Population genetics of plaice (pleuronectes platessa L.) in Northern Europe
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Chapter 6

LOW EFFECTIVE POPULATION SIZE AND INBREEDING IN PLAICE (Pleuronectes platessa): AN ABUNDANT BUT OVEREXPLOITED FLATFISH

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Abstract
We used DNA from archived otoliths to estimate effective population size ($N_e$) in plaice (Pleuronectes platessa) based temporal fluctuations in allele frequencies from samples collected between 1924 and 2002. Our results show that plaice $N_e$ is five orders of magnitude smaller than the estimated census sizes, i.e., at 20,000 in the North Sea and 2,000 in Iceland. Populations examined between 1924 and ca 1950 were in Hardy Weinberg Equilibrium. The emergence of heterozygote deficiencies from ca. 1970 (that are not attributable to sampling or technical artefacts, but attributable to inbreeding) coincide with the beginning of post-WWII, industrial-scale beam-trawling on the spawning grounds of the North Sea and around Iceland. We hypothesise that fishing-mediated reduction in both census and effective population sizes of plaice, in combination with disruption of mating behaviour at the spawning grounds has further reduced the effective population size thus tipping the balance and allowing inbreeding to emerge. Although the mechanism remains speculative, this is the first demonstration of probable fisheries-induced inbreeding and signals the need for understanding the social and mating behaviour in these fish.
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Introduction

The over-exploitation and subsequent collapse of most major fisheries in the world have emphasised the point that marine stocks are not inexhaustible. In 2002, 75% of the world’s marine stocks were fully exploited or overexploited (FAO, www.fao.org). Even after the collapse of major fisheries such as cod and herring (Hutchings 2000), the perception has remained that marine fishes are fundamentally resilient to large population reductions.

The fact that a ‘collapsed’ stock may still consist of millions of individuals, has led to complacency with regard to the potential effects of fisheries on the genetic health of affected species. The problem is that the census size of a population ($N$) is not the relevant measurement; it’s effective population size ($N_e$) that counts. $N_e$ refers to the number of individuals contributing to the next generation and can be much smaller than the census population size (Franckham 1995). In marine fish, $N_e$ is often several orders of magnitude smaller than $N$, producing $N_e/N$ ratios varying from $10^{-5}$ to $10^{-3}$ (Turner et al. 2002, Hauser et al. 2002, Hutchinson et al. 2003). With such small $N_e/N$ ratios, even species with a very large $N$ can suffer loss of genetic diversity due to fishing pressure as it has been shown for the New Zealand snapper (Hauser et al. 2002), the red drum (Turner et al. 2002) or the North Sea cod (Hutchinson et al. 2003).

Plaice is a common flatfish species inhabiting Northern European coastal waters and a main target of the North Sea beam-trawl fisheries (Rijnsdorp and Millner 1996). North Sea catches last century increased from a stable 55,000 t in the mid 1940s to a record 170,000 t in 1989, after which fisheries collapsed to 82,000 t in 2001 (Rijnsdorp and Millner 1996, ICES www.ices.dk). In Iceland a 50% decline of the stock has been observed this last 10 years (Anno. 2003).

Two recent genetic surveys of plaice populations in the Northern East Atlantic based on microsatellite loci and mitochondrial DNA, revealed weak but significant genetic differentiation (Hoarau et al. 2002, Hoarau et al. 2004) consistent with large scale homogeneity and recency of recolonisation of the North Atlantic over the past 15,000 years. Significant heterozygote deficiencies were also found in these surveys suggesting that mating was not random and that inbreeding might be a factor. Because genotyping (null alleles and large allele drop-out) and sampling artefacts (Wahlund effects) can often produce heterozygote deficiencies, extensive testing has
been performed in order to rule out these possibilities (Chapter 5). Testing for genotyping artefacts included reamplification of loci at lower temperatures, redesign of flanking primers for two of the six loci and an assessment of the proportion of expected null alleles using Brookfield’s method (1996). Size distribution of the genotypes was analysed using MICROCHEKER (van Oosterhout et al. 2003) to rule out the possibility of large allele drop-out (Chapter 5). Testing for sampling artefacts associated with a possible temporal Wahlund effect included a analysis of juveniles from ten separate cohorts. Even in this sampling regime, the finding of heterozygote deficiency persisted where no Wahlund effect was possible. The conclusion from all of these tests was that none of these factors could explain the observed excess of homozygotes.

Evidence for partial inbreeding was also the result of the cohort analyses (Chapter 5). Genetic evidence for kin structure, a prerequisite for inbreeding, was also shown in some of the cohorts. These results imply that social structure may exist and that effective population size in plaice must be small.

The aim of the present study was to estimate effective population size ($N_e$) in plaice, look for a possible reduction in genetic diversity ($H_e$) through time, and to determine whether inbreeding have only recently emerged. We use DNA extracted from archived otolith samples collected between 1924 and 2002 and six microsatellite loci.

Materials and methods

Sampling and DNA extraction

Two plaice populations were chosen—one from Iceland; and one from the North Sea. These two populations are genetically isolated (Hoarau et al 2002a, Hoarau et al. in press) and thus represent two independent samples.

Otolith samples from Iceland (64 samples from 1924, 1948 and 1972 each) and the North Sea (1950 and 1970, with $n=50$ and $n=64$ each) were obtained from the HAFRO (Reykjavik, Iceland) and RIVO (IJmuiden, The Netherlands) fisheries institutes. Data from 2002, (North Sea, $n=336$; Iceland, $n=186$), are from a previous study (Chapter 5).
Historical DNA was extracted from the dry tissues surrounding the otolith (Fig. 1) (Hutchinson et al. 1999) in a laboratory where no DNA-work had ever been done before. DNA-free pipettes and filtertips were also used to avoid contamination. Negative controls were used at every step from extraction to genotyping. Extracted DNA was of good quality with an overall amplification failure of only 1.4%, a level comparable to DNA extracted from muscle tissue (ca. 1% amplification failure).

![Fig. 1. Paper envelope and plaice otoliths.](image)

**Microsatellite loci**

Six microsatellite loci (PL92, PL115, PL142, PL167, List1001, List1003 were genotyped as previously described (Hoarau et al. 2002). As previously explained, these loci have been extensively tested for null alleles and large-allele drop-out (Chapter 5).

**Estimates of Ne**

Variance effective population sizes (Ne) were estimated using a likelihood based temporal method developed by Wang (2001). The variance effective population size is the size of an ideal population (i.e., one with no selection, random mating, and a Poisson-distributed reproductive success) that has the same properties with respect to allele frequency variance (genetic drift) as the actual population. An ML-approach is superior to those based on $F$-statistics because it can utilise more of the information in the data, i.e., the presence of many low-frequencies alleles, which are typical for microsatellite loci. We used the software MNE 1.0 (Wang 2001) with four points in time for the Iceland samples (i.e. 1924, 1948, 1972 and 2002) and three...
 points in time for the North Sea samples (i.e. 1950, 1970 and 2002). The accuracy of the $N_e$ estimate increases with the number of alleles, the sample size and the interval of sampling (Wang 2001). Based on the analysis of a real data set, Wang (2001) found that optimal accuracy was achieved when at least three time intervals (representing ca. 12 generations in this case) were used and 400 individuals. Two loci with 35 alleles in total were found to be adequate. Because sample size, number of alleles and sampling interval can compensate for one another to a certain extent, there is no absolute minimum value for any one parameter. In our case, the use of three to four time points covering 75 years and perhaps 20 generations, in combination with 828 individuals, six loci with 353 alleles gives us confidence that the estimates of $N_e$ for plaice are reasonably accurate.

**Diversity and departures from HWE**

Genetic diversity ($H_e$) was estimated using Genetix 4.04 (Belkhir et al. 2003). The occurrence of inbreeding was tested by comparing the mean observed Multi Locus Heterozygosity (number of heterozygous loci per individual) ($MLH_{obs}$) in the samples with the mean expected MLH ($MLH_{exp}$) under random mating (Chapter 5) and by computing the excess of homozygotes: $\left(MLH_{obs} - MLH_{exp}\right) / MLH_{exp}$. Significance level were determined using 1000 permutations (Chapter 5).

**Results and Discussion**

**Effective population size**

The ML-estimated $N_e$ for Iceland is 1733 individuals [1063; 3598 at 95% CI] and for the North Sea 19,535 individuals [3435; 70,000 at 95% CI]. Adult (> 3 years old) census sizes ($N$) are estimated to be $\sim10^8$ for Iceland (J Palsson, personal communication) and $\sim10^9$ for the North Sea (AD Rijnsdorp, personal communication).

The $N_e/N$ ratios for both populations are similar (ca. $2 \times 10^5$). Although this ratio is very small, it is within the range found for other marine fish with a similar life history (Hauser et al. 2002, Hutchinson et al. 2003, Turner et al. 2002). Thus, very low $N_e/N$ ratios appear to be common in marine fish characterised by high fecundity and high juvenile mortality (type III survivorship curves). Both of these factors can
lead to high variance in individual reproductive success and subsequently, to large discrepancies between $Ne$ and $N$ (Hedgecock 1994).

A high level of reproductive variance is assumed for plaice, with high levels of female fecundity (20,000-600,000 eggs·female$^{-1}$) (Rijnsdorp 1991) offset by high levels of daily mortality for eggs (up to 20%) (Rijnsdorp and Jaworski 1990) and juveniles (up to 4%) (Van der Veer et al. 1990). Therefore, it is likely that variance in plaice reproductive success is the main factor for the small $Ne/N$ ratio we observed.

Social structure and mating behaviour also increase the variance in reproductive success. The importance of mating behaviour is often overlooked in these types of commercial fish species mainly due to a lack of data. For plaice the commonly accepted model of free spawning in large, seasonal aggregations suggests little or no courting behaviour. In fact, very little is known about plaice mating behaviour in the wild due to technical unfeasibility of observation. However, recent observations of flatfish more generally, indicate complex mating behaviours including the possibility of female choice (Stoner et al. 1999, Carvalho et al. 2003).

Finally, as there is sexual dimorphism in maturation and growth, as well as a difference in the natural mortality and vulnerability to fishing, the heavy fishing pressure in the North Sea has removed the larger adult males specimens more than females. Consequently the sex ratio in plaice is slightly skewed towards females (Rijnsdorp 1994), which would further reduce of $Ne$.

**Genetic diversity through the 20th century**

Genetic diversity ($H_e$) has remained constant for both the Icelandic and North Sea populations (Fig. 1) despite increased fishing pressure on plaice over the last century. This was unexpected, as decreases in genetic diversity have been recorded for the overexploited New Zealand snapper and North Sea cod populations (Hauser et al. 2002, Hutchinson et al. 2003). For plaice, $Ne$ is probably still too large to detect any effect of exploitation on the genetic diversity. Indeed, plaice is still relatively abundant in the Northeast Atlantic and even though our estimates of $Ne$ are small, they are still one to two orders of magnitude higher than the $Ne$ estimated for the New Zealand snapper or the North Sea cod populations (Hauser et al. 2002, Hutchinson et al. 2003). Theoretically, a $Ne$ of 50 is all that is required to maintain short term genetic diversity and a $Ne$ of 500 individuals to maintain longer-term
stability. Our Ne estimates for plaice are above these threshold values suggesting that plaice is in no immediate danger of losing genetic diversity. However this conclusion should be taken with caution as a very recent bottleneck may not have had the time to lead to a detectable reduction of diversity.

**Inbreeding through the 20th century**

There is no evidence for a departure from HWE in either the North Sea or Icelandic populations in assays from samples taken ≤ 1950. The individual MLH distributions did not differ significantly from those expected under random mating. However, the finding of significant homozygote excesses ($P < 0.001$), indicative of inbreeding in samples from 1970 onward in both North Sea and Icelandic populations (Fig. 2) is particularly alarming.

![Fig. 2. Temporal changes in genetic diversity ($H_e$): dashed line; and inbreeding (define as excess of homozygotes: (MLH\textsubscript{exp}−MLH\textsubscript{obs})/MLH\textsubscript{exp})): solid line, open circle: non significant, filled circle: $P < 0.001$.](image-url)
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The apparent emergence of inbreeding coincides with the introduction of the beam trawl in the beginning of the 1960s. Although plaice has been heavily exploited for centuries (Van Neer et al. 2002), past fishing activities targeted mainly juveniles on the nursery grounds. Expansions of the fishery after WWII led to a greater focus on fishing mature individuals from spawning grounds. It is likely that increased fishing pressure on spawning aggregations, together with more efficient fishing gear and techniques, have led to unavoidable inbreeding in plaice, by reducing the size of spawning aggregations. If, as we hypothesise, kinship-based social structure is associated with the spawning grounds (Chapter 5), then fishing-mediated reductions in clan-sizes in combination with fishing-mediated disruptions of behaviour during the mating season, may force non-random matings to occur. Consequently, the rise of inbreeding after the 1950s is most likely related to the 3-fold increase in landings of plaice in the last fifty years, especially as fisheries activities account for 80% of plaice mortality (Rijnsdorp and Millner 1996).

Although our proposed mechanism for inbreeding remains speculative, the consequences of inbreeding for plaice populations are likely to be severe, potentially leading to population decline and eventual extinction (Saccheri et al. 1998). As inbreeding in plaice is less than ten generations old, inbreeding depression or a reduced fitness of inbred individuals could result (Charlesworth and Charlesworth 1987). The effects of inbreeding depression are expected to be most severe just after the onset of inbreeding notably as deleterious recessive alleles would not yet be purged by selection. Furthermore, previous results suggest inbreeding depression, is occurring (Chapter 5). An increase in the landings of “abnormal” fish (RIVO unpublished data) may also be indicative of possible inbreeding depression. A rigorous test is necessary but logistically difficult, as sufficient statistical power can be attained only by estimating several fitness components in thousands of individuals (David 1998).

Conclusion

We have shown that effective population sizes in plaice are small and have provided evidence for the emergence of inbreeding between 1950 and 1970. Although the mechanism underlying the observed results remains speculative, it is
clear that plaice stocks are not in Hardy-Weinberg Equilibrium and that a strong link exists between the onset of industrial scale fishing on the spawning grounds in the 1960s. Under the precautionary principle, we recommend that fishing on the spawning grounds be stopped.

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References


