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THE USE OF GENETIC TAGGING TO STUDY INTERACTION BETWEEN FARMED AND WILD ATLANTIC COD STOCKS

Jørstad[1]*, K.E., van der Meeren[2], T., Dahle[1] G., Paulsen[1], O.I., Svåsand[1], T., and Otterå[1], H.

[1] *Institute of Marine Research, PoBox 1870 Nordnes, NO-5817 Bergen, NORWAY*
[2] *Institute of Marine Research, Austevoll Research Station, NO-5392 Storebø, Norway*

*Corresponding author:
K.E. Jørstad Phone: +47 5523 6347 E-mail: knut.joerstad@imr.no

ABSTRACT

Escapement of farmed fish from the aquaculture is considered as a risk for negative genetic impacts on native gene pools. In order to investigate potential interbreeding between cultured and wild Atlantic cod, *Gadus morhua* L, a genetically marked cod strain was developed. These fish are homozygote for a rare allele in one of the glucosephosphate isomerase locus (*GPI-1*30*) expressed in white muscle tissue. Offspring from this strain were first used in large scale enhancement experiments at three locations in western Norway (1990 – 1994). As expected, the releases were followed by a significant increase of the overall frequencies of the marker allele in all locations, but the frequency declined rapidly thereafter. After about 10 years, however, significant higher frequency was only detected in one region, suggesting survival to maturation and some reproduction success of the released cod. Mature cod possessing the marker allele were collected from this region as basic for establishing a new farmed genetic tagged strain of cod. This strain was allowed to spawn in net pens in 2006 and 2007, and successful spawning was documented as well as leakage of fertilized farmed eggs and larvae into the natural environment. The survival and geographical spreading of the farmed offspring have been investigated, and recently, genetically marked fish of similar size as mature wild cod in the local area was observed. In addition, a new industrial scale study has been initiated based on the same cod strain. To year-classes (2007 and 2008), each consisting of 500 000 juveniles, are now farmed in a commercial cod facility. A comprehensive monitoring program has been established to detect escapees during the farming period. The first escapes, identified through the genetic tag, were found in the fjord area in November 2008, and a larger escapement was detected in early April 2009. A fraction of these fish were mature and offspring was detected throughout the 30 km long fjord system, including on local spawning sites of the wild cod.

INTRODUCTION

Interbreeding between escaped farmed fish and wild fish can potentially result in genetic changes in the wild populations that reduce their overall fitness (Utter et al., 1993; Utter and Epifanio, 2002; see also Hutchinson, 1997). Experimental documentation of harmful effects from interbreeding between cultured and wild stocks is limited, with exception of studies carried out Atlantic salmon (Einum and Fleming, 1997; McGinnity et al., 1997; Fleming et al., 2000; McGinnity et al., 2003). In this investigation, a significant reduction in the lifetime success of the offspring of farmed and hybrids were found compared to wild salmon offspring.

The cod farming industry in Norway consist of 507 cod licenses along the Norwegian coast, and the cod production is so far about 15 – 20 000 tonnes. The total production capacity, based on the licenses, is estimated to be about 300 000 tonnes (Innovasjon Noreg, 2006). Farmed cod have different behaviour compared to Atlantic salmon, and significant escapement from he net pens has been reported. Thus commercial cod farming represent specific challenges with respect to the physical design of farms as well as potential genetic threat to wild cod populations.

A genetically marked cod strain was developed in connection with the large-scale enhancement experiments carried out about 15 years ago at Institute of Marine Research (IMR), Bergen, Norway. All the fish in the marked broodstock were homozygotic for a rare allele in the polymorphic enzyme phosphoglucose isomerase (*GPI-1*30*) expressed in white muscle (Jørstad et al., 1991). Offspring of this cod have been used in a number of studies, including comparisons during early larval rearing experiments (Blom et al., 1994; van der Meeren et al., 1994; Suthers et al., 1999; van der Meeren and Jørstad, 2001) and several

release experiments (Jørstad et al. 1994; Otterå et al., 1999). The release activities and genetic aspects have been evaluated by Jørstad et al. (1999) and Jørstad (2004).

In this contribution we describe the application of genetic tagging in cod in several large scale experiments which are highly relevant to the present problems associated with genetic interaction between farmed cod escapes and local wild coastal cod populations. It includes earlier large scale cod experiments conducted 15- 20 years ago, as well as recent studies on farmed cod spawning in net pens and detection of genetically tagged escapes from industrial cod farming. The details describing materials and methods used in the presentation are given in the cited literature.

LARGE SCALE COD ENHANCEMENT STUDIES

The genetically tagged population (Jørstad et al., 1991) was originally developed for use in the large-scale cod experiments carried out within the Norwegian sea ranching program PUSH. Within this program, IMR conducted cod juvenile releases in three different areas (Fig. 1) in the coastal areas near Bergen. The cod studies were conducted in the period from 1990 and 1997, and cod juveniles were produced at the Parisvatn Field Station. For overview see Svåsand et al. (2000).

In general, the juveniles were mechanical tagged, however, in some cases a significant fraction of the released juveniles were offspring from the genetically tagged broodstock (Jørstad et al., 1994), and the recaptures could easily be identified in the large scale monitoring program conducted through the investigation period (Jørstad et al., 1994). An evaluation of the activities from a genetic point of view was carried out by Jørstad et al. (1999).

Collection of cod samples from the three release areas were conducted for about 5 years after the formal investigation period ended (1997), and the results from the genetic analyses were compared over the whole period of releases and the subsequent years of sampling, altogether 12 years. The frequency of the marker allele (*GPI-1*30*) in different years are shown in Figure 2. As expected, the frequency of the marker allele increased dramatically in all the populations after the release of genetically tagged juveniles. But the frequency quickly declined in a couple of years, and in two of the areas (Austevoll and Masfjorden) there was no detectable signal at the end of the sampling. In the Øygarden region, however, the marker allele continued to increase significantly in the population, and even homozygote (*GPI-1*30/30*) fish were found in the catches.

FARMED COD SPAWNING IN NET PENS

A new strain of genetically tagged cod was developed at IMR Austevoll Research Station in the period from 2002 to 2006. This was based on recaptured fish from the Øygarden region that was either homozygote (*GPI-1*30/30*) or heterozygote (*GPI-1*30/100*) for the marker allele. Broodstock fish were carefully selected based on least kin relationship after evaluation by DNA microsatellite analyses, and offspring from the collected broodstock fish were raised to maturation (F1 generation). The first spawning experiment was conducted with about 1000 F1 spawners in one net pen positioned in the inner part of Heimarkspollen, a nearly land lock fjord system in the Austevoll region (Fig. 1). Extensive sampling and monitoring of eggs and larvae revealed that a significant fraction of the larval population in the fjord system originated from the genetically tagged farmed cod in the net pen (Jørstad et al. 2008). Genetically tagged larvae were also found outside Heimarkspollen, demonstrating substantial advection to the surrounding areas. This was further confirmed in the 2007 spawning

experiment where larger fraction of the larvae from the net pen spawning was detected in the outer area adjacent to the inner fjord system (Fig. 3).

A monitoring fishery program were established with focus on revealing cod juveniles possessing the genetic marker, but only a few fish was identified. This was contrasted with the spawning season during spring 2009, where local fishermen in Austevoll collaborated and provided a larger sample size for testing for the genetic marker. These fish were kept alive and biopsy sample were collected for genetic analyses. The fish were T-floy tagged and released in the same area to allow them to spawn at the local spawning sites. Of a total 289 cod sampled 8 fish possessed the genetic marker, which correspond about 3% of the total material. The genetic tagged fish varied from 34 to 43 cm in total length, and represented about 5% of all fish within this size range, presumably constituting the recruits of the 2006 year class. The largest genetic tagged cod corresponded in size to the smallest mature wild cod found during the monitoring program.

ESCAPEMENT FROM COMMERCIAL COD FARMING

The genetically tagged broodstock was also used for large-scale production of cod juveniles for commercial cod farming. During spring 2007, fertilised eggs from natural spawning among selected broodstock fish of the genetically tagged F1 generation at IMR-Austevoll were transferred to the lagoon rearing facility at Parisvatn for incubation in the hatchery and subsequent release for startfeeding in the large-scale enclosure production system. About 500 000 juveniles were obtained from the lagoon and transported in June 2007 to a commercial cod farm in the Florø region, and then raised in net pens under standard farming conditions. The same approach was repeated in 2008, and this year about 600 000 genetic tagged juveniles were produced and transferred to the same cod farm.

A monitoring fishery was established in the adjacent fjord system, Norddalsfjorden, which is about 30 km long. The fist investigation was carried out in June 2008. Genetic analyses of the cod catches in November 2008 identified significant number of fish possessing the genetic tag, and these fish were distributed in a wide area of the fjord. Intensive sampling was conducted during the spawning period during spring 2009. Both local spawning and escapees from the farm were detected through identification of the genetic tag. Moreover, a new escapement was detected in the first part of April 2009. Genetic analyses of samples collected in the wild by local fishermen near the farm facility documented that about 60% of the fish had the genetic tag. Due to these escapements a larval sampling program was established and carried out from April to June 2009, covering the whole fjord system, including a local spawning site in the inner part of the fjord. Genetically tagged larvae were found in all areas and constituted about 1% of all collected larvae (869 specimens) distributed in the investigation area.

DISCUSSION

The first application of genetic tagging in the cod enhancement experiments in the 1990s clearly demonstrated the potential of the approach for study genetic interaction between farmed /released cod and wild cod stocks (Jørstad et al. 1997; Jørstad 2004). These studies has now been extended to include successful documentation of farmed cod spawning in net pens and spreading of offspring as fertilised eggs which hatch into viable larvae in the wild environment. And, recently, we have documented that a significant fraction of these larvae survive and reach a size corresponding to that of first-time spawners among the local wild cod. Although mature genetically tagged fish has actually not been observed, it is only a rhetoric question whether these fish will recruit to the spawning population in the area. In the

Austevoll experiment, no offspring from these fish have so far been detected. Since the fish from the 2006 experiment just barely entered the spawning stock in 2009, this was not unexpected. As we carry out an ongoing investigation, the impact of a potential contribution to the local spawning population of wild cod is expected to appear in the coming 2 or 3 years. Significant efforts will therefore be allocated in the monitoring program the next years.

The investigation dealing with the impacts of commercial scale cod farming is also an ongoing study, and has shown that genetic methods can be very efficient for detecting escapees from fish farms. The escaped cod are found throughout the fjord system, including on the local spawning grounds for coastal cod in the area. So far, offspring (larvae) from the escaped cod have been detected. Although the cod farmer uses continuous light to postpone maturation of the fish in the net cages (van der Meeren and Ivannikov, 2006), some fish do mature despite this light treatment. Thus, spawning in the net pens cannot be excluded as source for the genetically tagged larvae observed in the fjord. Studies on interbreeding between escaped farmed cod and wild cod are now in progress, and may be observed as increased frequencies of heterozygotes (*GPI-1*30/X*) of the rare allele.

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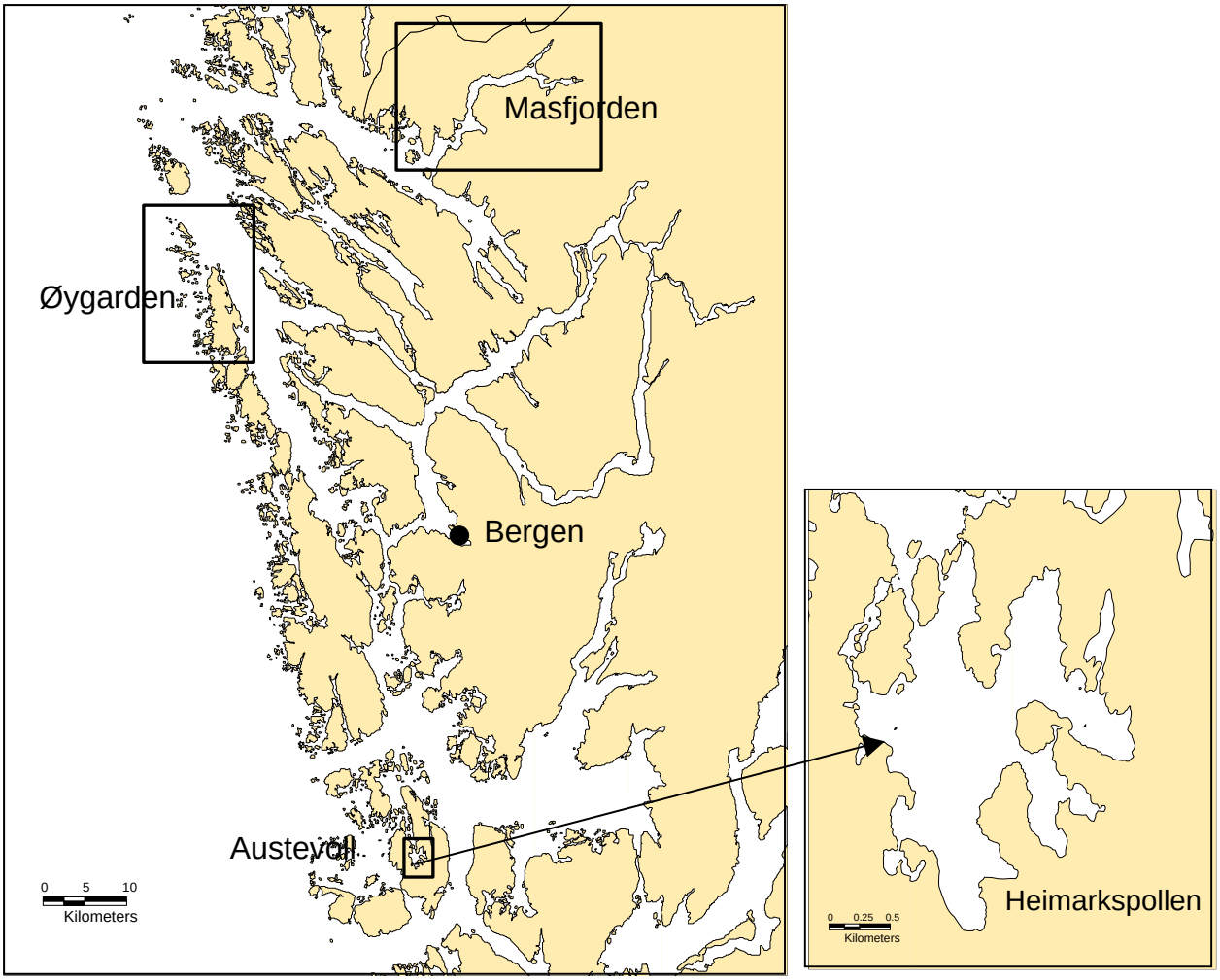

Figure 1. Cod enhancement experiments (1990 – 1997) in three geographic regions in western Norway. The number of released genetically tagged cod juveniles in the different areas, are given in Jørstad et al. (1999).

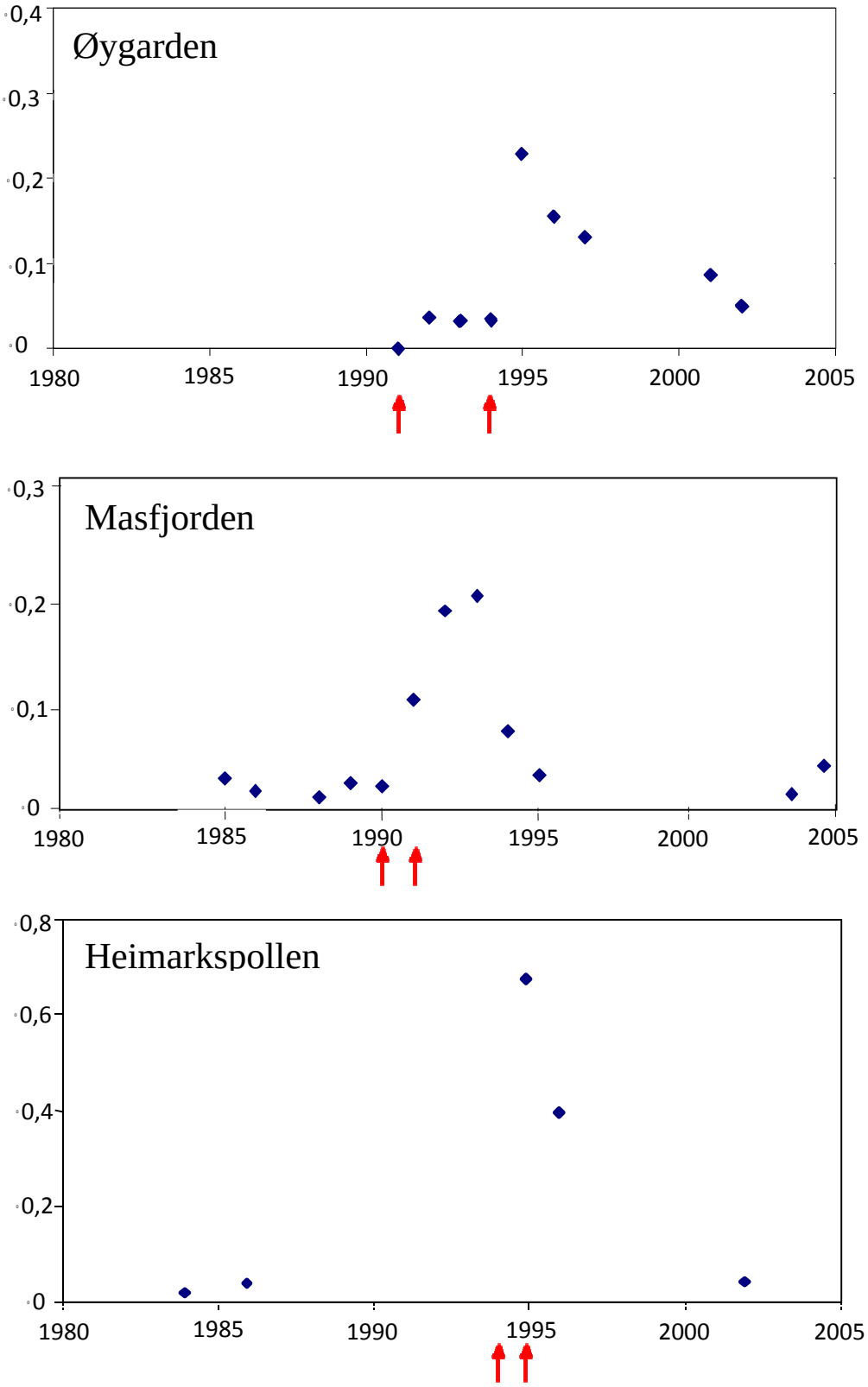

Figure 2. Frequencies of the marker allele (*GPI-1*30*) in the release areas in different years. The releases of genetically tagged cod juveniles are indicated by arrows.

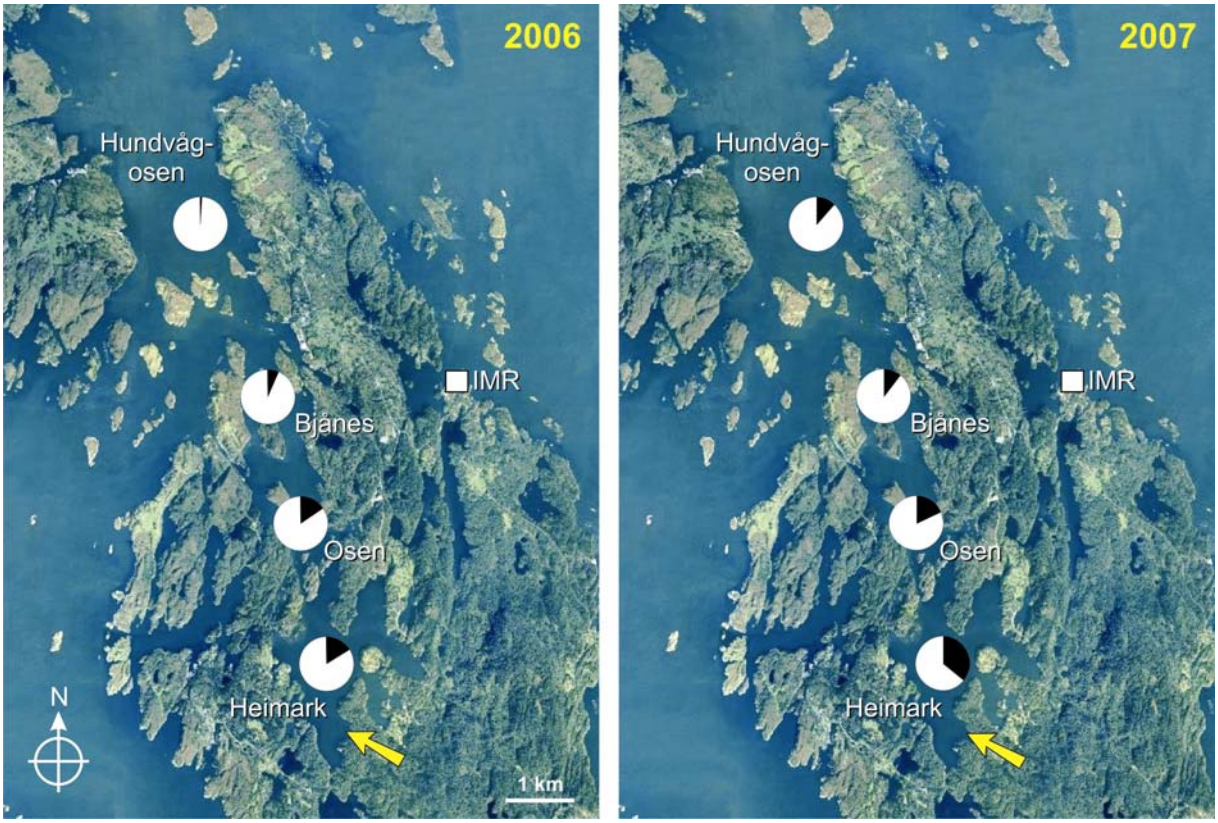

Figure 3. Cod spawning in net pens. Fractions of genetically tagged larvae (black) in larval samples collected from different areas in Austevoll in April 2006 and 2007. Spawning pens were located in inner part of Heimarkspollen (arrow) and took place from February to April in 2006 (one pen) and 2007 (two pens). Sampling was conducted during April (for details see Jørstad et al., 2008).