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ISBN 978-82-326-1574-1 (printed ver.)
ISBN 978-82-326-1447-9 (electronic ver.)
ISSN 1503-8181

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Thesis for the Degree of
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IMT-report 2016:116

Doctoral theses at NTNU, 2016:116

Printed by NTNU Grafisk senter

"*it's a will it's a way Rebekka!*"

my mother Ah-Lin Varne

Acknowledgements
This PhD was funded by the Faculty of Science and Technology with additional financial support provided by the EU-project Prevent escape, the NFR-projects iCoast and ECOCOAST.

To my supervisors I am sincerely grateful! Professor Jarle Mork my main supervisor who always with coolness would break down anything flashy and fancy to the core. Thoroughly teaching me that there are no short cuts and that everything has to be firmly grounded. Ingebrigt Uglem made my supervisor team complete with his energy, can-do attitude and emphasis on easily understandable communication of research results. Together it has been an exciting and demanding journey to reach the end of a PhD.

Thank you, Torild Johansen, for very helpful and constructive comments and for keeping my spirits up during the last stressful months. Jon-Ivar Westgaard gratitude goes to you for excellent genotyping. Kristina Kunz has eyes like an eagle and always helpful, even though she has been in the same situation as me; finishing her own PhD. Your help has been much appreciated. The scientist, the students and staff at Trondheim Biological Station created a fruitful marine research environment and a lot of fun times. During the years in Trondheim I have been with the people I consider my best friends. Thank you, you know who you are!

I also want to mention Møre & Romsdal County Authority, the aquaculture management section, who believed in me and offered me a permanent job in April 2015, and patiently let me take time off to finish my PhD.

My parents in law, Anne Christensen and Bjørn Backe, you have always welcomed me when I came to stay in your home for days and days. You have looked after the children and the professional advice from Bjørn when writing my thesis has been very helpful.

My parents Harry and Ah-Lin Varne; I am super grateful for your support and motivation throughout my years as a B.Sc., M.Sc. and a finally a PhD-student. Mum, your understanding and extensive help with the kids and in the house has been crucial. I could not have done it without you!

Finally I want to acknowledge and give my heartfelt thanks to Sigurd Backe, who has been with me from the start and has supported me during the ups and downs as a PhD-student. During these years I have been so lucky to become the mother of Oskar (5) and Rakel (3). Two amazing persons who have always kept me grounded and forced me to let go of the PhD challenges once I was home. Thank you for being my joy, I love you to the moon and back.

Isfjorden, January 2016, Rebekka Varne

List of papers

I. Ingebrigt Uglem, Marius Berg, **Rebekka Varne**, Rune Nilsen, Jarle Mork and Pål Arne Bjørn (2011). "Discrimination of wild and farmed Atlantic cod (*Gadus morhua*) based on morphology and scale-circuli pattern". *ICES Journal of Marine Science*, 68: 1928-1936.

II. Ingebrigt Uglem, Øyvind Knutsen, Olav Sigurd Kjesbu, Øyvind Johannes Hansen, Jarle Mork, Pål Arne Bjørn, **Rebekka Varne**, Rune Nilsen, Ingrid Ellingsen and Tim Dempster (2012). "Extent and ecological importance of escape through spawning in sea-cages for Atlantic cod". *Aquaculture Environment Interactions*, 3: 33-49

III. **Rebekka Varne**, Kristina Lore Kunz, Torild Johansen, Jon-Ivar Westgaard, Ingebrigt Uglem and Jarle Mork. (2015). "Farmed cod escapes and net-pen spawning left no clear genetic footprint in the local wild cod population" *Aquaculture Environment Interactions* 7:253-266

IV. **Rebekka Varne**, Torild Johansen, Jon-Ivar Westgaard, Kristina Lore Kunz, Ingebrigt Uglem, and Jarle Mork. "Escapees of farmed Atlantic cod (*Gadus morhua* L.); dispersal, presence and identification in a fjord system" [Manuscript]

Declaration of contributions

I. The project was initiated by I.U. The field work was carried out by R.N., **R.V**. and JM. Analyses conducted by I.U, who also wrote the manuscript together with RN with input from all co-authors.

II. I.U. initiated the review, coordinating and planning together with R.N and ØJK. ØJK facilitated collection of eggs. S.O.K estimate fecundity number of eggs analyzed in lab, input interpretation of fecundity data. Modelling I.E and Ø.J. Manuscript written by I.U, RN and ØJH with input from **R.V.** and other co-authors.

III. J.M. initiated the project. Field work done by J.M, K.K., **R.V.** Genotyping conducted by R.V., K.K. and J.I.W. Statistical analyses executed and manuscript written by R.V with comments from all co-authors.

IV. J.M and **R.V.** initiated the project. Field work by **R.V.**, K.K. and J.M. Additional samples and reports from fishermen organized by J.M. Genotyping by R.V and J.I.W. Otoliths read by J.M. Statistical analyses executed and manuscript written by R.V with comments from all co-authors.

List of abbreviations and glossary

Allele: alternative form of the same gene at a locus

Allozymes: forms of an enzyme encoded by different allelic genes (used as genetic markers)

Founder effect: loss of genetic variation when a new population is established by a very small number of individuals from a larger population

Gene: a segment of DNA that encodes a trait

Gene flow: defined as an immigration of individuals (genes) from one population to another, with subsequent reproduction

Genome: the whole genetic material of an organism

HW: Hardy-Weinberg; the principle states that genetic variation is constant in the absence of evolutionary forces

IMR: Institute of Marine Research, Norway

Introgression: successful hybridization of individuals from different gene pools

Locus: the site on a chromosome for a specific gene

Microsatellites: tandem repeats of a simple DNA sequence (used as genetic markers)

NCC: Norwegian coastal cod

NEAC: North East Arctic cod

$\mathbf{N_e}$: Effective population size; the size of an "ideal" population which has a genetic drift (random evolutionary force) equal to the one under study

Pelagic: situated in mid-water

RLFP: restriction fragment length polymorphism (used as genetic markers)

SNP: Single Nucleotide Polymorphism; variation in a single nucleotide (used as genetic markers)

TBS: Trondheim Biological Station, NTNU

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1 General Introduction

Atlantic cod has been traded for thousand years and is one of the best known cold-water white fish. For centuries Atlantic cod has been of great economic importance for Norway (Hylen et al. 2008). In later year's commercial aquaculture of cod emerged. To understand farming of cod, especially the genetic aspects, an introduction to the stock structure and life history is essential.

The distribution of Atlantic cod stretches along both sides of the North Atlantic Ocean, in the west from Cape Hatteras to Labrador, and in the east from Bay of Biscay to Svalbard. Cod are good swimmers and can cover large distances. During spawning they aggregate in specific areas and carry out intricate dances where the female chooses a male based on his performance in the "leks" (Nordeide & Folstad 2000, Rowe & Hutchings 2003). Its fecundity is high; one large female can spawn up to five million eggs in one season. Large and older females produce more and better quality eggs (Solemdal et al. 1993). After spawning, the eggs and later larvae have a pelagic stage which can last from weeks to months. This mobility in both juvenile and adult stages favors a substantial genetic interaction within the species (Mork 2000, Nielsen et al. 2009b, Eiríksson & Árnason 2013). Covering a vast geographical area the Atlantic cod is reported to consist of several stocks with distinct life history and different migration patterns.

The most prominent stock in Norway is the North East Arctic Cod (NEAC) which has an oceanic life history with yearly migrations from the feeding areas in the Barents Sea to the spawning grounds in mid- and northern Norway including the Lofoten islands. Along the coast and in the

fjords one finds the Norwegian Coastal Cod (NCC). NCC displays a more stationary pattern and covers a smaller geographical area. The majority of NCC returns to their original nursery fjord for spawning even if they may leave the fjords for feeding (Jakobsen, 1987).

In the last decades, the population level of NCC has been declining along the coast and restrictions have been implemented in the fishery. The cod farms had massive escape events of adult individuals and by spawning in the net pens, which raised concerns of potential harmful effects on coastal cod (Bekkevold et al. 2006). The need to quantify the actual impact became evident.

1.1 Cod genetics still awaiting consensus

Over the years there have been many studies designed to reveal the true genetic structure of cod (Møller 1968, Mork et al. 1982, Mork et al. 1985, Jørstad & Nævdal 1989, Nielsen et al. 2003, Westgaard & Fevolden 2007, Nielsen et al. 2009b, Eiríksson & Árnason 2013, Karlsen et al. 2013, Karlsen et al. 2014) but there are still a lack of consensus on several aspects. Despite, agreement existing on the genetic difference between Baltic cod and other stocks, and on the difference between the stocks in the western and eastern Atlantic (Mork et al. 1985; Pogson et al. 1995, Nielsen et al. 2003), there are ambivalent findings on other matters. For instance, while differentiations between smaller geographical ranges has been reported, they are dependent on type of marker used (Mork et al. 1985, Pogson et al. 1995, O'Leary et al. 2007, Reiss et al. 2009, Nordeide et al. 2011). The Norwegian cod management is stock-specific for NEAC and NCC,

which shows particularly large allele frequency differences for the restriction fragment length polymorphic (RFLP) marker *Pan*I. A difference between NEAC and NCC samples has also been reported when using nuclear genomic analyses (Moen et al. 2008, Karlsen et al. 2013), but not when analyzing mitochondrial genomes (Karlsen et al. 2014). Even though the genetic evidence may be arguable, NEAC and NCC are considered as different ecotypes and are managed as different stocks.

1.2 The history of cod farming

As cod fishery has been important along the Norwegian coast, there have been experiments trying to enhance the wild stocks. The history of cod stocking dates back to the 1880s, when the aim was to supplement wild stocks with hatched juveniles. The hatching of juveniles for stocking laid the groundwork for cod farming. Still, it took a hundred years before the onset of intensive production of cod for commercial purposes. Intensive production started in the UK and Norway in the early 1990s. A relatively successful production of juveniles was achieved, but aquaculture of cod was not seemed economic viable and the efforts seized (Svåsand et al. 2004).

Later, as the annual landings from the wild stocks were declining, cod was again considered a target species for aquaculture. There was a hope that it would become the same economic success as salmon aquaculture (Rosenlund & Skretting 2006), and that the production experience could easily be transferred. In 2002 cod farming reached a substantial quantity and was increasing until 2010 (Statistics-Norway 2014). The industry had a maximum production in 2010

with 21 240 tons. The same year the industry experienced a collapse, and by 2012 the production had dropped to half, thus the majority of the commercial cod farms were closed down (Statistics-Norway 2014). This collapse can mainly be explained by the cost of production being high and a simultaneous marked increase in the wild stock; the larger quotas of NEAC subsequently decreased the market price. The juvenile mortality was high and sexual maturing of cod in net pens slowed down the growth rate and decreased the quality of the meat. Hatching conditions of the eggs produced large amounts of cod with different degrees of deformities, which became very obvious to the public as cod are more prone to escaping than salmon. The initial expectation; that salmon aquaculture expertise could be directly applied to on another species was not fulfilled. Even though there has been a complete decimation of commercial farms, research is still ongoing trying to solve the challenges (Rillahan et al. 2011, Wold et al. 2014, Bangera et al. 2015, Li et al. 2015). The introduction of cod as a prominent aquaculture species seemed to be premature.

The results from an ongoing scientific breeding program, and the continuous development of aquaculture technology in general, have resulted in significant improvement (Puvanendran & Mortensen 2009, Zimmermann et al. 2012, Bangera 2014, Hansen et al. 2014). By 2014 all commercial cod aquaculture had ceased, but in 2015 one batch has been sold to a farmer by the "Norwegian Cod Breeding Program" (pers. comm. Atle Mortensen, NOFIMA). There is a potential that cod farming could rise again, and be an important future aquaculture industry, as farmed cod can provide a superior quality and an all-season supply.

1.3 Escaped farmed cod

The enhancement experiments served as a pointer to what could be expected of escaped farmed cod. A stock-enhancement study in western Norway (Austevoll) showed that cultured cod adapted quickly to the natural environment (Svåsand & Kristiansen 1990) and most of the cod remained in the area of release. Also, it was found that a larger size (> 20 cm) at release increased the probability of survival (Svåsand et al. 2000). These findings supported the concerns that escaped farmed cod would impact wild cod.

Later, several studies investigated cod from commercial broodstocks, with a life history in hatcheries and farms. Meager et al. (2012) found that hatchery reared cod (F_1-generation) have a behavioral phenotype distinct from wild cod. This was supported by evidence of significant changes in heart and brain morphology also in a F_1 -generation (Mayer et al. 2011). Although, large escape incidents were caused by weather conditions or human error, escapes through holes in the net pens were much more common in cod than in salmon (Moe et al. 2007, Jørstad et al. 2008, Hansen et al. 2009, Jensen et al. 2010). Juvenile farmed cod seemed to be out-competed by wild cod for food and shelter (Sverdrup et al. 2011) and farmed cod also showed lower anti-predator response (Meager et al. 2011). These findings suggested that farmed cod could be less adapted to a wild environment. Investigations using acoustic transmitters on released farmed cod showed that they had a random spread and were observed on local spawning grounds (Uglem et al. 2008). Farmed NEAC and NCC did however, not demonstrate significant differences in the spatiotemporal distribution after release (Uglem et al. 2010). A presence on the spawning

grounds would imply that farmed cod could participate in the wild spawning, which is supported by the equal spawning success of farmed and wild male cod in tank experiments (Wringe et al. 2015b). Though, it was also reported that mature farmed cod on the spawning grounds displayed a differing reproductive behavior (Meager et al. 2009, Jensen et al. 2010).

Domesticated animals, which are kept in large groups, will always have some healthy carriers of diseases, thus infections are expected to become a problem with large scale production (Jensen et al. 2010). As such, escaped farmed cod was seen as a potential source of pathogen transmission to wild cod (Øines et al. 2006). Since cod was a new aquaculture species the available diagnostic information about diseases caused by virus and harmful bacteria was limited. The experience so far from cod farming is that the bacterium *Francisella sp.* could have become a prominent pathogen (Bangera et al. 2014). How large this threat could be is unknown, since the industry collapsed in its infancy. Also, the development of vaccines, and the use of breeding to increase disease resistance will affect the severity the pathogens can have on the industry (Bangera et al. 2011, Bangera 2014, Bangera et al. 2014). Pathogens can also be counteracted with procedures as "all in all out" and zoning of production units (Simolin et al. 2002). The lice *Caligus elongatus* are relatively common in wild marine fish and are known to infest farmed fish (Heuch et al. 2007, Øines & Heuch 2007). Salmon lice *Lepeophtheirus salmonis* has caused major challenges for the salmon industry. However, the cod farms in Norway did not report significant problems with lice infestation (pers. comm. Inger Mette Hogstad, Norwegian Food Safety

Authority). Parasites was not a great concern in cod farming, as most parasites infest through food, and farmed cod are given processed feed (Heuch et al. 2011, Stene et al. 2012).

1.4 Escape by spawning

In contrast to the other aquaculture fish species in Norway, cod spawned in the net pens. The release of milt, roe and also fertilized eggs was an uninvestigated feature and the ecological effects were unclear (Jensen et al. 2010). This type of escape, named "escape by spawning", was considered to have a potentially massive impact on wild stocks, if the gametes or fertilized eggs reached the local spawning grounds (Bekkevold et al. 2006, Glover 2010, Uglem et al. 2012). A study from a landlocked poll outside Bergen, using genetically marked cod, found larvae from the net pens in and outside the poll, strongly suggesting successful net pen spawning and a potential for significant dispersal (Jørstad et al. 2008, Jørstad et al. 2014). The tendency of cod to spawn in the net pen was of great economic disadvantage for the farmers, as it delayed the attainment of slaughter weight. In cod maturation is linked to size more than age. NEA cod mature late, at 5 - 8 years (Hylen et al. 2008), coastal cod mature between 2 - 4 years (Godø & Moksness 1987), whereas farmed cod mature at 1 - 2 years of age (Karlsen et al. 2006). In farmed cod the onset of maturation usually started before the fish reached slaughter weight, and resulted in spawning in the net pens. Exposure to light was used to delay the onset of maturation, but showed variable success in postponing maturation in the net pens (Davie et al. 2007a, b, Korsøen et al. 2013). It is known that recruit spawners of cod are less successful than the repeat spawners (Trippel 1998). Farmed cod would only be able to spawn once and hence only be

recruit spawners. Cryptic inhibition by the ovarian fluids of cod has been suggested to inhibit hybridization between different wild populations in Canada (Beirão et al. 2015). The ovarian fluids in farmed females have also been indicated to inhibit fertilization (Beirão et al. 2014). In addition, farmed cod have been reported to have a lower egg quality (Salze et al. 2005), as the quality can be correlated to the temperature at oocyte maturation and ovulation (Hansen et al. 2012). This, coupled with the indications that farmed males have limited sperm fitness (Skjæraasen et al. 2009), could contribute to explain the lack of signs of introgression despite, escape by spawning at the cod farms (Uglem et al. 2012, Varne et al. 2015). The actual impact of escape by spawning will depend on the timing of net pen spawning to the wild cod spawning, in terms of possibility for direct introgression and survival of eggs and larvae according to the so-called Match-mismatch model for recruitment (Cushing 1990). Participating in a large communal spawning will also lower the risk of being preyed on in the pelagic stage. Even though the survival of escapees can be expected to be low; it could still result in significant genetic introgression if the number of escapees is large compared to the wild population.

1.5 Genetic signatures of farmed fish

When a species is introduced for industrial farming, breeding becomes an important tool to enhance commercial production traits. Breeding is the use of one or more subsamples from wild stocks (broodstock), which are then selected for specific traits. The selection process and caging will cause intentional and unintentional adaption to the farming environment. The unintentional adaption is also known as domestication (Evans et al. 2014). The broodstock will be subject to

changes by genetic drift with a magnitude depending on its genetically effective size (N_e). A small population will experience stronger genetic drift and rapidly change its genetic signature. This is also described as the Founder effect; where the subsample will have a lowered genetic variability and by chance alone could change in a different direction than the mother population (Crow and Kimura, 1970). There is evidence for a reduced reproductive success in wild salmon after enhancement stocking because of domestication in the hatched and released individuals (Araki et al. 2007). Domestication can happen in one generation and it has been shown that "*those with the greatest fitness in a captive environment produced offspring that performed the worst in the wild*" (Christie et al. 2012). The measurable extent of the genetic differentiation between the farmed and the wild populations will depend on the number of generations of selection, the size of the broodstock, and the genetic markers chosen.

In Norway there were two scientific breeding programs (Norwegian Cod Breeding Programme and an IMR program) and two commercial breeding programs for cod (Marine Breed AS and Havlandet AS). All used stroking of mature fish, which give the breeders more control than the use of mass-spawning in tanks (Armitage et al. 2007, Herlin et al. 2007). The programs were based on a combined family- and individual selection, as used in salmonids (Delghandi et al. 2003). The need to map the heritability of commercially important traits in cod resulted in several studies (Gjerde et al. 2004, Kolstad et al. 2006, Bangera et al. 2015). When the knowledge of commercial traits was still in its infancy, cod aquaculture drastically increased, and imported farmed cod from Scotland was also used (Glover et al. 2011). Genetic difference found

between breed, family, even net pens at the same farm, were attributed to founder effects and size sorting at the farm (Glover et al. 2010). Size sorting was essential as larger cod prey on the smaller specimens. Today (2015) there is only one broodstock of cod left in Norway, the "Norwegian Cod Breeding Program" which is kept at Havbruksstasjonen, Tromsø. The Marine Breed broodstock, which was the source for the farmed cod in this study, was later merged into Havlandet AS. The commercial broodstocks do not exist anymore (pers. comm. Synnøve Helland, NOFIMA).

1.6 Genetic effect of introgression

The genetic impact of farmed escapees on a local stock can be divided into indirect and direct genetic effects (Shaklee & Currens 2003, Ferguson et al. 2007). Indirect genetic effect is when escapees cause changes in the genetic composition of the local stock not attributed to hybridization. For example competition for food, transfer of pathogens, changes in the movement patters of wild individuals or modified selection regimes can cause a reduction of population size in the local stocks, which will facilitate a genetic alteration. This might subsequently cause the wild stock to become more vulnerable through lowered genetic variability, inbreeding depression and/or genetic loss, all of which reduces the capability for adaption (Utter 2003, Moreau & Fleming 2011). Direct genetic effect is hybridization of escapees and local individuals. This direct genetic effect is also called introgression. The genetic composition of a wild population has been shaped through thousands of generations by selection and immigration patterns. This results in an adaption to the local environment. A genetic

alteration by domesticated immigrants is considered a risk to long term survival for local populations. Negative impacts have been documented in salmon for supplementation programs, i.e. not breeding for commercial traits, potentially reducing the populations' long term fitness (Lynch & O'Hely 2001, Naylor et al. 2005, Araki et al. 2007, McGinnity et al. 2009, O'Toole et al. 2015). Genetic theory predicts that a hybrid will on average perform intermediate between the parents, so that *farm x wild* hybrids will have a lower fitness than pure wild. This concurs with empirical data (McGinnity et al. 2003, O'Toole et al. 2015). For newly domesticated species the negative effects of introgression are considered to be smaller the first years of breeding because the farmed-wild genetic differentiation is smaller. The negative impact will increase as domestication increases (Mork 1991), and the magnitude of introgression is positively correlated with the probability of extinction (Theodorou & Couvet 2004, Bourret et al. 2011). The true effect will be dependent on the actual amount of immigrants successfully mating and the strength of natural selection to purge hybrids (Hindar et al. 1991, Bekkevold et al. 2006, Baskett et al. 2013). Large, rare pulses of escapees would be more efficiently purged by natural selection than a low level leakage over a long time (Baskett et al. 2013). An investigation of 21 Norwegian salmon populations over three decades shows that there are different levels of introgression in rivers with similar escape pressures (Glover et al. 2012). The level of introgression has been suggested to be strongly influenced by the demography of the native populations (Heino et al. 2015).

1.7 Consequences of farmed cod escapees on the local cod stocks

The main aquaculture method in Norway is production in open net-pens located in sheltered coastal areas, mainly the same habitat as coastal cod. Up to the peak year of 2010, cod farming was increasing along the coast of Norway, and with it came the necessity to address its impacts on the local cod populations. Farmed cod that escaped from net-pens are likely to compete with wild cod for space and resources (Skjæraasen et al. 2007). This could change the predation regime in an ecosystem (Jensen et al. 2010). Presence of farmed cod could also cause disruption in local social behavior, especially on the spawning grounds (Bekkevold et al. 2006, Meager et al. 2010). Through escape by spawning, the farmed cod could affect local stocks at all ages, and is not limited to the escape of matured individuals. To get the full picture of the potential impact of farmed cod it was necessary to investigate all sides of escapee interaction with the wild stocks.

Cod is a purely marine fish with a lengthy pelagic stage and a life history very different from salmonids. A study of farmed cod interactions outside Bergen found significant signs of introgression from farmed cod, and also concluded that there has been farmed cod interbreeding in the wild (Jørstad et al. 2014). However, in the study from the Trondheimsfjord no robust signs of introgression was found (Varne et al. 2015). The genetic impact of farmed escapees seems to be dependent on a quick adaption of escapes to a life in the wild and an actual interaction with the local cod population. In cod, estimates of the genetic impact, based on the number of escapees seems to be inaccurate and has resonance with the results found in salmon (Heino et al. 2015).

Upon the start of cod farming in the Trondheimsfjord potential impact was assessed in an Environmental Impact Assessment (EIA), the first and only formal risk assessment for cod farming in Norway (Winther et al. 2007). The EIA advised to reduce the risk of contact between wild and farmed cod by avoiding areas close to wild spawning grounds, as well as areas with high aggregations of wild cod, particularly the juvenile areas. Also they recommended to avoid areas used for cod fishing (Winther et al. 2007). Avoidance of proximity to spawning areas and fishing grounds is implemented in the management today.

2 Objectives and project design

The objective of the current project was to investigate the fate of escaped farmed cod and detect any introgression in the local stock. The project was designed to integrate phenotypic and genetic approaches (Begg & Waldman 1999). Methodologies were tried out for phenotypic discrimination of wild and farmed cod, and baseline values for wild and farmed genetic characteristics were established. With this design, a genetic change in the wild stock in the direction of the farmed cod characteristics would be indicative of an introgression. The statistical ability of these tools to actually detect farmed cod specimens amongst wild cod in the post-escape period could also be assessed. Farmed cod from two different batches were tagged and released in simulated escape events in order to study dispersal and survival of escapees from the farm in the Trondheimsfjord estuarine system. Also, the presence, timing and abundance of pen-spawned pelagic cod eggs and larvae in the neighborhood of the cod farm were investigated.

The novelty of the project design was the Trondheimsfjord as a pristine location with a well-characterized local cod stock and a known source of farmed cod. In 2007, a cod farming plant was set up in the Trondheimsfjord. After the establishment of the farm, extensive pen spawning as well as two massive escape events were reported (Norwegian Directorate of Fisheries 2009). The number of cod which escaped was comparable to the number of natural spawners in the fjord (Sundnes 1980). These escape incidents were applied as real time large scale escape experiments.

2.1 Aims of the study:

I. Establish parameters to distinguish farmed cod by morphology and scale circuli patterns and estimate the rate of correct classification.

II. Evaluate the potential magnitude of escape by spawning on the wild stocks. Use existing knowledge of survival rates and quality of released eggs to predict post-escape survival. Information from different farms was used to determine the extent, frequency and timing of spawning in cod aquaculture. Use a model to simulate the potential distribution of eggs and larvae in the Trondheimsfjord.

III. Establish the genetic characteristics of the farmed cod in the Trondheimsfjord, and describe any genetic differentiation between local and farmed cod by comparing historical wild stock data and farmed samples. Investigate if genetic introgression was detectable in the local stock, and quantify any geneflow. Compare the genetic results with the results from a simulated escape experiment.

IV. Detect farmed escapees and describe their dispersal and presence in the fjord. The detection of escapees was done using visual categorization, otolith deposition patterns and genetic markers. Evaluate how the different methods compare and include age and recapture data.

3 Materials and methods

3.1 Background of samples in the study

The *Pre*-sample consisted of specimen from the local stock in the Trondheimsfjord collected before the onset of the cod farm. The sample was sourced from the database and tissue bank at Trondhjem Biological Station (TBS) and had been collected in the nursery area Borgenfjord in 2005, three years before the start-up of the farm. The sample was used as a genetic baseline representative for the local wild cod stock.

Surveys for pelagic cod eggs and larvae using plankton nets (100 cm diameter, mesh size 500 my, depth 50 m to surface) were undertaken in the areas surrounding the cod farm after receiving reports of spawning in the net pens at a time well outside the natural cod spawning season in the fjord. Escaped production specimens were identified in commercial net catches by an experienced local fisherman who visually categorized specimens as farmed or wild during a time interval of six months following the second escape event. He reported the sizes of his catches and the number of cod he visually classified as farmed in each catch. Another local experienced fisherman collected sample of presumably wild cod of approximalty the same size as the escaped cod.

The remaining samples, potentially containing escapees, came from trawl hauls with the NTNU vessel R/V “Gunnerus”. These were un-categorized samples as no visual classification was used

during the collection. The samplings were carried out approximately within one year after the farmed closed down.

Three years after the shutdown of "Frengen Havbruk" the *Post*-sample was collected by a local fisherman in the vicinity of the main spawning area. Only juvenile specimens were included in this sample, thus excluding any farmed escapees. Any genetic trace of farmed cod would then have originated from an introgression.

3.2 Simulated escape experiment

The farmed cod used in the tag and recapture experiment were collected from the net pens and put in tanks on R/V "Gunnerus". The tanks had continuous sea water flow and both placement of tanks and all handling procedures were executed on deck. The handling time of cod, out of water was on average five minutes. As quickly and as careful as possible the fish were length measured, fin clipped and tagged, before release into the fjord. The released cod were on average 46 cm. Tissues from the farmed cod used in the tag and recapture experiment were sampled for genetic analyses. The cod were tagged and released in two rounds following the two major escape events at the farm. In March 2009 the samples consisted mainly of cod from the first batch *Farm1* and were thus of the same size as the escapees (~1 kg). The next tag and tissue sampling in November 2009 consisted mostly of the second batch *Farm2*. Thus the dispersal ability of both batches and their genetic signatures were screened.

3.3 Morphological investigation

The morphological investigation was performed on samples from Frengen Havbruk in the Trondheimsfjord, a sample from a cod farm close to the island Mausund outside the fjord, as well as wild specimens from both sites. The fish were killed and placed on a light-grey board with rulers and the fins were pinned outstretched, before pictures were taken with a digital camera. The pictures were analysed using image analysis software.

The structure and deposition pattern of the otoliths are commonly used for distinguishing fish populations (Kerr & Campana 2014). Farmed specimens will usually get less distinct zonation while in farm conditions, with no significant differences in availability or type of food during a year. This feature was used as an indication of farmed or wild origin, together with a published description of the otoliths of the local cod stock (Ekli 1997). The otolith typing and age reading was done by an experienced person familiar with the local cod otoliths, making this type of categorization highly relevant for this study.

3.4 Genetic identification

In the present study four allozyme loci, nine microsatellite loci and one RFLP marker were used. All have previously been extensively screened in Atlantic cod, and included both assumed neutral markers and markers which showed signs of natural selection (Nordeide et al. 2011). The use of allozymes allowed direct comparison of the historical database at TBS (Mork et al. 1982). The DNA-markers chosen were the standard set used by IMR, which screens an extensive

amount of cod samples yearly (Delghandi et al. 2003, Westgaard et al. 2007). All DNA marker analyses were done in the same lab at the IMR laboratory in Tromsø.

Prior any advanced analyses, basic tests were performed on the wild cod samples to assess the quality of the markers. The basic test included test for genotyping error, conformance to HW, linkage disequilibrium and selection. Genotyping errors like null alleles, large allele drop-out and the miss-scoring of stutter peaks may result in apparent heterozygote deficiency in microsatellites, which can create a false, or inflate a real, genetic differentiation between samples.

3.5 Study area; the Trondheimsfjord

The Trondheimsfjord is the 7th longest fjord in Norway (130 km) with depths of more than 600 meters, with three main basins separated by shallower thresholds. It is hydrologically characterized by a typical estuarine circulation with an outgoing surface current driven by seven large rivers emptying into the fjord. The fjord is rich in fish species; more than one hundred were described already by Storm (1883). Many gadoid species spawn in the fjord, most of them in March-May, and the fjord is known to harbor local, self-sustaining populations of cod and herring (Mork 2000). The annual year-class strength of cod in the fjord is significantly correlated to the magnitude of the annual spring flood in April-June (Ekli 1997). This indicates that pelagic cod eggs and larvae as well as planktonic food organisms for larvae and codlings (mainly nauplii larvae and adults of the copepod *Calanus finmarchicus*), to an annually varying degree are

washed out of the fjord to coastal waters (Dahl 1899, Swenander 1906). Thus, strong cod year-classes occur more frequently after cold, dry winters with reduced spring floods.

3.6 The cod farm in the Trondheimsfjord and the broodstock

The hatchery used by *Frengen Havbruk* in the Trondheimsfjord had one egg supplier; *Marine Breed*. Their broodstock consisted of NCC which was sampled from five different locations along the Norwegian coast, not including the Trondheimsfjord cod stock (pers. comm. Terje Refstie, Aquaforsk Genetics). At the time of start-up (2006-2007) only the F_1 generation was available. During the three years of operation, *Frengen Havbruk* had two sea transfers of cod. They were from two different batches and contained 270 000 and 300 000 codlings, respectively. The codlings were transferred at approximately 13 cm and 65 gram and were originally set in two net pens. Later they were size sorted into additional net pens. The codlings were reported to be too small to spawn in the first six months in sea (pers. comm. Iver Tanem, farm manager). After three years of operation the farm closed down in April 2010.

4 Summary of papers

4.1 "Discrimination of wild and farmed Atlantic cod (*Gadus morhua*) based on morphology and scale-circuli pattern" (Paper I)

Environmental factors in hatcheries during early development can cause cranial, skeletal and skin deformities and change body proportions in cod, which were all observed in the farmed samples in this study (Fig. 1). The deformities ranged from subtle to very prominent. Using three morphometric measures representing dorsal fin size, neck curvature, and length of lower jaw, 100 % of wild and 95 % of farmed cod were classified correctly. Morphology substantiates the use of visual classification as a simple and fast method which is crucial for recapture fisheries.

The use of scale analyses is considered a robust method to distinguish between farmed and wild salmon. This study confirmed that scale circuli analyses can be used to distinguish between wild and farmed origin of cod. The analyses showed that 86 % of wild and 80 % of farmed individuals were correctly classified.

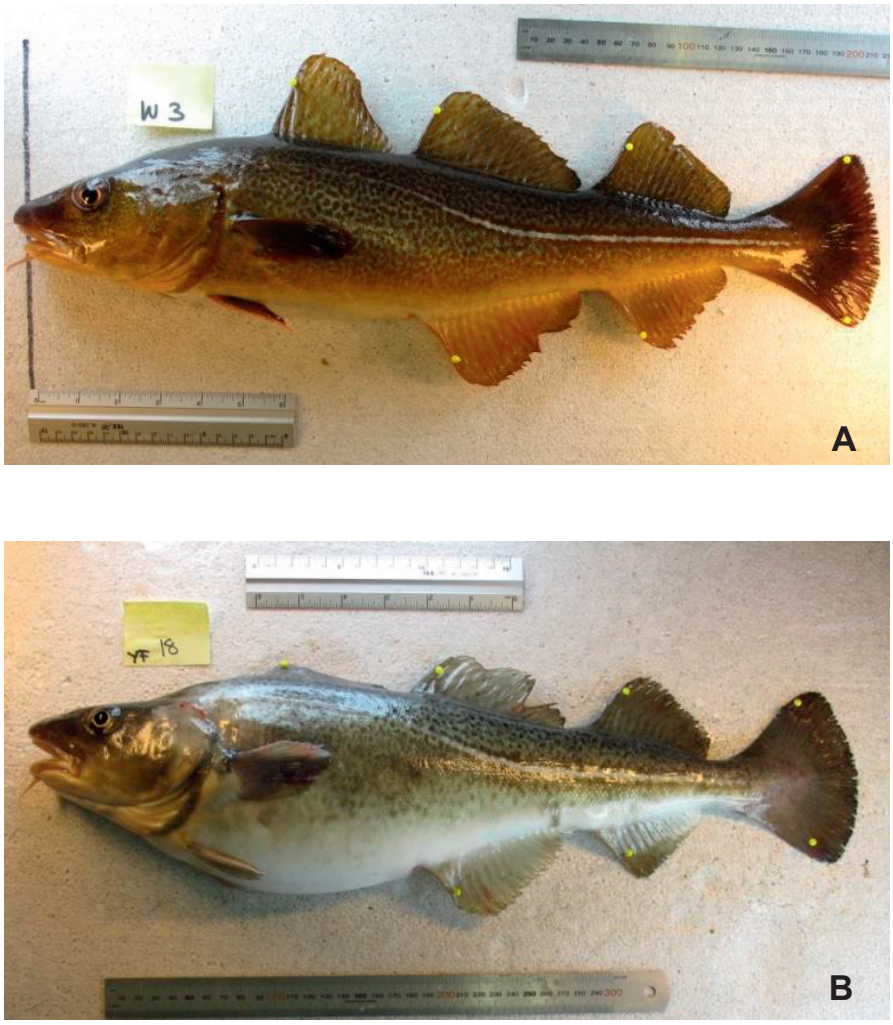

Fig. 1 A) Wild cod taken in cod pots sampled November 2009 B) Farmed cod from Frengen Havbruk AS (*Farm2* batch) sampled November 2009. Pictures were used for morphological analyses.

4.2 "Extent and ecological importance of escape through spawning in sea-cages for Atlantic cod" (Paper II)

Unlike aquaculture of salmon, farmed cod released large amounts of eggs and larvae to the ecosystem through spawning in the net pens. The knowledge of the impact of escape by spawning on wild cod was limited. Existing knowledge on cod eggs together with collected data was used to estimate the amount of gametes produced in cod farms. The study describes a model simulation of an escape by spawning where the farmed and wild eggs and larvae would mix in coastal systems and experience similar larval environments. The study can be seen as a worst case scenario to estimate the impact escape by spawning could have. The survival of escaped cod eggs until adult fish can vary significantly and will also be unpredictable. Match-mismatch to seasonal prey availability, purging by current systems, and impaired viability of farmed cod eggs and larvae can all tend to reduce the impact. Fjords have their specific current dynamics caused both by topography and by seasonal spring flood out-transport. Timing of spawning is crucial for the magnitude of the end effect, making it a complex task to estimate the overall impact.

The modelled impact was later not confirmed by the genetic studies. Even though the survival of cod from escape by spawning appeared to have been low in the Trondheimsfjord, a transport of eggs and larvae by the estuarine circulation may have had genetic impact on cod populations outside the Trondheimsfjord.

4.3 "Farmed cod escapes and net-pen spawning left no clear genetic footprint in the local wild cod population" (Paper III)

The design of the project took into account that genetic introgression from farmed to wild cod could happen in two ways; by spawning of farmed cod in net pens and by escaped farmed fish from the net pens which subsequently spawned together with wild relatives. The basis for the analyses was the demonstration that the farmed cod batches were genetically different from the local wild population in the Trondheimsfjord at several marker loci. However, after two massive escapes of mature individuals, in addition to escape by spawning and observed fertilized eggs likely stemming from the pens, there was no robust evidence that an introgression had taken place in the wild cod. This picture received support from the tag and recaptures experiment which indicated restricted survival of escapees. The match-mismatch hypothesis may explain the lack of contribution from escape by spawning. A lower gamete quality for first time spawners and for farmed cod in particular might further have decreased the survival rates for escape by spawning.

We find that our study is in line with recent findings in salmon, namely that introgression is not only depending on the amount of escapees, but is also very much dependent on survival and adaption of escapees as well as the healthy condition of the recipient local stock. The lack of evidence of introgression in the Trondheimsfjord does not exclude a genetic impact on cod outside the fjord.

4.4 "Escaped farmed cod (*Gadus morhua* L.); dispersal, presence and identification in a fjord system" (Paper IV)

The dispersal and presence of escaped farmed cod in the Trondheimsfjord were examined by two tag-recapture experiments. The experiments were executed shortly after each major escape incidents containing two different production batches, respectively. The recaptures showed a random and limited spatial dispersal in the fjord. The absence of recaptures after six months also suggests a low survival and/or ability to adapt to a natural habitat for the farmed cod. Reports from commercial cod catches indicated a high proportion of farmed cod in the first two months after escape. The result suggests that a recapture fishery of farmed cod will be efficient the first months after an escape.

Established genetic characteristics were used in assignment tests on samples of post-escape commercial and scientific catches. There were two batches of farmed cod, where the *Farm2* batch was the most admixed; the genetics were more overlapping with the wild sample than *Farm1*. This more admixed batch was the likely source of escapees in the samples. The admixture reduced the power of the individual genetic assignment, but results still showed the highest proportion of farmed cod in the samples visually classified as farmed by the fisherman. When comparing all methods the classification of individual specimens was contradictory, indicating some degree of misclassification by the different methods.

5 Discussion

5.1 Tag and recapture experiment

The classic method of tag and recapture gives indisputable evidence for spatial dispersal of fish. The method has been used on cod for decades to monitor the migration patterns and mapping the movements in the fjords and coastal areas. For wild cod and cod used in enhancement projects there are reports of recapture rates up to 30 % (Mork 1990, Svåsand et al. 2000), still for a NCC population a recapture success of 10 % is considered reasonable (Julliard et al. 2001, Olsen 2006) . In this study the farmed cod had a recapture rate of 4 %. The recapture rate depends on many factors like handling, the size of compensation given to the fishermen, species and location. The farmed cod tagged in this study lie in the low end of recapture success reported for coastal cod. Handling of the fish will always induce stress and a recovery time in a tank before release might have improved the survival of the released cod, as a mortality up to 10 % two days after tagging has been reported for Atlantic cod (Björnsson et al. 2011). Cod smaller than 20 cm are more prone to predation by larger predators and by cannibalism as cod can swallow prey up to 50% of their own length (Svåsand & Kristiansen 1990). The released cod were on average 46 cm, which reduced the probability of being killed by predation.

It has been shown that hobby-fishermen have an increased probability of detecting an anchor tag, as they handle fewer fish and assumingly have more time at disposition (Cadigan & Brattey 2006). All recaptures in this study, except one, were reported by hobby-fishermen. The two

commercial fishermen contributing to this study did not report of any tags even though processing a greater load. This could be attributed to the quick and random diffusion in the fjord causing the tagged specimen to avoid the area used by the fishermen. A similar tagging experiment with acoustic transmitters shows that farmed cod dispersed rapidly over larger areas (Uglem et al. 2008). The reports of high proportions of farmed cod in the catches in Varne et al. (2016) do not necessarily contradict the dispersal patterns of the tagged individuals. As only 200 specimen were tagged and released after the second escape event in September 2009 (42 000 escapees). It is very well possible that a majority of the escapees stayed in somewhat homogenous groups the first months after release, whereas some farmed cod dispersed further away. Overall, the findings support other studies that escaped farmed cod have a behavior deviating from wild cod and can be expected to be less adapted to a wild habitat.

5.2 Morphological and visual classification

Differences in morphological characteristics, scale circuli patterns and otolith patterns caused by environmental conditions in hatcheries and net pens result in visual and measurable characteristics of farmed cod specimens (Uglem et al. 2011). The only other study of morphological differences in F_1-generation of farmed cod reports of very similar results, and indicate that these differences could be descriptive of the response Atlantic cod have to a farmed environment (Wringe et al. 2015a).

The broodstock supplier Marine Breed reports low levels of deformities (pers. comm. Synnøve Helland, NOFIMA), while the results in Uglem et al. (2011) show a substantial amount. This can be attributed to the conditions in the local hatchery. As broodstock and hatching routines are improved, less deformity is expected. Such an improvement is reported in the current broodstock in the national breeding program for cod (pers.com Atle Mortensen, NOFIMA). The morphological classification described in Uglem et al. (2011) will probably be less efficient as broodstocks and hatchery conditions improve. Still, morphological effects caused by the farmed environment and diet will be evident (Abaad et al. 2015). For cod the most prominent visual signs are enlarged liver (swollen belly) and fin abrasion (Fig. 1 B). These features show a farmed origin, but they will only be present a limited time after escape. For cod the ratio of liver weight to whole body weight decreased from ~11 % to ~3 % percent after one year in the wild (Svåsand et al. 2004) .

In Atlantic cod, otoliths have been used to distinguish between fish from different stocks, e.g. NCC and NEAC (Stransky et al. 2007) and cod from Faroes plateau and Faroes bank (Cardinale et al. 2004). The exact age of a farmed specimen can be almost impossible to read (Arechavala-Lopez et al. 2012), however, the deposition patterns will give an indication of farmed or wild origin. In Varne et al. (2016) one sample was classified as wild or farmed by otolith deposition patterns, the results suggesting different degrees of correspondence to the other classification methods (27 % - 65 % correspondence).

5.3 Genetic information

Genetically, the initially restricted effective population sizes in farmed broodstocks are likely to be further reduced by selective breeding programs. In the present study this was exemplified by the comparatively large genetic differences between two batches of cod from the same farm. On the other hand, cod farming is a young industry, and the broodstocks are relatively recently established from wild populations. The farmed cod therefore contain the same alleles, or rather a subset of them, as the wild cod. This applied both to allozymes and microsatellites in the present study.

In classical population genetics, neutral markers, e.g. markers which are not under selection, are preferable for detecting whether populations are genetically isolated or are interacting. An introgression will be detected as a change in allele frequencies in the direction of the immigrant. If there are several immigrant sources with deviating allele frequencies, this could lead to a concealing effect. If such an effect is present, it could underestimate and disguise any introgression (Besnier et al. 2011). The loci in this study were checked for a concealing effect, and a possibility for such an effect was present in all allozymes and in two of the microsatellite markers (Varne et al. 2015).

All farmed species have broodstocks made up of samples originating from wild populations, of which there usually has been selection for production related traits (Gjedrem 2000). The farmed broodstock in this study has been subject to directional selection, and genetic drift is expected as

the broodstock is a small subsample of NCC. A genetic difference can also be enhanced by the size sorting in the farm. Overlapping allele frequencies with wild populations will be present, even after generations of selection (Skaala et al. 2004), and even more so for marine species with a pelagic life history (Ward et al. 1994). The use of highly polymorphic markers has facilitated the use of assignment tests (Excoffier & Heckel 2006). Using these tests one can match the multilocus profile of an individual to baseline populations, and estimate the probability of belonging (Glover 2010, Glover et al. 2010). For marine species the overlapping signatures of wild and farmed stocks can result in low or mixed assignment (Šegvić-Bubić et al. 2014). This can be interpreted as interbreeding, but it might also be a bias when testing low differentiated populations, especially when a limited number of markers are available (Brown et al. 2015). Loci under selection have been suggested to be better suited to discriminate farmed and wild animals (Glover et al. 2010). While this might be the case in some very specific situations, it should be noted that such markers do not usually lend themselves for formal statistical testing, since a valid null hypothesis cannot be formulated. Assigning escapees originating from newly established broodstocks can be challenging (Mäkinen et al. 2015), also parentage assignment has been suggested to be more efficient than population assignment for some situations (Bylemans et al. 2016).

5.3.1 Allozymes

Allozymes have shown low levels of differentiation throughout the species range (Mork et al. 1985). The farmed cod involved in this study, however, show large differences in allele frequencies between batches, likely signalizing high genetic drift in the small effective population size of the production units in the cod farming industry. This heterogeneity between the farmed batches caused some data analysis problems in the present study, since it represented a potential concealing effect at all loci. Allozymes were not assessed as effective for detection of introgression in this study.

5.3.2 Microsatellites

Microsatellites are small repetitive pieces of DNA, and pieces with different numbers of repeats represent "alleles" in the genetic analyses. A high number of alleles is common and adds to the analytical capabilities of microsatellites in evolutionary, as well as introgression studies. The common microsatellite set used in Norwegian cod stock management was used in the study. In this project all DNA marker analyses were executed in the same lab at the Institute of Marine Research in Tromsø, any bias between laboratories were then excluded. The results could also in the future easily be compared with the extensive database at IMR.

Extreme differences in the *Pan*I allele frequencies have been reported for the NCC and NEAC (Andersen et al. 2015), and *Pan*I is used as a marker to distinguish samples of these stocks (Fevolden & Pogson 1997). This marker has also been suggested to be a diagnostic marker for farmed and wild cod (Fevolden et al. 2009, Glover et al. 2010). The efficiency implies that the

farmed source contains NEAC genotypes and the wild have NCC genotypes. In this study, however, the broodstock turned out to originate from NCC, so the *Pan*I marker was not as useful. Still, the analyses showed significant differentiation for this marker (Varne et al. 2015). Analyses of *Pan*I were available from historical samples covering 35 years of the local Trondheimsfjord cod stock (Karlsson & Mork 2003). Karlsson and Mork (2003) reported significant allele frequency heterogeneity between both sampling years, cohorts and sexes, and *Pan*I was regarded as so influenced by natural selection that is was not considered as a stable population characteristics. Actually, the temporal *Pan*I allele frequency fluctuations between year-classes in the Trondheimsfjord time series were larger than that between the *Pre* and *Post* samples in the present project (Varne et al. 2015). The historical data made the signs of a farmed introgression in *Pan*I very questionable.

The markers *Gmo8* and *Gmo19* have in previous studies shown homozygotes excess (Lage et al. 2004) and large allele drop out (Herlin et al. 2007). Dahle et al. (2006) reported an heterozygote deficiency, not only at *Gmo8*, *Gmo19*, but also in *Gmo2*, and *Gmo36*. Glover (2011) reported amplification failure in one case study at locus *Gmo19* and chose to exclude the marker. In this study we found significant evidence of null alleles at *Gmo19* in some samples, but the overall result was reasonable and *Gmo19* was included in the analyses (Varne et al. 2015). On the other hand, *Tch11* marker showed significant values for null alleles for both *Pre*- and *Post*-samples, which caused significant HW deviaton in the *Pre*-sample (Varne et al. 2015). The deviation was so large that using adjusted frequencies did not change the significant results. When such large

bias were present, the most reasonble action was to exclude *Tch11* from the analyses. Several studies, on the other hand, do not report of any such bias (O'Reilly et al. 2000, Delghandi et al. 2003, Nielsen et al. 2006, Poulsen et al. 2006, Wesmajervi et al. 2006, Westgaard et al. 2007, Wennevik et al. 2008, Nielsen et al. 2009a, Glover et al. 2011), which stress that in every study one has to carefully assess the markers used.

The decision to exclude specific markers was further facilitated by the fact that the statistical power was not appreciably reduced when using a reduced marker set (Varne et al. 2015). A similar situation was reported for herring, also using isozymes and microsatellites, where low levels of differentiation were concluded when excluding outlier microsatellites (Larsson et al. 2007).

5.4 Genetic and biological approaches to population identification

The lower genetic differentiation present in a species, the more important it is to use biological information to verify and substantiate the genetic results. This is particularly evident in marine species, as they often have pelagic life stages, which make substantial gene flow an expected and probable common feature. Migration behavior and historically stable and confined spawning areas are essential information for detecting natural genetic stock structure. For detecting genetic introgression from farmed relatives, baseline genetic data for the wild population are crucial.

5.5 Strengths and limitations of the study

The novelty and strength of this study was the Trondheimsfjord as a confined area; a possible interaction from outside the fjord was less probable. Also, historical data for the local stock was available, and there were only one farm with a known broodstock limited to two batches. The historical data was essential in the assessment of the genetic markers, and led to the exclusion of *Pan*I. The knowledge about the genetic sources increased the efficiency of the genetic assignment. The cod farm was present in the Trondheimsfjord for a limited time of three years, which made it possible to use age as an indicator for wild and potential farmed specimen, aiding in the assessment of the different classification methods.

A limitation of the study, especially for the genetic assignment, was that the farmed batch likely present in the samples, were the most admixed, subsequently reducing the power of the genetic assignment. Also the historical samples had not been screened for all microsatellites used in this study, which caused the baseline for wild cod stock to be limited to one sample which was analyzed in this study. The numbers of tagged and released cod were also relatively low, and resulted in a limited number of recaptures. If the reports from the fisherman, regarding the proportion of visually categorized cod, had been carried out for a longer time it could have shown if the proportion of farmed cod was stable and potentially could be used as an estimate for visual misclassification. When combining all categorization methods the correspondence dropped, indicating an unknown degree of misclassification. Describing farmed otolith

deposition patterns for larger data set could have made way for an improvement, and a possible verification of the method.

6 Conclusions

The main findings in the study were the absence of any robust evidence for a genetic introgression of farmed cod into the wild population, a significant morphological differences between farmed and wild cod, the indication that recapture fisheries for escaped cod are most effective the first two months after escape, and that the survival of escaped farmed cod seems to be limited.

The significant morphological difference substantiates a visual categorization, which can be done on-site by fishermen. The presence of farmed cod, in samples the fisherman visually categorized as farmed, was also confirmed by the genetic analyses. Though, on-site visual classification is likely to be most effective immediately after escape.

This study describes a large genetic difference between the two batches of farmed cod, of which originated from the same broodstock and had been selected for one generation only. The potential impact on the local stock was estimated to be high, as the farm had massive escape events and experienced escape by spawning. Still, no robust evidence of genetic introgression was found in the local Trondheimsfjord stock. The finding is inconsistent with a similar study outside Bergen, and underlines the complexity of detecting a genetic impact and the importance of a genetic and biological baseline data for detecting introgression.

7 Future perspectives

Marine species are more challenging to categorize as farmed or wild because of the high gene flow and low genetic differentiation present in many species. Especially in newly domesticated species the genetic markers available may not be involved in or linked to the domestication, and will not be diagnostic for farmed specimens. The use of genomic information can facilitate the identification of sites directly influenced by domestication, and SNP markers can provide higher statistical power. A current weak point is that there are few monitoring programs of local wild genetic signatures in coastal and fjord populations in Norway. Also not implemented is official monitoring of farmed broodstocks, but this is anyway challenging since different batches, size sorting, and sampling effect can cause significant genetic differences even between net pens in the same farm.

Although genetic characteristics are crucial for long term identification and detection of introgression, visual clues will be important on-site methods for easy and quick identification of escapees. Visual identification can be used by laymen or professional fishermen in recapture fisheries. These methods will be most efficient, and for some traits only visible, shortly after an escape. Scale circuli and otolith patterns are good options to detect farmed specimen, but cannot be used to connect an escapee to a specific farm.

The potential problem of escape by spawning should be assessed in all new aquaculture species, especially when the production units are in the same habitat as the wild counterpart.

The overall goal should be to minimize farmed escapees at all life stages, which is also in the interest of the aquaculture industry. The possibility to identify the source of an escapee would facilitate the management duties substantially, but it is doubtful that this can be achieved with existing population genetics tools. The use of high resolution tags like otolith fingerprinting and industrial scale internal tagging might be the most immediate way to go. Genetics will continue to be the best option for detecting long term impact on natural populations.

The fate of farmed cod escapees have been reported to cause both introgression and no introgression. Combining the information of the locations of the different farms could be used to find a best practice for choosing future cod farm locations. This information could also potentially be used for other marine aquaculture species with a pelagic phase.

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Number of references:141

9 Research papers I-IV

Paper I

ICES Journal of Marine Science (2011), 68(9), 1928–1936. doi:10.1093/icesjms/fsr120

Discrimination of wild and farmed Atlantic cod (*Gadus morhua*) based on morphology and scale-circuli pattern

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Uglem, I., Berg, M., Varne, R., Nilsen, R., Mork, J., and Bjørn, P. A. 2011. Discrimination of wild and farmed Atlantic cod (*Gadus morhua*) based on morphology and scale-circuli pattern. – ICES Journal of Marine Science, 68: 1928–1936.

Received 14 January 2011; accepted 22 June 2011; advance access publication 25 July 2011.

To evaluate the spatio–temporal distribution and ecological impacts of escaped farmed Atlantic cod (*Gadus morhua*), it is necessary that escapees can be traced in the wild. To do this, simple, reliable, and fast methods for determining the origin of cod are required. The aim of this proof-of-concept study was to evaluate whether simple analyses of scales and body morphology can distinguish between wild and farmed cod. Digital images of fish and scales from adult cod from two farms, and wild cod caught near these farms, were analysed by computer-based image analyses. By combining mean breadth of circuli and length-adjusted scale radius in a discriminant analysis, 86 and 80% of wild and farmed fish, respectively, were correctly classified. Moreover, using three simple morphometric measures representing dorsal fin size, neck curvature, and length of lower jaw, 100 and 95% of wild and farmed cod, respectively, were classified correctly. To validate these discrimination methods further, an expanded analysis of additional farmed and wild cod populations is required. The results pave the way for the development of a reliable and standardized methodology for classification of the origin of cod caught in the wild.

Keywords: aquaculture, Atlantic cod, fish escape, *Gadus morhua*, morphological variation scale analyses.

Introduction

In 2009, almost 20 000 t of farmed Atlantic cod (*Gadus morhua*) were produced in Norway (Norwegian Directorate of Fisheries, 2010). Knowledge of the ecological and genetic impacts of cod farming is still sparse, but the potential for negative ecological consequences is significant (Bekkevold *et al.*, 2006). Escaped farmed cod are present in the spawning areas of wild cod during the spawning season, and wild and escaped cod are likely to interbreed (Uglem *et al.*, 2008; Meager *et al.*, 2009). Within their sea cages, farmed cod can also produce viable larvae that subsequently mix with larvae from wild cod in the areas around the cod farms (Jørstad *et al.*, 2008). Hence, cod farming may result in unfavourable genetic changes in wild populations of cod similar to that found for Atlantic salmon (*Salmo salar*; Hindar *et al.*, 2006). Further, escaped farmed cod may transmit pathogens to wild populations (Øines *et al.*, 2006) and also increase the predation pressure on wild salmon smolt (Brooking *et al.*, 2006) and other fish species.

It has been suggested that farmed cod are more prone to escape from marine net-pen farms than, for instance, Atlantic salmon (Moe *et al.*, 2007). Estimates of escaped farmed cod were not recorded systematically until 2004, but Moe *et al.* (2007) estimated that up to 6% of the annual farmed stock may have escaped during the years 2000–2005. Between 2004 and 2009, a total of 1.13 million farmed cod escaped in Norway (Norwegian Directorate of Fisheries, 2010). On average, this corresponds to 1.1% of the farm stock at the end of each year (Norwegian Directorate of Fisheries, 2010). The proportion of escaped fish in cod farming has so far been higher than in salmon farming, where, on average over the years 2004–2009, 0.2% of the farmed stock at the end of each year was reported to have escaped (Norwegian Directorate of Fisheries, 2010).

To map the spatio–temporal distribution and possible ecological impacts of escaped farmed cod, it is necessary to be able to trace escapees in the wild. To do this, simple, reliable, and fast methods for determining the origin of cod are required. The importance of simple determination of the origin of cod caught in the wild is illustrated by frequent reports in the Norwegian media during recent years regarding catches of abnormal and assumed escaped farmed cod. In many of these cases, it has hitherto been difficult to verify that such fish were of farm origin, because genetic samples were not taken. Analyses of scales and body morphology can distinguish farmed and wild salmonids with a relatively high degree of certainty (Lund and Hansen, 1991; Fleming *et al.*, 1994; Fiske *et al.*, 2006). The primary intent of this proof-of-concept study, therefore, was to evaluate for Atlantic cod whether simple analyses of scales and body morphology have the potential for determining origin. This was done by analysing digital images of fish and scales of farmed cod from two farms and wild cod near the same farms, using computer-based image analyses.

Material and methods

Farmed Atlantic cod were sampled randomly from two fish farms, one located outside the island of Frøya, close to Mausund

Table 1. Length, weights, *K*-factors, and sex ratios for the Atlantic cod used in the (top panel) scale and (bottom panel) morphological analyses.

Location	Type	Date	*n*	Mean length (mm) ± s.d.	Mean weight (g) ± s.d.	Mean *K*-factor ± s.d.	Sex ratio (%) (male:female)
Frøya	Wild	3 November 2009	30	456 ± 55	947 ± 359	0.96 ± 0.06	53.3:47.7
	Farmed	5 November 2009	30	370 ± 55	619 ± 286	1.12 ± 0.18	56.7:43.3
Ytterøya	Wild	9 April 2010	49	580 ± 41	2 100 ± 434	1.06 ± 0.12	61.2:38.8
	Farmed	9 March 2010	50	541 ± 40	1 953 ± 480	1.23 ± 0.24	40.0:60.0
Frøya	Wild	3 November 2009	49	444.3 ± 63.0	902.8 ± 378.2	0.98 ± 0.12	55.1:44.9
	Farmed	5 November 2009	100	380.4 ± 58.1	675.8 ± 307.2	1.12 ± 0.17	53.0:47.0
Ytterøya	Wild	9 April 2010	50	580.8 ± 40.2	2 097.0 ± 429.9	1.06 ± 0.12	60.0:40.0
	Farmed	9 March 2010	50	545.0 ± 40.7	1 953.2 ± 480.2	1.19 ± 0.17	40.0:60.0

(63°52′10″N 08°38′52″E), and one at the island of Ytterøya in the inner part of the Trondheimsfjord (63°40′01″N 11°02′93″E; Table 1). The farmed cod from Frøya and Ytterøya were some 1.3 and 2.5 years old (±3 months) when they were sampled. The background of the farmed cod is unknown because they originated from a mixed broodstock consisting of both coastal cod and Northeast Arctic cod. Wild cod of approximately the same size as the farmed fish were sampled near the farms (<15 km) using fykenets and cod pots (Table 1). Cod captured in the wild were evaluated visually as being wild if they lacked obvious culture-related traits, i.e. neck or mouth deformities, fin damage, or other morphological features typical for escaped cod. The probability that escaped farmed cod were determined to be wild fish was judged to be low, but it is not possible to rule out completely the possibility that some of the wild-caught fish were, in fact, escaped farmed cod. No escape incidents were reported from the farm at Frøya, but two larger escape incidents were reported for the Ytterøya farm before the sampling of wild cod.

Length and weight were measured to the nearest millimetre and gramme for each fish. Otoliths of all wild fish were removed and stored dry in marked paper envelopes for subsequent estimation of age. Before age determination, the otoliths were broken through the nucleus, and age zones were classified as translucent or opaque according to the method outlined by Williams and Bedford (1974). Most wild fish from Frøya were between 2 and 4 years old, and most of the wild fish from Ytterøya were between 3 and 5 years old (Table 2).

Table 2. Estimated ages from otoliths for wild cod from Frøya and Ytterøya, with age categories representing the number of fish up to 1 year older than a given age.

	Number of fish at age (years)				
Location	1+	2+	3+	4+	5+
Frøya ($n = 49$)	2	36	10	0	1
Ytterøya ($n = 50$)	0	1	28	15	6

Scale analyses

Scales were sampled from the same position on all fish, above the lateral line under the third dorsal fin (Figure 1), by first using the blunt side of a knife to remove mucus and then the sharp side to remove between 30 and 60 scales. The scales were dried and stored in marked paper envelopes to await analysis. Before measurement of circuli pattern, the scales were rolled onto a translucent film. The scale-circuli patterns were analysed by capturing digital images of the scales using a Leica Z6-APO macroscope. The images were then examined by computer-based image analysis (ImagePro plus, Media Cybernetics, Inc.). Scale radius was measured from the centre to the edge of the scale (Figure 1). The distances between individual circuli were measured along the same axis as that used to measure the scale radius (Figure 1).

Morphological analyses

After capture/collection, the fish were killed and immediately stored on ice (<5 h) until they were photographed, before onset of rigor mortis, using a digital camera (Canon G10) mounted on a tripod. The fish were placed on a uniform, light-grey background, with the true left side of the fish up, and illuminated from four sides to avoid shadows. All fins were extended to their natural shape and held in place with needles. An object of known dimension (25 × 100 mm) was placed close to each fish to ensure correct calibration in the subsequent image analyses. Altogether, 19 morphological measures (Figure 1, Table 3) were recorded as x–y coordinates using the image analysis software ImageTool (V. 3.0, UTHSCSAN, http://ddsdx.uthscsa.edu/dig/). Repeatability was determined by measuring ten wild and ten farmed fish from Frøya five times. The average coefficient of variation (*CV*) of all measurements was 2.7%.

The morphological measures were selected to represent relatively easily measured parameters for basic differentiation between farmed and wild fish, and not primarily for describing body shape in detail. As farmed cod often have damaged fins (Hatlen *et al.*, 2006), the areas of the three dorsal fins, the caudal fin, and the two ventral fins, as well as pectoral fin length, were measured (Figure 1, Table 3). Further, the angle between the anterior fin root of the posterior dorsal fin and the tip of the second fin ray of this fin and the posterior fin root was measured. This angle is easy to measure in a field situation, without digital image analysis. Distances between fins may vary among different cod stocks (B. J. McAdam, pers. comm.), possibly as a result of variation in environmental conditions during ontogeny. Hence, the distances between dorsal, ventral, and caudal fin roots were also measured (Figure 1, Table 3). A large proportion of farmed cod have deformities in the most cranial vertebrae, which may result in both abnormal upward and downward curvature in the cranial region (Grotmol *et al.*, 2005; Fjelldal *et al.*, 2009). To be able to evaluate morphological variation in the head region, five distances were measured (Figure 1, Table 3). In addition, the angle from the lowest point on the dorsal side of the head to the highest point posterior and anterior of the lowest point was measured (Figure 1, Table 3). The different morphological

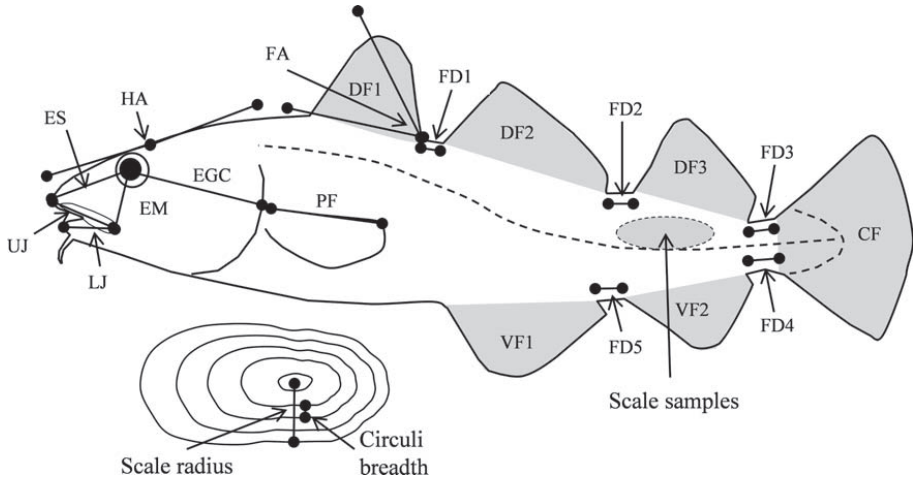

Figure 1. Morphological and scale measures (abbreviations listed in Table 3). The area from where the scale samples were taken is indicated under the last dorsal fin.

Table 3. Abbreviations and description of morphological measures, component loadings, percentage of variance, and eigenvalues for the PCs (with varimax rotation).

Code	Description	PC1	PC2	PC3
SE	Distance from the snout to the centre of the eye	0.935		
EGC	Distance from the centre of the eye to the end of the gill cover	0.848		
PF	Length of the pectoral fin	0.836		
EM	Distance from the centre of the eye to the corner of the mouth	0.922		
LJ	Length of the lower jaw	0.862		
UJ	Length of the upper jaw	0.875		
DF1	Area of dorsal fin 1	0.821		
DF2	Area of dorsal fin 2	0.893		
DF3	Area of dorsal fin 3	0.928		
CF	Area of caudal fin	0.948		
VF1	Area of ventral fin 1	0.910		
VF2	Area of ventral fin 2	0.917		
FD1	Distance from dorsal fin 1 to dorsal fin 2		0.454	
FD2	Distance from dorsal fin 2 to dorsal fin 3		0.589	
FD3	Distance from dorsal fin 3 to the caudal fin		0.561	
FD4	Distance from ventral fin 2 to the caudal fin		0.693	
FD5	Distance from ventral fin 1 to ventral fin 2		0.734	
HA	Angle to the lowest point on neck, to indicate neck deformity			0.829
Percentage of variance		53.8	11.0	8.3
Eigenvalue		9.69	1.97	1.5

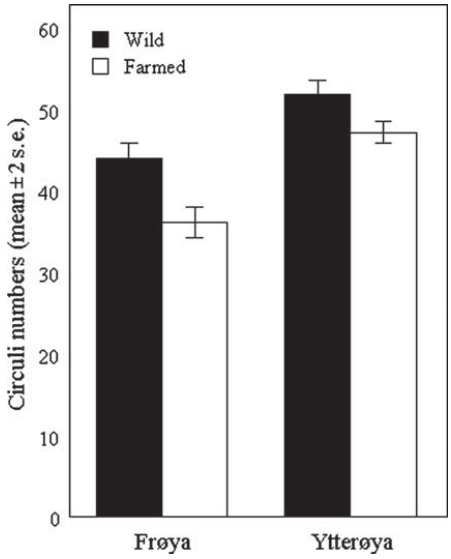

Figure 2. Mean number of circuli per scale for farmed and wild Atlantic cod from two locations, Frøya and Ytterøya.

measurements are hereafter referred to according to the codes described in Figure 1 and Table 3.

Data analysis

In a practical situation, use of scale-circuli patterns to determine the origin of a fish could take place without knowing the exact age of the fish, because age determination based on scale-circuli

patterns is usually impossible because of the presence of unclear seasonal zones for most of the farmed fish. Hence, further discrimination between wild and farmed cod using scale measures is not based on age or data on seasonal zone spacing. The circuli numbers varied both among groups and individuals, and cumulative circuli breadths were only calculated for the first 40 circuli, because almost 90% of the fish had at least this number of circuli. Univariate GLM type III sums of squares analyses with gender, fish type (farmed or wild), and location (Frøya or Ytterøya) as fixed factors, and fish length as covariates, were used for testing whether the scale parameters varied in relation to gender and fish size. Gender was not significantly associated with any of the three scale measures ($F < 1.23$, $p > 0.27$). Scale radius ($F = 41.4$, $p < 0.001$) and circuli number ($F = 42.4$, $p < 0.001$) were significantly related to fish length, so were length-adjusted before further analysis. Mean sclerite distance was not associated with fish length ($F = 3.73$, $p = 0.06$). Discriminant analysis based on selected scale parameters was used to classify fish as either farmed or wild.

All morphological measures were length-adjusted according to the method outlined by Reist (1986), i.e. by transforming all measures in the allometric equation

$$\tilde{Y}_i = \ln Y_i - b(\ln X_i - \ln X_{mean}), \quad (1)$$

where $\tilde{Y}_i$ is the natural logarithm of the correlated trait for fish i, Y_i the original unadjusted measurement, X_i the measured length of the individual, X_{mean} the mean length for all fish, and b the allometric coefficient (the slope of the relationship between ln Y and ln X). These transformations were made separately for each location and for farmed or wild fish. The length-adjusted measures from Equation (1) were further standardized to a mean of zero and s.d. of 1 (Z-standardization). Whether length-adjusted morphological measures were associated with age of the wild fish was tested using one-way ANOVA for ages 2+ and 3+ from Frøya, and ages 3+ and 4+ from Ytterøya (Table 2). The sample sizes of other ages were judged to be too low to be included in such analyses (Table 1). Apart from the relative caudal fin area for wild fish from Ytterøya ($F = 5.74$, $p = 0.02$), none of the morphological measures were associated with age for either Frøya ($F < 3.2$, $p > 0.08$) or Ytterøya ($F < 2.34$, $p > 0.13$). Univariate GLM type III sums-of-squares tests with fish type (farmed or wild) and location (Frøya or Ytterøya) as fixed factors were used to test for differences between morphological measures for farmed and wild fish. To minimize the number of parameters, all morphological measures apart from the angle between the anterior fin root of the posterior dorsal fin and the tip of the second fin ray of this fin and the posterior fin root (i.e. FA; Figure 1) were analysed using principal component analysis (PCA) with varimax rotation. The angle FA was not included in this analysis because, in principle, it is the same measure as DF1, i.e. both measures represent the area of the first dorsal fin. All PCAs with eigenvalues >1.00 were considered to be significant (Chatfield and Collins, 1980). Discriminant analysis, based on individual scores for different PCs and also on selected morphological parameters, was then used to classify fish into either farmed or wild categories. The data were analysed using PASW Statistics (SPSS, v. 18.0.2), with the significance level established at $p < 0.05$.

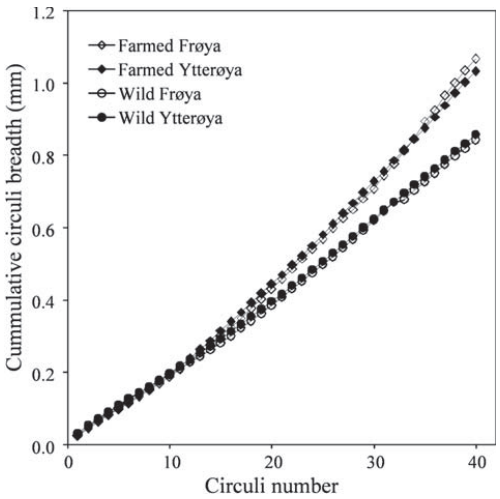

Figure 3. Cumulative circuli breadth (first 40 circuli) for farmed and wild Atlantic cod from Frøya and Ytterøya.

Results

Scales

The number of circuli per scale was lower for fish from Frøya than from Ytterøya ($F = 115.6$, $p < 0.001$), and the examined farmed fish also had fewer circuli than wild fish ($F = 49.9$, $p < 0.001$; Figure 2). There was no significant interaction between fish type and location ($F = 3.3$, $p = 0.073$) with respect to numbers of circuli per scale.

The cumulative circuli breadth did not differ between farmed and wild fish until circuli 14 (Figure 3; $F = 5.0$, $p = 0.026$). From circuli 14 to circuli 40, the cumulative circuli breadth was significantly larger for farmed fish than for wild fish (Figure 3; circuli 40, $F = 36.5$, $p < 0.001$). Location was significantly associated with variation in cumulative circuli breadth for circuli 40 ($F = 16.3$, $p < 0.01$). Further, there was a significant interaction

Table 4. Data from discriminant analysis for scale and morphological parameters, including the proportion of wild and farmed Atlantic cod being classified correctly.

								Correct classification (%)	
Model	***F***	**Eigenvalue**	**Canonical correlation**	**Wilk's λ**	**χ^2**	**d.f.**	***p*-value**	**Wild**	**Farmed**
Mean circuli breadth and length-adjusted scale radius	1	0.51	0.58	0.66	64.7	2	<0.001	86.1	80.0
PC1, PC2, and PC3	1	3.16	0.87	0.24	346.4	2	<0.001	97	96
LJ, HA, and FA	1	4.03	0.9	0.2	395	3	<0.001	100	95

Original classification and cross-validation is identical. The codes for the morphological parameters are described in detail in Figure 1 and Table 3.

Table 5. Means and s.d. for the size-correlated morphometric parameters, with results from univariate GLM analyses.

	Morphometric data (size-correlated according to Reist, 1986)								Univariate GLM statistics					
									Location		Type		Location × Type	
Measure	Wild Frøya (mean ± s.d.)		Farmed Frøya (mean ± s.d.)		Wild Ytterøya (mean ± s.d.)		Farmed Ytterøya (mean ± s.d.)		*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
FD1	2.38	0.17	2.38	0.31	2.47	0.29	2.71	0.34	28.4	<0.001	9.6	0.002	9.8	0.002
FD2	2.54	0.20	2.55	0.23	2.65	0.33	2.80	0.25	30.1	<0.001	5.2	0.023	5.1	0.025
FD3	2.76	0.21	2.89	0.17	2.85	0.30	2.92	0.32	9.2	0.003	3.9	0.049	0.9	0.356
FD4	2.81	0.22	2.89	0.15	3.07	0.33	2.95	0.21	29.6	<0.001	0.6	0.446	12.7	<0.001
FD5	2.77	0.22	2.82	0.20	3.00	0.36	2.77	0.28	7.1	0.008	6.7	0.010	16.2	<0.001
SE	3.78	0.06	3.46	0.07	4.14	0.08	3.83	0.06	1 662.7	<0.001	1 236.0	<0.001	0.6	0.442
EGC	4.27	0.07	3.92	0.23	4.50	0.07	4.35	0.06	253.1	<0.001	149.5	<0.001	22.6	<0.001
PF	4.19	0.08	3.76	0.26	4.35	0.16	4.30	0.06	200.1	<0.001	92.3	<0.001	55.6	<0.001
EM	3.51	0.10	3.22	0.07	3.82	0.09	3.59	0.07	993.4	<0.001	561.6	<0.001	9.5	0.002
LJ	3.36	0.09	2.94	0.13	3.63	0.18	3.26	0.15	244.0	<0.001	456.4	<0.001	2.3	0.127
UJ	3.64	0.11	3.26	0.14	3.86	0.14	3.59	0.10	256.4	<0.001	362.4	<0.001	9.7	0.002
DF1	7.54	0.14	6.36	0.42	7.80	0.14	6.95	0.33	99.9	<0.001	572.5	<0.001	16.0	<0.001
DF2	7.87	0.14	7.15	0.33	8.03	0.13	7.89	0.15	209.9	<0.001	190.0	<0.001	90.4	<0.001
DF3	7.46	0.15	6.99	0.14	7.67	0.14	7.68	0.13	570.6	<0.001	156.6	<0.001	166.2	<0.001
C	8.48	0.10	8.06	0.10	8.86	0.08	8.82	0.09	2 057.9	<0.001	340.1	<0.001	230.7	<0.001
VF1	7.23	0.14	6.81	0.12	7.36	0.18	7.43	0.12	414.9	<0.001	95.1	<0.001	183.4	<0.001
VF2	7.78	0.14	7.23	0.16	7.95	0.18	7.72	0.16	244.9	<0.001	337.3	<0.001	55.1	<0.001
HA	5.18	0.02	5.23	0.05	5.18	0.04	5.26	0.02	9.2	0.003	180.1	<0.001	15.9	<0.001
FA	3.94	0.14	3.24	0.39	3.97	0.16	3.17	0.38	0.2	0.678	328.6	<0.001	1.4	0.230

Abbreviations are described in detail in Figure 1 and Table 3.

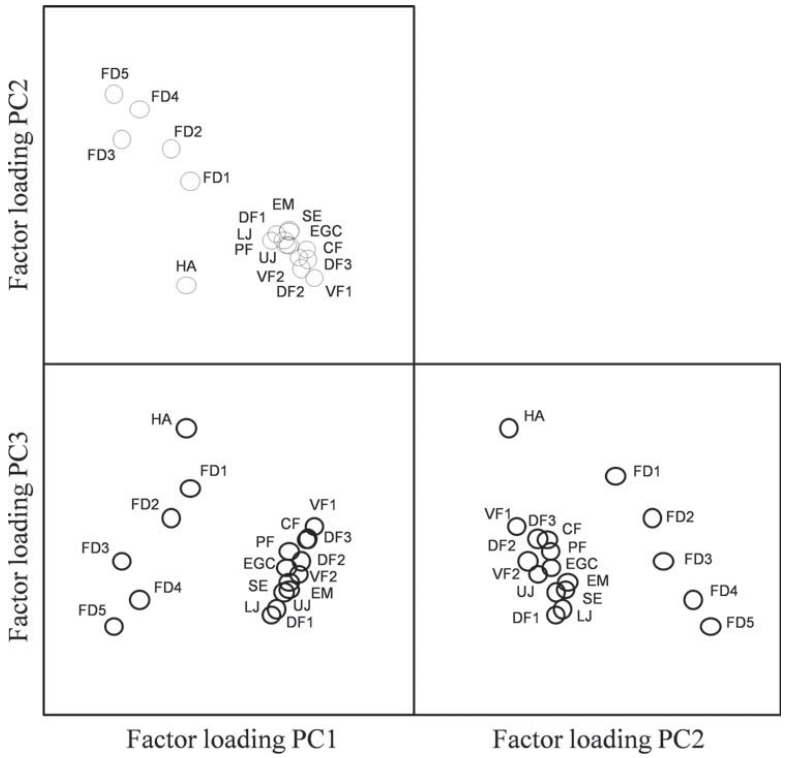

Figure 4. Factor loadings for the three significant PCs in relation to the morphological measures (abbreviations listed in Table 3).

for location and type for circuli 40 ($F = 11.0$, $p = 0.001$), indicating that the cumulative circuli breadth was slightly higher for farmed cod from Frøya than from Ytterøya, whereas the cumulative circuli breadth tended to be lower for wild fish from Frøya than from Ytterøya (Figure 3).

Mean circuli breadth and length-adjusted scale radius differed between wild and farmed fish (Univariate GLM type III sums-of-squares analysis, fixed factors: location and fish type; mean circuli breadth: $F = 67.7$, $p < 0.001$; scale radius: $F = 43.9$, $p < 0.001$). However, variation in length-adjusted circuli numbers per scale was not associated with fish type (Univariate GLM type III sums-of-squares analysis, fixed factors: location and fish type, $F < 0.1$, $p = 0.87$). Mean circuli breadth and length-adjusted scale radius were, therefore, selected for evaluation of the possibility of using scale parameters to discriminate between farmed and wild cod. A discriminant analysis with these parameters showed that 86.1 and 80% of wild and farmed fish, respectively, were correctly classified (Table 4).

Morphology

Apart from FD4 and FA, all morphological measures differed significantly among locations and farmed and wild fish (Table 5). FA did not differ between the two locations, whereas FD4 did not differ between farmed and wild fish (Table 5). Apart from FD3, SE, LJ, and FA, there were significant interaction effects for location and fish type for the other 15 morphological parameters (Table 5).

A combination of three principal components (PCs) explained 73.2% of the variation in size-adjusted body morphology variables (Table 3). The first PC comprised parameters describing the head region of the fish and fin areas, and PC2 represented the distances between fins (Figure 4, Table 3). PC3 represented variation in HA (Figure 4, Table 3). The relationship among the factor scores indicates morphological variation both between and within the different wild and farmed fish groups (Figure 5). In particular, the farmed fish from Frøya appeared to differ from the other groups with respect to variation in PC1, i.e. different measures in the head region and fin areas (Figure 5). A discriminant analysis of the individual scores of these three PCs showed that 97 and 96% of wild and farmed fish, respectively, were classified correctly (Table 4).

Three parameters were selected for considering the possibility of using a few simple measurements to discriminate between farmed and wild cod. The primary selection criterion was that these parameters would be easy to measure in the field. FA was selected because (i) it was highly correlated with the area of the posterior dorsal fin, (ii) there was no difference between locations, and (iii) there were no significant interaction effects between location and type. Likewise, LJ was selected because there was no interaction effect among location and type. HA

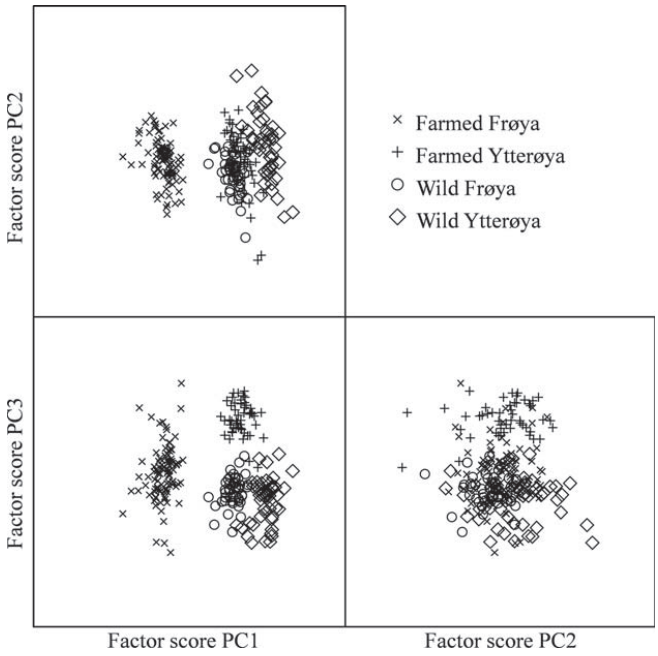

Figure 5. Individual factor scores for the three significant PCs in relation to location and fish type.

was selected because a large proportion of farmed cod have abnormal curvatures in the neck region. A discriminant analysis with FA, LJ, and HA showed that 100% of the wild fish were classified correctly and that 95% of the farmed fish were classified correctly (Table 4).

Discussion

This proof-of-concept study shows that variation in scale-circuli pattern and body morphology has the potential to distinguish between wild and farmed Atlantic cod, concurring with the results of earlier studies on farmed and wild Atlantic salmon (e.g. Lund and Hansen, 1991; Fleming *et al.*, 1994). Both morphological and scale analyses are commonly used for determination of proportions of escaped Atlantic salmon in Norwegian commercial and recreational fisheries and have therefore proven to be valuable management tools (Fiske *et al.*, 2006).

The variation in scale-circuli pattern and morphological traits between farmed and wild cod could be caused by a range of factors. In the same way as for Atlantic salmon (Fiske *et al.*, 2006), the differences in scale-circuli pattern between wild and farmed cod is most likely associated with varying growth patterns caused by variation in external and/or internal factors at different life stages. Initially, the growth patterns of the two groups are relatively similar. Later, farmed cod appear to grow faster than wild fish, as indicated by an increasingly larger cumulative circuli breadth and relatively larger-scale radius for farmed than for wild cod. It is reasonable to assume that the faster growth of farmed cod is a result of greater food availability through an abundance of artificial fish food, an energetically less costly life style because of a life in captivity, and optimal physical conditions during early life stages in intensive culture compared with their wild counterparts. However, genetic factors cannot be ruled out, although attempts to reveal genotypic differences in the growth of wild cod have provided inconsistent results (Mork *et al.*, 1984; Jørstad and Nævdal, 1994; Gjerde *et al.*, 2004; Jørstad *et al.*, 2006).

The morphological differences between wild and farmed cod caused by the culture process might be of both a relatively permanent nature and a direct cause of the duration of the cultivation period. For instance, deformations in neck curvature are probably determined in early life (Grotmol *et al.*, 2005; Fjelldal *et al.*, 2009) and will be persistent throughout the entire lifespan of the fish. On the other hand, the degree of fin damage would most likely increase throughout the culture period through, for example, social interactions or handling (e.g. Kindschi *et al.*, 2001; Person-Le Ruyet and Le Bayon, 2009). Morphological variation among different cod populations may also be related to phenotypic plasticity caused by environmental or genetic variation (Marcil *et al.*, 2006). Hence, it is important to bear in mind the fact that both scale and morphological parameters of farmed and wild cod may vary as a result of both environmental and genetic factors. Indeed, the results from this proof-of-concept study indicate that both the morphology and scale-circuli patterns vary both between and within farmed and wild fish populations.

Our results suggest that there is a need to examine more farmed and wild fish populations, as well as several year classes and ages, before a functional and reliable methodology for discrimination between wild and farmed cod can be developed. Another factor that must be taken into account during development of such

methodologies is that the occurrence of production-related deformities and damage to farmed cod may decrease over time because of the ongoing breeding of farmed cod and optimization of production methods. As many of the morphological traits examined in the current study are production-related deformities and damages, perhaps the opportunity to make reliable distinctions between wild and farmed fish based on morphology will be reduced in future. Moreover, calibration of methods for distinguishing between wild and farmed fish based on scale and morphological traits needs to be accompanied by genetic analyses to ensure that cod caught in the wild are truly wild-origin fish. As large numbers of farmed cod escape each year, either as fish or as fertilized eggs, genetic analysis is a prerequisite for verifying the origin of wild fish (Glover *et al.*, 2011). Finally, it will also be necessary to verify the precision of methods for distinguishing between escapees and wild fish through blind tests, i.e. testing datasets not originally used to develop the statistical models. However, the results from the current proof-of-concept study show that scale and morphological analyses have the potential to distinguish between wild and farmed cod.

Discrimination between escaped farmed cod and wild cod may also be achieved by other means than scale and morphological parameters. For instance, recent developments within genetics have led to increasingly more efficient and less costly ways not only for distinguishing between farmed and wild fish, but also for determining the actual farm from which an escapee originated (Glover *et al.*, 2008, 2010; Glover, 2010; Karlsson *et al.*, 2011). However, Glover *et al.* (2011) observed that some morphologically characterized wild-caught cod closely resembled escapees when screening wild and farmed cod for ten microsatellite loci and the *Pan I* locus. Therefore, it may be difficult to distinguish wild and farmed fish based on neutral or nearly neutral genetic markers in cases where cod are farmed in the same region as their broodstock or where escapees originate from several sources (Glover *et al.*, 2011). Further, trace-element composition in scales and otoliths has proven to be effective in distinguishing between wild and farmed salmon (Veinott and Porter, 2005; Adey *et al.*, 2009). Such analysis may also find application in cod. In addition, variation in fatty acid composition in body tissues could be used as a tool for determining origin because the commercial fish feed used in aquaculture would affect the fatty acid composition of farmed fish contra wild fish, which feed on natural organisms (Fernandez-Jover *et al.*, 2007).

Although the alternative methods for recognizing fish origin may have greater precision than scale and morphological analyses, they also require advanced technological equipment and a level of professional expertise not always readily available. Therefore, often, the selection of a method for distinguishing between wild and farmed fish will be a trade-off between reliability, processing speed, costs, and practical applicability. Sometimes a field-based determination of the origin of a fish may be an advantage. Also, methods that can be used by non-professionals will be useful. For instance, the results from this proof-of-concept study indicate that a large proportion of Atlantic cod may be correctly classified as either farmed or wild based on three simple morphological measures that could be collected either from images or from live fish after anaesthesia. If it is possible to develop a standardized methodology for using scales and/or morphological traits for distinguishing escaped farmed cod from wild fish, this would represent a practical approach for evaluating the origin of cod that also may supplement more advanced methods.

Acknowledgements

We thank the workers at the cod farms at Mausund, Frøya, and Ytterøya, Ronny Jakobsen from the Norwegian Institute of Food, Fisheries and Aquaculture Research, and Mamiko Mitamura and Iver Tanem for their support and help during the course of the study and John G. Taylor for language correction. The research was funded by the Norwegian Research Council projects EcoMA and iCoast (Project number: 192429) and the EU-project PreventEscape (project number: 226885).

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Paper II

Vol. 3: 33–49, 2012
doi: 10.3354/aei00049

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published online November 14

OPEN ACCESS

Extent and ecological importance of escape through spawning in sea-cages for Atlantic cod

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ABSTRACT: The culture of certain fish species to sizes at which they can reproduce has led to the escape of fertilised eggs or 'escape through spawning'. To investigate the extent and ecological importance of spawning in sea-cages for Atlantic cod *Gadus morhua* (L.), we (1) evaluated the extent, frequency and timing of spawning in cod culture; (2) analysed the quality of eggs released from farms in terms of variation in fatty acids; (3) modelled the distribution of eggs and larvae from a commercial cod culture site; and (4) predicted the post-escape survival of eggs through summarizing existing knowledge on survival rates of different life stages. Collectively, our results indicate that cod farming has the potential to produce large amounts of eggs and larvae through spawning in cages, with numbers of eggs spawned being 4 to 5 times higher in the second than in the first year. Our scenarios suggest that a typical sea-cage with 60 000 fish may produce 1.4 to 21 tons of 3 yr old first generation farmed cod through spawning in sea-cages. The quality of escaped eggs and larvae is likely to be sufficient for larvae to survive until the first feeding, while survival until adulthood, though difficult to predict, may be high under favourable conditions. Simulations indicate that eggs and larvae from farms may mix with those of wild fish during the spawning season, and thus experience comparable larval environments. However, several implementable management measures exist that will diminish the extent of egg escape in future cod farming.

KEY WORDS: Atlantic cod · *Gadus morhua* · Aquaculture · Escape · Spawning in farms

INTRODUCTION

As the demand for protein increases to support a growing global human population, the production of fish in sea-cage aquaculture systems has expanded. While modern, industrial sea-cage aquaculture has been built around salmonids, which do not spawn in sea-cages, the culture of fish species that are marine, pelagic broadcast spawners and that may reproduce within sea-cages has recently increased. These include Atlantic cod *Gadus morhua* (Jørstad et al. 2008) and sea bream *Sparus aurata* (Dimitriou et al. 2007). While the extent of escapes of adult fish is relatively well documented in some countries (e.g.

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Jensen et al. 2010) and the potential for negative ecological and/or genetic consequences to occur through escape of adult farmed fish is considered to be important (Bekkevold et al. 2006), knowledge of the extent and ecological effects of 'escape through spawning' by active reproduction within sea-cages by fish is sparse (Jørstad et al. 2008, van der Meeren et al. 2012).

In Atlantic cod farms, some fish mature during the first year of culture, while the majority mature during the second year (Svåsand et al. 1996, Hansen et al. 2001, Karlsen et al. 2006, Taranger et al. 2006, 2010). Consequently, almost the entire culture stock in any particular farm has the potential to spawn in sea-cages. Larvae from genetically marked farmed cod that spawned in a sea-cage have been found up to 8 km from an experimental farm during the natural spawning season of cod (Jørstad et al. 2008). Preliminary results also indicate that offspring from spawning in the cages survive until reproductive age (van der Meeren et al. 2012). If spawning occurs within commercial cod farms where numbers of animals are far greater, the contribution of 'escaped' larvae to cod recruitment within a particular fjord or area may be substantial. Thus, it is not unlikely that cod farming could result in genetic changes in wild cod populations, as occurs for Atlantic salmon (Naylor et al. 2005, Hindar et al. 2006, Skaala et al. 2006, Ferguson et al. 2007), but not only through the escape of farmed fish from sea-cages.

The extent and effect of spawning within commercial sea-cages is largely unknown, even though it is unquestionable that farmed cod have the potential for producing large numbers of 'escaped' eggs and larvae (van der Meeren et al. 2012). The effects of spawning in sea-cages will depend not only on the numbers of eggs and larvae that escape, but also on their quality and survival, which are influenced by both innate and environmental factors such as broodstock nutrition, timing of spawning, and dispersal routes following spawning (Kjørsvik et al. 1990). Further, the ecological effect of this type of escape will depend on the degree to which local adaptations exist in wild cod stocks, in the same way as is assumed to be the case for wild salmonids (e.g. Glover et al. 2012).

We evaluated the extent and ecological importance of escape through spawning in sea-cages for Atlantic cod by (1) evaluating the extent, frequency and timing of spawning in commercial cod culture; (2) analysing the quality of eggs released from farms using fatty acid profiles as proxy indicators; (3) modelling the distribution of eggs and larvae from a commercial cod culture site; and (4) predicting the post-escape survival of eggs through summarizing existing knowledge on survival rates of different life stages. Finally, we evaluated the need and possibility for implementing mitigative strategies for reducing or preventing escape of eggs.

MATERIALS AND METHODS

Extent, frequency and timing of spawning of Atlantic cod

Fish were sampled from 7 farms distributed from western to northern Norway (Fig. 1), prior to or during the spawning season of wild cod, from late 2009 to early 2010 and February and March 2011. Fish that had been in sea-cages for ~1 and 2 yr were sampled from 5 (Røsnes, Lyngen, Gildeskål, Frøya and Austevoll) and 4 (Ytterøya, Røsnes, Lyngen and Tysfjord) farms, respectively. The samples were used to determine sex ratio, reproductive status (see below for assessment), gonad size, fecundity and timing of spawning. Morphometric measures included total

Fig. 1. Locations in Norway where Atlantic cod *Gadus morhua* were farmed (black circles) and location of capture of wild fish for fatty acid analysis (black star)

Table 1. *Gadus morhua*. Summary of morphometric data (means ± SD) of 2 yr old female Atlantic cod from 3 farms used for fecundity analyses. Only females with vitellogenic oocytes were used in the fecundity analyses and statistical analyses were thus performed only for these females. Different superscript letters: differences among farms, same letters = no difference (1-way ANOVA with Tukey's post hoc tests, df = 2, $p < 0.05$)

Farm	Egg stage	N	Total length (cm)	Weight (g)	Fulton's *K*	Ovary weight (g)	GSI
Røsnes	Vitellogenic	31	58.3 ± 3.8[a]	2961 ± 366[a]	1.50 ± 0.21[a]	420.3 ± 118.5[a]	16.9 ± 5.5[a]
Tysfjord	Vitellogenic	22	57.3 ± 3.1[a]	2635 ± 410[b]	1.39 ± 0.10[b]	346.5 ± 99.9[a]	15.2 ± 4.0[a]
	Hydrated	4	57.5 ± 2.9	2614 ± 208	1.40 ± 0.29	489.5 ± 201.0	23.5 ± 10.1
Ytterøya	Previtellogenic	6	59.6 ± 3.1	2535 ± 341[c]	1.19 ± 0.05	66.7 ± 30.5	2.7 ± 1.1
	Vitellogenic	17	57.1 ± 4.6[a]	2306 ± 536	1.22 ± 0.04[c]	439.7 ± 202.6[a]	29.7 ± 32.1[a]
	Hydrated	3	54.2 ± 1.9	1910 ± 211	1.20 ± 0.01	582.3 ± 175.2	47.4 ± 23.0
	Spawned	3	56.2 ± 5.7	2189 ± 737	1.20 ± 0.02	316.3 ± 221.6	15.9 ± 7.4

length, whole body weight and gonad weight. Standard Fulton condition index [K = (body weight / body length^3) × 100] and gonadosomatic index [GSI = (gonad weight / total body weight) × 100] were calculated and a maturation index (values 1 to 4, with undeveloped gonads = 1 and ripe gonads = 4, while values 2 and 3 represented intermediate stages) recorded based on visual examination of gonad size and appearance.

Fecundity and hatching time was examined for 2 yr old fish from 3 farms following the methods outlined in Thorsen & Kjesbu (2001) and Kjesbu et al. (2010) (Table 1). In short, fecundity and spawning time was determined by collecting ovarian tissue that was fixed in 3.6% phosphate-buffered formaldehyde. Automated image analysis (ImageJ, available at: http://rsb.info.nih.gov/ij) was subsequently used for measuring oocyte diameter for oocytes being >200 µm. Of the 200 normal (vitellogenic) oocytes measured per sample, the largest 10% were defined as the leading cohort (LC) and the corresponding mean oocyte diameter used to predict time of start of spawning (calendar day) according to Kjesbu et al. (2010). Individual potential fecundity (i.e. standing stock of oocytes, F_p) was determined from oocyte packing density (number of oocytes g^{-1}), estimated from the mean diameter of all 200 oocytes measured in each sample, multiplied by whole ovary weight (Thorsen & Kjesbu 2001). As F_p decreases throughout gonadal development due to atresia (Kjesbu et al. 2010), the standing stock of oocytes immediately before spawning would be lower than the F_p calculated from samples collected at various points in time prior to spawning. To adjust for atresia a linear regression between calculated F_p and LC (F_p = –8676LC + 10 000 000, $r^2 = 0.28$) was used to estimate the standing stock of oocytes (oocytes at spawning = F_p – [(800 – LC) × 8676]) for an assumed average LC oocyte diameter at spawning (800 µm). Realised fecundity (i.e. the mean number of eggs spawned of the potential fecundity as recorded immediately before spawning, F_R) was then predicted using data on actual proportions of eggs spawned by farmed cod of the same size and condition as examined in the present study (Kjesbu et al. 1991).

Table 2. *Gadus morhua*. Overview of Atlantic cod females (means ± SD) used for analyses of fatty acids in stripped eggs

Location	N	Date	Total length (mm)	Weight (g)
Røsnes farm	14	15 Mar 2010	568 ± 53	2705 ± 632
Lyngen farm	15	25 Mar 2010	516 ± 21	1858 ± 227
Malangen wild	15	23 Mar 2010	795 ± 80	4483 ± 1422

Evaluation of egg quality of farmed and wild Atlantic cod

Variation in lipids and fatty acid compositions may be used as indicators of egg and larval quality and viability in teleost fish, including Atlantic cod (e.g. Sargent et al. 1995, Pickova et al. 1997, Salze et al. 2005, Fuiman & Ojanguren 2011, Lanes et al. 2012). Thus variation in fatty acid profiles were analysed for eggs from cod females sampled from 2 farms in northern Norway (Fig. 1, Table 2) during March 2010. Simultaneously, wild females were sampled from Malangen bank, a well-known spawning area outside Tromsø (Fig. 1, Table 2), located ~80 km away from the nearest farm to minimize the risk of sampling escaped farm cod. Escapees can usually be

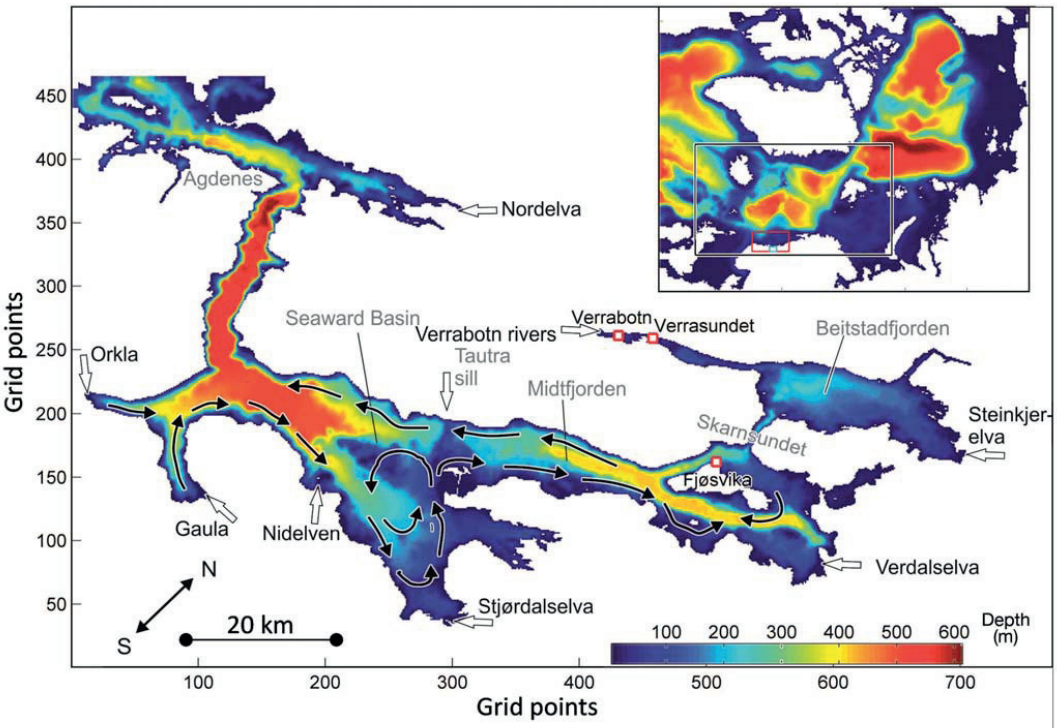

Fig. 2. Trondheimsfjorden model area. Colour scaling: depth in m (red: ~600 m to blue: shallow). Red squares: release sites for Atlantic cod *Gadus morhua* eggs, where Verrabotn and Verrasundet are natural spawning grounds, and Fjøsvika (at Ytterøya) is the fish farm. Arrows: position of rivers and the Tautra Sill. River names are in black and other names in grey. Upper right corner inset: nesting system of the model, where larger inset generates input to the one inside (i.e. black rectangle to inside of red rectangle, to cyan rectangle which is the model area of Trondheimsfjorden)

separated from wild fish by morphology (Uglem et al. 2011). None of the wild cod examined in the current study exhibited traits that regularly are found in farmed cod (e.g. deformed mouth, worn dorsal fins or neck deformities). To be certain that wild cod were analysed, fish with even minor signs of one of these morphological deformities/traits commonly seen in escaped farmed cod were not sampled.

Only ripe females (i.e. females with running eggs) were used. Eggs were sampled by applying a gentle pressure on the abdomen. The eggs (5 to 10 g) were stored at −80°C from sampling until analysis. The storage vials were flushed with nitrogen to avoid oxidation of samples before analysis.

Fatty acids were measured with a capillary gas chromatograph (Perkin Elmer, Autosystem XL) according to Kjørsvik et al. (2009). Duplicates of each sample were analysed and mean values were used in subsequent analyses.

Dispersal of Atlantic cod eggs from farms

To evaluate if escaped cod eggs and larvae mix with those of wild cod, we used a hydrodynamic model that simulated egg and larval drift from 2 spawning areas for wild fish and from a cod farm in Trondheimsfjorden. A 3D coupled numerical model system (SINMOD) was used to simulate the currents in the area of interest and a large scale model (20 km) covering the Arctic Ocean, the Nordic Seas and part of the Atlantic Ocean provided boundary conditions for a model with a resolution of 4 km. This model provided in turn boundary conditions for the shelf model with resolution of 800 m. This nesting technique was extended further to 160 m resolution (Fig. 2). Hydrodynamic variables (velocities, temperature and salinity) were imposed from the coarser model into the model with higher resolution through the open boundaries according to Slagstad & McClimans (2005).

Trondheimsfjorden consists of 3 main basins: Seaward Basin, Midtfjorden and Beitstadfjorden (Fig. 2). The initial field was interpolated from the larger 800 m model that calculated the boundary conditions. This smooth initial field had some ice in the fjord, so we ran a spin-up period of >2 mo (January to March 2009) to remove ice and obtain more realistic structures of the hydrography. The buoyancy of cod eggs is relatively independent of normally encountered temperature ranges due to thermal expansion in the egg being nearly equal to that of sea water (Sundnes et al. 1965), and the vertical distribution of the eggs should not be much affected by temperature as long as there is no ice cover to prevent wind mixing.

Egg releases were simulated from March 15 until April 30 from 3 locations: the 2 most important natural spawning grounds in Trondheimsfjorden (Verrabotn and Verrasundet; J. Mork unpubl. data) and 1 farming location (Fjøsvika at Ytterøya) (Fig. 2). As the farmed cod stock may spawn 10 to 100 times more eggs than the wild cod stock in Trondheimsfjorden (J. Mork unpubl. data), we 'released' 5 times more eggs in the model from the fish farm compared to the spawning grounds. At the natural spawning grounds, 10 eggs were released from 11 to 20 m over the bottom in 1 m intervals; at the fish farms, 100 eggs were released from 5 to 25 m depth in 0.2 m intervals. In sum 120 eggs were released every half hour until Day 47, which totals >270 000 eggs over the spawning season.

The buoyancy of eggs was calculated according to Dallavalle (1948) and was valid for Reynolds numbers between 0.5 and 5. We found this more realistic than Stokes formula, which is better suited for still water conditions where viscous forces are more important compared to turbulent forces (Sundby 1997, Knutsen et al. 2001). Eggs diameter and density were defined as 1.4 mm and 1.026 (Kjesbu et al. 1992), respectively, and were assumed to have this buoyancy for 84 day degrees before hatching (J. Mork unpubl. data). Afterwards, larvae and juveniles were treated as passive particles for a total drift time of up to 6 mo.

Prediction of survival of eggs from farmed Atlantic cod

The results from the current project and available data on hatching and survival rates of cod were used to develop 2 simple scenario models that predict survival of escaped eggs and larvae under various conditions. Using data on the sex ratio in farms, proportion of mature individuals after ~1 and 2 yr in the sea (farmed cod are usually slaughtered before they reach 3 yr of age) and number of eggs hatched per female, we estimated the total number of yolk-sac larvae produced by a typical farmed female. Existing knowledge on subsequent survival of cod larvae, juveniles and sub-adults was used to estimate survival to 3 yr old fish (Table 3). Based on results from the current study (see below) we assumed that 50 and 100% of the females spawn after ~1 and 2 yr in the sea, respectively. The average sizes of 1 and 2 yr old farmed females were set to 1 and 2.5 kg, respectively. Data on survival from spawned egg to later life stages incorporates both fertilization and hatching rate (Table 3), assuming that sperm availability in

Table 3. *Gadus morhua*. Survival estimates of Atlantic cod used in the scenario models. Fishing mortality (Z; d^{-1} or yr^{-1}) was either incorporated in the total mortality estimates or assumed to be zero for younger stages. Survival % is calculated from Z and indicates the survival through different life stage periods as indicated in the first column. NCC: Norwegian coastal cod, NEAC: North-east Arctic cod. *Italics*: values used in the scenario models

Life history stage, survival	Z	Survival (%)	Cod origin	Source
Model 1				
Spawning to hatching (16 d)	0.186 d^{-1}	*5.1*	NCC	Kristiansen et al. (1997)
Hatching to 1 yr old fish	13.02 yr^{-1}	*0.000223*	NCC	Kristiansen et al. (1997)
1–2 yr of age	0.55 yr^{-1}	*57.7*	NCC	Julliard et al. (2001)
1–2 yr of age	1.31 yr^{-1}	27.0	NCC	Larsen & Pedersen (2002)
Mean (survival: 1–2 yr)		*42.4*	NCC	
2–3 yr of age	1.05 yr^{-1}	35.0	NCC	Julliard et al. (2001)
2–3 yr of age	1.33 yr^{-1}	26.4	NCC	Kristiansen et al. (2000)
2–3 yr of age	0.45 yr^{-1}	63.8	NCC	Pedersen & Pope (2003)
Mean (survival: 2–3 yr)		*41.7*	NCC	
Model 2				
Spawning to hatching		*10.0*	NEAC	Fossum (1988), Sundby et al. (1989)
0–90 d	34.7 yr^{-1}	*0.0184*	NEAC	Sundby et al. (1989)
90–180 d	8.1 yr^{-1}	*13.5*	NEAC	Sundby et al. (1989)
180 d–3 yr of age	0.8 yr^{-1}	*12.3*	NEAC	Sundby et al. (1989)

farms is not a limiting factor. Finally, existing data on fish body growth rates was used to estimate biomass production from eggs spawned in sea-cages (Froese & Pauly 2012). Potential density-dependent processes were not taken into account; however, if they occur our survival and biomass estimates should be regarded as overestimates. Fishing mortality is either incorporated in the total mortality estimates or assumed to be zero for younger stages.

Two scenario models were developed. The first model (Model 1) simulates survival of eggs and larvae from farms located in sheltered coastal locations (Table 3). This model is in part based on results from a study where genetically marked farmed cod were allowed to spawn under controlled conditions (Kristiansen et al. 1997). This marker made it possible to separate larvae and juveniles from farmed and wild cod spawning simultaneously in the same area and to estimate the survival of eggs from farmed cod until 1 yr of age. Survival rates from 1 to 3 yr of age are based on several studies where survival in the wild was estimated for Norwegian coastal cod (NCC), which was also used in Kristiansen et al. (1997) (Table 3). The second scenario model (Model 2) is based on estimated mean survival rates between 1979 and 1988 for North-east Arctic cod, which is the major population of Atlantic cod in western and northern Norway (Sundby et al. 1989). The values estimated in the 2 scenario models were number and biomass of surviving 3 yr old fish, escaped as spawned eggs, per farmed fish (both males and females). The basic model was:

$$R_{3yr} = W_F \times \text{Spawning proportion} \times \text{Relative } F_R \times S_{3yr} \quad (1)$$

where R_{3yr} is the number of 3 yr old fish produced per farmed female, W_F is the average weight of farmed females just prior to spawning in kg, Spawning proportion is the assumed average percentage of either 1 or 2 yr old females spawning in sea-cages, Relative F_R is the mean estimated F_R kg^{-1}, and S_{3yr} is the assumed survival for the 2 scenarios until 3 yr of age (Table 3).

When estimating the number of 3 yr old 'semi-wild' fish produced per farmed fish, the sex ratio was taken into account since the basic model estimates number of fish per farmed female only. Hence, the biomass per fish in the farm was estimated by multiplying numbers of first generation cod produced per farm fish with the calculated gross average weight of cod along the Norwegian coast at 3 yr of age (Froese & Pauly 2012).

RESULTS

Extent, frequency and timing of spawning of farmed Atlantic cod

The average sex ratio determined for 6 farms (N = 4821) was close to 50:50 (females: males, 50.2:49.8%). The maturation rates for females examined during the first year in the sea (first spawning season) varied from 46.5 to 86.2% for the 4 farms without artificial light (Table 4). Maturation rates for males from the same farms were higher with >87% of the males being mature in 3 of these farms (Table 4). Artificial light was applied to only 1 of the examined farms (Gildeskål). Cod in this farm had been transferred to sea-cages 2 to 3 mo later than the other farms and were therefore also smaller; no mature fish were found in the samples from Gildeskål (Table 4). Almost all fish that were sampled during the second spring (i.e. second spawning season) across 4 sea-cage farms (n = 252; Ytterøya, Røsnes, Lyngen and Tysfjord farms) were mature, with the exception of 4 males from the Ytterøya farm.

Gonadal developmental stage for fish from the Ytterøya farm (Fig. 1) indicated that a higher proportion of males were ripe on 2 November 2009 com-

Table 4. *Gadus morhua*. Percentages of 1+ yr old Atlantic cod that had ripe gonads (males and females separately) and would spawn during the first 12 to 14 mo in sea-cages. Additional details given are sampling date, usage of artificial light in sea-cages, husbandry period at sea, length and weight of cod and sample size (N)

Farm	Sampling date	Artificial light	Approx. period in sea (yr)	Length (mean ± SD, mm)	Weight (mean ± SD, g)	Females		Males	
						N	Mature (%)	N	Mature (%)
Austevoll	8 Mar 2011	No	~1.3	422 ± 27	932 ± 147	29	86.2	24	95.8
Frøya	5 Nov 2009	No	~1	401 ± 67	751 ± 343	47	48.9	53	50.9
Gildeskål	10 Mar 2011	Yes	~0.8	372 ± 33	372 ± 216	49	0	51	0
Røsnes	15 Feb 2011	No	~1	447 ± 30	1126 ± 254	54	57.4	49	87.8
Lyngen	23 Feb 2011	No	~1	444 ± 30	1110 ± 259	43	46.5	57	89.5

pared to females (Fig. 3). The gonad stage index peaked in the last measurement, indicating that much of the spawning took place in March and April at the Ytterøya farm. Time of start of spawning was estimated through measurements of oocyte size for 3 farms (assumed average ocean temperature: 5°C) (Fig. 4). In the 2 northernmost farms, most females commenced spawning in April. In the Ytterøya farm, the spawning season was more lasting; some females started spawning in February, whereas most initiated spawning in March and April. Because Atlantic cod are batch spawners with an individual spawning period of 3 to 6 wk (Chambers & Waiwood 1996, Kjesbu et al. 1996), is it is reasonable to assume that spawning in sea-cages mainly occurred from March to June in the 2 northernmost farms, and also during February in the southernmost farm.

The potential, realised and relative fecundity varied among farms (Table 5). Females from the Røsnes farm were more productive compared to females from the 2 other farms. The overall mean number of eggs estimated to be spawned per kg female was 629 400 (± SD 219 700). In addition, F_p decreased with increasing leading cohort oocyte diameter (Univariate GLM, LC: $F = 15.9$, df = 1, $p < 0.001$; Farm: $F = 9.3$, df = 2, $p < 0.001$). When farm and LC were taken into account, F_p increased with increasing body weight ($F = 36.5$, df = 1, $p < 0.001$), while it was not significantly associated with somatic condition ($F < 0.1$, df = 1, $p = 0.98$).

Evaluation of egg quality of farmed and wild Atlantic cod

Fatty acid composition of eggs varied both between wild and farmed fish, and between the 2 farms included in the analysis (Fig. 5). A discriminant analysis on the 4 significant principal components showed that all females could be classified

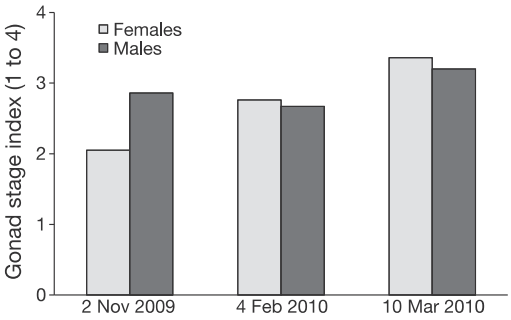

Fig. 3. *Gadus morhua.* Gonad stage index for Atlantic cod at the Ytterøya farm at different points of time. Gonads: undeveloped (1) to ripe (4)

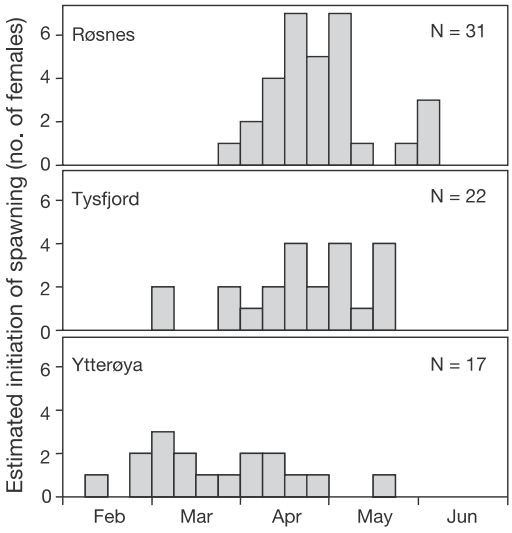

Fig. 4. *Gadus morhua.* Estimated initiation of time of spawning for 2 yr old female Atlantic cod at 3 farms by date. Average ocean temperature assumed to be 5°C

Table 5. *Gadus morhua.* Summary of fecundity data (means ± SD) of the 2 yr old female Atlantic cod from 3 farms used for fecundity analyses. Only females with vitellogenic oocytes were used. Potential fecundity: standing stock of maturing oocytes per female, Relative fecundity: potential fecundity per gram wet body weight, Realised fecundity: standing stock of oocytes before spawning corrected for subsequent atresia. Different superscript letters: differences among farms, same letters = no difference (1-way ANOVA with Tukey's post hoc tests, $p < 0.05$)

Farm	N	Oocyte diameter (µm)	Leading cohort oocyte diameter (µm)	Potential fecundity × 1000 (F_p)	Relative fecundity (F_p g^{-1})	Realised fecundity (oocytes g^{-1})
Ytterøya	17	565 ± 83^b	712 ± 86^b	3226 ± 1198^b	1379 ± 380^b	656 ± 272^{ab}
Tysfjord	22	512 ± 60^a	632 ± 72^a	3688 ± 1120^b	1382 ± 317^b	511 ± 184^b
Røsnes	31	491 ± 47^a	622 ± 60^a	4870 ± 1052^a	1651 ± 313^a	699 ± 179^a

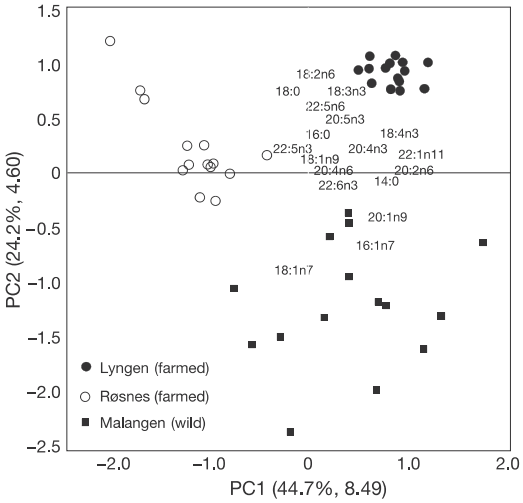

Fig. 5. *Gadus morhua*. Principal component (PC) analysis of fatty acids in eggs from farmed and wild Atlantic cod in relation to their origin. Individual factor scores and loadings for PCs 1 and 2 (with % variation explained and eigenvalues in parentheses)

correctly according to their origin on the basis of variation in fatty acid profiles (Table 6). Eggs from farmed fish contained proportionally more saturated and polyunsaturated fatty acids (PUFAs) than wild fish, whereas the levels of monounsaturated fatty acids were highest in eggs from wild fish (Fig. 5, Table 7). The proportions of total n-3 PUFAs did not vary significantly among the 3 groups, but the levels of eicosapentaenoic acid (EPA, C20:5n3) and n-3 docosapentaenoic acid (DPA, C22:5n3) were higher in eggs from farmed fish, while the level of docosahexaenoic acid (DHA, C22:6n3) was highest for wild fish (Table 7). The proportions of total n-6 PUFAs were approximately twice as high for eggs from farmed fish compared to wild fish (Table 7). This was basically due to the levels of linoleic acid (LA, C18:2n6) and n-6 docosapentaenoic acid (DA, C22:5n6) in eggs from farmed fish that were 2 to 4 times higher than for wild fish (Table 7, Fig. 6). Arachidonic acid (AA, C20:4n6) levels did not vary among the groups (Table 7, Fig. 6), while fish from the Røsnes farm had less eicosadienoic acid (EA, C20:2n6) compared to the 2 other groups (Table 7). As a consequence of the high n-6 PUFA levels, the n-3:n-6 ratio for eggs from wild fish was approximately twice that of farmed fish (Fig. 6; $F = 53.7$, $p < 0.001$). The DHA:EPA ratio from wild fish eggs was higher than for eggs from the 2 farms (Fig. 6; $F = 30.6$, $p < 0.001$), but there was little variation in EPA:AA ratio among the 3 groups (Fig. 6; $F = 0.1$, $p = 0.9$). The egg lipid content (proportion of dry wt ± SD) was not significantly different between eggs from the Røsnes farm (11.7 ± 1.5%) and wild fish (12.2 ± 1.0%), but eggs from the Lyngen farm (13.1 ± 0.8%) contained higher levels of lipid than eggs from wild fish ($F = 5.7$, $p = 0.007$).

Dispersal of Atlantic cod eggs from farms

The applied hydrodynamics model showed that the first eggs from the natural spawning ground of Verrasundet enter Beitstadfjorden after 4 d, while the eggs from Verrabotn, located further into this narrow branch of Trondheimsfjorden, did so after ~14 d (Figs. 2 & 7). Overall there is an anticyclonic circulation in this fjord system and particles coming through Verrasundet typically have an anticyclonic path into central Beitstadfjorden. In contrast to this, after 20 d, the first eggs from the Fjøsvika farm (Ytterøy) exit the fjord at Agdenes (Figs. 2 & 7). At this time, there was a high concentration of farmed eggs from the Tautra sill and inwards to Skarnsundet (Figs. 2 & 7). In Beitstadfjorden, mixing of eggs from all 3 sites took place at this point and about a third of the eggs were from the farm (Figs. 2 & 7). At 28 d after simulated initiation of spawning, eggs from the farm were spread all over the fjord except from Verrasundet, and their concentration was higher along the northern side of the fjord. At this time, however, farmed eggs start to enter Verrasundet as well, while 22% of all naturally spawned eggs still remained within this fjord arm.

Table 6. *Gadus morhua*. Test statistics from the discrimination analysis regarding separation of farmed and wild Atlantic cod on the basis of 4 significant principal components (PCs)

Model	*F*	Eigenvalue	Canonical correlation	Wilk's λ	χ^2_2	df	p	Correct classification (%)		
								Ytterøya farm	Røsnes farm	Malangen wild
Ovaries	1	37.3	0.98	0.01	206	8	<0.001	100	100	100
(PC1, 2, 3, 4)	2	3.8	0.89	0.21	62	3	<0.001			

Table 7. *Gadus morhua.* Fatty acid composition (% of weight excluded unknown fatty acids) of total lipids from running (unfertilized) eggs from broodstock of Atlantic cod from 3 farms. Means of duplicates and SD. Significant differences ($p < 0.05$) determined by 1-way ANOVA with Tukey's post-hoc tests indicated by differing letters in the 2 rightmost columns; same letter = no difference; smallest (a) to largest (c) mean. NS: not significant. R: Røsnes farm, L: Lyngen farm, M: Malangen bank, W: wild cod, F: farmed cod

Fatty acid	Røsnes (F) Mean	SD	Lyngen (F) Mean	SD	Malangen (W) Mean	SD	$p < 0.05$ R / L / M	W vs. F
C14:0	1.72	0.11	2.00	0.13	2.04	0.30	a b b	NS
C16:0	21.74	0.44	20.38	0.35	19.30	0.54	c b a	b a
C18:0	2.86	0.43	2.71	0.28	1.56	0.26	b b a	b a
Total saturated	26.32	0.35	25.09	0.35	22.90	0.56	c b a	b a
C16:1n7	2.87	0.30	3.21	0.33	4.37	0.67	a a b	a b
C18:1n9	12.34	0.51	11.83	0.51	11.85	0.85	NS	NS
C18:1n7	3.92	0.13	3.13	0.15	4.95	0.82	b a c	a b
C20:1n9	0.88	0.10	1.82	0.13	3.30	0.89	a b c	a b
C22:1n11	0.25	0.06	0.70	0.07	0.73	0.23	a b b	NS
C24:1	0.33	0.15	0.54	0.09	0.44	0.14	a b a	NS
Total monoenes	20.59	0.89	21.23	0.62	25.63	0.84	a a b	a b
C18:3n3	0.30	0.01	0.51	0.02	0.24	0.05	b c a	b a
C18:4n3	0.39	0.04	0.72	0.03	0.57	0.12	a c b	NS
C20:4n3	0.30	0.02	0.33	0.02	0.32	0.03	a b b	NS
C20:5n3	18.22	0.89	19.09	0.59	16.46	1.15	b c a	b a
C22:5n3	1.66	0.12	1.48	0.07	1.39	0.36	b c a	b a
C22:6n3	27.51	1.16	26.02	0.88	29.62	2.07	b a c	a b
Total n-3 PUFA	48.38	1.01	48.16	0.57	48.61	1.42	NS	NS
C18:2n6	2.67	0.08	3.21	0.10	0.86	0.15	b c a	b a
C20:2n6	0.13	0.04	0.22	0.02	0.20	0.02	a b b	NS
C20:4n6	1.63	0.16	1.74	0.12	1.63	0.66	NS	NS
C22:5n6	0.28	0.02	0.36	0.02	0.17	0.10	b c a	b a
Total n-6 PUFA	4.71	0.16	5.53	0.22	2.86	0.70	b c a	b a
Total PUFA	53.09	0.89	53.68	0.51	51.47	0.98	b b a	b a
Unknown	4.01	0.34	4.67	0.47	5.08	0.58	a b b	NS

After 56 d, eggs from the 2 spawning grounds were mixed over much of the fjord, but there were also localized pockets where little mixing occurred. Their concentration north of Skarnsundet was high, and they tended to follow the northern side of Midtfjorden outwards beyond the Tautra sill. Some were caught in eddies in the Seaward Basin and spread, while most continued along the northern side of Trondheimsfjorden toward the coast. About 6% of the eggs from the farm had been advected out of the fjord, while 0.1% of the naturally spawned eggs had been exported at this time. Drift time out of the model area from the farm was on average 29 d, with a range of ~17 to 44 d. From the 2 spawning grounds inside Verrafjorden, the average drift time out of the model area was 36 d, with a range of ~28 to 41 d.

Prediction of survival of eggs from farmed Atlantic cod

The scenario model based on estimated survival rates of both farmed and wild coastal cod (Model 1) resulted in a considerable lower number of 3 yr old 'semi-wild' cod due to spawning in cages compared to the model which was based on estimated survival rates of North-east Arctic cod (Model 2, Table 8). For instance, Model 1 and 2 result in 1140 and 17 280 three-yr old cod, respectively, if applied to a typical sea-cage with 60 000 cod (Table 8). The biomass produced by spawning in sea-cages is thus much higher for Model 2 compared to Model 1, and lower for spawning during the first season compared with the second (Table 8).

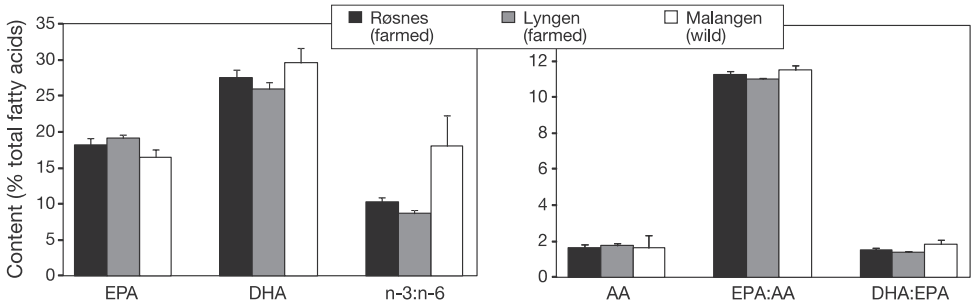

Fig. 6. *Gadus morhua.* Proportions (means ± SD) of eicosapentaenoic (EPA), docosahexaenoic (DHA) and aracidonic (AA) acids, and n3:n6, EPA:AA and DHA:EPA ratios of total fatty acids of Atlantic cod eggs. Unknown fatty acids were excluded

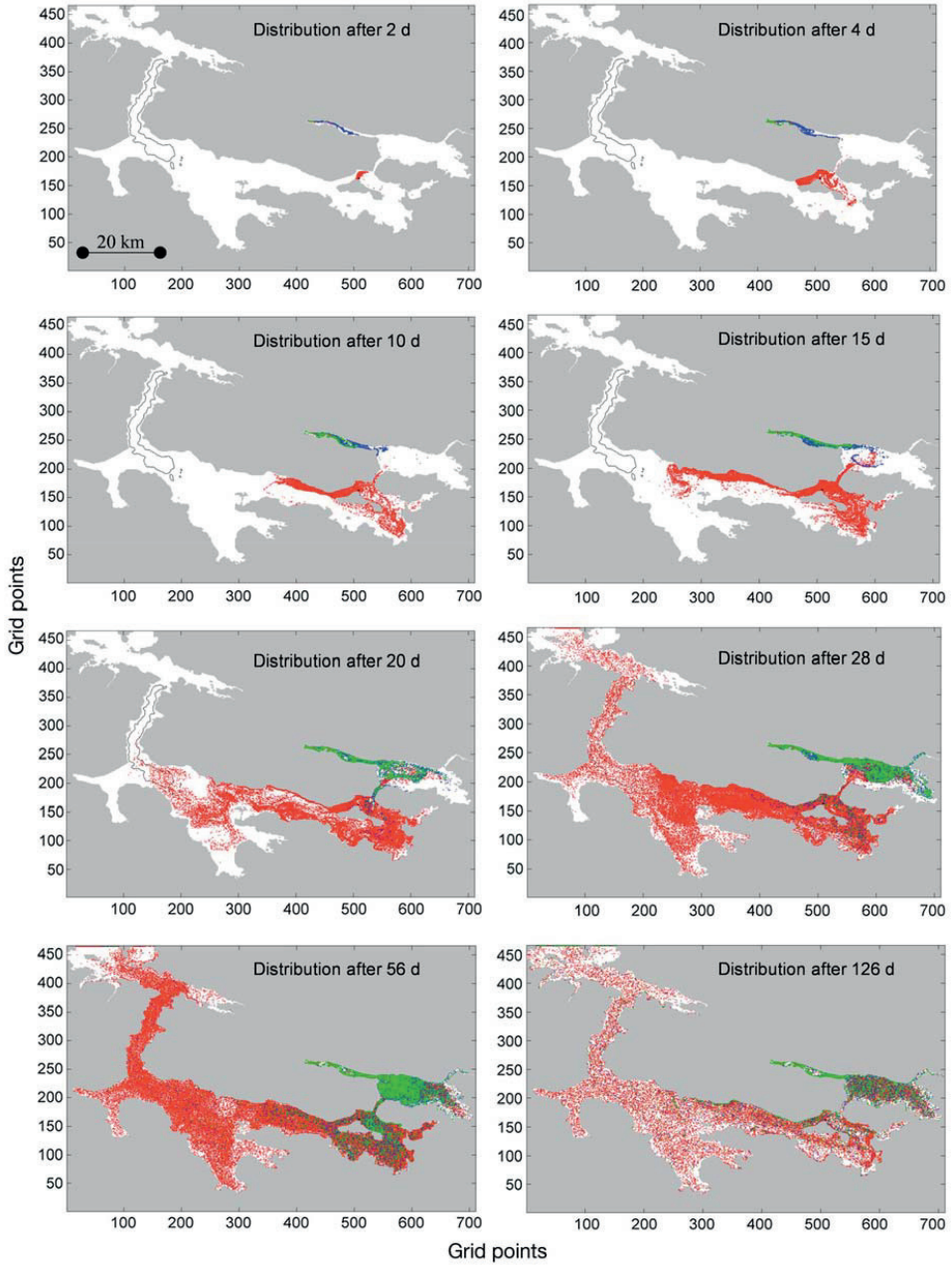

Fig. 7. *Gadus morhua*. Spread of simulated escaped and wild spawned Atlantic cod eggs and larvae in Trondheimsfjorden after 2, 4, 10, 15, 20, 28, 56 and 126 d. Eggs spawned in Verrabotn (green), Verrasund (blue), and farmed eggs (red). Farmed eggs become distributed effectively over most of the fjord, while the naturally spawned eggs have a significantly more restricted distribution at the beginning. On Day 126 the particle density was greatly reduced compared with Day 56 due to export of particles from the fjord. See Fig. 2 for details of Trondheimsfjorden

Table 8. *Gadus morhua.* Overview of results from the scenario models. Number and biomass of 3 yr old Atlantic cod escapees originating from spawning in cages presented either per farm fish or for a 'typical' sea-cage holding 60 000 fish

Spawning year	Per farm fish		Per sea-cage	
	Number	Biomass (kg)	Number	Biomass (kg)
Model 1				
First year	0.003	0.004	180	219
Second year	0.016	0.019	960	1169
Model 2				
First year	0.048	0.058	2880	3508
Second year	0.240	0.292	14400	17539

DISCUSSION

We have demonstrated that a large proportion of farmed Atlantic cod mature in industrial sea-cages both the first and second year of sea-based culture and that the fecundity of farmed cod is high. Furthermore, while the fatty acid composition of farmed and wild cod eggs differed, farmed cod eggs are likely to produce viable larvae upon escape from cages. Egg dispersal modelling suggested that mixing of farmed and wild cod eggs will occur in most parts of a fjord ecosystem and that progeny from both types of fish will experience similar environments, given that farmed cod spawn during the wild cod spawning season. Farms examined during this study were spread over a broad latitudinal range of cod farming; thus the results are likely to be relevant at an industry-wide scale. Taken together with previous studies that have indicated that escaped farmed cod eggs can survive until reproductive age (van der Meeren et al. 2012), our data show that spawning and egg release from sea-cages is a significant process that may have ecological repercussions for these ecosystems unless the extent of spawning within sea-cages is reduced.

Extent, frequency and timing of spawning of Atlantic cod

The natural spawning season of cod in Norway is late winter and early spring. Farmed cod also spawn during this period, although specimens that experience artificial light regimes may spawn at different times compared with wild cod (Hansen et al. 2001, Karlsen et al. 2006, Taranger et al. 2006). However, to evaluate the ecological importance of escape of farmed cod through spawning in sea-cages, further knowledge on the extent, frequency and timing of spawning is required.

Our results showed that between 47 and 86% of the females reared under natural light conditions were sexually mature after ~1 yr in the sea. In the only farm where artificial light was applied, no fish matured during their first spring in sea-cages. This might be a result of the artificial light inhibiting maturation or that these fish were transferred to sea 2 to 3 mo later than the fish from the other farms. If cod juveniles are introduced into sea-cages the preceding summer (or later), the fish could be too small for females to be mature during the first spawning season, while some of the males could mature at weights as low as 300 g (Taranger et al. 2010). However, the maturation ratio of farmed cod during the first year (1+ fish) may be high even when artificial light is used. According to Trippel et al. (2008), application of continuous 24 h light within sea-cages compared to natural photoperiod resulted in a lower, but still high, percentage of mature females (73 versus 90%) and males (90 versus 100%). Results from the current study also show that all females and almost all males were mature after 2 yr in the sea across the commercial farms investigated, which ground-truths similar results obtained in experimental settings (Svåsand et al. 1996, Hansen et al. 2001, Karlsen et al. 2006, Taranger et al. 2006, 2010).

The spawning seasons of the cod examined in the current study concur with previous observations for farmed cod (e.g. Otterå et al. 2006) and overlap with the natural season. In Norwegian waters, wild cod usually spawn during the winter and spring months (January to May), with considerable variation among populations. Typically, populations of cod spawn over a period of <3 mo (Brander 1994, Chambers & Waiwood 1996, Kjesbu et al. 1996), and initiation of spawning may depend on environmental or genetic variation (Otterå et al. 2006). The finding that initiation of spawning varied among the examined farms may thus be both a result of environmental variation or that the origin of the farmed cod differed. As for other fish species with a pelagic egg stage, timing of spawning is likely to be an adaptation to maximize recruitment; too early or too late spawning may mean that the start-feeding larvae will miss the zooplankton bloom, i.e. the match–mismatch hypothesis (e.g. Ellertsen et al. 1981, Taggart & Frank 1990, Pepin & Myers 1991, Sundby 2000). Zooplankton occurrence was not monitored in the present study; it is therefore difficult to assess whether the spawning of farmed cod took place outside or inside the optimal window for spawning at the different locations. Nevertheless, spawning in cages occurred over a relatively long period, which in part would imply some degree of

overlap. In our assessment of spawning season, the cod were reared under natural light conditions at all except for one farm. Manipulation of light conditions with artificial light sources in cages may delay the spawning time by several months (e.g. Hansen et al. 2001, Trippel et al. 2008, Taranger et al. 2010), potentially causing farmed cod to spawn outside the natural spawning season.

The standing stock of oocytes immediately before spawning (realised fecundity) corresponded to ~630 000 eggs kg^{-1} body weight across the 3 examined farms. The fecundities we determined from commercial farms are similar or higher than earlier measurements of relative fecundity for farmed cod from experimental farms (Kjesbu et al. 1991, Kjesbu & Holm 1994, Karlsen et al. 1995). Moreover, the estimated relative fecundity was higher than the typically recorded relative fecundity of wild cod (Botros 1962, Kjesbu 1989, Kjesbu et al. 1998). This is most likely related to the good nutrition provided during gonadal maturation for farmed cod (Kjesbu et al. 1991, Wroblewski et al. 1999).

Quality of farmed and wild Atlantic cod eggs

The quality of eggs and larvae is determinative for juvenile fish survival (e.g. Tocher 2010) and thus also to which degree farmed cod may produce 'escaped adult cod' through spawning in cages. We used the variation in biochemical composition of eggs as a proxy for egg quality. Egg fatty acid compositions can be affected by broodstock diet in various fish species (Tocher 2010), which in turn is important for production of high quality eggs and larvae (Tandler et al. 1995, Izquierdo et al. 2001, Pavlov et al. 2004, Salze et al. 2005, Fuiman & Ojanguren 2011, Lanes et al. 2012). We used stripped, unfertilized cod eggs since we knew the origin of the eggs down to an individual level and since the male contribution through sperm should have little immediate effect on the biochemical composition of the eggs.

Fatty acid profiles of eggs from wild and farmed cod differed significantly, most likely as a result of their different diets. Recently, the fish feed industry has developed feeds that contain substantial amounts of vegetable-derived oils and meals of terrestrial origin that consist mainly of n-6 rather than n-3 PUFAs. Sunflower, soya bean, palm or rapeseed oils are used extensively in fish feed production and result in high concentrations of oleic (18:1n9) and linoleic (18:2n6) acids (Pickova & Mørkøre 2007, Turchini & Torstensen 2009). Cod eggs from farmed females contained 3 to 4 times more linoleic acid than eggs from wild fish, which supports the assumption that eggs of farmed cod are affected by parental diet.

Fatty acid compositions of the total lipids were relatively similar to those previously reported for cod eggs (e.g. Salze et al. 2005, Lanes et al. 2012) with respect to the total levels of saturated, monounsaturated and polyunsaturated fatty acids. However, several differences with respect to the levels of highly unsaturated fatty acids (HUFAs) were detected. HUFA levels have been reported to be related to egg and larval viability. In marine fish, including cod, 3 HUFAs have been shown to be particularly important for fertilization rates, hatching and early survival: EPA (20:5n3), DHA (22:6n3) and AA (20:4n6) (Pickova et al. 1997, Sargent et al. 1999a,b, Pavlov et al. 2004, Salze et al. 2005, Lanes et al. 2012). DHA is important for pelagic fish eggs and larvae (including cod) because neural tissues, such as brain and eyes, contain very high levels of it (Sargent et al. 1995, 2002). Furthermore, the levels of AA and the DHA:EPA ratio in eggs are usually positively correlated with egg and larval quality criteria, while the EPA:AA ratio is negatively correlated with performance of early life stages (Pickova et al. 1997, Salze et al. 2005, Lanes et al. 2012). We detected that wild cod eggs had higher levels of DHA and a greater DHA:EPA ratio compared with eggs from farmed cod. There was, however, no variation in EPA:AA ratio or AA levels among the wild and farmed cod. This finding contrasts with the results of Salze et al. (2005) who found significant differences among wild and farmed fish in AA levels, the EPA:AA ratio, and also egg performance. Lanes et al. (2012) also found that AA levels in cod eggs from wild broodstock were higher compared with eggs from farmed broodstock, but did not demonstrate any correlation between AA levels and egg quality parameters. The AA levels of wild fish found in the present study were ~2 times lower than those reported previously, but comparable with the levels found for farmed fish (Salze et al. 2005). However, variation in AA levels might also be under strong genetic influence and represent interpopulation variation in lipid composition (Pickova et al. 1997).

Thus, in agreement with previous studies, our results indicate that eggs from wild cod might be of better quality than farmed cod (Salze et al. 2005, Lanes et al. 2012), even though there was no difference in the levels of AA between wild and farmed cod. The lack of variation in AA may on the other hand suggest that the viability of eggs and larvae of farmed cod is not critically inferior to that of wild fish.

This is further supported by the fact that cod on aquaculture diets both mature and spawn viable eggs that hatch, and fish survive to 3 yr of age in the wild and contribute to the recruit pool in fjord and coastal populations of cod (Jørstad et al. 2008, van der Meeren et al. 2012). The ability to conclude whether viability differs between eggs and larvae from wild and farmed cod is nevertheless restricted because other biochemical components in cod eggs not measured in the current study also influence egg quality, such as vitamins and the proportion of major lipid classes (Salze et al. 2005, Lanes et al. 2012).

Dispersal of Atlantic cod eggs from farms

Wild cod often spawn in the same areas over time (Wright et al. 2006). It is likely that these areas are due to evolutionary selection processes and result in maintenance or dispersal of eggs and larvae such as to maximize subsequent survival and growth. Cod farms can be located close to spawning sites for wild cod. Simulations derived from the 3D dispersal model indicated that escaped eggs and larvae from a cod farm located within a fjord mixed with their wild counterparts. If the distributions of eggs and larvae from wild and farmed cod overlap in time and space, it is reasonable to assume that farmed and wild cod eggs and larvae would experience similar early life history environments. As long as egg and larval quality is similar, comparable survival rates between wild and farmed eggs may be expected. The possibility for farmed and wild larvae to mix will, however, most likely vary among locations and years. Potential may exist to reduce the degree of mixing of farmed and wild eggs in fjords if farming locations can be established whereby escaped eggs are rapidly transported out of the fjord system.

Survival of eggs from farmed Atlantic cod

Assumed that half of the females spawn during their first year in the sea and all during their second, Model 1 predicts that 333 farmed fish produce one 3 yr old 'semi-wild' cod during the first year in the sea, while 63 fish are required during the second year. Correspondingly, Model 2 predicts that 21 farm fish would produce one 3 yr old escapee during the first spawning season, while only 4 farm fish would produce one 3 yr old escapee in the second season. These results indicate that the number of adult cod that originate from escaped eggs is 4 to 5 times higher for spawning during the second season in the sea compared with the first. This results from larger body size and higher fecundity and maturation rate. A further implication from the 2 scenarios is that survival will vary greatly under different conditions. The number of 'semi-wild' cod that survive to adulthood under disadvantageous conditions could be low, while spawning in sea-cages could produce considerable numbers of first generation adults under more optimal conditions. However, due to the wide range of variables that may influence egg quality, larval survival, recruitment and survival to adult size, the magnitude of farmed cod entry into wild cod populations through spawning in sea-cages would in practice be unpredictable. Nevertheless, in fjord systems or areas housing local cod stocks where cod farming is significant and where catches of wild cod may be only in the order of 10s to 100s of tons, the impact of spawning in sea-cages could be considerable. For instance, our scenarios suggest that a typical sea-cage with 60 000 fish may produce 1.4 to 21 t of first generation farmed cod through spawning in sea-cages. Given most cod farms have multiple cages of this size, the level of escape through spawning that this estimate suggests is significant when compared to known wild cod biomasses in specific fjord systems (e.g. Masfjord: total estimated wild cod biomass = 28 t; Salvanes & Ulltang 1992). At this scale, recruitment of 3 yr old first generation farmed cod spawned in sea-cages has the potential to swamp recruitment through wild cod spawning.

The survival estimates from the 2 scenario models should be regarded as coarse estimates of possible survival rates of eggs and larvae until 3 yr of age due to the many sources for variation in the underlying data. For instance, the proportions of mature females will vary among farms and whether artificial light has been used to manipulate maturity, which in turn will influence if eggs hatch during periods where food for the developing larvae is present. Fecundity will also vary among farms and ages and the accuracy of estimated survival rates for species with pelagic larvae are in general limited by numerous sources for variation due to methodological constraints and spatio-temporal variation in environmental parameters. In addition, the survival estimates may be overestimated, as it is likely that the survival of larvae from spawning during the first season would be lower than for spawning during the second season (e.g. Solemdal et al. 1993). This also concurs with recent experiences from the national cod breeding program in Norway, where first time spawners have resulted in much lower egg numbers and reduced

production output compared to older broodstock (Ø. J. Hansen pers. comm.). Furthermore, the survival estimates are likely to be overestimates as sperm quality, and subsequent fertilisation success, is reduced for farmed males compared to wild males (Skjæraasen et al. 2009, Butts et al. 2011).

Potential ecological effects of escaped Atlantic cod eggs

Whether the escape of farmed cod through spawning in sea-cages results in negative ecological effects depends on several factors. Farmed cod experience strong artificial selection during their larval phase under intensive culture conditions and also through selective breeding for optimal aquaculture strains. This may change the genetic variability of farmed versus wild cod, which in theory may alter the fitness of wild cod through interbreeding between farm escapees and wild fish. However, occurrence of negative genetic effects due to egg escape depends on the existence of local adaptations in cod populations. Such adaptations have been difficult to demonstrate for Atlantic cod. While growing evidence exists for genetic and spatial divergence over the geographic range of Atlantic cod (Hauser & Carvalho 2008, Knutsen et al. 2011, Skjæraasen et al. 2011), no conclusive evidence of a link between genetic differences and significant local adaptations exists. The genetic variability of cod is large and the potential for gene flow is high compared to other aquacultured species, such as Atlantic salmon *Salmo salar*. The probability that heritable local adaptations exist is therefore likely to be lower for cod than salmon. Nevertheless, we cannot exclude the potential for negative genetic effects, especially as intensive selective breeding to create optimal strains for aquaculture is underway. Further, cod are more prone to escape than salmon (Moe et al. 2007), and the possibility for escape of relatively large numbers of farmed cod exists, either through egg escape (present study) or escape of farmed fish (Bekkevold et al. 2006).

Paradoxically, escape of farmed cod through spawning in cages may increase 'wild' cod numbers, particularly if local adaptations are of minor importance and fitness is related to phenotypic plasticity. Demographic effects may result: increased recruitment may lead to greater numbers of smaller cod with lower growth rates due to density dependent competition. Correlative evidence exists that this process has occurred in Greece with escape through spawning of sea bream *Sparus aurata* (Dimitriou et al. 2007). However, the many environmental conditions in which farmed–wild hybrid fishes may be at a disadvantage in the wild could mean that any potential benefits of farmed–wild gene flow will be outweighed by its costs in natural situations in the long term (Hindar et al. 1991, Naylor et al. 2005, Hutchings & Fraser 2008).

Conclusions and recommendations to reduce the escape of Atlantic cod eggs from sea-cages

Commercial cod farming as a whole has the potential to produce large amounts of eggs and larvae through spawning in sea-cages. The quality of these eggs and larvae may be sufficient for an unknown proportion of the larvae to survive until first feeding. Farmed and wild eggs and larvae mix in coastal ecosystems and experience similar larval environments. Survival of escaped cod eggs until adult fish may vary significantly and will also be unpredictable. Whether or not escape of cod eggs will generate significant ecological effects is difficult to foresee, but the results from this and other studies indicate that egg escape, in combination with escape of juveniles and adults (e.g. average of 213 000 fish yr^{-1} from 2005 to 2009 in Norway; Jensen et al. 2010), may lead to noticeable effects in areas with intensive cod farming and small (reduced) wild fish populations.

The scenario models we developed assumed that farmed cod spawned in sea-cages both at 1 and 2 yr of age. As a result of increased growth rates in commercial cod farming during the last 2 to 3 yr due to selective breeding and improved production methods, it is now common that the desired slaughter size is reached before the second spawning season. This is an advantage for the farmers as the quality and price of farmed cod is higher before the second spawning compared to after spawning. Thus, if specific actions are required to mitigate potential future issues that may occur due to egg escape, a simple, realistic and profitable action to drastically reduce the risk of effects is to make slaughtering of fish before the second spawning season mandatory. In addition, photoperiod manipulation will decrease maturation ratio and fecundity, and delay the spawning time of farmed cod, both under controlled and commercial conditions (Hansen et al. 2001, Taranger et al. 2006, Trippel et al. 2008). As spawning during the first season in the sea produced 4 to 5 times fewer eggs than spawning during the second season, photoperiod manipulation will further reduce the potential for unwanted ecological effects of spawning in

sea-cages. Combined, these actions will reduce the escape of cod eggs significantly.

Recent research has shown that production of triploid Atlantic cod may practically eliminate the risk of egg escape, as gamete production by triploid females is delayed and dramatically lowered compared to diploid females (e.g. Feindel et al. 2011). This is promising as it indicates that triploid females will not mature before harvest, and growth rates will increase through reduced investment in gonad production. However, problems such as initially higher mortality, greater fingerling costs, maturation of triploid males and consumer acceptance need to be solved before production of triploid fish is taken up by industry (Triantafyllidis et al. 2007, Feindel et al. 2010). Technical solutions aimed at physically preventing spawned eggs entering the sea have also been suggested. Such solutions could involve the use of closed sea-cages or mechanical filters to remove eggs. However, development of closed cages is still at an early stage and is presently economically nonviable due to high technological and operational costs.

Acknowledgements. Funding was provided by the European Union 7th Research Framework project 'Assessing the causes and developing measures to prevent the escape of fish from sea-cage aquaculture' (Prevent Escape; Grant agreement no. 226885) and the Norwegian Research Council projects EcoMA and iCoast.

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Editorial responsibility: Marianne Holmer, Odense, Denmark

Submitted: April 12, 2012; Accepted: October 1, 2012
Proofs received from author(s): November 8, 2012

Paper III

Vol. 7: 253–266, 2015
doi: 10.3354/aei00153

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published online November 18

Farmed cod escapees and net-pen spawning left no clear genetic footprint in the local wild cod population

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ABSTRACT: This study investigated a potential genetic introgression from farmed to wild cod *Gadus morhua* L. in the Trondheimsfjord, Norway. During the first 2 yr of operation of a cod farm in the inner part of the fjord, 2 large escape events and extensive pen spawning were reported. Analyses of 4 allozyme markers revealed no significant changes in allele frequencies between samples of wild cod before and after cod farming, although prominent allele frequency differences were demonstrated between wild and farmed samples. Analyses of 10 DNA markers showed a significant change between pre- and post-farming samples, due to contradictory allele frequency differences at *Tch11*, *Pan* I and *Gmo132*. Excluding those 3 markers due to null alleles (*Tch11*) and selection (*Gmo132* and *Pan* I), the DNA markers paralleled the non-changed allele frequency signal from the allozymes. The topographies of the allozyme- and DNA-based dendrogram of the samples were congruent. Recaptures of tagged and released farmed cod indicated a seemingly random diffusion throughout the fjord and ended after approx. 6 mo. During an ongoing pen spawning, plankton net surveys sampling for cod eggs in the surroundings of the cod farm suggested the eggs originated from the farm. No larvae were present in the plankton samples. The apparent absence of introgression is explained relative to fitness and survival of pen-spawned larvae and adult escapees, and to a purging effect of the estuarine circulation of the Trondheimsfjord.

KEY WORDS: Atlantic cod · *Gadus morhua* · Aquaculture · Escapes · Introgression · Microsatellite DNA · Allozymes

INTRODUCTION

Commercial farming of cod *Gadus morhua* L. in net pens has a relatively short history in Norway, starting with a small-scale operation ca. 1990, but not reaching a substantial quantity before 2002 (Statistics Norway 2013). Frequent pen wreckage and escape incidents raised the similar concerns to those with salmonid aquaculture regarding harmful effects of genetic introgression by escapees into locally adapted wild populations (Bekkevold et al. 2006, Moe et al. 2007). These concerns are supported by the findings that adult escaped cod have appeared on the spawning grounds of wild relatives (Wroblewski et al. 1996, Uglem et al. 2008), and that escapees may take part in the annual reproduction process (Meager et al. 2010). In addition, and in contrast to salmonids, cod are known to spawn in the net pens (Uglem et al. 2012). Furthermore, in the

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Publisher: Inter-Research · www.int-res.com

landlocked fjord Heimarkspollen near Bergen, Norway, cod larvae from net pen spawning were found up to 8 km from the net pen (Jørstad et al. 2008), thus representing a potential genetic introgression of farmed cod into natural cod populations (Bekkevold et al. 2006, Jørstad et al. 2008, Glover 2010, Uglem et al. 2012). Jørstad et al. (2013) showed that genetically marked cod dispersed throughout a fjord system, and documented the presence of juveniles and successful reproduction either by spawning in net pens or by escapees participating on local spawning grounds. Farmed cod showed substantial genetic differences between farm cohorts and among farms, a feature which potentially can be used for identification of escaped cod (Glover et al. 2010). In late 2007, a cod farming facility was established in the inner part of the Trondheimsfjord (Fig. 1A). In December 2008 and September 2009, the cod farm experienced 2 major escape events due to pen wreckage, in which 25 000 and 42 000 individuals escaped, respectively (Norwegian Directorate of Fisheries 2009) (Fig. 2). In less than 1 yr the number of cod which escaped was comparable to the annual number of natural spawners in the fjord, as estimated by Sundnes (1980). The cod farm was operational until April 2010, and was the first and so far only cod farm in the fjord. For the first time, large amounts of adult cod of a non-indigenous origin had both spawned in net pens and escaped to the genetically well characterized Trondheimsfjord cod population. Genetic and biological characteristics of the Trondheimsfjord cod stock have been monitored thoroughly in a time series maintained by the Trondhjem Biological Station (TBS) since 1974 (Mork 1976, Mork et al. 1980, 1982, 1983, 1985, Mork & Sundnes 1985, Mork & Giæver 1999, Karlsson & Mork 2003, 2005). The present experimental conditions allowed both the monitoring of pen spawning and the set-up of a simulated escape event with tagged farmed cod for monitoring of recaptures. We hypothesized that by using the genetic characterization of the farmed cod, the level of genetic introgression might be estimated by comparing the genetic characteristics of the wild cod stock before and after cod farming in the fjord. Whether escapes from commercial scale farms result in changes in the genetic composition of wild stocks has not been examined in Norway yet.

MATERIALS AND METHODS

The Trondheimsfjord (Fig. 1A) is situated in mid-Norway and is the third longest and seventh deepest fjord in Norway. Based on results from previous tagging–recapture experiments (Sundnes 1980) and population genetic studies (Mork et al. 1983, 1985, Karlsson & Mork 2005), the cod in the Trondheimsfjord proper is regarded as a largely self-recruiting stock which receives and exports very few adult individuals to adjacent coastal areas. The main spawning area is located in the inner areas including Verrasundet (Mork et al. 1982), and the nursery areas of juveniles cover most of the shallow parts of the fjord, including the

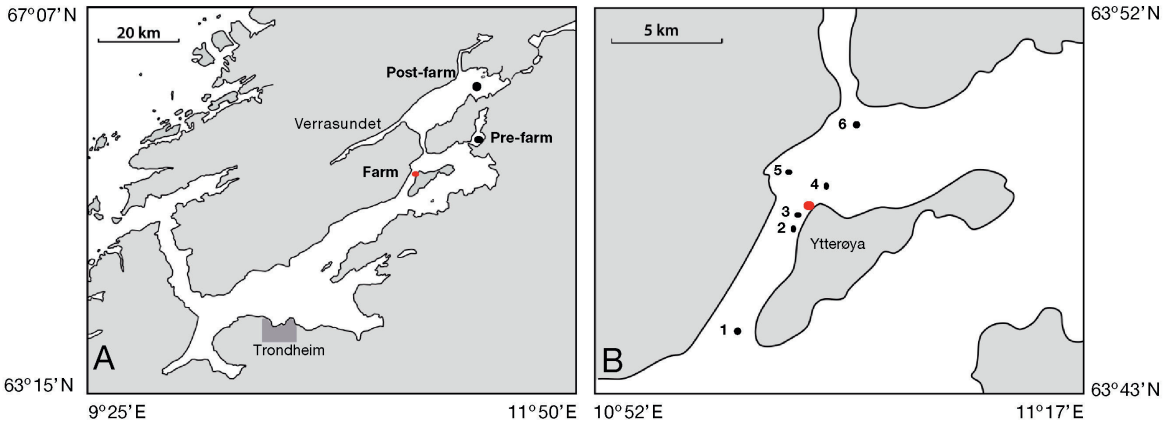

Fig. 1. (A) Locations of pre-farm (sampled in 2005 in Borgenfjorden) and post-farm (sampled in 2013 in Beitstadfjorden) sampling sites (black circles) in Trondheimsfjord. The location of the cod farm, near the island of Ytterøya, is indicated by a red circle. Verrasundet, in the innermost part of the fjord is the main wild cod spawning area in Trondheimsfjord. (B) Sampling areas of plankton net hauls for cod eggs (black circles). The downstream and upstream locations are indicated by numbers 1 and 6, respectively; other hauls were taken in the vicinity of the cod farm. See Table 9 for exact coordinates of net hauls

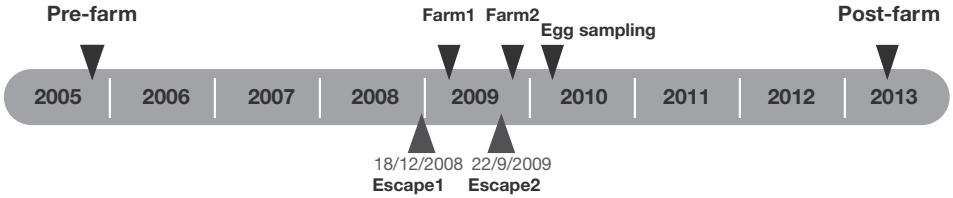

Fig. 2. Timeline of events in the study. Pre- and post-farming samples were taken several years prior to and after the period when the farm was operational (from December 2007 to April 2010). Escape1 was reported to consist of 25 000 cod and Escape2 of 42 000 cod

shallow side-arm Borgenfjorden (Fig. 1A). Average age at maturity for the Trondheimsfjord cod is 4 yr (Ekli 1997) and the normal spawning season for the Trondheimsfjord cod is from March to May with a peak in April (Mork 1976, Sundnes 1980). A total of 895 wild and farmed cod individuals from the Trondheimsfjord were included in this study. Genetic and biological characteristics of wild cod before the presence of farming activity were based on the reference sample (hereafter called Pre-farm) (Table 1), which was caught with a bottom trawl operated from the NTNU research vessel Harry Borthen I in the Borgenfjord (Fig. 1A), a local spawning ground and nursery area for cod. Samples of farmed cod (Farm1 and Farm2) were taken after each of 2 major escape incidents (Fig. 2, Table 1). A post-farming reference sample of wild cod was taken 3 yr after the termination of the cod farm (hereafter called Post-farm) (Table 1). Biological and genetic data as well as preserved tissues were available in the databases and collections at TBS (Kunz 2011). Farmed cod were obtained directly from the pens located near the Ytterøya Island in the inner Trondheimsfjord (63° 47′ 57.48″ N, 11° 02′ 55.08″ E) (Fig. 1A). The supplier of cod eggs to the hatchery, Fosen Akvasenter (later renamed 'Atlantic cod juveniles'), confirmed that their brood stock consisted of cod taken from 5 different areas along the coast of Norway, not including the Trondheimsfjord. The eggs were from the first generation of selection (F1 generation) (T. Refstie pers. comm.). Fosen Akvasenter had supplied the farm with codlings (mean length = ~13 cm) in 2 batches: the first at the end of 2007 and the second in early 2008 (I. Tanem pers. comm.). Biological data and tissues samples were collected from both batches to establish a comprehensive genetic and biological signature of the farmed fish. The sampling was performed using the RV Gunnerus; the first cruise in March 2009 (Farm1) and the second in November 2009 (Farm2). In the simulated escape experiment, the tagging and release of a total of 400 farmed cod (Table 1) was executed from RV Gunnerus while moored to the net pens, and their individual post-release behaviour was mapped from recapture reports managed by the Institute of Marine Research (IMR) in Bergen, Norway.

To evaluate the degree of 'escape by spawning' from the farm pens, plankton net hauls for cod eggs and larvae were conducted on locations in the vicinity of the farm at a time when the farm staff reported ongoing pen spawning. The Post-farm sample was collected in cylindrical pots (60 × 180 cm, stretched mesh 2.5 cm) at 10–20 m depths, 3 yr after the commercial cod farm was closed down (Table 1).

Table 1. Sampling and DNA marker information for Atlantic cod *Gadus morhua*, including sample name (locations of samples shown in Fig. 1A), and sampling date. Total (N): total number of individuals genotyped, Bio (N): individuals with full biological data and tissue samples, Allo Loci: number of allozyme marker loci, DNA Loci: number of DNA marker loci, Tagged (N): number of individuals used in tagging experiment (only fin clips and length information available). nd = no data available

Sample	Location	Date (dd/mm/yyyy)	Total (N)	Bio (N)	Allo Loci	DNA Loci	Tagged (N)
Pre-farm	Borgenfjorden	03/10/2005	192	192	4[a]	10	nd
Farm1	Ytterøya	25/03/2009	263	63[a]	4[a]	10	200
Farm2	Ytterøya	02/11/2009	248	48[a]	4[a]	10[a]	200
Post-farm	Beitstadfjorden	01/06/2013	192	192	4	10	nd

[a]Data from Kunz (2011)

Biological data

Biological data included age, weight, total length, sex, and gonad maturation stage (Table 2). From tagged cod, only fin clips and total body length were collected. Age was determined by otolith reading according to Rollefsen (1933). The gonad maturation stage categories (1 = immature, 2 = maturing, 3 = running and 4 = spent) followed Sivertsen (1935).

Table 2. Biological sample information for Atlantic cod *Gadus morhua* including age (yr), mean weight (g), mean total length (cm), and gonadic stage range (GS) according to Sivertsen (1935). nd: no data available

Sample	Age (yr)	Weight (g)	Length (cm)	GS	% females
Pre-farm	2–4	419	34.6	1–3	48
Farm1	2.5	1073	43.7	1–4	35
Farm2	2.2	nd	48.2	2–4	40
Post-farm	≤2	247	30.3	1	39

Tissue samples and genetic markers

Informative tissue samples for known polymorphisms in cod (muscle, liver, and heart) were taken immediately after death and kept frozen at –20°C during the cruises. For long-term storage, tissue samples were transferred to an ultra-low temperature freezer (–70°C) at TBS after the cruise. Fin clips for DNA analyses were preserved in 96% ethanol and kept at room temperature. Methods for tissue sampling and storage, and tissue extract preparations using all tissue types, electrophoresis conditions, and allozyme genotyping followed Mork et al. (1983). Four allozyme loci were screened: lactate dehydrogenase (*LDH-3**), phosphoglucose isomerase (*PGI-1**), isocitrate dehydrogenase (*IDHP-1**) and phosphoglucomutase (*PGM-1**). The enzyme staining recipes followed the protocols of Aebersold et al. (1987). The microsatellite set analysed in this study included *Gmo2* and *Gmo132* (Brooker et al. 1994), *Gmo3*, *Gmo8*, *Gmo19*, *Gmo34* and *Gmo35* (Miller et al. 2000), *Tch11* and *Tch13* (O'Reilly et al. 2000). The nuclear RFLP locus *Pan* I (Pogson & Fevolden 2003) was genotyped together with the microsatellites according to Stenvik et al. (2006).

DNA extraction and amplification

DNA isolation of the Farm2 sample was performed using the HotSHOT genomic DNA preparation method outlined in Truett et al. (2000). The remaining samples were isolated using Omega E-Z 96 Tissue DNA kit (Omega Bio-Tek). DNA was extracted from muscle tissues samples or fin clips from the tagged and released specimen. All microsatellites were labelled with fluorescent dye at the forward primer. The *Pan* I alleles were fluorescence-labelled according to Stenvik et al. (2006). The PCR was performed in a 2.5 µl volume and comprised 1× Multiplex PCR kit (Qiagen) and 0.1–1.0 µM primer. The PCR profile for both multiplexes consisted of an initial denaturation step at 95°C for 15 min followed by 22 cycles at 95°C for 30 s, 56°C for 3 min and 72°C for 1 min. The PCR reaction was completed with a final elongation step at 60°C for 30 min. Separation of the PCR products was conducted on a 3130*xl* Genetic Analyser (Applied Biosystems). The software Gene Mapper® 4.0 (Applied Biosystems) was used for allele scoring and all alleles were visually inspected.

Pen spawning

The concentration of pen-spawned cod eggs in the vicinity of the farm was investigated by plankton net sampling on 18 February 2010. The sampling was designed to detect the general drift direction of the pelagic eggs relative to the location of the cod farm (Fig. 1B). The plankton net sampling (diameter = 100 cm, mesh size = 1 mm, surface hauls = 50 m) was performed at 6 locations spanning the vicinity of the farm as well as locations upstream and downstream from the farm (Fig. 1B). The concentration of eggs refers to the number of eggs in each standardized haul. Cod eggs were identified by their visual appearance (translucent, visible embryonic cells, no oil drop) and size (diameter = ~1.5 mm) using a stereo microscope as described by Mork et al. (1983).

Tagging and recapture experiment

Two simulated farmed cod escape experiments were performed, each including 200 farmed cod (~2.5 yr old) (Table 2) which were tagged and released from alongside the farm pens in March and November 2009 (Table 1). Both Lea hydrostatic tags (n = 100) and Dart tags (n = 300) were attached in front of the first dorsal fin. Tagging procedures were approved by the Norwegian Animal Research Authority. Recaptures were performed mostly by the public; tag reporters received a small compensation when tags carrying information of the recapture site were returned to the IMR.

Statistical analyses

MICRO-CHECKER was used to test the microsatellite loci for null alleles and stuttering (Van Oosterhout et al. 2004). The number of alleles, observed and expected heterozygosity, unbiased Nei's genetic distance, and the fixation index (F_{ST}) were calculated

using GenAlEx (Peakall & Smouse 2006, 2012). Candidate loci for positive, neutral and balancing selection under the infinite allele mutation model were detected by the F_{ST}-outlier detection method implemented in LOSITAN (Antao et al. 2008). Using default parameters, outlier analyses were also redone after removing detected outlier loci ('Neutral' mean F_{ST} and 'Force mean' F_{ST} option). The R package HIERFSTAT (Goudet 2005) was used to estimate allelic richness; defined as the rarefied allelic count per locus and population. The significance of the differences in allelic richness was tested using Kruskal-Wallis ANOVA, where the mean allelic richness over all loci was tested between all samples (Kruskal & Wallis 1952, McDonald 2009). Exact tests for Hardy-Weinberg (HW) equilibrium, linkage disequilibrium (LD), and exact G-tests for genic differentiation were performed using the web version of Genepop 4.2 (Raymond & Rousset 1995, Rousset 2008). All analyses were executed with the default Markov chain parameters and overall p-values were calculated by Fisher's method. Bonferroni procedures were used to correct for multiple tests (Rice 1989). Reducing the degrees of freedom (df) in Rows by Columns (R × C) chi-square tests, by pooling all the alleles except the most common allele, provided a higher test power (Wright 1978). The frequency of the overall most common allele in the samples (all others pooled) were used to investigate possible concealing effects, such as the Post-farm wild cod sample potentially having been affected by 2 escaped batches (Farm1 and Farm2) with different allele frequencies. This procedure was executed for all markers. MEGA ver. 6 was used to construct the unweighted paired-group method with arithmetic mean (UPGMA) dendrograms (Tamura et al. 2013). Bootstrap replications (n = 10 000) were executed in POPTREEW to compute the probability of confidence of the UPGMA dendrograms (Takezaki et al. 2014). Because of difference in level of polymorphism, which leads to different power in statistical tests and interpretation of F_{ST}, the allozymes and microsatellites were analysed separately (Estoup et al. 1998). STRUCTURE 2.3.4, a Bayesian, Markov chain Monte Carlo (MCMC) program was used to cluster individuals based on estimated levels of individual admixture (Pritchard et al. 2000, Hubisz et al. 2009). An admixture model (MCMC 100 000 iterations, 200 000 burn in, 10 iterations, k = 1–5) analyses were completed using DNA loci only, both 10 loci and a reduced set of 7 loci. The number of populations (k) that best describes the data material was determined using STRUCTURE Harvester (Evanno et al. 2005).

Statistical power

The power of the marker set to detect genetic differentiation was estimated using POWSIM 4.1 (Ryman & Palm 2006). An effective population size (N_E) of 5000 and generations of drift ranging 0–15 were used in the set up. Simulations were run 10 000 times for each number of generations of drift. To find a value of F_{ST} corresponding to 50, 80 and 95% probability of detection, a linear regression between the nearest simulated points were used. The POWSIM simulations were used for the full marker set including allozyme and DNA loci, and a DNA marker set which excluded *Tch11*, *Gmo132* and *Pan* I. To test the power on the dataset used, Post-farm sample genotypes were replaced with 5, 7, 10, and 20% Farm1 and Farm2 genotypes, respectively. Pairwise F_{ST}, with probability of being significantly different to zero based on 999 permutations, were calculated for each pair of Pre-farm and 'replaced' Post-farm samples using the AMOVA function in GenAlEx (Peakall & Smouse 2006, 2012).

RESULTS

The wild cod specimens in the Pre-farm group were 2–4 yr old, while those in both of the farmed batches were a little more than 2 yr old (Table 2). The Post-farm sample consisted of juveniles of 2 yr or younger, thus born 3 yr after the termination of the farm (Table 2). The majority of the Farm1 individuals, which were sampled in March 2009, had running gonads (stage 3) (Table 2). Farm2, which was sampled in November 2009, consisted mostly of cod with maturing or spent gonads (i.e. in stages 2 and 4) (Fig. 3).

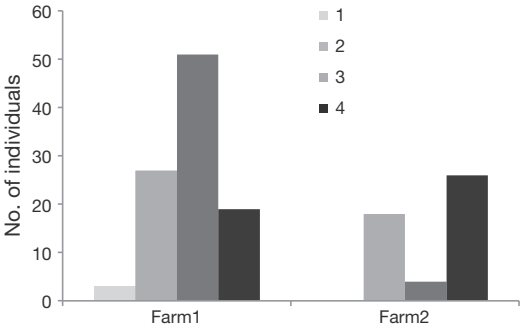

Fig. 3. Gonadic maturation stages (1 = immature, 2 = maturing, 3 = running and 4 = spent) in farmed Atlantic cod samples (mean age = 2.4 yr). Both samples were taken in 2009; Farm1 in March and Farm2 in November

Based on weight information provided by the farm company, the 2008 escape was from the Farm1 batch and the 2009 escape from Farm2 batch.

Statistical analyses

Altogether 13 and 221 alleles were detected for the allozymes and microsatellites, respectively, in the Pre-farm, Farm1, Farm2, and Post-farm samples. The scoring success was 96–100% for allozyme loci and 94–96% for DNA marker loci.

Allozyme markers

All 4 allozyme loci were in HW equilibrium in all farmed and wild cod samples (Table 3); individual locus results are given in Table S1 in the Supplement at www.int-res.com/articles/suppl/q007p253_supp.pdf. No significant LD was detected for any pair of loci in any sample (Table S1). The allele frequencies at the 4 loci in the 2 farmed samples were not significantly different from each other (see Bonferroni adjusted exact G-tests p-values in Table 4). However, the allele frequencies in the Farm1 sample differed significantly from the Pre-farm sample, and the Post-farm sample differed significantly from the Farm2 but not from the Pre-farm or the Farm1 (Table 4). Heterozygosities did not differ significantly between farmed and wild cod (Table S1). Several measures of genetic diversity showed no significant differences between the 4 groups (Kruskal-Wallis test; p = 0.69) (Table 3). Chi-squared R × C contingency table tests of allele frequencies (i.e. testing for the most common allele and pooled remaining alleles) in the allozyme loci of Pre-farm and Post-farm samples revealed no significant p-value at any locus (Table 5). The largest unbiased Nei's genetic distance (D = 0.017) was between the 2 farmed samples (Table 6).

Table 4. p-values from pairwise exact G-tests of genic differentiation across all loci and Atlantic cod samples (Fisher's method). Significant p-values after Bonferroni correction are shown in **bold** (adjusted p-value = 0.009). Above diagonal: microsatellites excluding *Gmo132*, *Tch11* and *Pan* I. Below diagonal: allozymes

	Pre-farm	Farm1	Farm2	Post-farm
Pre-farm		**<0.001**	**<0.001**	0.578
Farm1	**<0.001**		**<0.001**	**<0.001**
Farm2	0.438	0.009		**<0.001**
Post-farm	0.345	**0.001**	**<0.002**	

DNA markers

Tests for the reliability of the different DNA markers were carried out prior to the main data analyses. Significant evidence of the presence of null alleles was found in the Pre-farm and Farm1 samples at *Gmo2* and *Tch11*, respectively. The Post-farm sample showed evidence of null allele presence at 3 loci: *Gmo2*, *Gmo19* and *Tch11*. Tests for positive selection fell out significant only for *Gmo132*; this result was consistent using either the 'Neutral mean F_{ST}' or 'Force mean F_{ST}' option in LOSITAN. Pooled results from all markers in each sample revealed several significant (after Bonferroni correction) deviations from Hardy-Weinberg genotype composition (F_{IS}) (Table 7). In the Pre-farm sample, the significant deviation was caused by the locus *Tch11* (Table S2 in the Supplement). Adjustments for the presence of null alleles did not remove the significance. The Post-farm sample showed significant deviation from HW equilibrium at *Gmo8* (Table S2). Farm1 had 4 and Farm2 had 6 loci in HW disequilibrium (Bonferroni adjusted p-values = 0.0003) (Table S2). The Pre-farm sample showed LD (p < 0.0001) for 2 pairs of loci; *Gmo8*/*Gmo19* and *Gmo8*/*Tch13*. The Post-farm sample showed no significant LD. Nine and 11 pairs showed significant LD in Farm1 and Farm2, respectively (p < 0.0001).

Table 3. Genetic characterization of Atlantic cod samples by allozyme loci. N: number of allozyme-genotyped individuals, H_{obs}: observed heterozygosity, H_{exp}: expected heterozygosity, F_{IS}: Wright's F_{IS} value, N_A: number of alleles, N_{PA}: number of private alleles, *AR*: allelic richness. Mean values are given ± SE

Sample	N	H_{obs}	H_{exp}	F_{IS}	N_A	N_{PA}	*AR*
Pre-farm	192	0.305 ± 0.10	0.305 ± 0.10	–0.009	2.75 ± 0.48	0.25 ± 0.25	2.38 ± 0.32
Farm1	63	0.323 ± 0.12	0.325 ± 0.11	0.032	1.75 ± 0.25	0.00 ± 0.00	1.75 ± 0.25
Farm2	48	0.336 ± 0.12	0.311 ± 0.10	–0.046	2.25 ± 0.25	0.25 ± 0.25	2.22 ± 0.22
Post-farm	192	0.320 ± 0.09	0.318 ± 0.09	0.032	2.75 ± 0.48	0.25 ± 0.25	2.45 ± 0.29

Table 5. Frequency of the most common allele (100) at allozyme loci in the Atlantic cod samples. A potential concealing effect was possible at all loci since the 2 farmed samples displayed higher and lower frequency values than the Pre-farm sample. 'Direction of Post-farm to Farm' denotes whether the allele frequency in the Post-farm sample is as expected if caused by farmed cod. 'Pre vs. Post χ^2 p-value' denote p-value for Chi-square test between Pre-Farm and Post-farm samples using the most common allele and pooled remaining alleles. N: number of allozyme-genotyped individuals

Sample	N	*LDH-3**	*IDH-1**	*PGM-1**	*PGI-1**
Pre-farm	192	0.635	0.844	0.984	0.685
Farm1	63	0.500	0.713	1.000	0.733
Farm2	48	0.628	0.865	0.969	0.594
Post-farm	192	0.591	0.828	0.964	0.716
Direction of Post-farm to Farm		Farm1	Farm1	Farm2	Farm1
Pre- vs. Post-farm χ^2 p-value		0.208	0.559	0.070	0.344

Of these, 2 pairs occurred in both samples: *Gmo19*/*Gmo2* and *Gmo8*/*Gmo2* (Table S2). Mean allelic richness was nominally but not significantly lower in the 2 farm samples compared to the wild cod (Table 7). Among all DNA marker loci there were no significant differences in allelic richness among the samples (Kruskal-Wallis test; adjusted H = 3.0, df = 3, p = 0.392). Observed heterozygosities showed similar values in wild and farmed cod (Kruskal-Wallis test for homogeneity of mean heterozygozity for all loci among all samples; adjusted H = 3.0, df = 3, p = 0.396, Table 7). The difference in the number of private alleles over all loci and all 4 samples was not significant (Kruskal-Wallis test; adjusted H = 2.4, df = 3, p = 0.497).

Table 6. Nei's unbiased genetic distances between Atlantic cod samples for allozyme and DNA markers. Column values (above diagonal): DNA markers excluding *Gmo132*, *Tch11* and *Pan* I. Row values (below diagonal): allozymes

	Pre-farm	Farm1	Farm2	Post-farm
Pre-farm		0.055	0.048	0.000
Farm1	0.011		0.048	0.051
Farm2	0.002	0.017		0.046
Post-farm	0.000	0.006	0.005	

Allele frequency relationships among samples

The 2 farmed cod samples differed in allele frequencies at several DNA marker loci. Only 2 loci (*Gmo35* and *Pan* I) were in opposite directions relative to the corresponding values in the Pre-farm sample, potentially creating a concealing effect at these 2 loci in an introgression situation (Table 8). Both Farm1 and Farm2 differed significantly from Pre-farm and Post-farm ($p < 0.001$) in all tests. Using the full marker set in an exact G-test for genic differentiation, the Pre-farm and Post-farm samples showed significant differences ($p < 0.002$); however the significance was caused by *Tch11* and *Pan* I. p-values changed following the exclusion of *Pan* I (p = 0.042), and after exclusion of *Tch11* (p = 0.043). Excluding both *Tch11* and *Pan* I resulted in no significant allele frequency differences between Pre-farm and Post-farm (p = 0.578) (Table 4). Noteworthy, the change between Pre-farm and Post-farm frequency of the most common allele at *Tch11* was in the opposite direction of what would be expected if caused by a farm fish introgression. The allele frequencies at the DNA loci in the 4 samples were subjected to UPGMA cluster analysis and dendrogram construction using Nei's unbiased genetic distance (Table 6). The topography of the dendrogram from allozymes and the reduced DNA marker set were basically similar and confirmed graphically the genetic relationships among the 4 samples (Fig. 4).

For the markers which were not affected by a potential concealing effect, the frequency of the most common allele in the Post-farm sample changed towards the farmed cod at 5 of 8 loci, of these only *Gmo132* was significant (pooling of alleles, chi-square test p-value = 0.004) (Table 8). For the 2 markers for

Table 7. DNA marker characteristics for all 10 loci. N = number of genotyped Atlantic cod individuals, H_{obs}: observed heterozygosity, H_{exp}: expected heterozygosity, F_{IS}: Wright's F_{IS} values (all samples were significantly different from HW equilibrium; exact HW conformance test $p < 0.001$ for all samples), N_A: number of alleles, N_{PA}: number of private alleles, *AR*: allelic richnes. Mean values are given ± SE

Sample	N	H_{obs}	H_{exp}	F_{IS}	N_A	N_{PA}	*AR*
Pre-farm	192	0.684 ± 0.087	0.708 ± 0.092	0.022	18.3 ± 3.7	1.8 ± 0.6	15.8 ± 2.9
Farm1	96	0.683 ± 0.095	0.683 ± 0.092	0.008	10.9 ± 1.9	0.6 ± 0.3	10.9 ± 1.8
Farm2	192	0.634 ± 0.101	0.680 ± 0.106	0.077	15.2 ± 3.0	1.5 ± 0.5	14.0 ± 2.6
Post-farm	192	0.663 ± 0.093	0.702 ± 0.102	0.041	17.3 ± 3.2	1.0 ± 0.3	15.2 ± 2.6

Table 8. Number of genotyped Atlantic cod individuals, and frequency of the most common allele at DNA marker loci in the samples. 'Potential concealing effect' is the possibility of a concealing effect when the 2 farmed samples display both higher and lower frequency values than Pre-farm (Y = yes, N = no). 'Direction Post-farm to Farm' denotes whether the allele frequency in the Post sample is as expected if caused by farmed cod. 'Pre vs. Post-farm χ^2 p-value' denote p-value from Chi-square test for pooled alternative alleles at each marker for Pre-farm vs. Post-farm samples. Significant p-values after Bonferroni correction are shown in **bold** (adjusted p-value = 0.005)

Sample	N	*Gmo2*	*Gmo3*	*Gmo8*	*Gmo19*	*Gmo34*	*Gmo35*	*Gmo132*	*Tch11*	*Tch13*	*Pan* I
Allele:		107	191	124	145	98	126	116	172	93	A
Pre-farm	192	0.251	0.862	0.226	0.143	0.609	0.270	0.471	0.124	0.185	0.929
Farm1	96	0.234	0.898	0.122	0.214	0.747	0.298	0.188	0.070	0.126	0.875
Farm2	192	0.242	0.916	0.217	0.296	0.628	0.265	0.136	0.086	0.120	1.000
Post-farm	192	0.240	0.886	0.201	0.147	0.587	0.243	0.403	0.135	0.189	0.981
Potential concealing effect		N	N	N	N	N	Y	N	N	N	Y
Direction Post-farm to Farm		Y	Y	Y	Y	N	Farm2	Y	N	N	Farm2
Pre- vs. Post-farm χ^2 p-value		0.288	0.078	0.156	0.573	0.308	0.198	**0.004**	**<0.001**	0.512	**<0.001**

which a concealing effect could not be ruled out (*Gmo35* and *Pan* I), the frequency of the most common allele in the Post-farm sample indicated that Farm2 potentially might have had the strongest impact, though only *Pan* I was statistically significant (Table 8). STRUCTURE Harvester suggested that k = 2 best described the dataset. The individual admixture analyses in STRUCTURE clustered mainly the Pre-farm and Post-farm individuals together, and the farmed samples in the other cluster for both the full DNA marker set and the reduced set (Figs. S1 & S2 in the Supplement). There was evident similarity in the clustering proportions of Pre-farm and Post-farm (Table S3).

Statistical power of the marker sets

POWSIM showed that the total marker set containing 4 allozymes, 9 microsatellites and 1 RLPF had an 80% probability to detect differentiation at F_{ST} = 0.0010. For F_{ST} = 0.0015, the probability was 95%. After assessing the reliability of the markers, the reduced DNA loci set on which we based our conclusions had a similar power of detecting differentiation with an 80% probability at F_{ST} = 0.0009 and 95% probability at F_{ST} = 0.0014. The marker set showed a 95% probability of detection for a simulated introgression of 10% farmed genotypes, and a 50% probability of detection for a 5% simulated farmed introgression (Fig. 5, Table S4 in the Supplement).

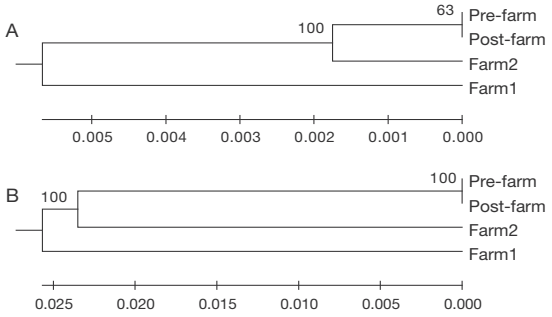

Fig. 4. UPGMA dendrograms based on Nei's unbiased genetic distances between the 4 Atlantic cod samples for (A) allozymes (sum of branch length = 0.0131), and (B) DNA markers excluding *Gmo132*, *Tch11* and *Pan* I (sum of branch length = 0.0748). Bootstrap value (%) shown at node

Pen spawning

Pelagic cod eggs in early development stages were found in plankton net samples taken at various dis-

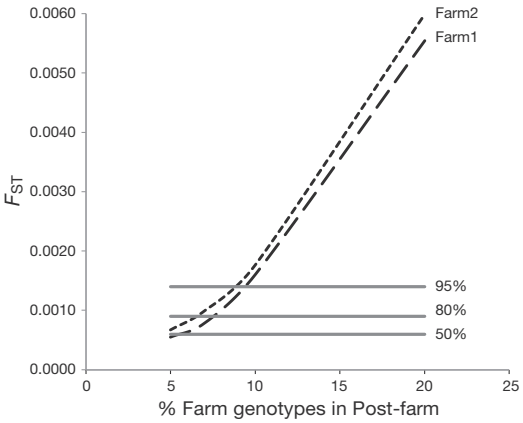

Fig. 5. Fixation index (F_{ST}) values from pairwise tests of Pre-farm and Post-farm Atlantic cod samples, where Post-farm individuals have been replaced by 5, 7, 10, and 20% Farm1 and Farm2 individuals, respectively. Levels of probability detection at 95%, 80% and 50% are drawn at F_{ST} levels 0.0014, 0.0009 and 0.0006, respectively

Table 9. Number of pelagic Atlantic cod *Gadus morhua* eggs in vertical plankton net hauls (1 mm mesh, 50–0 m depth) at various positions downstream, in the vicinity of, and upstream of the cod farm at Ytterøya in the Trondheimsfjord. Geographical coordinates for 6 separate plankton net hauls are given. Characterization is the location of the plankton net sampling site relative to the cod farm and in lieu of the estuarine current direction in the fjord

Location no.	Position	No. of cod eggs	Characterization
1	63° 44′ 50.30″ N, 10° 58′ 59.43″ E	75	Downstream
2	63° 47′ 57.54″ N, 11° 02′ 26.28″ E	14	Vicinity
3	63° 48′ 12.06″ N, 11° 02′ 48.30″ E	42	Vicinity
4	63° 48′ 24.00″ N, 11° 03′ 13.80″ E	15	Vicinity
5	63° 48′ 31.86″ N, 11° 02′ 04.50″ E	8	Vicinity
6	63° 49′ 35.16″ N, 11° 05′ 01.08″ E	2	Upstream

tances from the farm pens on 18 February 2010. The concentration of eggs was higher in the vicinity and downstream of the farm pens than upstream (Table 9). At the time of sampling, extensive net spawning was occurring as noted by the farm staff and by gonad inspections on cod taken from the pen (Fig. 3). The early development stages of the eggs in the plankton net samples confirmed a recent and hence off-season spawning.

Recaptures from simulated escape experiments

A total of 17 recaptures were taken between 11 and 191 days after release, which corresponds to a 4% recapture rate. Recaptures occurred throughout the Trondheimsfjord, both upstream and downstream of, but mostly in the vicinity of the release site. Average distance of the recapture site was 20 km, and the maximum distance was 70 km coastwards from the release site.

DISCUSSION

The present panel of assumingly reliable allozyme and DNA markers did not detect any genetic change in the local population post cod-farming activity. The statistical power of this marker set, used to detect genetic differences with a significant biological meaning (Ryman et al. 2006, Waples 1998), was estimated by POWSIM to be high. Certain scenarios of genetic introgression are particularly difficult to analyse (Glover et al. 2011); small genetic differences between the donor and the recipient, multiple donor sources, and multiple introgression events in the recipient are all examples of such scenarios. Additionally, the severity of the introgression is of crucial importance (Baskett et al. 2013). The situation in the Trondheimsfjord in this study was relatively simple in these respects, since the potential donor source was temporal, transient and well characterized, and there was no previous farming history. This study benefitted also from the extensive time series on the biological and genetic characteristics of the local cod stock in the Trondheimsfjord. The 2 batches of farmed cod showed significant genetic differences between each other as well as to the wild population. Such genetic heterogeneity present within a farmed cod source was also reported by Glover et al. (2010). The farmed cod microsatellite loci showed multiple cases of LD, and most loci were not in HW equilibrium (Table S2). Such observations are not unexpected in domestic cod populations, where small effective population sizes, non-random matings, and continuous sorting by size are common (Glover 2010).

Assessment of the allozyme markers

Mork & Sundnes (1985) reported a higher survival in juvenile cod for double heterozygotes of LHD and PGI, which they suggested was evidence of selection, possibly in form of heterosis which at equilibrium will stabilize allele frequencies. In the present material there were no over-representation of double heterozygotes or other LD, and we considered the allozyme loci to be reliable in that respect. No genetic differentiation was found between Pre-farm and Post-farm samples at any allozyme locus. The genetic distances between the farmed samples were larger than between the farmed and wild cod groups (Table 6). Historically, allozyme markers have shown low differentiation over the entire species range of Atlantic cod (Mork et al. 1985). The potential presence of any concealing effect of the allozyme loci supported the decision to perform separate statistical analyses of allozymes and DNA loci.

Assessment of microsatellites and *Pan* I

The DNA type genetic markers in this study have been widely used in studies of cod population genetic

structure (Fevolden & Pogson 1997, Knutsen et al. 2003, Skarstein et al. 2007, Westgaard & Fevolden 2007, Wennevik et al. 2008, reviewed in Nordeide et al. 2011), as well as in studies on genetic aspects of cod farming (Delghandi et al. 2003, Dahle et al. 2006, Fevolden et al. 2009, Glover et al. 2010, 2011). There is published evidence for selection at *Gmo34* (Westgaard & Fevolden 2007), but the material in this study did not signal selection effects at this locus, which also is not represented in the locus panel for the aforementioned Trondheimsfjord time series (see 'Introduction'). *Pan* I was included in the present study because it was a potentially efficient marker (Glover et al. 2010) if the brood stock of the farmed cod contained sufficient representatives from the Northeast Arctic cod. The Northeast Arctic cod stock is known to have *Pan* I allele frequencies very different from Norwegian coastal cod (NCC), including the Trondheimsfjord cod (Karlsson & Mork 2003, 2005, Sarvas & Fevolden 2005, Westgaard & Fevolden 2007, Wennevik et al. 2008). However, the farmed cod in this study turned out to have *Pan* I allele frequencies similar to NCC, which reduced its potential as a key marker for introgression. For the *Gmo132* and *Pan* I DNA markers, the local Trondheimsfjord cod has shown selection effects in the form of significant HW imbalance as well as temporal instability and sex differences in allele frequencies based on the time series for the Trondheimsfjord cod (Karlsson & Mork 2003, 2005). Both *Gmo132* and *Pan* I showed significant allele frequency differences between Pre-farm and Post-farm samples when pooling alleles and employing a chi-square test. However, the observed allele frequency differences for *Gmo132* and *Pan* I are actually within the range of their natural temporal fluctuations in the Trondheimsfjord as reported by Karlsson & Mork (2003, 2005). It is generally accepted that *Gmo132* and *Pan* I are under selection in Atlantic cod (reviewed in Nordeide et al. 2011). In analyses of sample heterogeneity based on the full DNA loci set (exact G-tests), the prominent sources of significant allele frequency differences between Pre-farm and Post-farm samples were *Tch11* and *Pan* I. *Tch11* showed significant presence of null alleles and deviated strongly from HW equilibrium in the Pre-farm sample, signaling its unsuitability in the present analyses of introgression (Waples 2015). Also, the frequency of the most common allele for *Tch11* was higher in Post-farm than Pre-farm, in contrast to the 2 farmed samples, which both had a lower frequency of this allele than the Pre-farm wild sample (Table 8). This implies that the contribution to differentiation at *Tch11* could not be due to an impact from farmed cod. Furthermore, Dahle et al. (2006) reported particularly high differentiation and null alleles in *Tch11* (Dahle et al. 2006), and Glover et al. (2010) reported a high gene diversity and F_{ST} value for *Tch11*, Although many other studies employing *Tch11* have not reported unusual characteristics of this marker (O'Reilly et al. 2000, Delghandi et al. 2003, Nielsen et al. 2006, Poulsen et al. 2006, Wesmajervi et al. 2006, Westgaard & Fevolden 2007, Wennevik et al. 2008, Nielsen et al. 2009, Glover et al. 2011). In this study, the very directions of the nominal Pre- to Post-farm allele frequency changes at *Tch11* and *Pan* I as potential effects of introgression from Farm1 and Farm2 gene pools were contradictory and did not tell a consistent story (Table 8). This evidence suggested that more reliable conclusions were obtained from analyses which left out these 2 DNA loci from the genetic marker set (cf. Larsson et al. 2007, Eiríksson & Árnason 2013).

Apparent lack of genetic contribution from pen spawning to the wild stock

The date of pelagic egg sampling (18 February) was before the natural spawning period, during March–May, of the wild cod and most other gadoids in the Trondheimsfjord (Sundnes 1980). Thus, no wild cod eggs were expected in the plankton net samples on that date. Furthermore, there are no known natural spawning sites for cod in the close vicinity of the cod farm location. At the time of sampling, extensive net spawning was occurring as noted by the farm staff and by gonad inspections of cod samples taken from the pen. The only other gadoid eggs in the fjord which might be found in February are those from the early spawner saithe, *Pollachius virens*, which have eggs that are easily distinguished from cod eggs by their much smaller, non-overlapping diameter (Mork et al. 1983). Estuarine circulation in the Trondheimsfjord is known to create a relatively strong net outgoing (coastward) current through the Nordviksund passage, where the cod farm was located (Jacobson 1983). The tidal movement and temporal local eddies might affect the course of pelagic egg drift from the pens to some extent. However, the expected net effect of these drivers in this part of the Trondheimsfjord is an outgoing transport of pelagic eggs in the upper water layers, where the newly spawned cod eggs reside. The results from the planktonic egg survey supported this expectation, in that the abundance of cod eggs was higher close to the net pens than farther off, and much higher downstream than upstream from the cod

farm (Table 9). Assuming a passive pelagic drift of the net pen spawning products (i.e. eggs, larvae and later on codlings) during a 5 mo long pelagic stage, offspring spawned in the mid-part of the Trondheimsfjord, where the cod farm was located, may be transported out of the fjord before settling on the bottom. The extent to which this occurs has been shown to vary annually, depending on the strength of the annual spring flood in the fjord (Dahl 1899, Swenander 1906). A significant negative correlation between the magnitude of the spring flood and the year-class strength of cod in the Trondheimsfjord was reported by Ekli (1997). Together, these factors would indicate that farm-spawned eggs and later, larvae, may eventually be carried out of the fjord by the outgoing estuarine currents during their pelagic stage. To the degree that such transport takes place, it would tend to reduce, but not exclude the possibility of a genetic introgression in the local cod stock by net-spawning. Uglem et al. (2012) simulated egg dispersal for a 46 d period after spawning for this cod farm and fjord system, and indicated that the probability of eggs being carried out of the fjord by the estuarine circulation was 60 times higher for eggs spawned from the fish farm compared to those spawned from the main wild cod spawning site located further inwards in the fjord. During the entire pelagic period (eggs, larvae and pelagic codlings) until settling, and which in this study also includes the time of the annual spring flood, the probability of drifting out of the fjord is likely to be higher. The survival of cod larvae from pen spawning would depend on the presence of suitable food items (normally live nauplii larvae of crustaceans) in high concentrations within 1–2 d after hatching. The 'match-mismatch' model of fish larvae survival (Cushing 1990) implies that the annual spawning event of wild cod populations must be tuned to the annual plankton blooms in the fjord; otherwise, the larvae would starve and die. The present data on the pen-spawning in the Ytterøya Farm show that large parts of the pen spawning occurred before the commencement of the annual spring plankton bloom and the wild cod spawning period in the fjord, and that the available planktonic prey at that point in time might be critically scarce. This would negatively affect the survival of the pen-spawned larvae. Reports also exist of a generally lower hatching success in fertilized eggs of farmed cod than wild cod (Salze et al. 2005, Puckrin et al. 2013). In the present pen spawning, the egg quality was unknown; however, a lower egg quality would have contributed to the lack of a detectable genetic impact of farmed cod net-spawning on the wild cod stock in this fjord system.

Dispersal of escaped farmed cod

The tagging experiment, simulating an escape of cod from the farm pens, showed a rapid geographical dispersal of tagged cod and relatively few recaptures, which all were taken within a limited period of time (~6 mo). The geographical pattern of recaptures indicated a non-directional diffusion of the released farmed cod throughout most parts of the Trondheimsfjord. These results are in line with previous observations in other Norwegian fjords (Skjæraasen et al. 2011). Meager et al. (2011) suggested that farmed cod will have a lower survival in the wild due to weaker anti-predator responses than wild cod. According to Sverdrup at al. (2011), farmed cod also have lower competitive capacity than their wild relatives. This would tend to reduce an introgression impact from escapees, which would depend on survival until the spawning season, and on competitive fitness in the reproduction process. In the first escape incident in December 2009 the majority of individuals were spawning (stage 3) (Fig. 3), which is well before the natural spawning time in March–May. For the second escape incident, the farmed cod were either maturing (stage 2) or spent (stage 4) (Fig. 3). Since the escape was 7 mo prior to the peak spawning in April, it is possible that this batch were in sync with the natural spawning time. However, the low survival/presence indicated by recapture rates makes it less likely that a large part of the escaped cod survived until the natural spawning time in the fjord. Concerning escapees which might have survived until the natural spawning season, several studies have shown that escaped farmed cod can be present on spawning grounds (Wroblewski et al. 1996, Uglem et al. 2008, Skjæraasen et al. 2011, Jørstad et al. 2013), and that farmed cod thus have the potential to participate in the spawning (Meager et al. 2009, 2010, Skjæraasen et al. 2010). However, while it has been suggested that farmed females may effectively take part in the natural spawning, farmed males may show limited success based on sperm characteristic, morphology and behaviour (Skjæraasen et al. 2008, 2009, 2010). Therefore, to the extent that escaped farm cod have survived and participated in natural spawning in the Trondheimsfjord in this study, the genetic effect of this would not be fully proportional to their nominal numbers. The possibility that farmed eggs, larvae and adult might have left the fjord and caused some degree of genetic impact outside the fjord was not investigated in this study.

CONCLUSIONS

This study on potential interactions between farmed and wild cod in the Trondheimsfjord after extensive pen-spawning and massive escape events did not leave robust evidence of a genetic introgression from farmed cod to the local wild cod stock. This result does not exclude the possibility that an introgression did take place, but that it was either cleansed rapidly by natural selection or was too small to be detected by the markers used. Also, a genetic signature of introgression might have been weakened by the concealing effects at some of the genetic markers due to opposite impacts from 2 different batches of farmed cod with different genetic characteristics. The time of the net-pen spawning of farmed cod was found to be out of phase with the natural annual cod spawning and plankton production cycle in the fjord, and the larvae from pen-spawning may thereby have suffered mass deaths. An expected downstream transport of the pelagic eggs, larvae and codlings out of the fjord with the estuarine circulation would further reduce local genetic impact. Tagging–recapture results indicated that escaped farmed cod might not have survived long enough, and/or were not tuned to participate effectively in natural spawning. The summed effects of these factors may explain the apparent lack of genetic effects on the local wild cod stock.

Acknowledgements. We are grateful to Kathrine Michalsen at IMR for administration of the tagging reports, and to the manager and CEO of the cod farm Frengen Havbruk for cooperation. The crew on board RV Gunnerus of the Norwegian University of Science and Technology (NTNU) is acknowledged for skilled and wholehearted efforts in the field work. This project was part of the research program iCoast funded by the Norwegian Research Council (project number 122429) and the Norwegian Research Council project ECOCOAST: Kystøkosystemer i endring, and part of the PhD thesis of R.V. which were funded by the Faculty of Science and Technology at NTNU, Trondheim, Norway. Additional financial support was provided by the PreventEscape EU-project (project number 226885).

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Editorial responsibility: Ian Fleming, St. John's, Newfoundland and Labrador, Canada

Submitted: March 2, 2015; Accepted: October 12, 2015
Proofs received from author(s): November 13, 2015

Paper IV

Title:

"Escape of farmed Atlantic cod (*Gadus morhua* L.); dispersal, presence and identification in a fjord system"

Running page head: "Tracking escaped farmed cod by mark-recapture, visual and genetic group assignment"

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Key words: aquaculture, escapes, mark-recapture, microsatellites, otolith

Abstract

The dispersal behaviour and presence of escaped Atlantic cod (*Gadus morhua* L.) from production net pens in the Trondheimsfjord, Norway, were studied by tag-recapture experiments, genetic assignment and visual classification of body- and otolith characteristics in commercial and scientific catches. Recaptures from a total of 400 released farmed cod have been reported to show a random and limited spatial dispersal in the fjord and ceased after 6 ± 2 months. Similar limited dispersal and short presence were displayed when investigating reports of the abundance of farmed cod in commercial catches after a large escape incident. On-site classification was executed by experienced fishermen based on external morphological clues. The precision of the fishermen's classification was found to be fair when checked in the lab against otolith deposition patterns. The reports from the commercial catches indicate that a high presence of farmed cod were more likely in the first three months after escape. Genetic assignment procedures using six common microsatellite markers and the RFLP *Pan* I were applied on samples of commercial and scientific catches after the same escape incident. Two batches of farmed cod have been reported to be in the farm, where the most admixed batch was the likely source of escapees in this study. The admixture reduced the power of the individual genetic assignment. However, the overall genetic assignment yielded results which concurred with the non-genetic information with respect to escape time and dispersal pattern.

Introduction

Norwegian farming of Atlantic cod (*Gadus morhua* L.) as a commercial industry began around 1990 (Statistics-Norway 2014). The production volume peaked in 2010, but has currently ceased to a very low level (Statistics-Norway 2014). Frequent pen wrecks and escape incidents raised strong concerns during the peak years, similar to those expressed for salmon (*Salmo salar* L.) farming (Moe et al. 2007, Jensen et al. 2010). Compared to salmon,

cod escaped in larger proportions from net pens (Moe et al. 2007). Farmed cod show significant morphological differences to wild individuals (Uglem et al. 2011, Wringe et al. 2015) and substantial genetic differences within and among farms (Glover et al. 2010). The latter represents the precondition for genetic assignment to identify escaped farmed cod (Glover et al. 2011). However, when the origin of the broodstock were unknown, included local wild cod or when the farms contained several different genetic strains, distinguishing escapees and wild cod were more challenging (Glover et al. 2011). In comparison, the outline in the Trondheimsfjord was more transparent as both the biological and genetic outline for the wild and farmed cod has been described (Varne et al. 2015). The wild population in the Trondheimsfjord have been monitored for decades also in the fjord there was only one cod farm which was in operation for three years (Varne et al. 2015). From 2008 to 2009 the farm had two major escape events with a total of 67 000 reported escapees (Varne et al. 2015). The broodstock origin of the farmed cod which had been bred for one generation consisted of individuals from Norwegian coastal cod (NCC) populations (Varne et al. 2015). The farm was located in the inner part of the fjord near the main spawning Mork et al. 1982) and nursery area of the local cod population (Fig. 1). The present study aimed at using the genetic and biological characteristics to identify escaped farmed cod caught by local fishermen and evaluate their post-escape physical dispersion and presence in the fjord. The tools and methods chosen for pursuing these goals were the distribution of tagged and released farmed cod, otolith structure, on-site visual classification and genetic assignment methodology.

Material and methods

Samples of 244 cod from various locations in the Trondheimsfjord were collected during 2009-2011 (Table 1). The farm in the Trondheimsfjord (N63˚47’57.48’’, E11˚02’55.08’’) was operational from 2007 to 2010 and had two major escape incidents; the first on December 18th 2008 with 25 000 escapees and the second on September 22nd 2009 with 42

000 escapees (Varne et al. 2015). Varne et al. (2015) reported that the two escape events consisted of two batches with significant genetic differences, respectively. The batches were named *Farm1* (2008 escapees) and *Farm2* (2009 escapees). Samples collected in this study were categorized on-site in three groups; fish visually categorized as farmed (*F1* and *F2*), as wild (*W*) and uncategorized random specimen (*R1*, *R2*, *R3*). Sample *F1* and *F2* were collected and categorized by an experienced fisherman. The fisherman used the area east of the farm and collected cod from his catches he perceived as farmed. Visual signs of domestication like fin erosion and for cod enlarged livers are specially pronounced immediately after escape (Latremouille 2003, Uglem et al. 2011, Meerbeek et al. 2012). Neck deformities are reported in Uglem et al. (2011), as one of the three main morphometric measures for the classification of farmed cod with a 95 % correct classification rate. Sample *F1* was taken over a time span of 1-3 months after the 2009 escape, and *F2* was taken over a time span of 3-6 months after the same escape event. Sample *W* contained specimens visually categorized by another experienced fisherman as wild cod and was collected in the inner parts of the fjord in April 2010 (Table 1). The *W* sample consisted of individuals approximately the same size range as the escaped farmed cod, and the morphometric data registered of sample *W* has also been used in Uglem et al. (2011) as a reference sample for wild cod. In March 2011, two samples were taken by bottom trawl on two cruises with RV "Gunnerus" of NTNU; from an area further away from the farm (*R1*) and from the spawning area Verrabotn (*R3*; Table 1). Also in March 2011 samples *R2* was collected in cylindrical pots (60 x 180, stretched mesh 2.5 cm) in the nursery area Borgenfjorden by a local fisherman (Table 1).

Biological data

For sample *F1*, *F2* and *R3* only the fish head were collected; after decapitation the samples were kept frozen (-20º C) until sampling of tissues and otoliths at Trondhjem Biological Station (TBS), NTNU. Sample *W* contained tissue samples, otoliths and morphometric data

which included total body length (Uglem et al. 2011). Sample *R1* and *R2* contained full biological information which consists of; tissue samples, otoliths, total body length, weight, sex, and gonad maturation. Age was determined by otolith reading according to Rollefsen (1933) and used to exclude individuals born before 2007 from being of farmed origin. The annual zone deposition pattern of the otoliths was used as an indicator of farmed origin in sample *F2*. The first opaque deposition zone was interpreted as the time in the farm. The characteristic of the deposition patterns and shape in local wild cod in the Trondheimsfjord have been described in Ekli (1997) and were used for comparison. Age was determined for samples *F2*, *W*, *R1* and *R2*.

Simulated escape and presence of escapees in the fjord

Two hundred individuals from each batch of farmed cod (*Farm1*, *Farm2*) were tagged and released 97 and 41 days, respectively, after the reported escape incidents at the farm. Prior to the release, the farmed cod sampled from the net pens were kept in tanks with sea water flow on board RV "Gunnerus". The specimens were length measured, fin clipped and tagged before release into the sea at ~50 m from the net pens. Using a stainless steel pointer the tags were attached in front of the first dorsal fin (Varne et al. 2015). The *Farm1* sample was tagged with Lea hydrostatic tags (N=100) and Dart tags (N=100), the *Farm2* sample were all tagged with Dart tags (N=200). Both tags were clearly visible and contained address of return to the Institute of Marine Research (IMR). The sampling, tagging and release were executed in one day. Procedures were approved by the Norwegian Animal Research Authority. Additional information of the presence of escaped farmed cod comes from reports of on-site classification of farmed cod in commercial cod fishery in the vicinity of the farm from 20th October 2009 to 25th January 2010.

Genetic samples

Samples from muscle tissue or finclips were preserved in 96 % ethanol in individual tubes; the samples were kept at room temperature until DNA analyses. The microsatellites screened were *Gmo2* and *Gmo132* (Brooker et al. 1994), *Gmo3*, *Gmo8*, *Gmo19*, *Gmo34* and *Gmo35* (Miller et al. 2000), *Tch11* and *Tch13* (O'Reilly et al. 2000). As described in Stenvik et al. (2006) the nuclear RFLP locus *Pan* I (Pogson & Fevolden 2003) was genotyped together with the microsatellites. The reference samples from Varne et al. (2015) included one group of wild cod collected before the start of the farm (*Pre*; N = 192*)*, and samples drawn from each of the two batches of farmed cod *Farm1* (N = 96) and *Farm2* (N = 192). The DNA isolation and amplification were carried out with the same procedures as in Varne et al. (2015).

Statistical analyses

Air distance from release site to recapture site were used to calculate maximum, minimum, average and median distances. Estimated survival was based on time of last recapture. A Kruskal Wallis test was used to check for significant differences between the two farmed batches for distance and days before recapture. The relation between days before recapture and distance at recapture were analysed for with a linear correlation. MICRO-CHECKER was used to test the genetic dataset for null alleles and stuttering (Van Oosterhout et al. 2004). The numbers of alleles, number of effective alleles, observed and expected heterozygosity , overall F_{ST} and pairwise F_{ST} with probability if significant difference from 0 based on 999 permutations were calculated using GenAlEx (Peakall & Smouse 2006, 2012). Candidate loci for positive selection under the infinite allele mutation model were detected by the F_{ST}-outlier detection method by Beaumont and Nichols (1996) and implemented in Lositan (Antao et al. 2008). Genepop 4.2 web version (Raymond & Rousset 1995, Rousset 2008) was used for exact test for Hardy-Weinberg equilibrium (HWE) and detection of linkage disequilibrium (LD). All analyses were executed with the default Markov chain

parameters and overall p-values were calculated by Fisher's method. Bonferroni procedures were used to correct for multiple tests (Rice 1989). Varne et al. (2015) excluded *Gmo132*, *Tch11* and *Pan*I by reason of observed selection, null alleles and history of selection in the local cod stock. Since we used the same baseline samples the analyses were conducted excluding the same markers: *Gmo132*, *Tch11* and *Pan*I. Assignment tests were conducted in Geneclass2 (Piry et al. 2004) by comparing the multilocus genotypes to reference genotypes. The power of assignment was found by self-assignment of individuals to baseline populations using the Bayesian method by Rannala and Mountain (1997) and assignment threshold was set to 0.05. A probability computation to baseline population when simulating 100 000 individuals using the Bayesian simulation (Rannala & Mountain 1997) was also executed. The threshold for assignment (p-value) of the Monte-Carlo resampling Type I error (false rejection from baseline sample) were set to 0.05, 0.01 and 0.001. A Discriminate Analysis of Principal Components (DAPC) was executed by the R package Adegenet (Jombart 2008). In a DAPC analyses group membership for an individual are derived from the position of the genotypes on the discriminant factors (Jombart et al. 2010). The baseline samples *Pre, Farm1* and *Farm2* were analysed together with a pooled sample containing wild and potentially farmed individuals. STRUCTURE 2.3.4 was used to cluster individuals by individual admixture proportions (Pritchard et al. 2000, Hubisz et al. 2009); the program clusters individuals to achieve HWE in each cluster. STRUCTURE Harvester by Evanno et al. (2005) was then used to find the k best describing the data. The program was forced to use *Pre*, *Farm1* and *Farm2*, as learning samples. Since the wild and farmed cod showed relatively low levels of differentiation, the allele frequencies were set to be correlated (Nielsen et al. 2003).

Results

Otoliths and age as indicators to farmed origin

The samples consisted of 7 age groups (2-9 years) and 9 cohorts (2002-2009: Table 2). The *F2* specimens, visually categorized as farmed cod, where identified to be hatched in 2007 or later. The appearance of the *F2* otolith structure indicated that 25 of 37 were of farmed origin. According to the age distribution in the sample visually categorized as wild (*W*), 28 individuals could potentially be *Farm1* escapees whereas only one was young enough to originate from the second escape event (*Farm2*). In sample *R1*, all individuals were born in 2006 or 2007 making them potential candidates to be of farmed origin. The cod in sample *R2* had the largest age range (2-9 years) and could potentially contain six *Farm1* and six *Farm2* specimens (Table 2).

Dispersal and presence of farmed cod

Seventeen reported recaptures corresponds to a recapture rate of four percent (Varne et al. 2015). There were no reports of recaptures outside the fjord and most were caught in the inner parts of the fjord-system (Fig. 1). The recaptures consisted of 12 *Farm1* and five *Farm2* specimens (Fig. 2). There was no difference between Lea and Dart tags for *Farm1* specimen with six recaptures for each tag type. Average time before recapture was 99 ± 63 days and the maximum reported time was 194 days (Fig. 2). Distances from release site were from < 1 km to 70 km, with an average of 13 km (median = 14 km). There were no significant differences between *Farm1* and *Farm2* for days before recapture and distance (Kruskal Wallis $p = 0.60$ and $p = 0.53$, respectively, Table 3). This was consistent when analysing the recaptures as a pooled sample ($R^2 < 0.01$, linear correlation, Fig. 3). The fisherman had a total of 54 catches of which he reported the number of cod he categorized as farmed or wild. The percentage of cod visually classified as farmed in the commercial catches dropped after approximately three months (Fig. 4). The percent of farmed cod in catches ranged from 9 - 100 % with an average

of 45 % and a median of 38 %. The size of the catches ranged from 16-500 individuals (average = 119, median = 76).

Genetic characteristics

The success of DNA marker scoring for the 10 markers ranged between 92 % and 99 %. Altogether 185 alleles were detected. Null alleles were indicated at *Gmo19* in sample *F1* and at *Gmo2* in sample *R2*; this constituted 3.3 % of the material and was not considered as substantial. Test for positive selection fell out significant only for *Gmo132*. Allele frequencies for the most common allele in all samples may be found in Appendix Table 5. Test for HWE following Bonferroni corrections were significant in three samples; *F1* (*Gmo19*), *F2* (*Gmo8*) and *R2* (*Gmo19*). Sample *F1*, *R1* and *R2* were monomorphic at the *Pan*I marker for allele Pan^A. Detailed summary statistic for all samples and loci can be found in Appendix Table 6. Mean overall F_{ST} value for the dataset was 0.0217 ±0.004. Pairwise F_{ST} comparisons showed a significant differentiation after Bonferroni adjusted p-value for all *Farm1* and *Farm2* comparisons except the comparisons to *F1* (Appendix Table 7).

Assignment tests and clustering

The self-assignment to reference population using Geneclass2 had an average correct assignment score of 78 %. *Farm1* showed the highest self-assignment score, then *Pre*, whereas *Farm2* had the lowest score (Table 4). It is noted that the miss-assigned wild (*Pre*) individuals were more likely *Farm2* then *Farm1* (Table 4). Sample *F1* and *F2* had the highest assignment to farm, with 59 and 49 % respectively. When simulating individuals for self-assignment to exclude population of origin, no reference sample was excluded for any individual at any threshold. The DAPC result resonance the results from Geneclass2 as *Farm1* shows the largest differentiation and *Pre* and *Farm2* have the largest overlap (Appendix Fig. 5). Cluster one has a low proportion of *Pre* individuals and higher proportion of farmed, the pooled samples collected in this study (MID) have a higher proportion of individuals in cluster one than *Pre*, indicating a presence of farmed cod in the samples

(Appendix Table 8). STRUCTURE showed a relatively high membership proportion for the reference samples *Pre* (0.978) and *Farm1* (0.982). *Farm2* had a more admixed clustering, where the largest cluster were a *Farm2* cluster (0.496) and the other membership proportions were divided between the *Pre* and *Farm1* cluster (0.284 and 0.221, respectively). The highest assignment to farm were found in *F1* (32%) and *F2* (29%) by using STRUCTURE.

Combination of the methods (visual, otolith and genetic)

The Geneclass2 assignment result was used to compare the genetic categorization to the other classification methods because of the better self-assignment score. For the *F2* sample it was possible to compare the appearance of the otoliths to the other classification methods. In the sample the visual and otolith structure classification had the highest correspondence at 65 %, while the visual and genetic classification corresponded in 51 % of the individuals. The correspondence dropped to 28 % when comparing genetic classification and otolith structure. The lowest correspondence was found when combining all three classification types; visual, otoliths and genetic classification (27 %). For the samples *W*, *R1* and *R2* age was used to detect potential farmed cod and confirm wild origin when the otoliths showed more than two deposition zones. In sample *W* one of the five individuals suggested to be *Farm1* by Geneclass2 could be excluded to so because of age. This indicates a possible presence of *Farm1* specimen in the *W* sample, but such a presence was not supported by the recapture data. Eleven individuals were assigned as *Farm2* in sample *W*, when collating with the age none of the assignments to *Farm2* could be correct. This indicates that *W* likely consists of wild cod, as categorized by the visual classification. Sample *R1* had one and six individuals assigned to *Farm1* and *Farm2*, respectively (Table 4). In *R1* the age of the cod assigned as *Farm1* contradict the genetic assignment result, but this was not the case for the individuals assigned as *Farm2*. The absence of *Farm1* specimen in R1 is in line with short period of recorded recaptures. Sample *R2* had 12 individuals which based on age could be of farmed

origin, in this case only one individual was assigned to *Farm1* and three to *Farm2*. For one genetic assigned farmed individual in *R2* the result was in agreement with age data. The number of genetic assigned farmed cod was within the range of expected misclassification in sample *R1* and *R2*, which makes a presence of farmed cod uncertain.

Discussion

Quality of age data and visual classification

Otolith shape of Atlantic cod has been shown to be a significant phenotypic stock separator (Cardinale et al. 2004). On the other hand the exact age of a farmed specimen can be difficult or impossible to read (Arechavala-Lopez et al. 2012); still the presence of deposition zones will give an indication of years in the wild. Some of the cod in sample *F2*, which were on-site categorized as farmed, were age-read to be younger than the escaped farmed cod. The otoliths showed no signs of having lived a life in the wild for more than one year, so it is reasonable to assume that the young age is caused by the difficulty of distinguishing the years in the farm. This difficulty of precise age reading for farmed fish can pose a problem if one needs to distinguish farmed generations. In this study, the age of the farmed fish were known and the collected escapees had approximately the same size, still the otolith reading classified them to be three different year-classes. If escape history for the farm had not been available these cod could be assumed to be the result of interbreeding between escapees in the wild. The otolith categorization had the highest proportion of farmed specimens and thus had the highest correspondence to the visual classification. We consider age as a reliable indicator of the cod born before 2006 as wild in this study. The ability of detecting escapees on-site is crucial for recapture fishery (Uglem et al. 2010). Experienced fishermen are likely to distinguish wild and farmed fish immediately after release. But the most obvious farm signs may be lost after some time in the sea; still any pronounced deformities will be permanently present. Our results suggest that a recapture fishery can be done effectively by local fishermen using on-

site classification, if it is initiated immediately after escape events. The reports from the commercial catches indicate that the farmed escapees are more likely to stay in homogeneous groups in the first three months after escape. Later the reduced proportion of farmed cod in the catches indicates an overlapping distribution with the wild stock and a likely high mortality rate. The ongoing work on improved hatching conditions for farmed cod has led to a lower degree of deformities (pers comm. Atle Mortensen, NOFIMA). Thus future visual classification of farmed cod could rely on induced signs by the farmed environment like body shape and fin damage. Time after escape will then be crucial for the on-site detection of escapees by this method.

Dispersal ability and presence of farmed cod

An overall trend was that *Farm1* seemed to have a higher recapture rate than *Farm2*. This could be attributed to the time of release, early spring for *Farm1*, which could suggest more favourable conditions than for the *Farm2* individuals, which were released in November. The farm and fisherman reported that the second escape event (*Farm2* batch) consisted of individuals of larger weight, seemingly in better condition, and also escaped in larger numbers than *Farm1*. This suggests that the time of escape could be an important factor for the ability of survival in the wild and also for newly domesticated species. The last recapture were registered after six months (194 days). These findings are in line with a similar study, where the last recapture were reported nine months after release (Skjæraasen et al. 2011). In contrast to the tagged wild cod in the same study, of which recaptures were reported until the study ended three years after release (Skjæraasen et al. 2011). Based on this we suggest survival to be low after 6 - 9 months in the wild. Sample *F1* and *F2* were taken in the vicinity of the farm approximately 11 and 14 months after the *Farm1* escape, thus we consider the presence of *Farm1* individuals to be less likely in the material. This is also confirmed by the age of the *F2* catch, because a *F1* individual would have shown additional deposition zones

(Table 2). Both in this material and in Skjæraasen et al. (2011), farmed cod showed random and limited dispersal distance. Uglem et al. (2008; 2010) also observed a rapid and random dispersal of farmed cod escapees, but considered recapture fisheries near the farms to be possible immediately after escape. Similar patterns were found in Canada but the farmed recaptures showed that they were capable of traveling longer distances than the studies in Norway (Zimmermann et al. 2013). Earlier tag-recapture experiments in the Trondheimsfjord using wild cod have shown that there are minimal interactions by adult cod with the surrounding coastal areas (Sundnes 1980). The few recaptures outside the fjord were only registered north of the fjord, indicating a coherence with the northward coastal current (Sundnes 1980). Air distance from release site to recapture site were used in the calculations, which is a coarse estimate of the actual travelled distance and dispersal area, still it gave a good indication that the escapees were likely to stay in the fjord. This suggests that any impact of escaped farmed cod could be reduced by recapture fishery, because escapees stay in the vicinity of the farm and in relatively homogenous groups immediately after escape.

Genetic detection of escapees

The use of DNA markers to distinguish invader populations is efficient when allele frequency information for all participating stocks is available, which is the case in the present study. Furthermore, significant genetic differences between the two farmed cod batches and between the farmed and local wild cod have been reported (Varne et al. 2015). Analyses excluding three markers (*Gmo132*, *Tch11*, *Pan* 1) were performed to avoid strong LD, significant selection and significant null alleles as the same reference populations was used in this study and in Varne et al. (2015). The *Pan* I marker could potentially be a discriminating marker had the farmed brood stock contained individuals from NEAC. The farmed cod in this material, however showed similar *Pan* I frequencies as the local coastal cod and several samples were monomorphic, reducing its diagnostic power. The reference population *Farm1*

showed a higher genetic differentiation to the wild reference sample *Pre* than *Farm2* (Varne et al. 2015). The likely source of farmed cod in samples *F1* and *F2* turned out to be *Farm2*, the most admixed batch, and thus most challenging to assign correctly. For genetic classification the differentiation in the material is in the low end of successful analyses (Putman & Carbone 2014). The most prominent genetic differentiation for Atlantic cod exists between the North Sea and the Baltic cod stock. In such a respect the farmed F_1-generation and the wild local stock in this study show a similar differentiation as the North Sea and Western Baltic (F_{ST} = 0.0221, Nielsen et al. 2003). Available life history information, and comparison of the genetic signature with the otolith structure and age of the individuals, gave us the possibility to verify parts of the material. Fish from sample *F1* and *F2*, which were visually categorized as farmed, displayed a lower amount of null alleles, HW-disequilibrium, and LD than the farmed reference samples; this might be explained by the small and variable sample sizes and a potential visual misclassification of wild cod. It is also noted that the wild baseline *Pre* consist of one sample from one year which would narrow the genetic baseline. The farmed cod had also been size-sorted during the years in captivity which might have enhanced the differentiating genetic signature of the strains. When there was a high presence of farmed cod in a sample, it was possible to distinguish samples with sufficient statistical significance. But with the type and number of markers used in this study the genetic differentiation was not strong enough to accurately distinguish single farmed individual. The correspondence with other biological measures is also inconsistent which indicates some degree of misclassification by the different methods.

Individual assignment and clustering analyses

Individual assignment analyses in Geneclass2 were performed using a multilocus genotype method (Rannala & Mountain 1997), which handles HW-disequilibrium better than the other programs used (Renshaw et al. 2006). Assignment tests require information of reference

populations, and a $F_{ST} \geq 0.05$ is recommended for effective application (Cornuet et al. 1999). The presence of farmed cod originating from farms outside the fjord cannot be excluded, but is unlikely. The relatively low differentiation between sample *Pre* and *Farm2* reduced the efficiency of the assignment test. The self-assignment score (78 %) were still reasonable and in line with results from Glover et al. (2011) for a similar case (75 %). Geneclass2 assigned individuals mostly to *Farm2*, this is coherent with the available life history of which *Farm2* were the likely source for the samples. Sample *W* was taken eight months after the last recapture of the tagged and released *Farm1* individuals and we consider the presence of *Farm1* in this sample is less likely. The genetic assignment also corroborates the visual categorization that *W* contained wild fish, as only one individual was assigned as *Farm1*. The assignment analyses indicate that sample *R1, R2* and *R3* could contain farmed fish, but since this result is not supported when age is taken into account, this might be an effect of misclassification. As there were large differences in sample size, the ability of Geneclass2 to reduce such source of bias might have strengthened the analyses (Putman & Carbone 2014). DAPC uses a categorisation based on PCA, as such it does not use a genetic model and will not be sensitive to HW-disequilibrium. Still the genetic differentiation might have been too low as the clusters of the baseline populations were overlapping, which would underestimate farmed escapees. In STRUCTURE a three-population model showed the best fit to the data, which corresponds well to the baseline populations from Varne et al (2015); wild and two batches of farmed cod. There was a violation of an assumption in STRUCTURE; the learning samples were in effect admixed since we cannot exclude the presence of wild type alleles in the farmed cod. Also the pronounced HW-disequilibrium and LD in the farmed samples will weaken the power of STRUCTURE, since the algorithm try to cluster the individuals to HW-equilibrium. Additionally, the program is not designed to handle strong LD. This may explain the difficulty to distinguish *Farm1* and *Farm2* individuals using STRUCTURE.

Conclusion

As aquaculture industry is increasing, the cultivation of newly domesticated species will be more common. Especially when the species is naturally occurring in the surrounding environment there will be a need for an accurate detection of escapes. The escaped farmed cod remained in the inner parts of the fjord and showed a limited presence after ~6 months. The use of genetic tools for the detection of escapees has offered a seemingly quick and reliable solution, but the classical tools are only as good as the samples, and require comprehensive baselines of the populations in question for a good detection result. We assessed three commonly used genetic data programs for detecting escapees and compared the results to dispersal data and biological information. Even though the genetic signatures of the samples were in the low end for a successful result they still performed reasonably well on a group level. The assignments of the reference populations were good, except for the most admixed farmed batch (*Farm2*). Of the genetic programs used the Geneclass2 analyses gave the best fit to the biological information. We consider the genetic detection not strong enough to classify single individuals with a high accuracy. It is noted that the farmed source likely to be in the samples in this study, were the least differentiated batch and situations with more differentiated strains could significantly increase the power of the genetic detection. For newly domesticated species and when baseline for the wild stock and brood-stock sources are missing we stress the importance of using stable markers and a large marker set. The use of SNP might have provided larger statistical power necessary to categorize single specimen (Martinsohn & Ogden 2009). Also the use of internal physical tags for farmed specimen (e.g. PIT tags) would give hard evidence for the connection of escapees to specific farms. Our results show that visual classification on-site is reliable when deformities are common and the sings of farm life are present. When the presence deformities are reduced and time passes after an escape the use of other classification methods will be crucial. For farmed cod the

limited presence in the system after six months indicate a reduced impact potential on the wild stock.

Acknowledgement

We are grateful for the excellent work by the fishermen Tor Hynne and Steinar Pettersen, and the always professional and helpful crew on RV "Gunnerus" of NTNU. Also all fishermen that contributed by sending tags with information of recapture location deserve to be mentioned. Gratitude goes also to Kathrine Michalsen at IMR, Bergen who administrated the tagging reports. This study was part of the research program iCoast funded by the Norwegian Research Council (project number 122429) and received additional financial support by the PreventEscape EU-project (project number 226885). This paper is also part of the PhD thesis of one of the authors (RV) which were funded by the Faculty of Science and Technology at NTNU.

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Figures

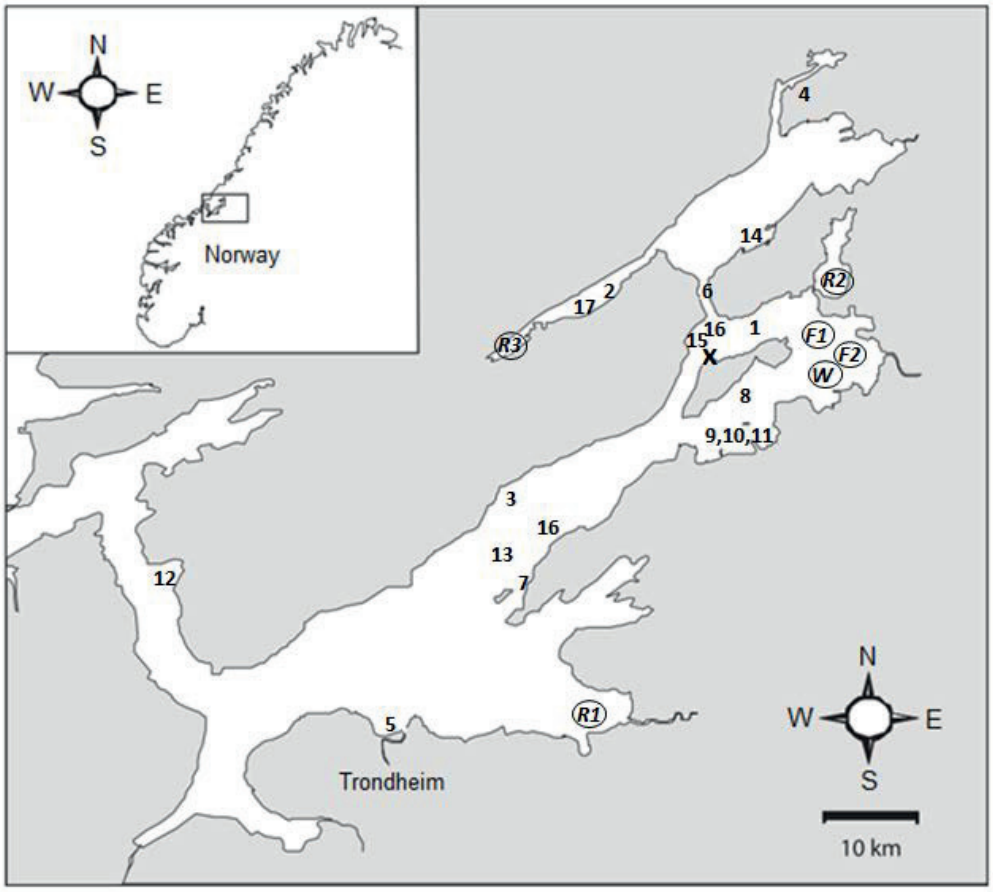

Fig. 1 Map of the Trondheimsfjord; recaptures and samples. Recaptures numbered chronologically by recapture date. Location of farm at black X. Samples *F1*, *F2*, *W* in Levangerfjord, *R1* in Stjørdalsfjord, *R2* in the nursery area Borgenfjorden, *R3* in the spawning area Verrabotn. Map from Karlsson & Mork 2005.

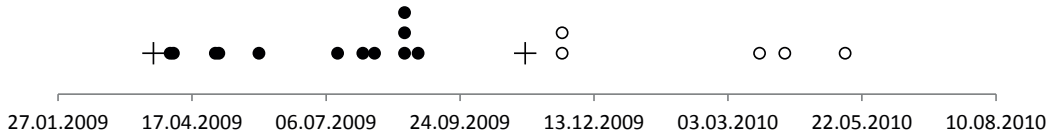

Fig. 2 Recaptures according to date. + = first release on the 25.3.2009, and second release on the 2.11.2009. One circle corresponds to one individual. Black circles are recaptures from the first release (batch *Farm1*), unfilled circles = recaptures from the second release (batch *Farm2*).

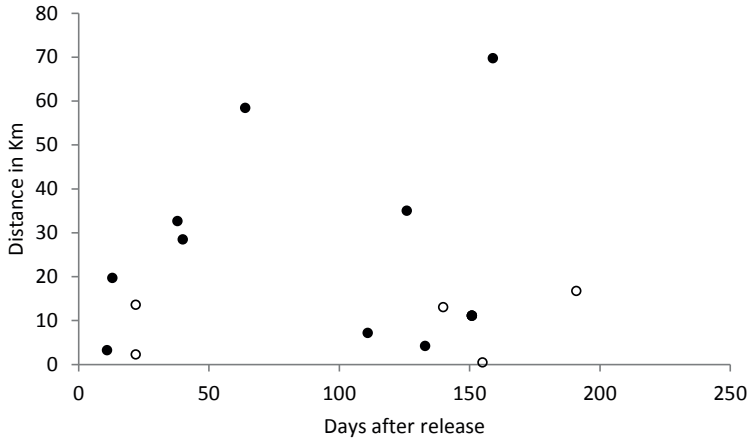

Fig. 3 Scatter-plot of days after release vs. distance in kilometre from the release site. Filled circles = *Farm1*, unfilled circles = *Farm2*

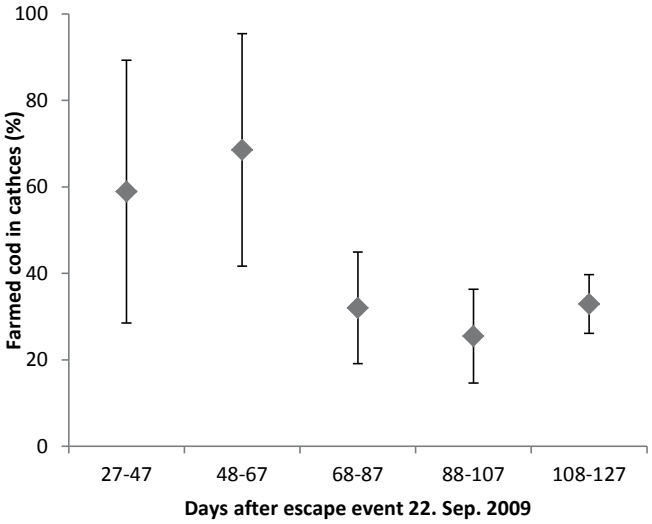

Fig. 4 Percentage of visually classified farmed cod in commercial catches after the second escape event (22. September 2009). Diamonds show mean of visually classified farmed cod and whiskers display standard deviation. Number of catches in the each 20 day period had an average of 10.8 and a median of 12.

Tables

Table 1 Sample = abbreviations for the different samples, Location = area samples were taken, Date = for *F1* specimen were collected over a period in autumn 2009, *F2* specimen were collected during winter 2010, other dates refers to the exact day the fish was caught. Collector = two experienced local fishermen or scientific staff on board research vessel, Total (N) = total number of sampled fish, Bio. Data = available biological data, DNA Loci = the number of DNA markers utilized, Visual classification = on-site classification by fishermen for sample *F1*, *F2* and *W*. * = published biological and morphological data in Uglem et al. 2011, ** = published genetic signature in Varne et al. 2015, *Farm1* and *Farm2* total N include individuals used in tag and release experiment.

Sample	Location	Date	Collector	Fishing tool	Total (N)	Bio. Data (N)	DNA data	Visual classification
*Pre***	Borgenfjord	03.10.2005	RV "Harry Borten"	Trawl	192	192	192	-
*Farm1***	Cod Farm	25.03.2009	RV "Gunnerus"	Hand-net	263	63	96	-
*Farm2***	Cod Farm	02.11.2009	RV "Gunnerus"	Hand-net	248	48	192	-
F1	Levangerfjord	01.12.2009	Fisherman	Gill-net	27	NA	27	Farmed
F2	Levangerfjord	01.01.2010	Fisherman	Gill-net	37	NA	37	Farmed
*W**	Levangerfjord	27.04.2010	Fisherman	Fyke-net, cod pots	50	50*	50	Wild
R1	Stjørdalsfjord	03.03.2011	RV "Gunnerus"	Trawl	28	28	28	-
R2	Borgenfjord	19.03.2011	Fisherman	Cod pots	23	23	23	-
R3	Verrabotn	31.03.2011	RV "Gunnerus"	Trawl	79	NA	79	-

Table 2 Age distribution in samples. Cohort 2006 are candidates for *Farm1* escapees, 2007-2009 are cohorts for *Farm2* escapees. Age not determined for sample *F1* and *R3*.

Catch	(N)	No. individuals in each cohort							
		2002	2003	2004	2005	2006	2007	2008	2009
F1	27								
F2	37						6	10	21
W	50			6	14	28	1		
R1	28					3	25		
R2	23	3			7	6	2	4	
R3	79								

Table 3 List of batches, the number of recaptures for each batch, average time in sea before recapture and average air distance from release site.

Tagged batch	Release date	Recaptures (n)	Average time (days)	Average distance (km)
Farm1	24.03.2009	12	96 ± 58	24 ± 22
Farm2	02.11.2009	5	106 ± 73	12 ± 7

Table 4 Geneclass2 results. Reference populations = self-assignment test for reference populations from Varne et al. (2015). Assignment in samples = Assigned to sample population according to the reference populations. Individuals that did not score for any loci are not included in N.

		N	*Pre*	*Farm1*	*Farm2*	Correct assigned (%)
Reference populations	*Pre*	192	**152**	11	29	79
	Farm1	96	8	**80**	8	83
	Farm2	192	36	16	**140**	73
						Assigned to farm (%)
Assignment of samples	*F1*	22	9	5	8	59
	F2	37	19	9	9	49
	W	49	33	5	11	33
	R1	27	19	1	6	26
	R2	20	16	1	3	20
	R3	76	48	11	17	37

APPENDIX

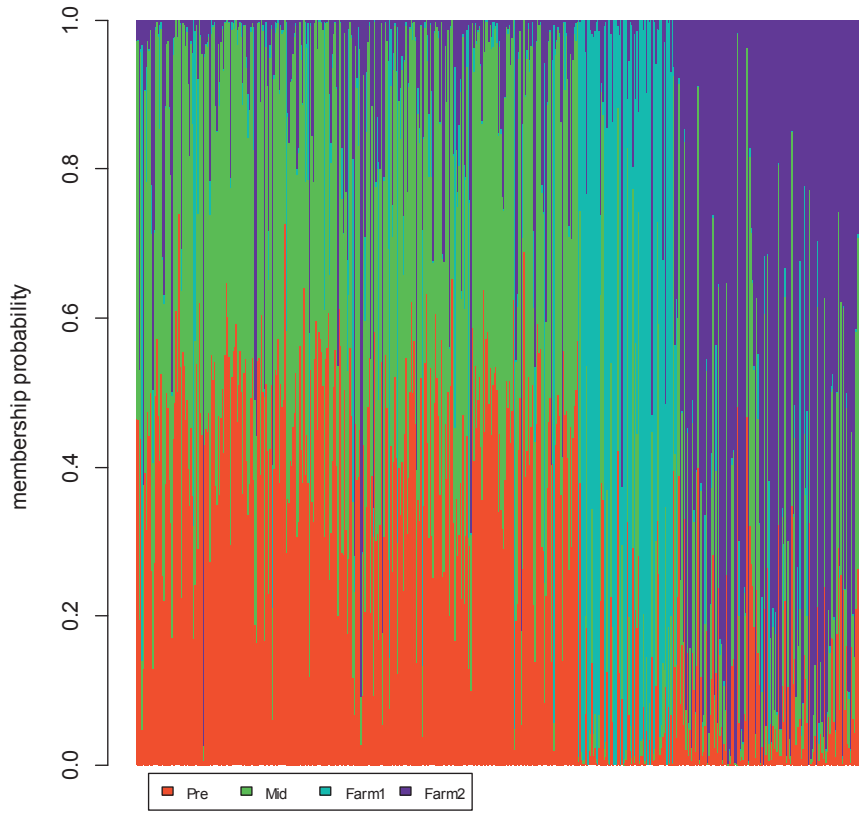

Fig. 5 APPENDIX. Compoplot of DAPC analyses using 4 clusters. Dataset analyzed as 4 samples; *Pre, Mid, Farm1 and Farm2*. Mid is the pooled sample consisting of: *F1, F2, W, R1, R2, R3*. Each vertical line represents one individual. X-axes corresponds to samples in order *Pre* (n = 192), *Mid = F1* (n = 27), *F2* (n = 37), *W* (n = 50), *R1* (n = 28), *R2* (n = 23), *R3* (n = 79), *Farm1* (n = 96) and *Farm2* (n = 192).

Table 5 APPENDIX Allele frequencies of the most common allele for all loci in all samples.

Sample	Area	N	*Gmo2*	*Gmo3*	*Gmo8*	*Gmo19*	*Gmo34*	*Gmo35*	*Gmo132*	*Tch11*	*Tch13*	*Pan* I
			107	191	124	145	98	126	116	172	93	*A*
F1	Ytterøya/Verdal	27	0.184	0.868	0.214	0.238	0.658	0.262	0.105	0.190	0.184	1.000
F2	Ytterøya/Verdal	37	0.250	0.922	0.095	0.214	0.609	0.405	0.234	0.125	0.219	0.932
W	Ytterøya	50	0.219	0.872	0.167	0.188	0.656	0.271	0.333	0.117	0.198	0.970
R1	Stjørdalsfjorden	28	0.192	0.808	0.231	0.167	0.712	0.241	0.260	0.148	0.173	1.000
R2	Borgenfjorden	23	0.300	0.925	0.250	0.175	0.550	0.300	0.450	0.075	0.200	0.952
R3	Verrabotn	79	0.273	0.824	0.200	0.181	0.713	0.274	0.452	0.125	0.219	0.993
		Σ 244										

Table 6 APPENDIX Summary statistics. N = number of individuals genotyped, N_A = number of alleles. N_{AE} = number of effective alleles, H_{exp} = expected heterozygosity, H_{obs} = observed heterozygosity, $F_{IS\ W\&C}$ = inbreeding coefficient Weir and Cockerham method, P_{HWE} = probability for loci to be in HW-equilibrium, LD = number of significantly linked loci per locus (Bonferroni adjusted p-value 0.001), Global $F_{ST\ W\&C} = F_{ST}$ over all samples, *F1&F2 vs.rest* F_{ST} = hierarchical $F_{ST\ W\&C}$ when material was divided into presumed farmed and presumed wild samples.

Catch	Variable	*Gmo2*	*Gmo3*	*Gmo8*	*Gmo19*	*Gmo34*	*Gmo35*	*Gmo132*	*Tch11*	*Tch13*	*Pan* 1
F1	N	19	19	21	21	19	21	19	21	19	22
	N_A	11	5	14	11	5	8	14	15	16	1
	N_{AE}	5.641	1.318	8.481	5.919	2.117	5.690	7.600	9.587	10.314	1.000
	N_{PA}	-	-	-	-	-	1	-	-	-	-
	H_{exp}	0.684	0.211	0.952	0.571	0.579	0.857	0.947	1.000	1.000	0.000
	H_{obs}	0.823	0.241	0.882	0.831	0.528	0.824	0.868	0.896	0.903	0.000
	$F_{IS\ W\&C}$	0.1945	0.1529	-0.0554	0.3343	-0.0703	-0.0155	-0.0640	-0.0923	-0.0806	monomorph
	P_{HWE}	0.0390	0.1197	0.4197	**0.0004**	0.2095	0.0591	0.8253	0.0831	0.9971	monomorph
	LD	-	-	2	2	-	2	-	-	-	-
F2	N	32	32	37	35	32	37	32	36	32	37
	N_A	14	3	22	17	7	9	18	18	18	2
	N_{AE}	7.474	1.171	12.445	9.646	2.444	4.206	8.359	11.571	9.309	1.144
	N_{PA}	-	-	1	-	-	-	-	-	-	-
	H_{exp}	0.750	0.156	0.892	0.857	0.656	0.784	0.750	0.889	0.875	0,135
	H_{obs}	0.866	0.146	0.920	0.896	0.591	0.762	0.880	0.914	0.893	0,126
	F_{IS}	0.1497	-0.0544	0.0439	0.0582	-0.0950	-0.0146	0.1636	0.0411	0.0356	-0.0588
	P_{HWE}	0.1707	1.0000	**0.0000**	0.0954	0.8403	0.5150	0.0360	0.1099	0.3778	1.0000
	LD	-	-	-	-	-	-	-	-	-	-

Catch	Variable	***Gmo2***	***Gmo3***	***Gmo8***	***Gmo19***	***Gmo34***	***Gmo35***	***Gmo132***	***Tch11***	***Tch13***	***Pan* 1**
W	N	48	47	48	48	48	48	48	47	48	50
	N_A	15	3	23	19	6	7	20	18	23	2
	N_{AE}	7.067	1.291	11.607	11.607	2.133	5.696	6.649	11.750	9.996	1.062
	N_{PA}	-	-	1	-	-	-	-	-	-	-
	H_{exp}	0.854	0.255	0.896	0.833	0.521	0.792	0.833	0.851	0.958	0.060
	H_{obs}	0.859	0.225	0.914	0.914	0.531	0.824	0.850	0.915	0.900	0.058
	F_{IS}	0.0156	-0.1231	0.0302	0.0985	0.0301	0.0503	0.0297	0.0805	-0.0544	-0.0208
	P_{HWE}	0.9584	1.0000	0.0739	0.0365	0.5417	0.2416	0.4793	0.6787	0.0221	1.0000
	LD	-	-	-	-	-	-	-	-	-	-
R1	N	26	26	26	27	26	27	25	27	26	27
	N_A	11	3	17	21	5	7	16	16	19	1
	N_{AE}	6.438	1.484	9.455	13.376	1.860	5.786	8.065	10.881	10.242	1.000
	N_{PA}	-	-	-	-	-	-	-	-	-	-
	H_{exp}	0.885	0.308	0.769	0.889	0.538	0.704	0.880	0.963	0.962	0.000
	H_{obs}	0,845	0,326	0,894	0,925	0,462	0,827	0,876	0,908	0.902	0.000
	F_{IS}	-0.0277	0.0762	0.1590	0.0581	-0.1457	0.1676	0.0158	-0.0416	-0.0460	monomorph
	P_{HWE}	0.4320	0.1010	0.0528	0.0516	0.3262	0.2786	0.3669	0.2298	0.6321	monomorph
	LD	-	-	-	-	-	-	-	-	-	-
R2	N	20	20	20	20	20	20	20	20	20	21
	N_A	11	2	14	13	5	7	13	16	13	2
	N_{AE}	6.061	1.161	8.000	8.163	2.667	4.819	4.188	13.333	7.692	1.100
	N_{PA}	-	-	-	-	-	-	-	-	-	-
	H_{exp}	0.650	0.150	0.900	0.850	0.600	0.900	0.750	0.850	0.950	0.095
	H_{obs}	0.835	0.139	0.875	0.878	0.625	0.793	0.761	0.925	0.870	0.091
	F_{IS}	0.2458	-0.0556	-0.0029	0.0569	0.0656	-0.1104	0.0404	0.1065	-0.0665	-0.0256
	P_{HWE}	0.1306	1.0000	0.3937	0.2448	0.6665	0.3314	0.5825	0.3640	0.7550	1.0000
	LD	-	-	-	-	-	-	-	-	-	-

Catch	Variable	***Gmo2***	***Gmo3***	***Gmo8***	***Gmo19***	***Gmo34***	***Gmo35***	***Gmo132***	***Tch11***	***Tch13***	***Pan* I**
R3	N	75	71	70	72	75	73	73	72	73	74
	N_A	14	5	30	19	6	9	23	21	21	2
	N_{AE}	6.425	1.449	11.980	11.245	1.884	5.353	4.454	13.787	9.148	1.014
	N_{PA}	1	-	1	-	-	1	1	-	1	-
	H_{exp}	0.867	0.324	0.943	0.792	0.480	0.822	0.822	0.847	0.822	0.014
	H_{obs}	0.844	0.310	0.917	0.911	0.469	0.813	0.775	0.927	0.891	0.013
	F_{IS}	-0.0197	-0.0380	-0.0215	0.1379	-0.0164	-0.0038	-0.0530	0.0935	0.0841	monomorph
	P_{HWE}	0.7663	0.6134	0.2811	**0.0003**	0.3054	0.1196	0.9317	0.5188	0.0572	monomorph
	LD	-	-	-	-	-	-	-	-	-	-
Global F_{ST}		0.0025	0.0039	0.0043	0.0000	0.0025	0.0000	0.0197	0.0000	0.0000	0.0000
F1&F2 vs.rest F_{ST}		0.0007	0.0070	0.0068	0.0029	0.0000	0.0051	0.0295	0.0037	0.0000	0.0078

Table 7 APPENDIX Pairwise comparisons all samples including baseline samples *Pre*, *Farm1* and *Farm2*. F_{ST} values below diagonal. Probability (P-value) based on 999 permutations above diagonal. Bonferroni adjusted p-value = 0.0014.

	Pre	***F1***	***F2***	***W***	***R1***	***R2***	***R3***	***Farm1***	***Farm2***
Pre		0.011	0.008	0.394	0.486	0.443	0.049	**0.001**	**0.001**
F1	0.0076		0.445	0.247	0.350	0.279	0.090	0.002	0.007
F2	0.0063	0.0000		0.197	0.058	0.454	0.002	**0.001**	**0.001**
W	0.0001	0.0021	0.0021		0.429	0.459	0.479	**0.001**	**0.001**
R1	0.0000	0.0016	0.0065	0.0000		0.450	0.473	**0.001**	**0.001**
R2	0.0000	0.0030	0.0000	0.0000	0.0000		0.129	**0.001**	**0.001**
R3	0.0019	0.0045	0.0083	0.0000	0.0000	0.0037		**0.001**	**0.001**
Farm1	0.0192	0.0147	0.0137	0.0157	0.0149	0.0220	0.0155		**0.001**
Farm2	0.0176	0.0096	0.0189	0.0126	0.0107	0.0150	0.0140	0.0180	

Table 8 APPENDIX. DAPC 4 clusters. 90 retained PCs. Samples *Pre* (n = 192), *Mid* = *F1* (n = 27), *F2* (n = 37), *W* (n = 50), *R1* (n = 28), *R2* (n = 23), *R3* (n = 79), *Farm1* (n = 96) and *Farm2* (n = 192).

	1	2	3	4
Pre	5	69	54	53
Mid	28	64	48	69
Farm1	30	15	12	31
Farm2	56	23	43	37

Doctoral theses in Biology
Norwegian University of Science and Technology
Department of Biology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos Zoology	Breeding events of birds in relation to spring temperature and environmental phenology
1978	Egil Sakshaug	Dr. philos Botany	"The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton"
1980	Arnfinn Langeland	Dr. philos Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation
1984	Eivin Røskaft	Dr. philos Zoology	Sociobiological studies of the rook *Corvus frugilegus*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos Zoology	Biochemical genetic studies in fish
1985	John Solem	Dr. philos Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains
1985	Randi E. Reinertsen	Dr. philos Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds
1986	Bernt-Erik Sæther	Dr. philos Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach
1986	Torleif Holthe	Dr. philos Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna
1987	Helene Lampe	Dr. scient Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires
1987	Olav Hogstad	Dr. philos Zoology	Winter survival strategies of the Willow tit *Parus montanus*
1987	Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway
1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*

1987	Bjørn Åge Tømmerås	Dr. scient Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction
1988	Hans Christian Pedersen	Dr. philos Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care
1988	Tor G. Heggberget	Dr. philos Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure
1988	Marianne V. Nielsen	Dr. scient Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*)
1988	Ole Kristian Berg	Dr. scient Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.)
1989	John W. Jensen	Dr. philos Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth
1989	Helga J. Vivås	Dr. scient Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*
1989	Reidar Andersen	Dr. scient Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture
1990	Bengt Finstad	Dr. scient Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season
1990	Hege Johannesen	Dr. scient Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmion (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams
1990	Tor Jørgen Almaas	Dr. scient Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues
1990	Magne Husby	Dr. scient Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*
1991	Tor Kvam	Dr. scient Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway
1991	Jan Henning L'Abêe Lund	Dr. philos Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular
1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991	Trond Nordtug	Dr. scient Zoology	Reflectometric studies of photomechanical adaptation in superposition eyes of arthropods
1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway
1991	Odd Terje Sandlund	Dr. philos Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism

1991	Nina Jonsson	Dr. philos Zoology	Aspects of migration and spawning in salmonids
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks
1992	Arne Vollan Aarset	Dr. philos Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient Zoology	Habitat shifts in coregonids.
1993	Yngvar Asbjørn Olsen	Dr. scient Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels ans some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993	Kjetil Bevanger	Dr. scient Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Botany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)
1994	Inga Elise Bruteig	Dr. scient Botany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses
1994	Morten Bakken	Dr. scient Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*
1994	Arne Moksnes	Dr. philos Zoology	Host adaptations towards brood parasitism by the Cockoo

1994	Solveig Bakken	Dr. scient Botany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1994	Torbjørn Forseth	Dr. scient Zoology	Bioenergetics in ecological and life history studies of fishes.
1995	Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions
1995	Hanne Christensen	Dr. scient Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*
1995	Svein Håkon Lorentsen	Dr. scient Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition
1995	Chris Jørgen Jensen	Dr. scient Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995	Martha Kold Bakkevig	Dr. scient Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport
1995	Vidar Moen	Dr. scient Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations
1995	Hans Haavardsholm Blom	Dr. philos Botany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden
1996	Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae
1996	Ola Ugedal	Dr. scient Zoology	Radiocesium turnover in freshwater fishes
1996	Ingibjørg Einarsdottir	Dr. scient Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines
1996	Christina M. S. Pereira	Dr. scient Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation
1996	Jan Fredrik Børseth	Dr. scient Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics
1996	Gunnar Henriksen	Dr. scient Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region
1997	Gunvor Øie	Dr. scient Botany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae
1997	Håkon Holien	Dr. scient Botany	Studies of lichens in spurce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters
1997	Ole Reitan	Dr. scient Zoology	Responses of birds to habitat disturbance due to damming
1997	Jon Arne Grøttum	Dr. scient Zoology	Physiological effects of reduced water quality on fish in aquaculture
1997	Per Gustav Thingstad	Dr. scient Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher
1997	Torgeir Nygård	Dr. scient Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as
1997	Signe Nybø	Dr. scient Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway

1997	Atle Wibe	Dr. scient Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry
1997	Rolv Lundheim	Dr. scient Zoology	Adaptive and incidental biological ice nucleators
1997	Arild Magne Landa	Dr. scient Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation
1997	Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*
1997	Jarle Tufto	Dr. scient Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997	Trygve Hesthagen	Dr. philos Zoology	Population responces of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997	Trygve Sigholt	Dr. philos Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997	Jan Østnes	Dr. scient Zoology	Cold sensation in adult and neonate birds
1998	Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins
1998	Thor Harald Ringsby	Dr. scient Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998	Erling Johan Solberg	Dr. scient Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998	Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity
1998	Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro
1998	Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach
1998	Bente Gunnveig Berg	Dr. scient Zoology	Encoding of pheromone information in two related moth species
1999	Kristian Overskaug	Dr. scient Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999	Hans Kristen Stenøien	Dr. scient Botany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999	Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway
1999	Ingvar Stenberg	Dr. scient Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999	Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis
1999	Trina Falck Galloway	Dr. scient Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)

1999	Marianne Giæver	Dr. scient Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gradus morhua*) in the North-East Atlantic
1999	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*
1999	Ingrid Bysveen Mjølnerød	Dr. scient Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999	Stein-Are Sæther	Dr. philos Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999	Katrine Wangen Rustad	Dr. scient Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999	Per Terje Smiseth	Dr. scient Zoology	Social evolution in monogamous families:
1999	Gunnbjørn Bremset	Dr. scient Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999	Frode Ødegaard	Dr. scient Zoology	Host spesificity as parameter in estimates of arhrophod species richness
1999	Sonja Andersen	Dr. scient Zoology	Expressional and functional analyses of human, secretory phospholipase A2
2000	Ingrid Salvesen	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000	Ingar Jostein Øien	Dr. scient Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000	Pavlos Makridis	Dr. scient Botany	Methods for the microbial econtrol of live food used for the rearing of marine fish larvae
2000	Sigbjørn Stokke	Dr. scient Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000	Odd A. Gulseth	Dr. philos Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000	Pål A. Olsvik	Dr. scient Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000	Sigurd Einum	Dr. scient Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001	Jan Ove Evjemo	Dr. scient Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species
2001	Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forset systems
2001	Ingebrigt Uglem	Dr. scient Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001	Bård Gunnar Stokke	Dr. scient Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient Zoology	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)

2002	Mariann Sandsund	Dr. scient Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr. philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and the Ral GTPase from *Drosophila melanogaster*
2002	Henrik Jensen	Dr. scient Biology	Causes and consequenses of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr. scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr. scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr. scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr. scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr. scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo Salar* L.) parr and smolt
2004	Torkild Bakken	Dr. scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)
2004	Ingar Pareliussen	Dr. scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar
2004	Tore Brembu	Dr. scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr. scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities

2004	Hanne T. Skiri	Dr. scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*)
2004	Lene Østby	Dr. scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr. scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr. scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr. scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr. scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr. scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr. scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	ph.d Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr. scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr. scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyrid hormone and vitamin A concentrations
2005	Christian Westad	Dr. scient Biology	Motor control of the upper trapezius
2005	Lasse Mork Olsen	ph.d Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	ph.d Biology	Implications of mate choice for the management of small populations
2005	Ariaya Hymete Sahle Dingle	ph.d Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	ph.d Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	ph.d Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana
2005	Kjartan Østbye	Dr. scient Biology	The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation
2006	Kari Mette Murvoll	ph.d Biology	Levels and effects of persistent organic pollutans (POPs) in seabirds, Retinoids and α-tocopherol – potential biomakers of POPs in birds?
2006	Ivar Herfindal	Dr. scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006	Nils Egil Tokle	ph.d Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus*
2006	Jan Ove Gjershaug	Dr. philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia

2006	Jon Kristian Skei	Dr. scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006	Johanna Järnegren	ph.d Biology	Acesta Oophaga and Acesta Excavata – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	ph.d Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	ph.d Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	ph.d Biology	Wildlife conservation and local land use conflicts in western Serengeti, Corridor Tanzania
2006	Anna Maria Billing	ph.d Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	ph.d Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	ph.d Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	ph.d Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	ph.d Biology	Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor* - a study on possible competition for the semi-essential amino acid cysteine
2007	Kasper Hancke	ph.d Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	ph.d Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	ph.d Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	ph.d Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, (*Mamestra brassicae* L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007	Snorre Henriksen	ph.d Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	ph.d Biology	Spatial Ecology of Wolverines in Scandinavia
2007	Vedasto Gabriel Ndibalema	ph.d Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania
2007	Julius William Nyahongo	ph.d Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania
2007	Shombe Ntaraluka Hassan	ph.d Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	ph.d Biology	Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning
2007	Anne Skjetne Mortensen	ph.d Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008	Brage Bremset Hansen	ph.d Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem

2008	Jiska van Dijk	ph.d Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	ph.d Biology	The ecology and behaviour of the Masai Ostrich (Struthio camelus massaicus) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	ph.d Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, (*Taeniopygia guttata*)
2008	Sølvi Wehn	ph.d Biology	Biodiversity dynamics in semi-natural mountain landscapes - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	ph.d Biology	"The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations"
2008	Katarina Mariann Jørgensen	Dr. scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008	Tommy Jørstad	ph.d Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	ph.d Biology	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	ph.d Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	ph.d Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania
2008	Ragnhild Lyngved	ph.d Biology	Somatic embryogenesis in *Cyclamen persicum.* Biological investigations and educational aspects of cloning
2008	Line Elisabeth Sundt-Hansen	ph.d Biology	Cost of rapid growth in salmonid fishes
2008	Line Johansen	ph.d Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009	Astrid Jullumstrø Feuerherm	ph.d Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease
2009	Pål Kvello	ph.d Biology	Neurons forming the network involved in gustatory coding and learning in the moth *Heliothis virescens:* Physiological and morphological characterisation, and integration into a standard brain atlas
2009	Trygve Devold Kjellsen	ph.d Biology	Extreme Frost Tolerance in Boreal Conifers
2009	Johan Reinert Vikan	ph.d Biology	Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches
2009	Zsolt Volent	ph.d Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009	Lester Rocha	ph.d Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009	Dennis Ikanda	ph.d Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania
2010	Huy Quang Nguyen	ph.d Biology	Egg characteristics and development of larval digestive function of cobia (*Rachycentron canadum*) in response to dietary treatments - Focus on formulated diets
2010	Eli Kvingedal	ph.d Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype

2010	Sverre Lundemo	ph.d Biology	Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe
2010	Iddi Mihijai Mfunda	ph.d Biology	Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. Tha Case of Serengeti Ecosystem, Tanzania
2010	Anton Tinchov Antonov	ph.d Biology	Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis
2010	Anders Lyngstad	ph.d Biology	Population Ecology of *Eriophorum latifolium*, a Clonal Species in Rich Fen Vegetation
2010	Hilde Færevik	ph.d Biology	Impact of protective clothing on thermal and cognitive responses
2010	Ingerid Brænne Arbo	ph.d Medical technology	Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans
2010	Yngvild Vindenes	ph.d Biology	Stochastic modeling of finite populations with individual heterogeneity in vital parameters
2010	Hans-Richard Brattbakk	ph.d Medical technology	The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits
2011	Geir Hysing Bolstad	ph.d Biology	Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy
2011	Karen de Jong	ph.d Biology	Operational sex ratio and reproductive behaviour in the two-spotted goby (*Gobiusculus flavescens*)
2011	Ann-Iren Kittang	ph.d Biology	*Arabidopsis thaliana* L. adaptation mechanisms to microgravity through the EMCS MULTIGEN-2 experiment on the ISS:– The science of space experiment integration and adaptation to simulated microgravity
2011	Aline Magdalena Lee	ph.d Biology	Stochastic modeling of mating systems and their effect on population dynamics and genetics
2011	Christopher Gravningen Sørmo	ph.d Biology	Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in Arabidopsis thaliana
2011	Grethe Robertsen	ph.d Biology	Relative performance of salmonid phenotypes across environments and competitive intensities
2011	Line-Kristin Larsen	ph.d Biology	Life-history trait dynamics in experimental populations of guppy (*Poecilia reticulata*): the role of breeding regime and captive environment
2011	Maxim A. K. Teichert	ph.d Biology	Regulation in Atlantic salmon (*Salmo salar*): The interaction between habitat and density
2011	Torunn Beate Hancke	ph.d Biology	Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology
2011	Sajeda Begum	ph.d Biology	Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh
2011	Kari J. K. Attramadal	ph.d Biology	Water treatment as an approach to increase microbial control in the culture of cold water marine larvae
2011	Camilla Kalvatn Egset	ph.d Biology	The Evolvability of Static Allometry: A Case Study
2011	AHM Raihan Sarker	ph.d Biology	Conflict over the conservation of the Asian elephant (*Elephas maximus*) in Bangladesh
2011	Gro Dehli Villanger	ph.d Biology	Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals
2011	Kari Bjørneraas	ph.d Biology	Spatiotemporal variation in resource utilisation by a large herbivore, the moose

2011	John Odden	ph.d Biology	The ecology of a conflict: Eurasian lynx depredation on domestic sheep
2011	Simen Pedersen	ph.d Biology	Effects of native and introduced cervids on small mammals and birds
2011	Mohsen Falahati-Anbaran	ph.d Biology	Evolutionary consequences of seed banks and seed dispersal in *Arabidopsis*
2012	Jakob Hønborg Hansen	ph.d Biology	Shift work in the offshore vessel fleet: circadian rhythms and cognitive performance
2012	Elin Noreen	ph.d Biology	Consequences of diet quality and age on life-history traits in a small passerine bird
2012	Irja Ida Ratikainen	ph.d Biology	Theoretical and empirical approaches to studying foraging decisions: the past and future of behavioural ecology
2012	Aleksander Handå	ph.d Biology	Cultivation of mussels (*Mytilus edulis):*Feed requirements, storage and integration with salmon (*Salmo salar*) farming
2012	Morten Kraabøl	ph.d Biology	Reproductive and migratory challenges inflicted on migrant brown trour (*Salmo trutta* L) in a heavily modified river
2012	Jisca Huisman	ph.d Biology	Gene flow and natural selection in Atlantic salmon
	Maria Bergvik	ph.d Biology	Lipid and astaxanthin contents and biochemical post-harvest stability in *Calanus finmarchicus*
2012	Bjarte Bye Løfaldli	ph.d Biology	Functional and morphological characterization of central olfactory neurons in the model insect *Heliothis virescens*.
2012	Karen Marie Hammer	ph.d Biology	Acid-base regulation and metabolite responses in shallow- and deep-living marine invertebrates during environmental hypercapnia
2012	Øystein Nordrum Wiggen	ph.d Biology	Optimal performance in the cold
2012	Robert Dominikus Fyumagwa	Dr. Philos Biology	Anthropogenic and natural influence on disease prevalence at the human –livestock-wildlife interface in the Serengeti ecosystem, Tanzania
2012	Jenny Bytingsvik	ph.d Biology	Organohalogenated contaminants (OHCs) in polar bear mother-cub pairs from Svalbard, Norway. Maternal transfer, exposure assessment and thyroid hormone disruptive effects in polar bear cubs
2012	Christer Moe Rolandsen	ph.d Biology	The ecological significance of space use and movement patterns of moose in a variable environment
2012	Erlend Kjeldsberg Hovland	ph.d Biology	Bio-optics and Ecology in *Emiliania huxleyi* Blooms: Field and Remote Sensing Studies in Norwegian Waters
2012	Lise Cats Myhre	ph.d Biology	Effects of the social and physical environment on mating behaviour in a marine fish
2012	Tonje Aronsen	ph.d Biology	Demographic, environmental and evolutionary aspects of sexual selection
	Bin Liu	ph.d Biology	Molecular genetic investigation of cell separation and cell death regulation in *Arabidopsis thaliana*
2013	Jørgen Rosvold	ph.d Biology	Ungulates in a dynamic and increasingly human dominated landscape – A millennia-scale perspective
2013	Pankaj Barah	ph.d Biology	Integrated Systems Approaches to Study Plant Stress Responses
2013	Marit Linnerud	ph.d Biology	Patterns in spatial and temporal variation in population abundances of vertebrates
2013	Xinxin Wang	ph.d Biology	Integrated multi-trophic aquaculture driven by nutrient wastes released from Atlantic salmon (*Salmo salar*) farming
2013	Ingrid Ertshus Mathisen	ph.d Biology	Structure, dynamics, and regeneration capacity at the sub-arctic forest-tundra ecotone of northern Norway and Kola Peninsula, NW Russia

2013	Anders Foldvik	ph.d Biology	Spatial distributions and productivity in salmonid populations
2013	Anna Marie Holand	ph.d Biology	Statistical methods for estimating intra- and inter-population variation in genetic diversity
2013	Anna Solvang Båtnes	ph.d Biology	Light in the dark – the role of irradiance in the high Arctic marine ecosystem during polar night
2013	Sebastian Wacker	ph.d Biology	The dynamics of sexual selection: effects of OSR, density and resource competition in a fish
2013	Cecilie Miljeteig	ph.d Biology	Phototaxis in *Calanus finmarchicus* – light sensitivity and the influence of energy reserves and oil exposure
2013	Ane Kjersti Vie	ph.d Biology	Molecular and functional characterisation of the IDA family of signalling peptides in *Arabidopsis thaliana*
2013	Marianne Nymark	ph.d Biology	Light responses in the marine diatom *Phaeodactylum tricornutum*
2014	Jannik Schultner	ph.d Biology	Resource Allocation under Stress - Mechanisms and Strategies in a Long-Lived Bird
2014	Craig Ryan Jackson	ph.d Biology	Factors influencing African wild dog *(Lycaon pictus*) habitat selection and ranging behaviour: conservation and management implications
2014	Aravind Venkatesan	ph.d Biology	Application of Semantic Web Technology to establish knowledge management and discovery in the Life Sciences
2014	Kristin Collier Valle	ph.d Biology	Photoacclimation mechanisms and light responses in marine micro- and macroalgae
2014	Michael Puffer	ph.d Biology	Effects of rapidly fluctuating water levels on juvenile Atlantic salmon (*Salmo salar* L.)
2014	Gundula S. Bartzke	ph.d Biology	Effects of power lines on moose *(Alces alces)* habitat selection, movements and feeding activity
2014	Eirin Marie Bjørkvoll	ph.d Biology	Life-history variation and stochastic population dynamics in vertebrates
2014	Håkon Holand	ph.d Biology	The parasite *Syngamus trachea* in a metapopulation of house sparrows
2014	Randi Magnus Sommerfelt	ph.d Biology	Molecular mechanisms of inflammation – a central role for cytosolic phospholiphase A2
2014	Espen Lie Dahl	ph.d Biology	Population demographics in white-tailed eagle at an on-shore wind farm area in coastal Norway
2014	Anders Øverby	ph.d Biology	Functional analysis of the action of plant isothiocyanates: cellular mechanisms and in vivo role in plants, and anticancer activity
2014	Kamal Prasad Acharya	ph.d Biology	Invasive species: Genetics, characteristics and trait variation along a latitudinal gradient.
2014	Ida Beathe Øverjordet	ph.d Biology	Element accumulation and oxidative stress variables in Arctic pelagic food chains: Calanus, little auks (alle alle) and black-legged kittiwakes (Rissa tridactyla)
2014	Kristin Møller Gabrielsen	ph.d Biology	Target tissue toxicity of the thyroid hormone system in two species of arctic mammals carrying high loads of organohalogen contaminants
2015	Gine Roll Skjervø	dr. philos Biology	Testing behavioral ecology models with historical individual-based human demographic data from Norway
2015	Nils Erik Gustaf Forsberg	ph.d Biology	Spatial and Temporal Genetic Structure in Landrace Cereals
2015	Leila Alipanah	ph.d Biology	Integrated analyses of nitrogen and phosphorus deprivation in the diatoms *Phaeodactylum tricornutum* and *Seminavis robusta*
2015	Javad Najafi	ph.d Biology	Molecular investigation of signaling components in sugar sensing and defense in *Arabidopsis thaliana*

2015	Bjørnar Sporsheim	ph.d Biology	Quantitative confocal laser scanning microscopy: optimization of in vivo and in vitro analysis of intracellular transport
2015	Magni Olsen Kyrkjeeide	ph.d Biology	Genetic variation and structure in peatmosses (*Sphagnum*)
2015	Keshuai Li	ph.d Biology	Phospholipids in Atlantic cod (*Gadus morhua* L.) larvae rearing: Incorporation of DHA in live feed and larval phospholipids and the metabolic capabilities of larvae for the de novo synthesis
2015	Ingvild Fladvad Størdal	ph.d Biology	The role of the copepod *Calanus finmarchicus* in affecting the fate of marine oil spills
2016	Thomas Kvalnes	ph.d Biology	Evolution by natural selection in age-structured populations in fluctuating environments
2016	Øystein Leiknes	ph.d Biology	The effect of nutrition on important life-history traits in the marine copepod *Calanus finmarchicus*
2016	Johan Henrik Hårdensson Berntsen	ph.d Biology	Individual variation in survival: The effect of incubation temperature on the rate of physiological ageing in a small passerine bird
2016	Marianne Opsahl Olufsen	ph.d Biology	Multiple environmental stressors: Biological interactions between parameters of climate change and perfluorinated alkyl substances in fish