

**Crossing barriers:**  
Genetic consequences of translocating  
wild cleaner fish for aquaculture

**Ellika Faust**  
Doctoral Thesis



UNIVERSITY OF GOTHENBURG

Department of Marine sciences  
Faculty of Science

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Crossing barriers: Genetic consequences of translocating wild cleaner fish for aquaculture

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*To my wife,  
for keeping me grounded and  
always reminding me of what is important*

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## ABSTRACT

Several species of fish show a symbiotic cleaning behaviour, where they assist other species by feeding and removing dead skin or ectoparasites. Some of these cleaner fish species are used as a low-cost parasite control in salmon aquaculture, often considered to be more environmentally friendly than other delousing methods. Approximately 54 million cleaner fish are annually used in Norwegian salmon farms. This has resulted in an increasing fishing pressure on wild cleaner fish populations. Together with long-distance translocation, this raises concerns of potential overfishing, anthropogenic introductions, and hybridisation.

Recently, increasing numbers of corksaw wrasse (*Symphodus melops*), have been reported in mid Norway, north of its described distribution range. This is an area heavily relying on the import and translocation of cleaner fish from Skagerrak-Kattegat, more than 1000 km away. Using genetic markers, I show that the new population is both a result of a northward range expansion, as well as a translocation of individuals from southern populations. Further investigation revealed that escapees and hybrids may constitute up to 20 % of the populations at the northern edge of the species distribution. In contrast, escapees and hybrids are rare in other parts along the Norwegian west coast, where salmon farming is also common. The set of genetic markers has a high power to detect escapees and hybrids, and can be applied to monitoring of wild populations.

Investigations of corksaw wrasse in Skagerrak-Kattegat revealed reduced genetic diversity, a weak pattern of isolation by distance, and surprisingly little population structure. This suggests a very recent colonisation and high connectivity among sites in this region. Along the Swedish west coast, I evaluate the potential effects of the newly established wrasse fishery on local ecosystems and populations, and provide a baseline to support ecosystem-based management for wrasse fisheries in Sweden. I also investigate the genetic population structure of lumpfish (*Cyclopterus lumpus*) across the Atlantic, which is the most commonly used cleaner fish species. I show that there is a lot more structure than previously known, and identify eight genetically divergent regions. Hierarchical analysis of these regions demonstrates additional local and cryptic substructure. The genetic markers used here can also be applied for management of wild lumpfish populations and the identification of population origin.

Overall, these findings provide important information both for aquaculture management and conservation of wild populations, and have implications for the increasing use and translocation of cleaner fish for parasite control in aquaculture. Moving genetic material between isolated populations could drastically alter the genetic composition and erode population structure, potentially resulting in loss of local adaptation and hampering natural range expansion. Although the ecological and evolutionary significance of escapees warrants further investigation, these results should be taken into consideration in the use of translocated cleaner fish.

**Keywords:** conservation genetics, population genetics, aquaculture escapee, hybridisation, corksling wrasse, lumpfish, cleaner fish translocation, sea lice, range expansion, fishery management

## POPULÄRVETENSKAPLIG SAMMANFATTNING

Det finns flera fiskarter som lever i symbios med andra fiskar och därmed drar nytta av varandra. Till exempel finns fiskarter som får sin föda genom att rengöra andra arter från död hud och parasiter. De fiskarter som har ett sådant rengöringsbeteende kallas ofta putsarfiskar. Vissa arter av putsarfisk, framförallt snultror och sjurygg, används för parasitbekämpning i laxodlingar. Där äter de laxlus, som annars skulle orsaka stora problem för laxen. I slutet av 2000-talet ökade efterfrågan på putsarfisk, eftersom laxlusen började bli resistent mot kemiska bekämpningsmedel. Sedan 2010 har användningen av putsarfiskar i norsk laxodling ökat exponentiellt: Nu används cirka 50 miljoner putsarfiskar inom norsk laxodling varje år, fördelat på ca 30 miljoner odlade och ca 20 miljoner fångade. Många putsarfiskar fångas i områden långt ifrån de laxodlingar där de används. Bland annat fiskas mycket snultror i Skagerrak, för att sedan transporteras levande i tankbilar till odlingar på norska västkusten, där lokala populationer antingen saknas, eller inte är tillräckligt stora för att kunna möta efterfrågan.

Under de senaste åren har norska fiskare sett att skärsnultror, en av de mest använda arterna av putsarfisk, har etablerat sig i nya områden norr om deras normala utbredning. Eftersom det finns många laxodlingar i området, uppstod frågan om det kunde vara ett resultat av att förflyttade fiskar hade rymt från odlingarna. Genetiska analyser visar att ett nytt bestånd av skärsnultra i Flatanger, norr om Trondheim, verkar vara ett resultat av att arten har börjat expandera norrut, men också av att förflyttade individer rymt från laxodlingar och börjat beblanda sig med lokala bestånd. Ytterligare studier visar att rymlingar och hybrider i vissa fall kan utgöra upp till 20 % av de lokala bestånden i de nordligaste områdena. Däremot är rymlingar och hybrider inte lika vanliga längs andra delar av den norska västkusten, även om laxodlingar också finns i dessa områden. De genetiska markörer som tagits fram i den här studien har en god förmåga att upptäcka både rymlingar och hybrider, och kan därmed användas för framtida övervakning av vilda bestånd.

Vidare studier visar att skärsnultror i Skagerrak-Kattegatt är genetiskt väldigt lika varandra, men har store genetiska skillnader mot fiskar på norska västkusten. Detta kan bero på att skärsnultrorna nyligen koloniserade Skagerrak-Kattegatt. Troligtvis är det också ett resultat av migration och genflöde mellan olika bestånd i området. Därför är det omöjligt att skilja på en skärsnultra från till exempel norska sydkusten och svenska västkusten. I den här avhandlingen utvärderar jag även det nyetablerade fisket av snultror på den svenska västkusten och dess möjliga effekter på ekosystemet. Genom att studera den genetiska variationen i bestånden, och snultrornas funktion i ekosystemet, får vi bättre kunskap för att utveckla en ekosystembaserad förvaltning.

Utöver snultror undersöker jag genetiska skillnader hos sjurygg, ofta kallad stenbit, i norra Atlanten. Sjurygg är den vanligaste arten av putsarfisk inom norsk laxodling, men används

även i till exempel Kanada och Storbritannien. Genetiska analyser visar att det finns många fler genetiskt skilda bestånd än vad man tidigare trott. Totalt kan vi identifiera åtta separata bestånd av sjurygg globalt. Mer detaljerad analys av de globala bestånden visar dessutom att olika geografiska områden kan ha flera lokala bestånd. Detta kan vara ett tecken på att sjurygg återvänder till samma områden på kusten när de leker. De genetiska markörer som vi tagit fram i denna studie kan användas i förvaltning av vilda sjuryggsbestånd, men även för att spåra enskilda individers ursprung.

När putsarfiskar med ett annat geografiskt ursprung rymmer och fortplantar sig med lokala bestånd, kan det få både genetiska och ekologiska konsekvenser. Till exempel kan nya genvarianter som är sämre anpassade till den lokala miljön, introduceras i det lokala beståndet. Evolutionära anpassningar, som tagit lång tid att utveckla, kan därmed snabbt gå förlorade. Rymlingarna kan också påverka andra arter genom ökad konkurrens om föda och boplatser, eller introducera nya sjukdomar och parasiter. Det är ett välkänt problem att fisk som rymmer från odlingar kan ha stora effekter på vilda bestånd. För att kunna bedöma risken med förrymda putsarfiskar är det viktigt att känna till ursprung av fisken. Resultaten i den här avhandlingen bidrar med viktig information om biologin och genetiskt skilda bestånd hos olika arter av putsarfisk för förvaltning av vilda bestånd. Även om ekologiska och evolutionära konsekvenser av förrymda putsarfiskar behöver utredas vidare, så bör resultaten från dessa studier beaktas i framtida användande av putsarfisk.

## LIST OF PAPERS

This thesis is based on the following papers, which are referred to by their roman numerals:

- Paper I**      **Faust, E.**, Halvorsen, K.T., Andersen, P., Knutsen, H. & André, C. (2018). Cleaner fish escape salmon farms and hybridize with local wrasse populations. *Royal Society Open Science* 5, 171752. <https://doi.org/10.1098/rsos.171752>
- Paper II**      **Faust, E.\***, Jansson, E.\*, André, C., Halvorsen, K.T., Dahle, G., Knutsen, H., Quintela, M. & Glover, K.A. (2021). Not that clean: Aquaculture-mediated translocation of cleaner fish has led to hybridization on the northern edge of the species' range. *Evolutionary Applications*, 14(6), 1572–1497. <https://doi.org/10.1111/eva.13220>
- Paper III**     Bourlat, S.J., **Faust, E.**, Wennhage, H., Wikström, A., Rigby, K., Vigo, M., Kraly, P., Selander, E. & André, C. (2021). Wrasse fishery on the Swedish West Coast: Towards ecosystem-based management. *ICES Journal of Marine Science*, 78, 1386-1397. <https://doi.org/10.1093/icesjms/fsaa249>
- Paper IV**     **Faust, E.**, Halvorsen, K.T., Jorde, P.E., Knutsen, H., Mattingsdal, M., Selander, E., Strand, Å., Wennhage, H., André, C. & De Wit, P. High genetic connectivity in an otherwise highly structured marine fish. [*Manuscript*]
- Paper V**      Jansson, E.\* , **Faust, E.\***, Bekkevold, D., Quintela, M., Durif, C., Halvorsen, K.T., Dahle, G., Pampoulie, C.S., Kennedy, J., Whittaker, B., Unneland, L., André, C. & Glover, K.A. (2022). Global, regional, and cryptic genetic population structure in a high gene flow transatlantic fish. *bioRxiv*. <https://doi.org/10.1101/2022.03.22.485384>

\* Shared first author

## MY CONTRIBUTION

I: Took part in planning and sampling. Processed and analysed all genomic samples. Performed statistical analysis of genomic data and interpreted results. Took the lead in writing the manuscript.

II: Took part in planning and sampling. Processed and analysed genomic samples and identified genomic markers for genotyping. Took part in genotyping. Performed statistical analysis and interpretation of results. Took the lead in writing the manuscript.

III: Took part in planning and sampling. Processed fish in the field during fish survey, and gut content for metabarcoding in the lab. Took part in the interpretation of the results and writing the manuscript.

IV: Took part in planning and sampling. Processed all genomic samples. Performed statistical analysis of genomic data and interpreted results. Took the lead in writing the manuscript.

V: Took part in planning and sampling. Processed and analysed genomic samples and identified genomic markers for genotyping. Took part in statistical analysis, interpretation of the results and drafting the manuscript.

## OTHER PUBLICATIONS NOT IN THIS THESIS

**Faust, E.**, André, C., Meurling, S., Kochmann, J., Christiansen, H., Jensen, L.F., Charrier, G., Laugen, A.T. & Strand, Å. (2017). Origin and route of establishment of the invasive Pacific oyster *Crassostrea gigas* in Scandinavia. *Marine Ecology Progress Series*, 575, 95–105. <https://doi.org/10.3354/meps12219>

Seljestad, G.W., Quintela, M., **Faust, E.**, Halvorsen, K.T., Besnier, F., Jansson, E., Dahle, G., Knutsen, H., André, C., Folkvord, A. & Glover, K.A. (2020). “A cleaner break”: Genetic divergence between geographic groups and sympatric phenotypes revealed in ballan wrasse (*Labrus bergylta*). *Ecology and Evolution*, 10(12), 6120–6135. <https://doi.org/10.1002/ece3.6404>

Green, L., Apostolou, A., **Faust, E.**, Palmqvist, K., Behrens, J.W., Havenhand, J.N., Leder, E.H. & Kvarnemo, C. (2021). Ancestral Sperm Ecotypes Reveal Multiple Invasions of a Non-Native Fish in Northern Europe. *Cells*, 10(7), 1743. <https://doi.org/10.3390/cells10071743>

Tallaksen Halvorsen, K., Skiftesvik, A.B., Durif, C., **Faust, E.**, Wennhage, H., André, C., ... Mortensen, S. (2021). Towards a sustainable fishery and use of cleaner fish in salmonid aquaculture. <https://doi.org/10.6027/temanord2021-545>

Green, L., **Faust, F.**, Hinchcliffe, J., Brijs, J., Holmes, A., Englund Örn, F., Svensson, O., Roques, J.A.C., Leder, E.H., Sandblom, E. & Kvarnemo, C. Invader at the edge - genomic origins and physiological differences of round gobies across a steep urban salinity gradient. *[In revision]*

Rieder, J., Jahnke, M., André, C., Christiansen, H., De Wit, P., **Faust, E.**, Green, L., Jonsson P.R., Laikre, L., Laugen A.T., Rafajlovic, M., Sandström, A., Tomasini, M. & Volckaert, F.A.M. Seascape genomics: improving marine biodiversity management by combining genetic knowledge with environmental and ecological information. *[Submitted]*

# INTRODUCTION

## BIOLOGICAL INVASION

Biological invasions have been highlighted as a global threat to biodiversity and biological communities in the marine environment, and is seen as one of the top conservation concerns (Molnar et al., 2008; IPCC, 2019). A biological invasion can be defined as the processes where organisms are introduced to a region beyond their native range and establishes a viable population, often with negative consequences for the local ecosystem (Ricciardi, 2013). Traditionally more attention has been focused on introduction of non-indigenous species or genetically modified organisms. However, introductions of non-native populations, which are genetically divergent from local populations, and captive-bred populations are far more common (Laikre et al., 2010). The invasion process comprises a sequence of events: 1) Arrival: The invading population have to overcome existing barriers and travel to a new region/environment. 2) Survival: Once having arrived, they need to survive, dealing with potential environmental mismatch or biotic resistance. 3) Reproduction: In order to establish, there has to be a high enough density of individuals for them to be able to reproduce, and offspring have to survive in the new environment. 4) Persistence: The population needs to become viable and self-sustaining, overcoming e.g., low genetic diversity. 5) Spread: Dispersal outside the point of introduction, establishing a new species range (Nentwig, 2008; Rius & Darling, 2014).

There are two main ways species and populations can spread to a new environment: with natural dispersal or with human-mediated dispersal, either directly or indirectly. Natural dispersal is normally limited by multiple barriers, among which geographical barriers may be the most evident. Humans have made it possible for species and populations to overcome many such barriers, either deliberately, e.g., for domestication or recreational hunting (Laikre et al., 2010), or accidentally, e.g., as stowaways in ballast water or escaping from aquaculture (Nentwig, 2008). Anthropogenic introductions have accelerated exponentially during the past millennium due to human activities, such as shipping, trading and aquaculture (Nunes et al., 2014). Traits such as high reproduction and dispersal rate is often considered important for organisms to be able to successfully colonise a new area. These two characteristics are common in many marine species, which can produce thousands of small larval offspring, and often have a high ability to disperse at one or multiple life stages (Kinlan et al., 2005). An inventory in 2013 found a total of 1 369 marine non-indigenous species had so far been reported along the European coast (Katsanevakis et al., 2013), a number that is likely to increase (Sardain et al., 2019).

Moving organisms outside their natural boundaries can lead to many potential problems and can have a diverse range of ecological, genetic, pathogenic and socio-economic impacts (Atalah & Sanchez-Jerez, 2020). Even if a species is already present, introduced populations may not be ecologically equivalent to native populations. These newcomers may vary strongly in their ecological impacts compared to the pre-existing population, for example through differences in prey consumption (Evangelista, Cucherousset, & Lecerf 2019). A successful

invader can affect the whole ecosystem, for example by altering local food webs or community structure, through competition, predation or even by changing the abiotic environment (Crooks, 2002). One incredibly successful invader is the pacific oyster (*Crassostrea gigas*), which is native to northeast Asia, but has been introduced worldwide for aquaculture purposes (Herbert et al., 2016). Pacific oysters are able to completely alter the abiotic environment they settle in by creating hard, and often large, reef like structures. By changing the environment, from what was once often a soft sandy bottom, they allow the potential for other organisms to move in and settle (Troost, 2010). Many introduced organisms do not arrive alone, but bring a number of hitchhikers, such as bacterial, viral, and parasitic disease agents (Korsnes et al., 2017). Although some of these might already exist in the environment, others will be novel and can quickly spread throughout the local ecosystem, potentially affecting local populations which have not been able to create any form of resistance (Tepolt et al., 2020). Just one example is the introduction of the rinderpest virus into sub-Saharan Africa. The virus, which was transmitted through domestic cattle, decimated native ungulates (McCallum & Dobson, 1995).

## ANTHROPOGENIC HYBRIDISATION

An important, but often understudied effect of anthropogenic introductions, is hybridisation between populations or species with incomplete reproductive isolation, i.e., anthropogenic hybridisation (McFarlane & Pemberton, 2019). The outcomes of such anthropogenic hybridisation depend on the levels of reproductive isolation between taxa. For instance, the introduced brook trout and the native bull trout in North America are known to interbreed, but first-generation hybrids are effectively sterile (Leary et al., 1993). Consequently, the wasted reproduction effort of hybridizing with the introduced brook trout, has led to the rapid displacement of the native bull trout. In taxa that are less reproductively isolated, e.g., populations of the same species or more recently separated species, genomes can sometimes recombine, and allele combinations can introgress from one or both taxa into the gene pool of the other (Largiadèr, 2007; Harrison & Larson, 2014). This introgression can be symmetric, with backcrossing into both gene pools, or asymmetric, with directional backcrossing into one of the gene pools (McFarlane & Pemberton, 2019). Asymmetric introgression can occur when mating with a certain taxon is more common, e.g., due to mating preference, postzygotic barriers, or different densities of the two taxa. Introgression can affect various portions of the genome, from localized breakthroughs located in small genomics regions (Le Moan et al. 2021, Fraisse et al. 2022), to genome-wide signatures of admixture (Simon et al. 2021). Admixed population can sometime result in a hybrid swarm, which over time can effectively replace parental taxa, or form new lineages with distinct ecological niches.

Genetic hybridisation with introgression can be of high concern in conservation. For instance, introgression of deleterious or maladapted alleles could have highly detrimental consequences for native taxa, often referred to as genetic pollution (Rhymer & Simberloff, 1996). This can also be a concern when populations of the same species hybridize, if the introduced population are genetically divergent from the local population. This can result in loss of genetic variation, loss of adaptive alleles, altered population composition and changed population structure (Laikre et al., 2010; Blakeslee et al., 2020). This genetic pollution is well exemplified by gene

flow from farmed fish escaping aquaculture, which has been identified as a serious threat to wild stocks (Atalah & Sanchez-Jerez, 2020). Studies of salmon have shown that escapees from open farming can have a large impact on local populations through hybridisation, leading to altered subdivision (Glover et al., 2012), genetic swamping, reduced genetic variation, and reduced fitness of wild populations (Bolstad et al., 2017; Glover et al., 2017).

## APPLICATION OF DNA-BASED METHODS

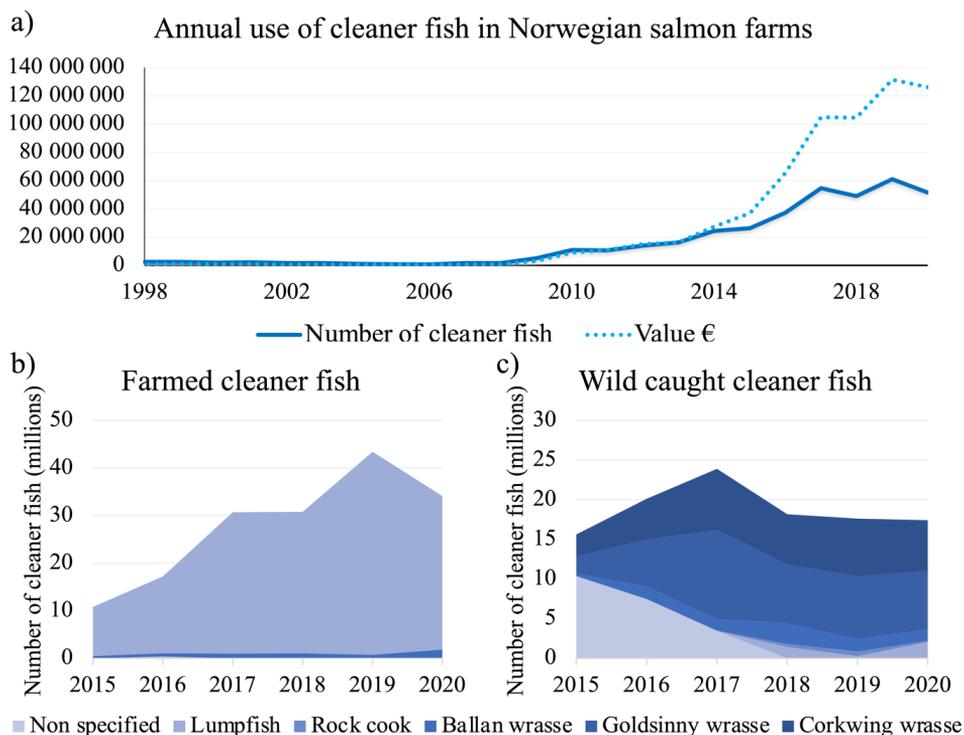
Genomic and genetic studies of biological invasions have become increasingly more common over the last few decades. Research fields such as phylogeography, metabarcoding and population genetics, have become popular approaches for understanding mechanisms and patterns associated with the establishment of non-native organisms. Genetic tools can among other things be used for the identification of newly established species or populations, or for understanding the pathways of invasions, e.g., by identifying source populations, the number of introductions, and subsequent dispersal. Studies of biological invasions with DNA-based methods can also improve our understanding of basic evolutionary processes, such as founder effects, secondary contacts, hybridisation and admixture. Consequently, these types of studies can simultaneously improve our understanding of evolutionary processes and aid management and conservation (Rius et al., 2015; Viard & Comtet, 2015; Viard et al., 2016). Although the need for genetic information has been incorporated into many management policies, the implementation of available genetic knowledge into regulation is still limited (Lundmark et al., 2019; Sandström et al., 2019; Laikre et al., 2020)

## CLEANER FISH AS PARASITE CONTROL

Salmonids are among the most intensively farmed fish in open-ocean and coastal aquaculture. Atlantic salmon is the fish species with the highest aquaculture production value in the world (Cai et al., 2019). Sea lice infestations are a major issue within salmonid aquaculture, in particular the salmon lice (*Lepeophtheirus salmonis*). Salmon lice has been estimated to cost the industry €300-360 million annually and has a greater economic impact than any other parasite (Costello, 2009a; Lafferty et al., 2015). Furthermore, increasing evidence demonstrates that the lice from aquaculture can cause significant mortality in wild fish populations (Costello, 2009b). Wild Atlantic salmon was recently added to the Norwegian red list of threatened species, largely due to the increasing threat of escaping conspecifics and the spread of the salmon lice (Hesthagen et al., 2021). Thus, finding a successful treatment that is effective, as well as safe for the fish and the environment, is of great importance for the aquaculture industry.

Several species of fish exhibit a natural symbiotic cleaning behaviour, removing ectoparasites from larger fish and other organisms (Baliga & Law, 2016). In the late 1980s it was discovered that the natural cleaning behaviour in some wrasse species could also be used to reduce infestations of sea lice in commercial salmon aquaculture (Bjordal, 1988; Darwall et al., 1992). Since the 1990s a small number of wild-caught wrasse have been used for sea lice control. The use of cleaner fish has seen a dramatic increase since 2008 (Figure 1), partially due to sea lice

developing resistance to widely used pharmaceutical treatments (Besnier et al., 2014; Kaur et al., 2017). Using cleaner fish is a low-cost method compared to other types of parasite control, and is often considered to be more environmentally friendly than other delousing methods (Liu & Bjelland, 2014). In 2014, lumpfish were also discovered to have potential use as a cleaner fish for parasite removal (Imsland et al., 2014). The number of cleaner fish used in Norway alone has increased from 1.7 million in 2008 to 61 million in 2019 and 51 million 2020 (Figure 1a).



**Figure 1.** The use of cleaner fish in Norwegian salmon and trout farms a) Between 1998 and 2020. b) Annual use of farmed cleaner fish by species between 2015 and 2020. c) Annual use of wild cleaner fish by species between 2015 and 2020. Non-specified refers to wrasse with no species name recorded. Source: Norwegian directorate of Fisheries.

Currently there are five species of cleaner fish being used for parasite control in Norwegian aquaculture: lumpfish (*Cyclopterus lumpus*), ballan wrasse (*Labrus bergylta*), goldsinny wrasse (*Ctenolabrus rupestris*), corkwing wrasse (*Symphodus melops*) and small amounts of rock cook (*Centrolabrus exoletus*) (Norwegian Directorate of Fisheries, 2021). Since 2017, when quotas for the Norwegian wrasse fishery were introduced, lumpfish became the most commonly used cleaner fish in Norwegian aquaculture with an annual input of ~34 million fish. The majority of lumpfish are farmed from wild broodstocks, while almost all wrasse are caught wild and directly transported to aquaculture facilities. Currently, the only commercially

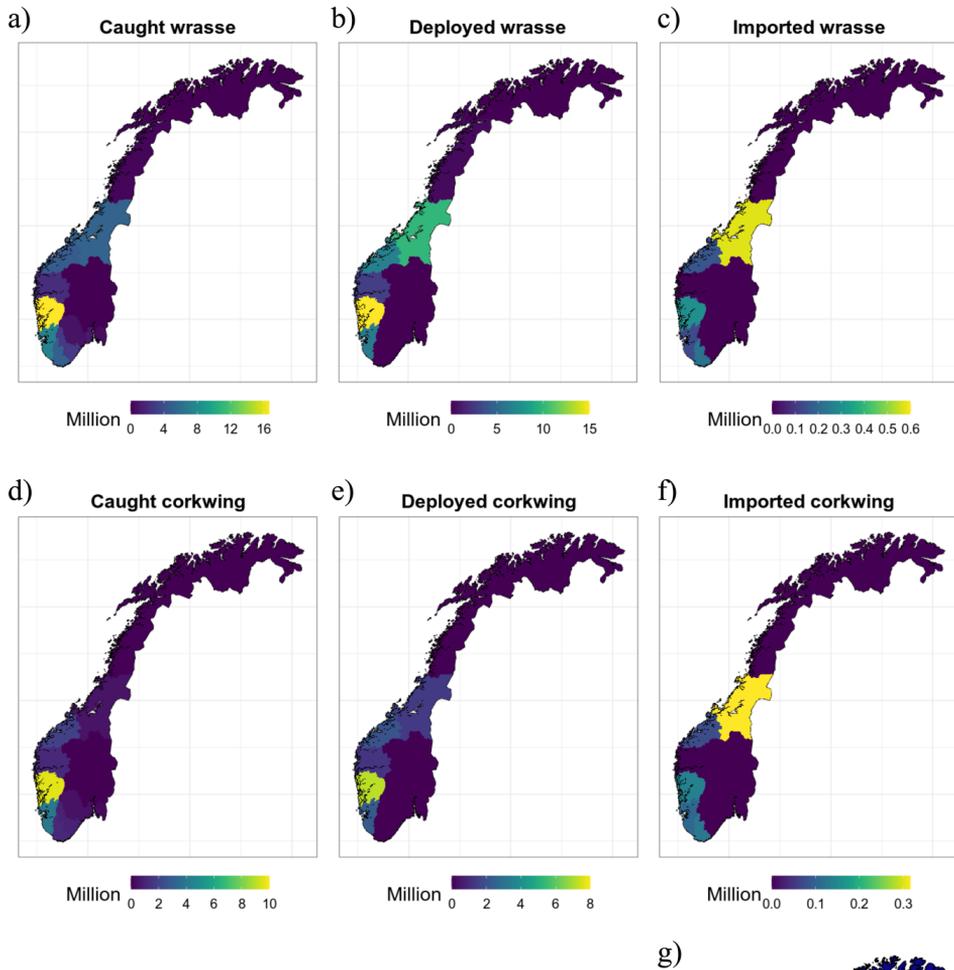
reared wrasse species is the ballan wrasse, although only on a very small scale (Figure 1b). Goldsinny and corkwing wrasse are, by far, the most commonly used wild-caught cleaner fish (Figure 1c). In 2020, 7.4 million goldsinny and 6.3 million corkwing wrasse were deployed as cleaner fish in Norwegian aquaculture (Norwegian Directorate of Fisheries, 2021).

The use of cleaner fish as parasite control in other parts of the world is still relatively small but is likely to increase (VKM 2019). While some countries, e.g., Canada, do not allow the use of wild-caught cleaner fish in open marine aquaculture (Boyce et al., 2018), others, such as the UK, apply a similar system to Norway with a mix of farmed and wild-caught cleaner fish. Currently, an estimated one million wrasse are harvested in southwestern England annually for live transport to salmon farms in Scotland (Devon & Severn, 2017; Riley et al., 2017). Other countries, e.g., Chile, are only starting to investigate the possibility of utilising cleaner fish for parasite control (Sánchez et al., 2018).

## WRASSE TRANSLOCATION AND FISHERY

In many areas the aquaculture demand for cleaner fish exceeds what can be supplied from local fish stocks. Consequently, large quantities of wild-caught wrasse are transported from other areas often hundreds of kilometres away (Figure 2). Since 2010, ballan wrasse, goldsinny wrasse and corkwing wrasse have been targeted by Swedish fisheries and 600 000 to one million wrasse are exported to Norway annually (Figure 2) (Andersson et al., 2021). Source and destination of imported wrasse was not recorded prior to 2017, when it became mandatory. During 2017 and 2018, the majority of imported wrasse was transported to the Trøndelag region in mid-Norway (Figure 2).

A recent report suggests that hybridisation between imported cleaner fish and local fish could cause genetic changes, with severe negative impact on local populations of corkwing and ballan wrasse, and potentially lead to reduced viability and adaptability of local goldsinny wrasse (VKM 2019; Halvorsen, Skiftesvik, et al., 2021). They assessed that there is a moderate risk of genetic change in all wrasse species as well as a moderate risk of negative impact from corkwing wrasse spreading beyond the species range. In this report, only wrasse imported from Sweden were addressed, however, much larger numbers of wrasse are being transported within Norway. Southern Norway, adjacent to the Swedish wrasse fisheries, has few fish farms but high densities of wild wrasse (Skiftesvik et al., 2014; VKM 2019). Approximately ~20% of all fished cleaner wrasse are captured in southern Norway annually, but in most years less than 1% of all cleaner fish are deployed in that area (Norwegian Directorate of Fisheries, 2021, 2022). In contrast to imported wrasse, there are currently no requirements to record the source or destination of cleaner fish that are caught in Norway, even though translocation distances can exceed a thousand km.



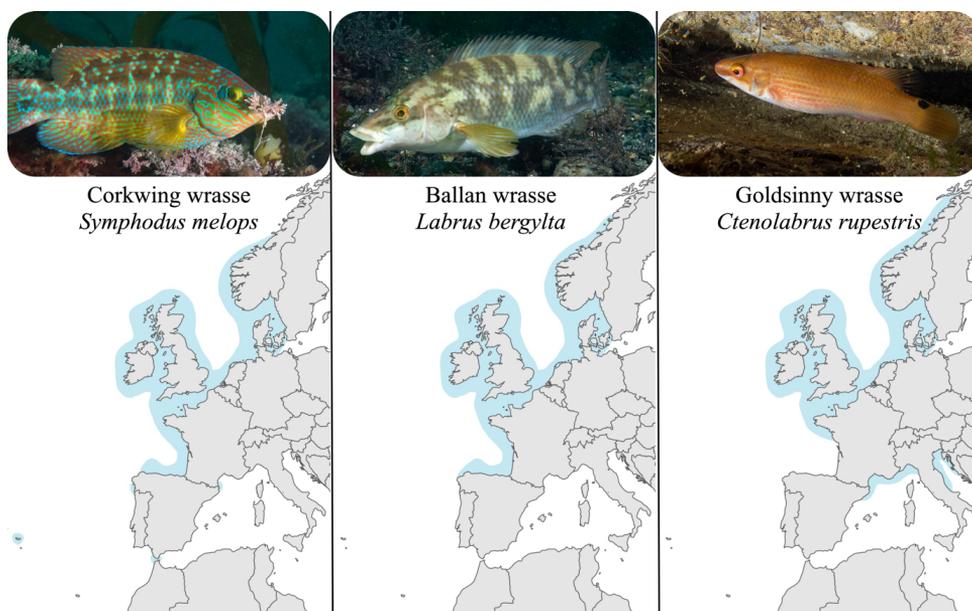
**Figure 2.** Map of Norway showing number of a) caught wrasse, b) wrasse deployed, c) destination of imported wrasses from Sweden, d) caught corkwing, e) corkwing deployed, f) destination of imported corkwing from Sweden, in 2017 and 2018 (total) for each county, g) Map of counties. Catch data and deployment data: Norwegian directorate of Fisheries. Data on imported wrasses from Sweden was provided by the Norwegian Environmental Agency. Note: For the sake of anonymity in reported deployment statistics, no species-segregated data for the south coast counties is reported and is thus not included in the above map. Note that different scales are used and data is displayed according to the 19 counties still in use in 2017-2018.

## STUDY SPECIES

### *Corkwing wrasse*

Corkwing wrasse (*Symphodus melops*) is a marine fish species of the family Labridae native to the eastern Atlantic, with a natural distribution from Morocco to mid-Norway (Figure 3) (Knutsen et al., 2013; VKM 2019). They can live up to eight to nine years (Darwall et al., 1992; Halvorsen et al., 2016; Uglem et al., 2000), and grow up to 24 cm in length, making it the second largest species of wrasse in Scandinavia (Halvorsen et al., 2016). Just as other wrasse species, corkwing inhabit rocky shores and reefs along the coast where they can often be found in shallow areas (Skiftesvik et al., 2014). Corkwing wrasse is a territorial and nest building species, with male parental care until eggs have hatched (Potts, 1985; Halvorsen et al., 2016). During the spawning season (May-July) nesting males display bright blue, green and red colours in order to attract females to their nests (Potts, 1974). Females are brown/grey in colour and much smaller in body size than the nesting males. A small proportion of males employ female mimicry and do not build nests but rather perform sneak spawning (Uglem et al., 2000). The male morphs are believed to be fixed for life and could potentially be genetically determined (Halvorsen et al., 2016). Some concern has been raised that current size limits in the Norwegian wrasse fishery may be sex selective, as nesting males grow faster and mature later than females and sneaker males (Halvorsen et al., 2016; Halvorsen, Sordalen, et al., 2017).

Earlier studies of corkwing wrasse have found a reduced genetic diversity in northern Europe aligned with a large genetic break between Atlantic and Scandinavian populations, likely caused by the populations undergoing bottlenecks as it expanded toward the northeast (Robalo et al., 2012; Knutsen et al., 2013). A second genetic break along the Norwegian coast was later discovered by Blanco Gonzalez et al (2016). They found that a long stretch of sandy beaches (<60 km long), which is an unsuitable wrasse habitat, separates south-eastern Skagerrak populations from populations in western Norway. Corkwing wrasse is a non-migratory fish species which lays benthic eggs and is dependent on the planktonic larval stage for dispersal (Darwall et al., 1992). Thus, this large unsuitable habitat might act as an environmental barrier for gene flow. Recent analysis of demographic history suggests that the genetic divergence between the populations might be a result of post-glacial recolonization and founder events separating the populations for more than ~10 kya, followed by a secondary contact (Mattingsdal et al., 2020). Given the low number of hybrids it is likely that the secondary contact is very recent or hybrids are actively selected against. Skagerrak populations southeast of the genetic break have a much lower genetic diversity than their north-western counterparts (Mattingsdal et al., 2020). The two populations also have different life histories. Fish belonging to the south-eastern population grow faster, mature earlier and rarely reach more than four years of age (Halvorsen et al., 2016). Furthermore, the ratio between nesting and sneaker males differs between the two regions, with few sneaker males in the south. However, as Norwegian fisheries only apply a minimum size limit, this could be a result of selective fishery where nesting males are likely to be targeted disproportionately (Halvorsen, 2017).



**Figure 3.** Distribution of three cleaner fish species. From left to right: corkwing wrasse, ballan wrasse and goldsinny wrasse. Redrawn from VKM Report 2019, and information in Halvorsen et al., 2021. Photos: Paul Naylor at marinephoto.co.uk.

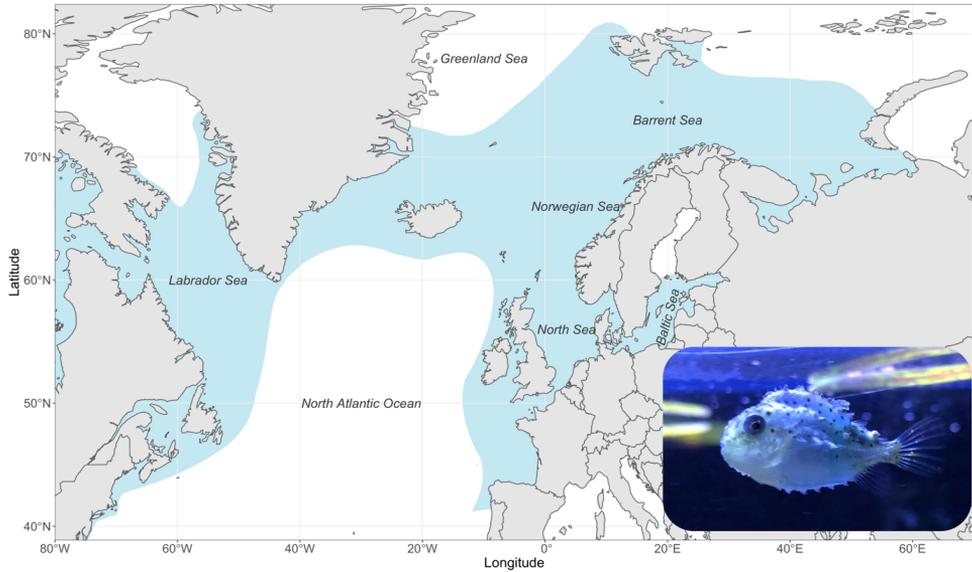
#### *Ballan wrasse*

Ballan wrasse (*Labrus bergylta*) can be found from North Africa to mid Norway, slightly further north than corkwing wrasse (Figure 3) (VKM 2019). This species is a sequential hermaphrodite, first maturing as a female at ~22 cm, to later change sex when reaching 34-40 cm (Darwall et al., 1992; Muncaster et al., 2013), which makes it the largest wrasse in Scandinavia. Males are territorial, providing parental care to the benthic eggs until hatching. Scandinavian populations are highly divergent from populations in southern Europe and the British Isles (D'Arcy et al., 2013). A genetic break on the southwestern coast of Norway was recently discovered (Seljestad et al., 2020), in the same place as in corkwing wrasse, but much weaker. Ballan wrasses have two colour morphs, spotted and plain. Large genetic differences between the morphs have been discovered in north-western Spain (Quintela et al., 2016), but no such difference could be observed in Scandinavian populations in later studies (Seljestad et al., 2020).

#### *Goldsinny wrasse*

Goldsinny wrasse (*Ctenolabrus rupestris*) has a similar distribution to corkwing and ballan wrasse, but can be found further north along the Norwegian coast (Figure 3) (VKM 2019). Goldsinny is the smallest of the Scandinavian wrasse and can reach 20 cm (Darwall et al., 1992), but they are often not larger than 16 cm (Halvorsen, 2017). Goldsinny males also defend territories, but in contrast to ballan and corkwing wrasse species, they do not build nests as the

females' eggs are pelagic (Hilldén, 1984). Like the other species of wrasse, goldsinny is genetically divided across the North Sea (Jansson et al., 2017). Within Scandinavia there is a clear pattern of isolation by distance, but in contrast to the other two wrasse species, there is no genetic break within Scandinavia (Jansson et al., 2020).



**Figure 4.** Lumpfish distribution redrawn from VKM Report 2019 and FAO species profile. Photo of juvenile lumpfish: Ellika Faust

### Lumpfish

Lumpfish (*Cyclopterus lumpus*) is semi-pelagic fish and can be found throughout the North Atlantic (Figure 4) (Davenport, 1985). Adult lumpfish spend most of their time in the upper water column in the open ocean, but migrate towards coastal areas to spawn (Eriksen et al., 2014). Similarly, to ballan and corkwing wrasse, lumpfish have benthic eggs which are cared for by the male (Davenport, 1985; Mitamura et al., 2012). Juveniles remain in shallow coastal waters for approximately 6-12 months until they migrate offshore. Low recapture rates and few age classes of spawning lumpfish, have led to the suggestion that post-spawning mortality may be high in the species (Kasper et al., 2014; Hedeholm et al., 2017). However, repeated reproduction has been observed in females, which in consecutive years returned to spawn in the same area (Kennedy et al., 2015, 2019), supporting theories of natal homing in the species.

Earlier studies have shown that lumpfish in the West Atlantic is genetically divergent from Icelandic and East Atlantic populations, and that the smaller lumpfish found in the Baltic Sea is divergent from both (Pampoulie et al., 2014; Garcia-Mayoral et al., 2016; Whittaker et al., 2018). Garcia-Mayoral et al. (2016) found latitudinal differentiation among populations in Northwest and Southwest Greenland. Lumpfish from the English Channel and Iceland have been discovered to be genetically divergent from each other and other eastern Atlantic samples

(Whittaker et al., 2018). There is some contradictory evidence whether there is structure among lumpfish found along the Norwegian west coast (Jónsdóttir et al., 2017; Whittaker et al., 2018).

## KNOWLEDGE GAPS

Approximately 54 million cleaner fish are deployed in Norwegian salmonid farms annually. However, the increasing fishing pressure on wild populations and the long-distance translocation raises concerns of potential stock depletion and anthropogenic introductions and hybridisation.

An increasing number of observations of corkwing wrasse have been reported in the Flatanger municipality in mid Norway, a region 130 km north of the previously described species range. The Flatanger region is an area densely populated with salmonid aquaculture farms, which rely heavily on the import of cleaner fish from southern populations. Thus, the question arises whether the newly established corkwing wrasse population in Flatanger could be a direct effect of imported cleaner fish. Risks associated with farmed fish escaping aquaculture is a highly debated issue. However, in contrast to salmonids, there are no monitoring programs nor action plans for how to prevent and or deal with escaping cleaner fish. Currently it is unknown how much cleaner fish escape, and whether there is a difference between regions in the number of escapees and the extent of genetic admixture with local populations.

The increasing demand for cleaner fish as parasite control in Norway has led to the establishment of a wrasse fishery on the Swedish west coast in 2010. Prior to the start of this fishery, wrasse were not considered commercial species and information on population densities and trophic interactions is scarce. Currently, there is not enough data to support an ecosystem-based management of the Swedish wrasse fishery. Furthermore, corkwing wrasse has been suggested to have extremely low genetic diversity and weak population structure south and east of the genetic break in southern Norway. So far only corkwing wrasse from Gullmarsfjorden in Sweden and a few locations along the Norwegian south-eastern coast have been investigated. The limited geographical sampling makes it difficult to define appropriate fisheries management units in Sweden and other countries in the North Sea - Baltic Sea transition zone.

Since a maximum-quota system was introduced in the Norwegian wrasse fishery in 2017, an average of 34 million lumpfish per year have been used as cleaner fish, exceeding the number of wild caught wrasse. Earlier genetic studies of lumpfish have revealed different degrees of population structure. To this date only smaller sets of microsatellite loci have been used, and sampling has often concentrated on one part of the distribution range. With the increased farming of lumpfish for cleaner fish use, and the translocation associated with it, it is urgent to improve our standing of connectivity and population structure in this species.

## THESIS AIMS

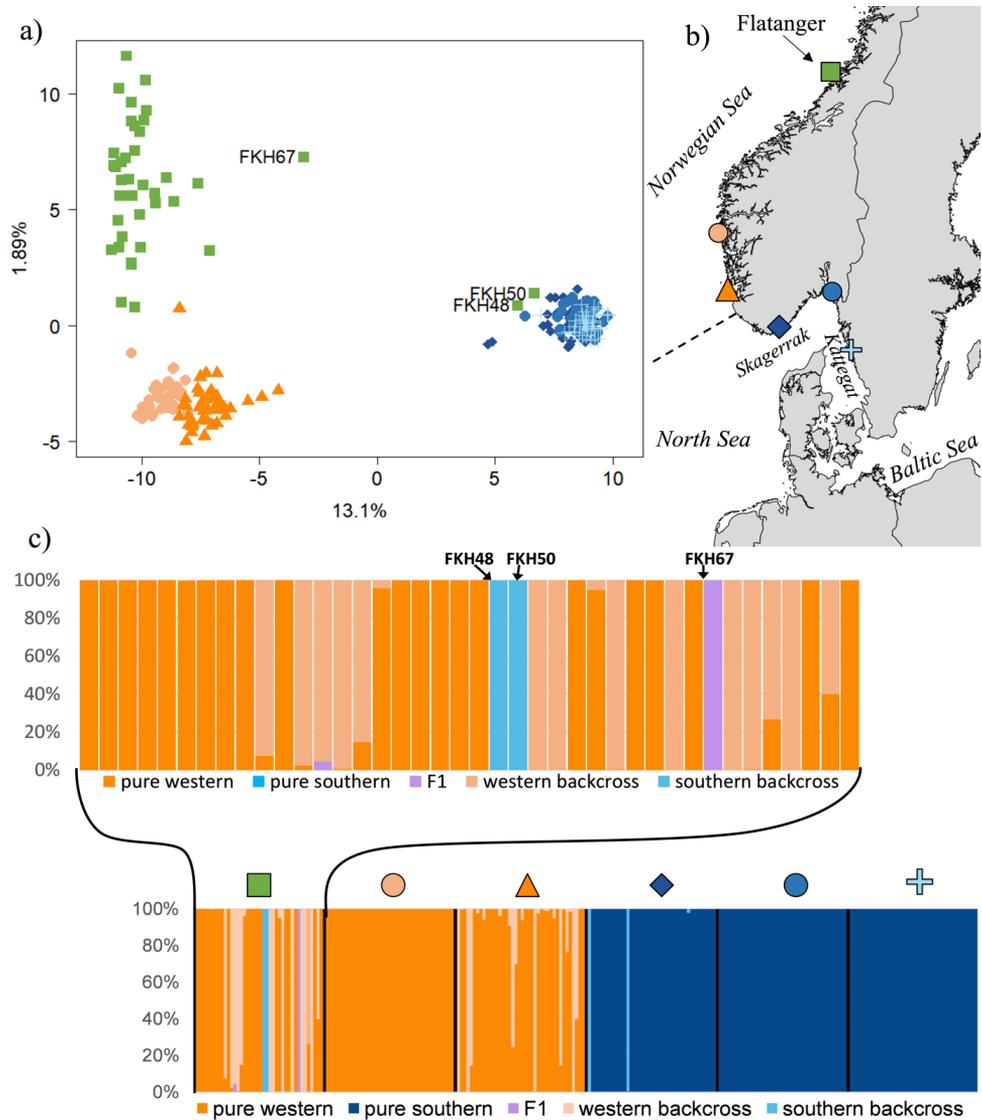
- PAPER I** Investigate whether the newly established corkwing wrasse population in mid Norway is a consequence of a northwards range expansion, cleaner fish escaping salmon farms, or a mix of both.
- PAPER II** Quantify and determine the geographic extent of corkwing wrasse escaping Norwegian salmon farms. Develop a tool for management to aid monitoring of corkwing wrasse escapees and introgression.
- PAPER III** Evaluate if the Swedish wrasse fishery has led to stock depletion, gain insights on the role of wrasse in trophic food web, and to provide knowledge and recommendations to support ecosystem-based management for wrasse fisheries in Sweden.
- PAPER IV** Investigate population structure, genetic diversity and connectivity of corkwing wrasse in the North Sea - Baltic Sea transition zone, with the aim to aid in defining biologically relevant management units.
- PAPER V** Improve on existing knowledge of connectivity and population structure of lumpfish across the entire north Atlantic, with a focus on the eastern Atlantic and the Norwegian coast where lumpfish are increasingly being used as cleaner fish.

## SUMMARY OF PAPERS

### PAPER I - Cleaner fish escape salmon farms and hybridize with local wrasse populations

In this paper we examined the origin of the recently established population of corksling wrasse (*S. melops*) in Flatanger in mid Norway, 130 km north of its natural distribution range. Flatanger municipality is an area in Norway with many salmonid farms that rely heavily on the use and long-distance import of cleaner fish such as corksling wrasse from Skagerrak. Reports have suggested that it is possible for cleaner fish to escape from salmon farms through tears in the net, slipping through the mesh, or even intentional release at the end of the season (Woll et al., 2013; Svåsand et al., 2017; Blanco Gonzalez & de Boer, 2017). However, corksling wrasse has also increased in abundance in other areas in Scandinavia, suggesting that warmer temperature might allow the species to expand in the north (Knutson et al., 2013). In this study we aimed to answer the question whether the newly established population in Flatanger was 1.) A direct result of these cleaner fish escaping aquaculture facilities and establishing a feral population, 2.) A result of the species expanding its range northwards, or 3.) Due to a combination of these two processes.

We used population genetics methods in order to answer this question. First, we sampled a total of 240 individuals from six different locations, one in Flatanger, two in southwestern Norway, where wrasse is harvested but used locally, and three locations on the Skagerrak–Kattegat coast, where almost all commercially captured wrasses are transported to distant salmonid farms. Second, we used the restriction-site-associated DNA sequencing method 2b-RAD (Wang et al., 2012) to identify SNPs and genotype the individuals. Genomic DNA was extracted from fin clips and prepared libraries were pooled with individual barcodes and sequenced on an Illumina HiSeq2500 platform. The bioinformatic analysis of the DNA sequences followed a modified de novo pipeline from Pierre de Wit (2016). After removing genotyping errors and uninformative polymorphisms, 4372 SNPs remained. We estimated population differentiation by calculating pairwise  $F_{ST}$ , and used clustering methods to estimate genetic differentiation among individuals. Finally, we investigated the occurrence of hybridisation with NEWHYBRIDS in the Flatanger location using 200 highly differentiated SNPs. Individuals were assigned to six different hybrid classes; pure western, pure southern, F1, F2, western backcross or southern backcross. We assessed accuracy and power to identify individuals of the different hybrid classes with the set of 200 SNPs by simulating and analysing data, based on western and southern allele frequencies.



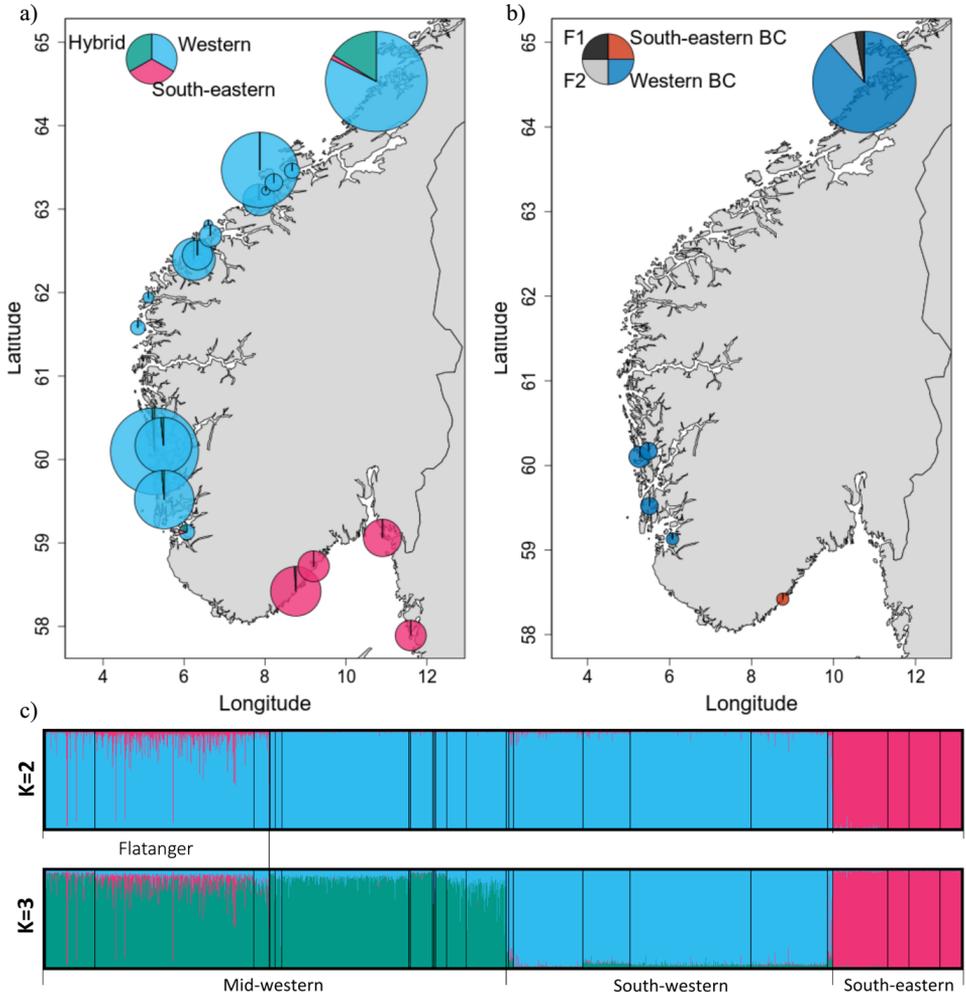
**Figure 5.** a) The first and second components of a principal component analysis of 240 corkwing wrasse from 6 locations based on 4357 SNPs. The first component explains 13.1% of the total variation and the second 1.89%. Each point represents one individual, and colour and symbols represent sampling sites. b) Map of sampling sites. Dashed line represents the genetic break. c) Hybrid analysis of all individuals (bottom) and individuals sampled in Flatanger (top) using the 200 SNPs with highest  $F_{ST}$  estimates in NEWHYBRIDS. Each vertical line represents one individual and its probability to belong to one of the six genotype classes; no F2 genotypes were present. Individuals are sorted from North (left) to South (right)

We found that Flatanger was overall more genetically similar to the western samples than to Skagerrak-Kattegat populations. This suggests that the species is going through a natural range expansion. However, individual based analysis revealed that some individuals were genetically much closer to the Skagerrak-Kattegat populations (Figure 5a). Two individuals clustered with the southern population and were identified as southern backcrosses, i.e., 75% southern genotype and 25% western genotype (Figure 5c). One individual was classified as a F1 hybrid, and an additional 12 individuals from Flatanger had a high probability of being western backcrosses, i.e., 75% western genotype and 25% southern genotype. In summary, we found that the Flatanger population is mainly a result of a northward range expansion, but there has also been considerable gene flow from southern populations in Skagerrak and Kattegat. Our results provide the first evidence that corkwing wrasse escape from fish farms and hybridise with local populations.

## PAPER II - Not that clean: Aquaculture-mediated translocation of cleaner fish has led to hybridization on the northern edge of the species' range

In **Paper I** we discovered that corkwing wrasse are able to escape and hybridise with local populations at the northern edge of the species distribution, and we could use genetic markers to detect these individuals. However, we only investigated a relatively small number of individuals from a single region. Thus, the geographical extent and magnitude of escapees and introgression is still unknown. We expanded upon our first study by genotyping a large number of wild caught corkwing wrasse along the Norwegian west coast in areas heavily relying on the use of cleaner fish. A second aim was to develop a suite of genetic markers that can be used by management stakeholders for future monitoring of escapees and hybrids in the wild. We used 2b-RAD sequences from **Paper I** and mapped them to the genome of corkwing wrasse (Mattingsdal et al. 2018). We then identified SNP loci with high divergence ( $F_{ST} > 0.4$ ) between western and southern samples, which were used for primer design, amplification and genotype calling, based on the low cost Agena MassARRAY iPLEX Platform (Gabriel et al., 2009). Similarly, to **Paper I**, accuracy, efficiency and power to correctly identify escaping individual hybrids was assessed by simulating data based on western and southern allele frequencies.

Genomic DNA was extracted from a total of 1955 unique individuals and 105 technical replicates, which were then genotyped for 106 SNPs. After filtering, the final data set consisted of 1766 unique individuals genotyped for 84 SNPs with a total of 2.9 % missing data. Genetic differentiation among sites was estimated by calculating pairwise  $F_{ST}$  and individual-based clustering methods. The frequency of escapees and hybrids was estimated with NEWHYBRIDS and accuracy and power were re-assessed with the 84 of SNPs remaining after filtering.



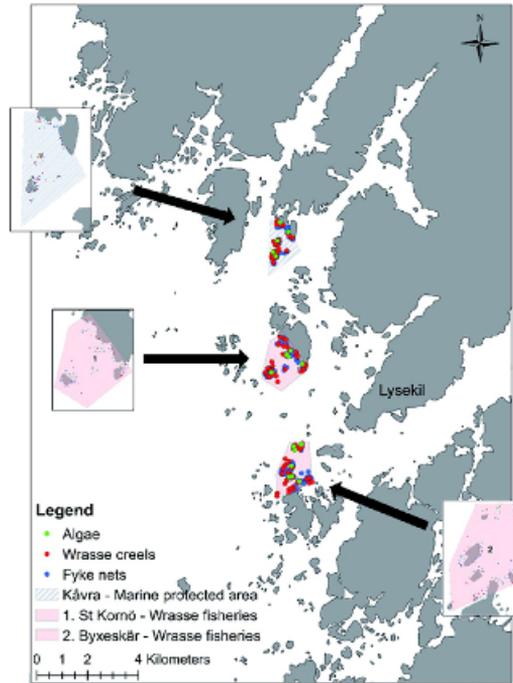
**Figure 6.** Assignment of 1766 corkwing wrasse based on 84 SNPs. Top: Map displaying proportion of corkwing wrasse from each sampling site classified by NEWHYBRID. a) Left map displays individuals classified as western, south-eastern or hybrid with >50% probability. b) Right map displays the proportion of hybrids assigned to the different hybrid classes F1, F2, backcross with western and backcross with south-eastern. Sizes reflect the relative number of individuals per sample. c) STRUCTURE plot for K = 2 (top) and 3 (bottom) with sampling location given as a priori. Each vertical bar represents one individual and the colour the proportion of that individual assigned to the different genetic clusters. Individuals are sorted from North (left) to South (right).

Results show that samples on the Norwegian west coast are overall similar to each other, but genetically distinct from Skagerrak samples. However, in addition to the previously known genetic break on the southwest tip of Norway, results suggest that there could also be a genetic discontinuity along the Norwegian west coast (Figure 6c). The panel of 84 SNPs had an accuracy above 95% and a power above 95 to correctly classify individuals as pure western, southern or hybrids. Of the 1519 corkwing wrasse successfully genotyped on the Norwegian west coast, 7 were identified as escapees and 79 as potential hybrids (Figure 6a-b). Almost all of the escapees and hybrids were collected at the northern edge of the population distribution in Flatanger in mid-Norway; the same region as investigated in **Paper I**. We found that escapees and hybrids might constitute up to 20 % of the local population in Flatanger but may be rare elsewhere. Overall, these results show that the relative frequency of escaped and hybridising individuals is still low in most regions on the Norwegian west coast.

### PAPER III - Wrasse fishery on the Swedish west coast: Towards ecosystem-based management

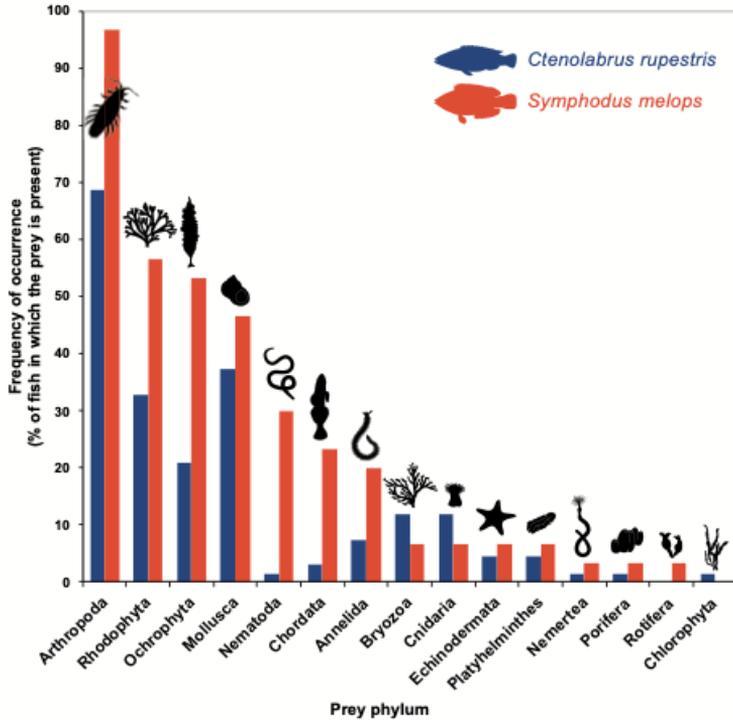
The high demand for cleaner fish in Norwegian salmon farms led to the establishment of wrasse fisheries on the Swedish west coast in 2010 (Andersson et al., 2021). This coincided with the closure of the eel fishery in 2011, which left a large fleet of fishermen with gear that could catch wrasse. The Swedish fishery is small-scale and fishermen can only fish for wrasse after receiving an exemption. Since the start of the fishery, ~14 such exemptions have been handed out for wrasse fishery annually (Andersson et al., 2021). Although five species of wrasse can be found in Sweden, only corkwing, goldsinny and ballan wrasse are caught commercially. In this study we aimed to gather some of the fundamental knowledge needed to implement an ecosystem-based approach to management of the new emerging fishery.

High site fidelity and narrow home range is a common trait in all three species targeted in Sweden, which suggests low levels of migration and very local populations (Skiftesvik et al., 2014; Halvorsen, Larsen, et al., 2021). A recent study found that catch per unit effort of corkwing and goldsinny was significantly higher inside marine protected areas (MPA), and corkwing wrasse inside the MPAs were larger and older (Halvorsen, Larsen, et al., 2017). To study if Swedish wrasse populations also show signs of depletion, such as lower density or average size in fished areas, we conducted a fishery-independent survey where we compared a 2.6 km<sup>2</sup> large MPA with two equally large unprotected areas where wrasse were being targeted by the fishery (Figure 7). Over a nine-day period in August in 2016 we deployed a total of 112 wrasse pots and 164 fyke nets. In each of the three areas, we also collected samples of the alga *Saccharina latissima* and associated fauna to investigate potential cascading effects of removing wrasse.



**Figure 7.** Map of the study area outside Lysekil on the Swedish west coast. Kåvra (hashed area) is a marine protected area where wrasse have not been fished for the last 30 years. Both St. Kornö and Byxeskär (pink areas) have been subjected to an active wrasse fishery since 2013. The blue dots show the positions for the fyke net deployments, the red dots the wrasse pots and the green dots represent the localities where *S. latissima* algae and associated fauna were sampled.

In addition to individual size and abundance, we also analysed the diet of goldsinny and corkwing wrasse to get a better understanding of their role as an intermediate predator, and the potential cascading effects of their removal on the ecosystem. The diet was analysed with metabarcoding, which allows the simultaneous identification of a wide array of prey species with high-throughput DNA sequencing, even from highly digested gut contents (Pompanon et al., 2012). Mitochondrial cytochrome oxidase 1 was amplified from the gut content DNA using metazoan primers. Taxonomy assignment was carried out using BLAST against the GenBank database, and resulted in a total of 3 933 assigned and unassigned amplicon sequence variants. Finally, we performed a mesocosm study to mechanistically compare how wrasse presence affects the algal belt ecosystem. Wrasse presence was manipulated in a simplified community, and fouling organisms were monitored over 16 days.



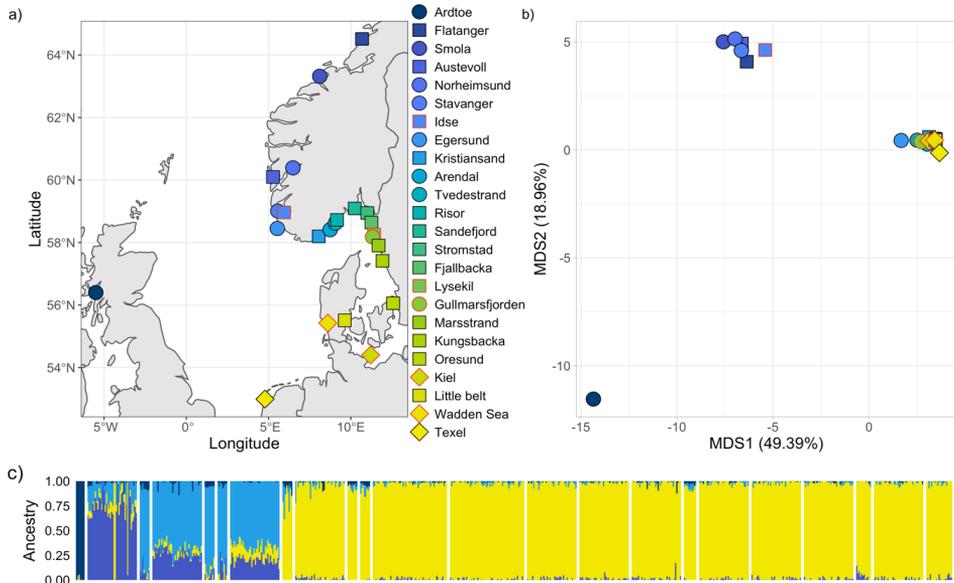
**Figure 8.** Bar chart showing frequency of occurrence (% of fish in which this amplicon sequence variant was found) of various phyla in the gut contents of goldsinny (*C. rupestris*) and corkwing wrasse (*S. melops*).

The fish survey data showed no clear evidence of depletion or size differences for any of the three wrasse species, ballan, corkwing and goldsinny wrasse, between fished and control areas. However, very few ballan wrasse were caught in both areas making it difficult to assess the effect of fishing on this species. Similarly, fouling organisms and associated fauna with *S. latissima* algae showed no differences between the protected area and the two fished localities. Using molecular methods, we were able to detect 189 prey species belonging to 15 phyla, obtaining information from even highly digested prey (Figure 8). We found that Arthropoda (Crustacea and Insecta) were the most commonly found prey taxa in the gut contents of wrasse, followed by algae in corkwing wrasse and Mollusca in goldsinny wrasse. The high presence of algae species suggests that algae might be grazed intentionally rather than consumed accidentally while searching for prey or building nests. Our results suggest that wrasse feed opportunistically and according to prey availability. However, there may be differences in feeding related to size and age which was not explored in this study. The mesocosm experiments confirmed that wrasse prey efficiently on small herbivorous gastropods and crustaceans, but also intermediate consumers as well as insects and primary producers.

## PAPER IV - High genetic connectivity in an otherwise highly structured marine fish

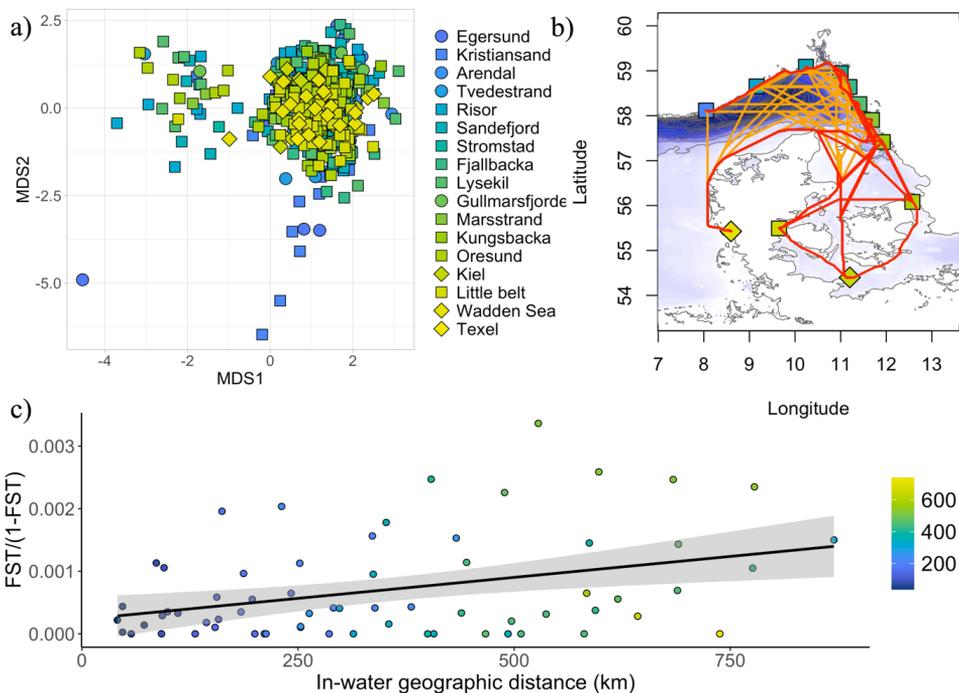
In **Paper I** and **Paper II** we found that corkwing wrasse south and east of the genetic break in southern Norway, had overall very low diversity and no sign of population structuring. This was in accordance with what has been shown in other studies of genetic population structure of corkwing wrasse in Europe (Robalo et al., 2012; Knutsen et al., 2013; Blanco Gonzalez et al., 2016; Mattingdal et al., 2020). However, similarly to previous studies, our sampling in **Paper I** and **Paper II** was limited to only a few sites south of the genetic break in Skagerrak-Kattegat. Consequently, the question remained, if the low diversity and lack of structure is true for the whole transition zone from the North Sea to the Baltic Sea, or if there was more structure or diversity to be found. This knowledge is important to disentangle the colonisation history of corkwing wrasse in Scandinavia, and for the development of a sustainable management of the new wrasse fishery. In order to investigate this, we sampled corkwing wrasse in Norway, Sweden, Denmark and Germany, from the Danish Wadden Sea to Kiel in the Baltic Sea. In addition to this, we also received an unusual sample of corkwing wrasse from Texel in the Netherlands. In this study we aimed to 1) investigate the population structure and connectivity of corkwing wrasse in Northern Europe, with a specific focus on the transition zone from the North Sea to the Baltic Sea, 2) use demographic analysis to assess if there is ongoing and/or historic gene flow.

In this study we combined new 2bRAD data from high resolution sampling, with data from **Paper I**, and whole genome shotgun sequencing (WGS) data from Mattingdal et al. (2020). The new 2bRAD data was mostly processed as described in **Paper I**, with the exception of samples from Kiel, Wadden Sea and Texel, which were sequenced on Illumina NovaSeq 600, instead of the previous Illumina HighSeq. Sequences were mapped to the corkwing wrasse genome (Mattingdal et al., 2018) and SNPs were called jointly using both 2bRAD and WGS data. Quality was assessed using identity across technical replicates. After data filtering 3849 SNPs remained for further analyses. Technical artefacts due to sequencing methods were investigated by analysing 19 pairs of individuals that were replicated across different pairs of platforms. Robustness of the combined data from different sources was further assessed by analysing overall population structure and comparing the results to earlier studies (Faust et al., 2018; Mattingdal et al., 2020). We estimated population structure by estimating pairwise  $F_{ST}$  and tested for isolation by distance with regression analysis of genetic distance and shortest waterway distance. Genetic differentiation among individuals was estimated and visualised using three individual-based clustering methods: sparse non-negative matrix factorization algorithm (sNMF), principal component analysis (PCA) and partial distance-based redundancy analysis (dbRDA), where technical artefacts could be partialled out. Finally, corkwing demographic history in the North Sea - Baltic Sea transition zone was assessed using the software “Moments” (Jouganous et al., 2017).



**Figure 9.** Sampling sites and major genetic patterns of corkwing wrasse in Northern Europe. a) Map of sampling sites. Shapes represent the sequencing method (circle = WGS data, square = 2bRAD HighSeq data and diamond = 2bRAD NovaSeq data). Red edges symbolise sites from which a subset of 3-4 individuals were re-sequenced using 2bRAD NovaSeq. b) Multidimensional Scaling Plot (MDS). c) Individual ancestry estimates using sNMF for  $K = 4$  which was deemed the best run in terms of prediction capability. Each vertical bar represents one individual; the colour represents the proportion of that individual assigned to the 4 different clusters. Individuals are grouped by samples, which are ordered from left to right as seen in the map legend.

Overall, we could clearly distinguish three regional clusters: British Isles, the west coast of Norway in Northern Scandinavia and the North Sea - Baltic Sea transition zone in Southern Scandinavia (Figure 9). In our target region, Southern Scandinavia, we found that technical artefacts due to sequencing methods gave a stronger signal than any biological pattern. Once partialled out, individual clustering did not reveal any further structure patterns (Figure 10a). However, we found that genetic distance was positively correlated with geographical distance, indicative of isolation-by-distance (Figure 10b-c). This was further confirmed with demographic modelling between two edge populations, in the North Sea and the Baltic. A model with a split and subsequent migration was the best fit for the data, suggesting that these separated populations are connected with migration. Our results suggest that populations in Southern Scandinavia are overall well connected, and can be managed as such. However, populations with a longer coastal distance may mainly be connected via stepwise migration through intermediate populations.

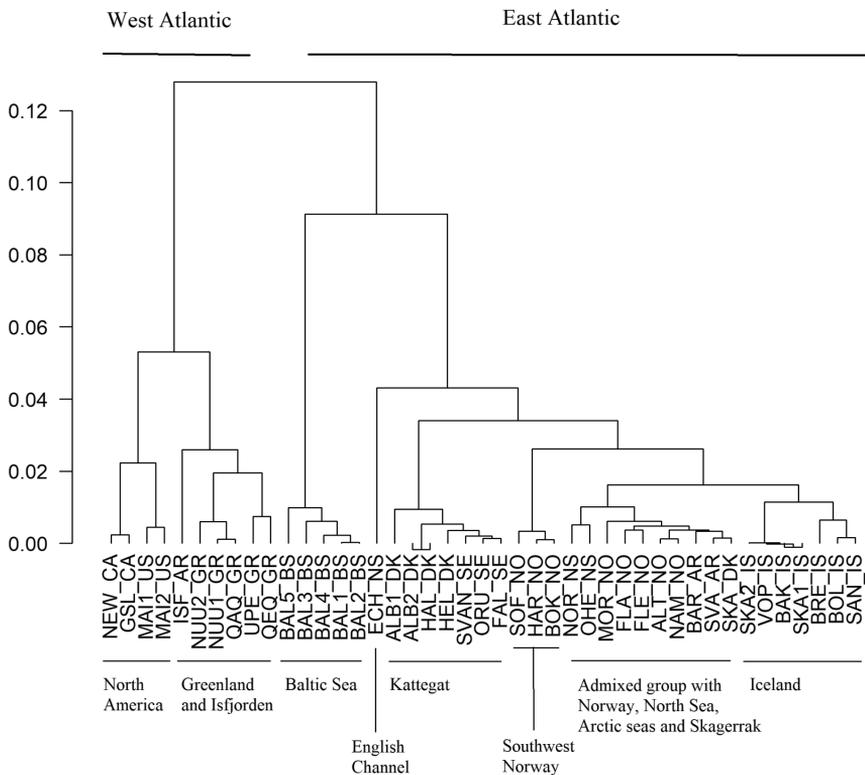


**Figure 10.** Genetic distance of corkwing wrasse in Southern Scandinavia. a) Multidimensional Scaling Plot (MDS) after removing batch effects of sequencing. Each point is an individual, colours represent sample origin and shapes sequencing method (circle = WGS data, square = 2bRAD HighSeq data and diamond = 2bRAD NovaSeq data). b) Bathymetry map of Southern Scandinavia showing samples sequenced using 2bRAD. Orange lines are the shortest path between samples with no depth constraint and red lines with a depth constraint of 0-50m. c) Scatterplot showing patterns of Isolation-by-Distance where genetic distance is positively correlated with geographical distance with depth restriction of 0-50 m ( $r = 0.36$ ,  $p = 0.038$ ). Colours represent geographical distance with no depth restriction ( $r = 0.31$ ,  $p = 0.024$ ). Single individuals from Texel and WGS data were not included in b-c.

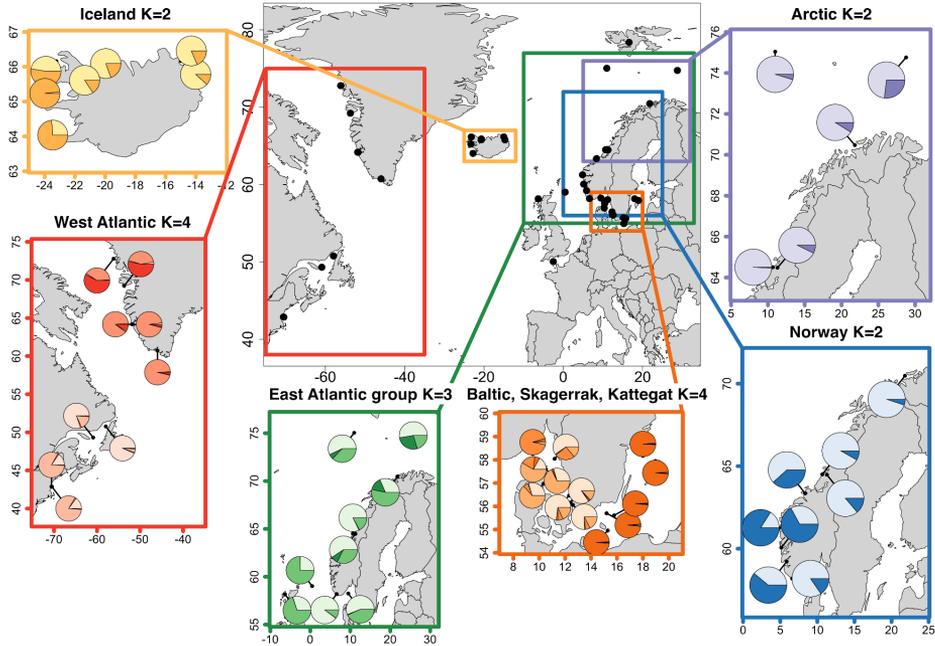
## PAPER V - Global, regional, and cryptic genetic population structure in a high gene flow transatlantic fish

Lumpfish (*C. lumpus*) has a long history of commercial exploitation, and several thousand tons are captured annually for their roe (Kennedy et al., 2019). Recently, a fishery for mature lumpfish to be used as broodstock for cleaner fish production has added further pressure on wild populations. In Norway alone, over 40 million farmed lumpfish are now sold annually to be used as cleaner fish (Norwegian Directorate of Fisheries, 2021). In addition to the already existing fishing pressure, escapees of translocated lumpfish from salmonid farms risk becoming another threat to local populations. In this study, we aim to improve our understanding of connectivity and population genetic structure of lumpfish across the entire

north Atlantic. We focus on the eastern Atlantic and the Norwegian coast where lumpfish are increasingly being used as cleaner fish. Here we used a two-step approach similar to **Paper II**. First, we used 4 393 genome wide SNPs identified with 2bRAD, as described in **Paper I, II** and **IV** to analyse the global population structure of 95 individuals from 10 locations. Based on these results we identified 139 discriminatory SNPs to be used in a geographically much more comprehensive study, where we genotyped 1 669 individuals from 40 locations. Genetic structure was analysed with pairwise  $F_{ST}$ , and clustering approaches were used to investigate and visualise the genetic differentiation among individuals on both global and regional scales.



**Figure 11.** Dendrogram of pairwise  $F_{ST}$  estimates between lumpfish samples, based on 139 SNPs. Sample codes are explained in detail in Table 1 Paper V, also on bioRxiv (<https://doi.org/10.1101/2022.03.22.485384>).



**Figure 12.** Regional STRUCTURE assignment of lumpfish based on targeted 139 SNPs. Squares represent separate structure runs for different regions where a set of samples, which due to their genetic and geographic closeness, were analysed together. Each pie chart shows each sample's assignment to the selected number of clusters ( $K$ ) averaged across individuals.

By combining genome-wide 2bRAD with a targeted SNP panel, we uncovered multiple layers of population structure across the global distribution of lumpfish. Both approaches identified a major split between the East and West Atlantic and a distinct Baltic Sea population. The extensive sampling revealed eight major clusters (Figure 11); North America, Greenland with Isfjorden from Svalbard, Baltic Sea, English Channel, Kattegat, Southwestern Norwegian fjords, Iceland and a mixed group in the East Atlantic. Once hierarchical clustering analysis was done within these regions, we found local and cryptic structure within the different regions (Figure 12). In Greenland and North America, we detected a pattern of a north-south divergence, and a west-east divergence in Iceland. We also detected several potential migrants and recent hybrids suggesting movement of individuals between regions that were otherwise genetically distinct. Overall, our results show that lumpfish is a highly structured species with many local stocks. The high levels of population structure are consistent with the suggested natal homing behaviour in lumpfish but could also be an indication of local adaptation. Further, we provide a baseline for future monitoring of wild populations and a panel of SNPs which could be applied for identification of wild or translocated lumpfish.

## DISCUSSION

With large-scale translocations, and an annual use of ~54 million cleaner fish in Norwegian salmonid farming, better knowledge of population structure, genetic diversity and connectivity is needed. In this thesis I report the first evidence of cleaner fish escaping salmon farms and hybridising with local populations. The genomic consequences of hybridisation between genetically distinct populations are hard to predict and depend on many factors, such as inbreeding, segregating genetic incompatibilities, and locally adapted alleles. Studies of Atlantic salmon have demonstrated significantly lowered fitness in hybrids between domesticated Atlantic salmon and wild populations (Skaala et al., 2012, 2019). Given the known life history differences between south-eastern and western populations of corkwing wrasse, we would expect to see both genetic and phenotypic effects of hybridisation. A recent free-mating experiment with western and south-eastern individuals, found that the western lineages contributed more to the next generation, i.e., produced more offspring (Blanco Gonzalez et al., 2019). However, in this study western individuals were moved to a southern environment, which is the opposite direction of common cleaner fish translocation. More work is needed to understand how translocated individuals from south-eastern populations will affect fitness in recipient populations. It is critical to assess phenotypic differences between individuals with native vs. south-eastern origins, and compare fitness between these groups in order to disentangle the ecological and evolutionary significance of hybridization.

As the corkwing wrasse population in Flatanger constitutes the northern boundary of the species distribution (Figures 3 & 5), it is likely to play an important role in northward range expansion as temperatures increase. Populations at the edge of a species distribution often experience environmental conditions similar to those just outside the species range. Thus, edge populations are often the most likely populations to carry genotypes that are able to colonise new habitats (Gibson et al., 2009). However, expanding populations will often also experience increased genetic load (Box 1). This is due to many factors, such as smaller effective population sizes, population structuring, increased drift, increased inbreeding and accumulation of deleterious mutations (Sexton et al., 2009; Allendorf et al., 2013; Peischl et al., 2013). This is often referred to as expansion load (Box 1), which can have profound effects on species, and is believed to be one of the processes maintaining species boundaries. Migration from the central population can benefit the edge population by reducing expansion load by bringing in new alleles and increasing levels of heterozygosity (Bridle et al., 2010; Allendorf et al., 2013). However, gene flow from foreign environments can also disrupt local adaptation and make edge populations more maladapted to the local environment (Kirkpatrick & Barton, 1997; Gilbert et al., 2017). Thus, it is possible that Flatanger populations will benefit from some migration from some populations, but could quickly become maladapted if introduced individuals come from a very different environment. If western populations are locally adapted to their environment, it is likely that the continued long-distance transfer of southern individuals will introduce maladapted alleles into the gene pool and thus work as a barrier to further range expansion.

### Box 1

**Genetic load:** the relative difference in fitness between the average genotype and the theoretically fittest genotype in a population. It can also be considered as a measure of the reduction in the mean fitness of a population, relative to a population composed entirely of individuals having optimal genotypes. The four primary sources for genetic load are mutation, segregation, drift and migration load.

**Mutation load:** the decrease in fitness due to the accumulation of deleterious mutations.

**Segregation load:** the decrease in fitness which occurs when combinations of alleles which have evolved together are segregated to less favourable combinations. This happens when heterozygotes have higher fitness than either of the homozygotes.

**Drift load:** accumulation of deleterious alleles due to random genetic drift. Alleles that are normally kept at low levels in the population due to mutation and selection, can by chance increase to much higher levels when genetic drift is high.

**Migration load:** a reduction in fitness caused by migrant individuals which are not well adapted to the local environment.

**Inbreeding load:** the reduction in fitness in inbred populations. This is caused by a combination of increased mutation load and segregation load.

**Expansion load:** the reduction in fitness as a result of genetic drift in the front of range expansion which can result in accumulation of deleterious mutations.

Southern corksiding wrasse are also translocated to salmon farms even further north than Flatanger, beyond the current range, where no wild corksiding populations are present. However, it is still unknown if cleaner fish are able to escape and survive in this environment, as well as what potential consequences this could have for local ecosystems. Although escaping cleaner wrasse would presently have no local populations to hybridise with, they may add new pressures in terms of competition, predation or even introduce new diseases or parasites to other species in the area (J. W. Treasurer, 2012; Wallace et al., 2015; Svåsand et al., 2017).

Currently, there are no regulations or records regarding the origin of the cleaner fish released into the net-pens along the Norwegian coastline. Since 2017, the source and destination of all wrasse imported from other countries must be reported by the transporting companies. In contrast, transporters do not have to report the source, or the destination of wild-caught cleaner fish caught within Norway. Unfortunately, country borders do not necessarily align with biological barriers. This is also true for corksiding wrasse populations, which are separated by a strong barrier at the southwestern tip of Norway, but show no signs of differentiation along Norway's national borders. In the transition zone from the North Sea to the Baltic Sea it is not possible to detect any genetic difference between individuals from Sweden, Denmark, Germany or south-eastern Norway. This means that a corksiding wrasse translocated from the

south-eastern coast of Norway poses the same genetic threats as an imported fish from e.g., Sweden. Ideally, cleaner fish should not be translocated across existing genetic barriers, where they may negatively affect local populations and ecosystems. Imported corksiding wrasse represents only a small fraction of fish being translocated, but currently there is no data on how many are being translocated within Norway. Consequently, due to the lack of reporting of translocations within Norway, it is not possible to properly assess how big of a threat translocation might actually be. Swedish wrasse populations show no signs of depletion with the current small-scale fishery. Our results suggest that populations in the transition zone from the North Sea to the Baltic Sea are well connected, and can be managed as such. However, this emerging fishery should be closely monitored for potential local changes in abundance or depletion, which could cause cascading effects on the ecosystem and stepping stones between populations separated by longer coastal distances.

In contrast to corksiding wrasse, lumpfish is highly structured also in southern Scandinavia. The results in this thesis demonstrate that lumpfish have a high level of genetic population structuring, despite the species transatlantic distribution range and high migration ability. Since 2017, an average of 34 million lumpfish are used as cleaner fish in Norway annually (Norwegian Directorate of Fisheries, 2021). This means that stock movements, where large numbers of hatchery-reared lumpfish are translocated among different regions across the Atlantic, represent additional risk to wild lumpfish. Given its global distributions, it is not unlikely that it too can escapee and survive in areas around salmon farms. Currently, the majority of the broodstock is still sourced from wild populations and escaping lumpfish from genetically distant populations could, similarly to wrasse, result in hybridisation and introgression into local lumpfish populations. The combination of a high degree of population structure and potential local adaptation, emphasises the need to manage the species on a regional level and avoid translocations between areas with genetically distinct populations.

In addition to the genetic risks, translocated cleaner fish may act as vectors of diseases, being asymptomatic carriers of bacterial, viral, and parasitic disease agents (Korsnes et al., 2017). An additional concern is the health and welfare of the cleaner fish and other ethical aspects. Many cleaner fish are killed during handling and transportation (up to 40%) or during other delousing procedures (Hjeltnes et al., 2019). A report by the Norwegian Veterinary Institute stated that this “effectively makes cleaner fish a ‘single use’ product, which in itself constitutes a welfare challenge for which both the industry and the authorities must find a better solution.” (Hjeltnes et al., 2018). While the loss of 20% of farmed salmonids is considered unacceptable, a cleaner fish mortality of 100% is not unusual in a production cycle (Hjeltnes et al., 2019).

## NOVELTY AND SIGNIFICANCE

This thesis provides the first evidence that translocated wild corksiding wrasse used as cleaner fish in salmon farms are escaping and hybridising with local populations (**Paper I, Paper II**). With genetic tools, I demonstrate that the recently established Flatanger population is mainly a result of an ongoing northwards range expansion, along with a significant genetic contribution from translocated southern populations. I show that escapees and hybrids may

constitute as much as 20 % of the Flatanger population (**Paper II**). In other parts along the Norwegian coast, where salmon farming is also common, I detected remarkably few escapees and hybrids. This suggests that introgression might be easier, or easier to detect, in smaller edge-populations than in higher-density areas. Finally, I developed a testing suite of 84 SNP-markers to identify escapees and hybrids, with the purpose to aid management and monitoring of wild populations of corkwing wrasse (**Paper II**).

Although I could not detect any signs of stock depletion of corkwing or goldsinny wrasse, or cascading effects on the ecosystem due the Swedish wrasse fishery (**Paper III**), I provide a baseline for future monitoring. This is especially important if the removal of wrasse on the Swedish coast were to increase. The genetic diversity and divergence of corkwing wrasse in Skagerrak has previously been estimated to be very low in comparison to other regions. In this thesis I show that this is not due to limited geographical sampling, but holds true along the whole transition zone from the North Sea to the Baltic Sea (**Paper IV**). Although populations show some isolation by distance, individual corkwing wrasse from e.g., Wadden Sea is genetically almost indistinguishable from fish in the southern Baltic Sea. This highlights the importance of transnational collaborations in fishery management, since genetic barriers do not necessarily align with national borders. In this thesis I show that lumpfish is a highly structured species, despite its transatlantic distribution range and high migratory ability (**Paper V**). This is consistent with natal homing behaviour and also indicative of local adaptation, and suggests that the species should be managed on a regional or even local basis. Translocation of lumpfish should be done with great caution, taking in account various risks related to escapees. The data in this thesis and the developed panel of SNPs can be used as a genetic baseline in future genetic studies, and aid in monitoring of wild lumpfish populations.

The use of cleaner fish for parasite control in other parts of the world is likely to increase in the coming years. This thesis complements previous work on how the use of lumpfish and wrasse in aquaculture can affect local populations and ecosystems due to fishery, and native populations around salmon farms due to translocation. Based on the results in this thesis, knowledge of existing population structure should be applied in management of the fishery and translocation of cleaner fish, to avoid overfishing and mixing stocks. Finally, monitoring should be prioritised in regions with large numbers of imported cleaner fish and/or with small populations, such as at the edge of the species range. Although the evolutionary and ecological significance of escapees warrants further investigation, the results from this thesis should be taken into consideration in the future fishery and translocation of cleaner fish.

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