention of juveniles within a hybrid zone between North Sea and Baltic Sea cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences, 62: 2219–2225.

Nielsen, E.E., Hansen, M.M., and Bach, L. 2001. Looking for a needle in a haystack: Discovery of indigenous salmon in heavily stocked populations. Conservation Genetics, 2: 219–232.

Nissling, A., and Westin, L. 1997. Salinity requirements for successful spawning of Baltic and Belt Sea cod and the potential for cod stock interactions in the Baltic Sea. Marine Ecology Progress Series, 152: 261–271.

Odense, P.H., Leung, T.C., Allen, T.M., and Parker, E. 1969. Multiple forms of lactate dehydrogenase in the cod, *Gadus morhua* L. Biochem. Genet, 3: 317–334.

Olsen, E., Lilly, G.R., Heino, M., Morgan, M.J., Brattey, J., and Dieckmann, U. 2005. Assessing changes in age and size at maturation in collapsing populations of Atlantic cod (Gadus morhua) Canadian Journal of Fisheries and Aquatic Sciences, 62(4): 811–823.

Olsen, E.M., Heino, M., Lilly, G.R., Morgan, M.J., Brattey, J., Ernande, B., and Dieckmann, U. 2005. Maturation trends indicative of rapid evolution preceded the collapse of northern cod.Nature, 428(6986): 932–935.

Olsen, E. M., Lilly, G. R., Heino, M., Morgan, M. J., Brattey, J. B., and Dieckmann, U. 2005. Assessing changes in age and size at maturation in collapsing populations of Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences, in press.

Palumbi, S.R. 2001. Humans as the world's greatest evolutionary force. Science, 293: 1786–1790.

Petersen, M.F., and Steffensen, J.F. 2003. Preferred temperatute of juvenile Atlantic cod Gadus morhua with different haemoglobin types at normoxia and moderate hypoxia. The Journal of Experimental Biology, 206: 359–364.

Pogson, G.H., and Fevolden, S. E. 1998. DNA heterozygosity and growth rate in the Atlantic cod *Gadus morhua*(L.). Evolution, 52: 915–920

Pogson, G.H., and Fevolden, S.E. 2003. Natural selection and the genetic differentiation of coastal and Arctic populations of the Atlantic cod in northern Norway: a test involving nucleotide sequence variation at the pantophysin (*Pan* I) locus. Molecular Ecology, 12: 63–74

Pogson, G.H. and Mesa, K.A. 2004. Positive Darwinian selection at the pantophysin (*Pan* I) locus in marine gadid fishes. Molecular Biology and Evolution, 21: 65–75.

Pogson, G.H. 2001. Nucleotide polymorphism and natural selection at the pantophysin (*Pan* I) locus in the Atlantic cod, *Gadus morhua*(L.). Genetics, 157: 317–330.

Pogson, G.H., Mesa, K.H., and Boutilier, R. G. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: A comparison of allozyme and nuclear RFLP loci. Genetics, 139: 375–385

Powers, D.A., and Schulte, P.M. 1998. Evolutionary adaptations of gene structure and gene expression in natural populations in relation to a changing environment: a

multidisciplinary approach to address the million-year saga of a small fish. J. Exp. Zool., 282: 71–94.

Pörtner, H.O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A., Johansen, T., Fischer, T., Knust, R., Lannig, G., Naevdal, G., Nedenes, A., Nyhammer, G., Sartoris, F.J., Serendero, I., Sirabella, P., Thorkildsen, S. and Haass, N.K., Kartenbeck, J., and Leube, R.E. 1996. Pantophysin is a ubiquitously expressed synaptophysin homologue and defines constitutive transport vesicles. Journal of Cell Biology, 134: 731–746

Reznick, D.N. *et al.* 1997. Evaluation of the rate of evolution in natural populations of guppies (Poecilia reticulata). Science, 275: 1934–1937.

Ridgway, G., Naevdal, G.N. 2004. Genotypes of *Mytilus* from waters of different salinity around Bergen, Norway. Helgoland. Mar. Res, 58: 104–109.

Ruzzante, D.E., Mariani, S., Bekkevold, D., André, C., Mosegaard, H., Clausen, L.A.W., Salvanes, A.G.V., Skjæraasen, J.E., and Trygve Nilsen, T. 2004. Sub-populations of coastal cod with different behaviour and life-history strategies. Mar. Ecol. Prog. Ser., 267: 241–251.

Salvanes, A.G.V., Skjaeraasen, J.E., and Nilsen, T. 2004. Sub-populations of coastal cod with different behaviour and life-history strategies. Marine Ecology-Progress Series, 267: 241–251.

Salvanes, A.G.V., and Hart, P.J.B. 2000. Is individual variation in competetive performance of reared juvenile cod influenced by haemoglobin genotype? Sarsia, 85: 265–274.

Schmalhausen, I.I. 1949. Factors of Evolution: The Theory of Stabilizing Selection. Blakiston, Philadelphia.

Schulte, P., Glemet, H.C., Fiebig, A.A., and Powers, D. 2000. Adaptive variation in lactate dehydrogenase-B gene expression: Role of a stress-responsive regulatory element. Proc. Nat. Acad. Sci. USA, 97: 6597–6602

Sick, K. 1961. Haemoglobin polymorphisms in fishes. Nature, 192: 894–896.

Sick, K. 1965a. Haemoglobin polymorphism of cod in the Baltic and the Danish Belt Sea. Hereditas, 54: 19–48.

Sick, K. 1965b. Haemoglobin polymorphism of cod in the North Sea and the North Atlantic Ocean. Hereditas 54: 49–69.

Slate J. 2005. Quantitative trait locus mapping in natural populations: progress, caveats and future directions. Mol. Ecol., 14: 363–379.

Smedbol, R.K., McPherson, A., Hansen, M.M. and Kenchington, E. 2002. Myths and moderation in marine “metapopulations”? Fish and Fisheries, 3: 20–35

Stearns, S. C., and Crandall, R. E. 1984. Plasticity for age and size at sexual maturity: a life-history response to unavoidable stress. In Fish reproduction: strategies and tactics, pp. 13–33. Ed. by R. J. Wootton. Academic Press, London.

Stearns, S.C., and Koella, J.C. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution, 40: 893–913.

Stenseth, N.C., Bjørnstad, O.N., Falck, W., Fromentin, J.M., Gjøsæter, J., Gray, J.S. 1999. Dynamics of coastal cod populations: intra- and intercohort density dependence and stochastic processes. Proceedings of the Royal Society of London Series B-Biological Sciences, 266: 1645–1654

Stenseth, N.C., Jorde, P.E., Chan, K.-S., Hansen, E., Knutsen, H., André, C., Skogen, M.D. and Lekve, K. 2006. Ecological and genetic impact of Atlantic cod larval drift in the Skagerrak. Proceedings of the Royal Society of London, B (in press).

Stokes, T. K., and Law, R. 2002. Fishing as an evolutionary force. Marine Ecology Progress Series, 208: 307–309.

Stokes, T.K., McGlade, J.M., and Law, R. 1993 The exploitation of evolving resources. Springer-Verlag, Berlin.

Svåsand, T., Kristiansen, T.S., Pedersen, T., Salvanes, A.G.V., Engelsen, R., Nævdal, G, and Nødtvedt, M. 2000. The Enhancement of cod stocks. Fish and Fisheries, 2: 173–205.

Tallmon, D.A., Luikart, G., Waples, R.S. 2004. The alluring simplicity and complex reality of genetic rescue. TRENDS in Ecology and Evolution, 19: 489–496.

Väinölä, R., and Hvilsom, M.M. 1991. Genetic divergence and a hybrid zone between Baltic and North Sea *Mytilus* populations (Mytilidae; Mollusca). Biol. J. Linn. Soc, 43: 127–148.

Varvio, S.L., Koehn, R.K., and Vainola, R. 1988. Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. Mar. Biol., 98: 51–60.

Vasemägi, A., Primmer, C.R. 2005. Challenges for identifying functionally important genetic variation: the promise of combing complementary research strategies. Molecular Ecology, 14: 3623–3642.

Waples, R. S., M. J. Ford, D. Schmitt. 2004. Empirical results from salmon supplementation in the Pacific Northwest: A preliminary assessment. *In* Bert, T. (Ed.) Ecological and Genetic Implications of Aquaculture Activities. Kluwer Academic Publishers.

Ward, R.D., Woodwark, M., and Skibinski, D.O.F. 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. J. Fish. Biol., 44: 213–232.

Ward, R.D., Woodwark, M., and Skibinski, D.O.F. 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. J. Fish. Biol., 44: 213–232.

Wilkins, N.P. 1971. Haemoglobin polymorphism in cod, whiting and pollack in Scottish waters. Rapp. P.-v. Réun. Cons. int. Explor. Mer, 161: 60–63.

Zakhartsev, M. 2001. Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developinga hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). Continental Shelf Research, 21: 1975–1997.

2.4 To assess the genetic effects of the introgression of farmed Atlantic salmon on wild salmon populations (ToR e)

This text was based on a working paper prepared by P. McGinnity and Eric Verspoor; adopted by WGAGFM at Newport, Ireland in 2006.

2.4.1 Introduction

Since its origin in 1969, salmon farming in the North Atlantic has increased production to c. 800 000 million tonnes in 2004, with Norway and Scotland being the major producers in Europe. NASCO (2005) reports that during the same period the international catch of Atlantic salmon has declined from the 10 000 tonnes reported in the 1960s to 2100 tonnes in 2005. Currently in the order of 2 million salmon escape from salmon farms each year in the North Atlantic, which is equivalent to about 50% of the wild pre-fishery abundance of salmon in this ocean. Escaped farm salmon comprise some 20–40% of the salmon in some North Atlantic areas and rivers with over 80% in some Norwegian rivers (Ferguson *et al.*, 2006). Farm salmon parr also escape from juvenile rearing units but the extent of this has been poorly studied.

Two decades of intensive research into the genetic impacts of farm escape salmon on natural populations has provided a substantial body of useful quantitative data. It is worth noting that

this has been one of the best and most successful examples of the application of genetics in answering a difficult fisheries management question. For comprehensive reviews of this research see, Youngson and Verspoor (1998), Naylor *et al.*, (2005), Ferguson *et al.*, (2006), and most recently Hindar *et al.*, (2006).

The objective of this paper is to briefly summarise the principal findings of twenty years of research into the genetic effects of the introgression of farmed Atlantic salmon on wild salmon populations, to report on attempts to incorporate the data arising from these studies into realistic life history impact models, to review some of the most recent research in the area, to summarise some of the implications of this research for the management of wild fisheries and to recommend useful avenues for future research.

2.4.2 Genetic impacts (taken directly from the summary presented in Ferguson *et al.*, 2006 with permission of authors)

Farm salmon are genetically different from wild stocks due to geographical origin, founding effects, and as a result of deliberate and accidental selection, and genetic drift, during domestication. Many farm salmon differences can be related to selection for faster growth and later maturity together with inadvertent changes affecting survival, deformity, feed conversion rate, spawning time, morphology, aggression, egg viability, egg production, and risk-taking behaviour.

Escaped salmon enter rivers generally adjacent to the site of escape but sometimes at considerable distances. These fish have been shown to breed, and interbreed with wild fish, although the greater reproductive success of farm females relative to males, and differences in behaviour, mean that more hybrids are produced than pure farm offspring.

Farm salmon have both indirect and direct genetic effects on wild populations. Indirect genetic effects occur due to behavioural, ecological, and disease interaction thereby reducing the effective population size of the wild population and increasing genetic drift. In particular competition with farm fish and hybrids, which are larger, can reduce wild smolt production. Direct genetic effects occur due to interbreeding with wild fish and backcrossing in subsequent generations.

Farm salmon offspring and hybrids show substantially reduced lifetime success with poorer survival in the early juvenile stages and again in the sea. This results in a loss of fitness (reduced recruitment) in individual wild populations. Since farm escapes are regular occurrences, such reductions in fitness are cumulative and potentially lead to an extinction vortex in 'weak' populations (i.e. on the verge of self sustainability).

Hybridisation and introgression can change the performance characteristics in wild populations with, for example, an increase in multi sea winter salmon in otherwise predominantly grilse populations, which may be desirable from an angling perspective in such rivers. However, given their reduced lifetime success, 'hybrids' do not compensate for the loss of wild recruitment resulting in a decrease in fitness in the population.

Hybridisation and introgression due to backcrossing will result in gene flow from farm to wild. As only a few farm strains are used throughout the industry, this gene flow will reduce the natural inter-population heterogeneity found in Atlantic salmon, thereby reducing the adaptive potential of the species.

Genetically modified (transgenic) salmon would be expected to result in the same genetic effects as non-modified ones, both with respect to changes in genetic structure and with respect to fitness. However, the negative impact on fitness is likely to be even greater.

2.4.3 Incorporating data into life history models

Experimental studies confirm that in at least some situations escaped farm salmon can have major negative impacts on wild populations. However the experimental work is confined to only a few of the potential escape interaction scenarios, which are likely to exist. As such existing information is still inadequate for providing robust scientific information on the management of farm escapes in many situations. In light of the length of time and cost of undertaking experimental studies of a range of escape scenarios the only realistic way forward is to develop predicative models which allow for risk assessment across the range if escape scenarios which could be expected to be encountered. This could range from a few farm escapes interbreeding with a large healthy population in which case it would be unlikely that there would be a large negative impact, to a situation where you have a large continuous input into a small depressed population. Furthermore in light of the fact that we know that farm escapes have this negative impact, the political will to support studies where we are deliberately releasing farm fish into control situations on a wide-scale is unlikely to be there and justifiably so. This means that the only realistic way forward to progress understanding and assess risk is through computer based modelling of the data that has already been collected or that will collected in the future from a few dedicated facilities. In the case of Atlantic salmon this is a very real option because of the detailed understanding that already exists regarding the population dynamics of wild populations and the good understanding of the genetic implications of the interbreeding of farm and wild fish. Furthermore, in recent years theoretical geneticists have begun to develop realistic multi-locus models of genetic structuring in populations (hybrid zone scenarios, etc.).

A recent example of the potential of modelling is Hindar *et al.,* (in press) where they provide a quantitative picture of the rapid change likely to occur in many wild populations as a consequence of farm escapes. Based on data from spawning and whole-river experiments, they model the future of wild salmon populations experiencing invasions of escaped farm salmon. Simulations with a fixed intrusion rate of 20% farm escapes at spawning suggest that substantial changes take place in wild populations within ten salmon generations. Low-invasion scenarios suggest that farm offspring are unlikely to establish in the population, whereas high-invasion scenarios suggest that populations are eventually composed of hybrid and farm descendants. Recovery of the wild population is not likely under all circumstances, even after many decades of no further escapes. They also observe that managers of wild fish will have problems finding broodstock of the original wild population after a few generations of high intrusion rates.

A recent initiative to examine the scope for modeling and the ways forward has been put in place as part of the recently funded EU GENIMPACT community action where a workshop will review this issue. The workshop will bring together researchers working on farm wild interactions in a range of European aquaculture species with modelers attempting to identify the key research questions and the most optimum approach to answering questions and also to develop research initiatives for future EU funding. For example, from the work of Hindar *et al.* (2006), as well as others (Gilbey *et al*., in prep; Bacon *et al.,* unpublished) have identified density dependent factors as being critical in providing realistic outputs from these models. Furthermore it is clear that the genetic model used also is critically important in determining the predictive power of these models.

2.4.4 Update of most recent research

Evolutionary change in farmed populations

Roberge *et al*. (2006) compared the transcription profiles of 3557 genes in the progeny of farmed and wild salmon from Norway and Canada grown in control conditions and showed that five to seven generations of artificial selection led to heritable changes in gene transcription profiles (see Box 1) the average magnitude of the differences being 25% and

18% for at least 1.4% and 1.7% of the expressed genes in juvenile salmon from Norway and Canada, respectively. Remarkably, genes showing significant transcription profile differences in both farmed strains all exhibited parallel changes. The authors of this paper suggest that these findings, along with the identification of several genes whose expression profiles were modified through artificial selection, suggest how gene flow from farm escapes may affect the genetic integrity of wild populations. It also suggests that we are closer to understanding the specific genetic differences between farmed and wild stocks that are responsible for the fitness differences seen in the wild that arise due to selective breeding and domestication. Once these are understood this information can be used to provide more realistic genetic models of interactions, which can be used in modelling exercises.

Potential for indirect genetic effects of farm escapes on natural salmon populations

While direct genetic effects of introgression between wild and hatchery-reared salmon have been demonstrated (McGinnity *et al.*, 2003), the impact of diseases originating from aquaculture (Håstein and Lindstad, 1991; Johnsen and Jensen, 1994; McVicar, 1997) on the genetic integrity of wild fish has not been addressed (E. deEyto, Marine Institute, Ireland, unpublished) compared genotype frequencies of Atlantic salmon (*Salmo salar*) surviving in a natural river six months after their introduction as eggs with frequencies expected from parental crosses. In order to distinguish between natural selection and other forces that might impact on genetic variation, they included eight putatively neutral microsatellite loci in the analysis as controls as well as immunogenetic loci (see Box 2) from both MHC Class I and class II. They found that Atlantic salmon MHC class II alpha genes were under selection in the wild, while the MHC class I-linked microsatellite or at eight non-MHC-linked microsatellite loci were not. They concluded that selection at the MHC class II locus was a result of an immune response, rather than any demographic event. They also showed that survival was associated with additive allelic effects rather than heterozygote advantage at the MHC class II locus. These results have implications for both the conservation of wild salmon stocks, and also the susceptibility of hatchery fish to disease. The authors concluded that natural or hatchery populations have the best chance of dealing with episodic and variable disease challenges if MHC genetic variation is preserved both among and within populations.

Indirect genetic effects on co-occurring wild sea trout

Several studies have documented the genetic effects of intra-specific hybridisation of reared and wild Atlantic salmon, most notably Youngson *et al.*, 1993. However, the effects of salmon aquaculture on wild congeners are less well understood. It is possible that diseases, introduced or increased in incidence by salmon aquaculture activities, have the potential to impact co-occurring wild sea trout (*Salmo trutta* L.). Coughlan *et al.*, (in press) have recently presented data that suggests that salmon farming and ocean ranching can have an indirect genetic effect (most likely mediated by disease) on cohabiting sea trout by reducing variability at major histocompatibility class I genes. Samples of DNA extracted from scales taken from sea trout in the Burrishoole River, in the west of Ireland, before and at intervals during aquaculture activities, were investigated. In these samples allelic variation at a microsatellite marker tightly linked to a locus critical to immune response (*Satr-UBA*) was compared with variation at six neutral microsatellite loci. A significant decline in allelic richness and gene diversity at the *Satr-UBA* marker locus, that was observed since aquaculture started (and which may be an indication of a selective response), was not reflected by similar reductions at neutral loci.

2.4.5 Management considerations (taken directly from Ferguson *et al.*, 2006)

- The Guidelines on Containment of Farm Salmon, developed by the North Atlantic Farming Industry and the North Atlantic Salmon Conservation Organization (NASCO) should be the minimum standard for the construction and

operation of fish farms. Research into further improving both technological and operation standards should be undertaken.

- Smolt rearing units should not outflow into salmon rivers (as already required in Norway).
- Marine cages should not be situated within 30km of salmon rivers.
- Where escapes occur, appropriate recovery plans and resources should be available for immediate deployment.
- Further investigations in the use of triploids and other bioconfinement methods should be undertaken.
- If it is intended to introduce sterile transgenic salmon in the industry in the future, research, should be undertaken, prior to permission being granted, to determine the ecological impact that such fish may have on wild populations.

Additional recommendations

- Building of realistic working simulation models, which can be used to assess risks of direct genetic interactions, which can be used to identify research priorities.
- Research into indirect genetic and ecological impacts associated with issues such as introduction disease and effects of density dependant population dynamics.
- Spatial and temporal studies.

Box 1.

Transcription profiles are the direct intensity measurements of "**gene expression"** levels for individual genes using DNA "**micro-array"** technology. "**Gene expression**" is the term used to describe the transcription of the information contained within the **DNA**, the repository of genetic information, into messenger RNA (mRNA) molecules that are then translated into the proteins that perform most of the critical functions of cells. A DNA "**micro-array"** is a tool for analysing gene expression that consists of a small membrane or glass slide containing samples of many genes arranged in a regular pattern. It works by exploiting the ability of a given mRNA molecule to bind specifically to, or hybridise to, the DNA template from which it originated. By using an array containing many DNA samples the expression levels of hundreds or thousands of genes within a cell can be determined simultaneously in a single experiment by measuring the amount of mRNA bound to each site on the array. With the aid of a computer the amount of mRNA bound to the spots on the microarray is precisely measured, generating a profile of gene expression in the cell.

Box 2

The genes of the major histocompatibility complex (MHC) encode proteins that play a crucial role in the vertebrate immune response and several lines of evidence suggest that MHC variability is maintained by pathogen-driven balancing selection.

2.4.6 References

Ferguson, A., Fleming, I., Hindar, K., Skaala, Ø., McGinnity, P., Cross, T., and Prodöhl, P. 2006. Farm escapes. *In* The Genetics of Atlantic Salmon: Implications for Conservation. Ed. by E. Verspoor, J. Nielsen and L. Stradmeyer. Blackwell, Oxford. (In press)

Håstein T., and Lindstad, T. 1991. Diseases in wild and cultured salmon: possible interactions. Aquaculture, 98: 277–288.

Hindar, K., Fleming, I. A., McGinnity, P., and Diserud, O. 2006. Genetic and ecological effects of salmon farming on wild salmon: modelling from experimental results. – ICES Journal of Marine Science, 63: xx-xx.

Johnsen, B.O., and Jensen, A.J. 1994. The spread of furunculosis in salmonids in Norwegian rivers. Journal of Fish Biology, 45: 47–55.

McGinnity, P., Prodöhl, P., Ferguson, A., Hynes, R., Ó Maoiléidigh, N., Baker, N., Cotter, D., O'Hea, B., Cooke, D., Rogan, G., Taggart, J., and Cross, T. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon *Salmo salar* as a result of interactions with escaped farm salmon. Proceedings of the Royal Society of London, Series B, 270: 2443–2450.

McVicar A.H. 1997. Disease and parasite implications of the coexistence of wild and cultured Atlantic salmon populations. *ICES Journal of Marine Science*, 54: 1093–1103.

NASCO, 2005. Report of the Twenty-Second Annual Meeting of the Council. CNL(05)50, North Atlantic Salmon Conservation Organization, Edinburgh. 591 pp.

Naylor, R., Hindar, K., Fleming, I. A., Goldburg, R., Williams, S., Volpe, J., Whoriskey, F., Eagle, J., Kelso, D., and Mangel, M. 2005. Fugitive salmon: Assessing risks of escaped fish from aquaculture. BioScience, 55: 427–437.

Roberge, C., Einum, S., Guderley, H., and Bernatchez, L. 2006. Rapid parallel evolutionary changes of gene transcription profiles in farmed Atlantic salmon. Molecular Ecology, 15: 9–20.

Youngson, A.F. and Verspoor, E. (1998). Interactions between wild and introduced Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences, 55: (1): 153–160

3 Group Business

3.1 Draft Resolutions for 2007

The WG considered ToRs for the 2007 meeting and prepared a draft set for consideration by ICES (Annex 3). Five items were proposed, with one being a carry over from the 2005 ToRs.

3.2 Meeting places in 2007 and 2008

In 2004 it was agreed that future meetings should plan for 3.5 days of business in order to allow for a full discussion of the ToRs. Over the past few years the ToRs are increasing in complexity and require more time for discussion in order to reach consensus on the report.

During discussions on meeting place in the year 2005 in Denmark, the WG responded positively to an offer from N. Kourti and P. Carreau from European Commission Joint Research Centre at Ispra, Italy to host the meeting in 2007. Since then NK has left the research centre and PC will leave in 2006. However, PC has promised that The European Science Centre will still host the meeting.

The WG finds it useful for planning purposes to determine meeting venues two years in advance. The 2008 meeting is provisionally planned for Pitlochry, Scotland UK at the invitation of E. Verspoor.

Annex 1: List of Participants

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Annex 2: WGAGFM Terms of Reference 2005

2005/2/MCC04 The **Working Group on the Application of Genetics in Fisheries and Mariculture** [WGAGFM] (Chair: E. Nielsen*, Denmark) will meet in Newport, Ireland, from 25–28 April 2006 to:

a) assess selected case studies and report on the current knowledge of the genetic basis of domestication processes in farmed fish and shellfish;

b) identify and provide recommendations on the technical and organisational requirements for establishing practical, functional and integrated international databases and supporting repositories for genetic stock identification;

c) synthesize the evidence and methods for detecting local (genetic) adaptation in marine fish and shellfish;

d) assess, through a case study of anadromous salmonids, the potential of genetic and spatial data analysis methods for resolving spatial boundaries of finfish and shellfish populations, and for gaining insight into the geographic and ecological factors controlling the development of population boundaries.

e) assess the genetic effects of introgression of farmed Atlantic salmon on wild salmon populations;

WGAGFM will report by 20 May 2005 for the attention of the Mariculture and Diadromous Fish Committees, ACME and ACE (WGECO).

Supporting Information

Priority:	The current activities of this Group will lead ICES into issues related to the ecosystem effects of fisheries and mariculture, especially with regard to the application of the Precautionary Approach. Consequently these activities are considered to have a very high priority.
Scientific Justification and relation to Action Plan:	Action Plan references: a) 2.5 b) 1.10,6.1, c) 1.10, 3.6 d) 1.10, 3.7 **a)** Many farmed species are not subjected to selective breeding programs, but the genetic impact of the process associated with aquaculture at the successive steps of the rearing (hatchery reproduction, nursery, on-growing) can be significant. Aside from the well documented reduction of genetic variability due to genetic drift, genetic effects can be due to hidden selective pressures associated to the farming environment or, conversely, to the absence of selective pressures encountered in the wild. Domestication is a process that will favour the establishment of the aquaculture in species of interest, but can be detrimental in case of population enhancement and conservation programs. Domestication effects are difficult to disentangle from intentional selection effects in species for which selective breeding programs are already established. Consequently, new aquaculture species, or those where no intentional genetic improvement has been initiated, are more likely to provide insight on domestication processes. Alternatively, in selected strains, genetics changes of non-selected traits can also be due to domestication processes. We aim to review the present state of knowledge of genetic aspects related to domestication of aquaculture species by examining study cases. (lead: P. Boudry, France) **b)** Most, if not all, commercial fish and shellfish species are composed of multiple, genetically distinct, and more or less reproductively separated populations. Understanding the spatial distribution and abundance of these stocks, and their contribution to regional stocks and fisheries, is crucial to the development of effective management programmes. The potential of GSI for increasing understanding of population structure and improving fisheries management has long been recognised, and it is now technically possible and practical for an increasing number of species. However, a major impediment to the implementation of GSI is the development of the needed baseline databases. Doing so represents a considerable technical and organisational challenge, given the amount of data involved, the need to standardise genetic typing and nomenclature across diverse research groups and analysis methods for screening. In almost all cases, the development and exploitation of such data bases will involve international networking and cooperation. Given the potential benefits to be gained in the future, how such data bases should be developed needs to be carefully considered before they are set up. This will ensure that mistakes are minimised. This is important as some may prove costly or difficult to rectify once made and hinder progress in exploiting this important source of management information. This initiative

	is supported by the regular working group members as well as the EU Joint Research Centre. (lead: E. Verspoor, Scotland) **c)** Although it is widely accepted that fish and shellfish stocks exhibit marked phenotypic variation in many traits, little is known of the genetic basis of such variability. The development of new markers and statistical theory has facilitated opportunities for estimating the nature and extent of the genetic component of this variation. This ToR will consider the range of options most appropriate for detecting local adaptation in marine fishes, including aspects of experimental design, applications of genomic technologies and quantitative genetic approaches. Such considerations will facilitate conservation of population diversity; more effective incorporation of population heterogeneity into stock assessment models and the translation of this information into management advice (lead: G. Carvalho, UK). **d)** The existence of population structuring in most finfish and shellfish stocks is widespread, with established knowledge of the extensive levels of population differentiation among marine and freshwater species. However, the actual spatial boundaries of populations and the underlying geographic and ecological factors governing the spatial limits of population needed to support population centred management approaches remain poorly understood. Advances in two areas of methodological development, now offer the possibility of addressing these issues. The first is development of GSI (Genetic Stock Identification) methodology, based largely on microsatellite marker analysis, which gives a high power for assigning individuals to populations (see ToR 2005b). The second is the availability of advanced spatial analysis using GIS (Geographical Information Systems) methods which has considerable potential as a means of interpreting patterns and distribution of genetic variation. Integration of knowledge acquired from geographical and genetic studies will allow us to gain a greater understanding of the critical factors which determine the manner in which species become distributed as reproductively isolated populations in their natural habitat, and to identify the boundaries between them. This may, for example, allow us to predict and model how extirpated or depleted populations can become re-established. It may also allow for more targeted, population sensitive exploitation. Inferences regarding population boundaries drawn this insight using geographical and environmental data will also facilitate the targeting of regions for directed sampling of specimens (spawners/juveniles/larvae etc.) required for the development of baseline data sets for GSI. (lead: T. Cross, Ireland; E. Verspoor, Scotland). **e)** This is in response to a request from NASCO.
Resource Requirements:	None required other than those provided by the host institute.
Participants:	The Group is normally attended by some 20–25 members and invitees of the WG Chair
Secretariat Facilities:	None required
Financial:	None required
Linkages To Advisory Committees:	ACME, ACE
Linkages To other Committees or Groups:	SIMWG , WGECO, WGMAFC, WGMASC
Linkages to other Organisations:	Linkage with the EC Joint Research Centre at Ispra, Italy.

Annex 3: Proposed Draft Resolutions for 2007

The **Working Group on the Application of Genetics in Fisheries and Mariculture** [WGAGFM] (Chair: E. Eg Nielsen, Denmark) will meet in Ispra Italy from 24–27 April 2007 to:

a) Update and review the available information on the genetics of the European Eel (*Anguilla anguilla*) including importance for recovery plans;
b) Critically review the potential application of genomics in fisheries management and aquaculture;
c) Identify and provide recommendations for the optimal extraction and storage of DNA from fish for molecular based studies;
d) Assess, through a case study of anadromous salmonids, the potential of genetic and spatial data analysis methods for resolving spatial boundaries of finfish and shellfish populations, and for gaining insight into the geographic and ecological factors controlling the development of population boundaries;
e) To identify the structural and institutional requirements for developing meta-data bases for genetics of fish species covered under the ICES remit**.**

WGAGFM will report by 11 May for the attention of the Mariculture and Diadromous Fish Committees, ACME and ACE (WGECO).

Supporting Information

PRIORITY:	The current activities of this Group will lead ICES into issues related to the ecosystem affects of fisheries and mariculture, especially with regard to the application of the Precautionary Approach. Consequently these activities are considered to have a very high priority.
SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:	Action Plan references: a)-2.5, -2.6 b) -2.5, - 1.10, c)-1.10 , d)-1.10,-3.7 e) 1.10 **Term of Reference a)** The European Eel (*Anguilla anguilla*) has shown a dramatic decline in the number of glass eel returns from the Sargasso Sea to the European Coasts. The European Commission will release an action plan for the Eel, which aims to strengthen the return rate of adult Eels to the Sargasso Sea and includes the development of national management plans. Detailed knowledge of the genetics of European Eel is required.: 1)To save the genetic diversity, 2) develop restocking programs to enhance the brood stock (already initiated in the River Elbe, Germany) and 3) enhance the efforts to succeed in artificially breeding the species. All these effort should in turn contribute to the recovery the stock. Accordingly it is important to update and review the available information on the genetics of European Eel, to identify existing knowledge gabs, define research priorities and provide appropriate advice for management. (lead: J. Trautner) **Term of Reference b)** At present a very dynamic development and application of genomics has been facilitated in a number of fields by the availability of new methodologies and tools, such as high throughput DNA sequencing and cDNA microarrays development. Genomic tools are already used in research on commercially important fish and shellfish species. Sequencing of complete genomes of cod, salmonids, flatfishes, sea bass and oysters has already been initiated. Microarray technology through the expression studies of thousands of genes at a time allow for identification of candidate genes involved in the function of multiple physiological, morphological and behavioural traits of interests in organisms and populations from different environments, which can be subject to selective pressure from e.g. fishery and aquaculture. This ToR will review these and other genomic technologies and pinpoint their potentially beneficial applications and implications for fisheries management and aquaculture. (lead: R. Wenne)

	Term of Reference c) It is widely accepted by members of the ICES WGAGFM that central data bases of genetic information and supporting repositories of samples are essential if we are to optimise the output of all molecular based studies of fishes. An important consideration when setting up these repositories will be the storage of the DNA samples themselves. A variety of techniques exist for extracting DNA and this Tor aims to review these methods and produce guidelines for optimal extraction and long term storage of DNA samples particular with respect to more valuable samples which cannot be replaced for example, ancient scale and otolith samples. (Lead M. O´Sullivan). **Term of Reference d)** The existence of population structuring in most finfish and shellfish stocks is widespread, with established knowledge of the extensive levels of population differentiation among marine and freshwater species. However, the actual spatial boundaries of populations and the underlying geographic and ecological factors governing the spatial limits of population needed to support population centred management approaches remain poorly understood. Advances in two areas of methodological development, now offer the possibility of addressing these issues. The first is development of GSI (Genetic Stock Identification) methodology, based largely on microsatellite marker analysis, which gives a high power for assigning individuals to populations (see ToR 2005b). The second is the availability of advanced spatial analysis using GIS (Geographical Information Systems) methods which has considerable potential as a means of interpreting patterns and distribution of genetic variation. Integration of knowledge acquired from geographical and genetic studies will allow us to gain a greater understanding of the critical factors which determine the manner in which species become distributed as reproductively isolated populations in their natural habitat, and to identify the boundaries between them. This may, for example, allow us to predict and model how extirpated or depleted populations can become re-established. It may also allow for more targeted, population sensitive exploitation. Inferences regarding population boundaries drawn this insight using geographical and environmental data will also facilitate the targeting of regions for directed sampling of specimens (spawners/juveniles/larvae etc.) required for the development of baseline data sets for GSI. (lead: T. Cross, E. Verspoor,). **Term of Reference e)** Studies of genetic variation provide valuable insight into the structuring of fish stocks into genetic populations, into the proportional contribution of different populations to mixed fisheries, and into the impacts on fisheries of exploitation and global climate change. Their value can be considerably enhanced by ensuring that the results of individual studies are integrated, temporally and spatially, and are accessible to the research community as a whole. The sooner it is done, the easier it is to integrate work and the greater the benefits that are realised. With the increasing number of DNA based studies of marine fish species, it is vital that integration be encouraged and supported. This can be achieved done by the development centrally administered, web accessible meta-data bases on existing primary data sets, DNA and tissue archives, and of those actively engaged in research on the various species. The structural and institutional requirements for establishing and maintaining maximally useful meta- genetic data bases to support genetic studies of fish stocks will be defined.
RESOURCE REQUIREMENTS:	None required other than those provided by the host institute.
PARTICIPANTS:	The Group is normally attended by some 15-25 members and guests
SECRETARIAT FACILITIES:	None required
FINANCIAL:	None required
LINKAGES TO ADVISORY COMMITTEES:	ACME, ACE
LINKAGES TO OTHER COMMITTEES OR GROUPS:	SIMWG , WGECO, WGMAFC, WGMASC
LINKAGES TO OTHER ORGANISATIONS:	Linkage with the EC Joint Research Centre at Ispra, Italy.
SECRETARIAT MARGINAL COST SHARE:	