Ecology and connectivity of coastal spawning areas of pleuronectidae as inferred from egg distribution and oceanographic modelling

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Abstract

Spawning areas are important for the recruitment of new individuals to the adult fish population thus alterations or losses of these areas may be critical for future recruitment. Coastal areas and coastal spawning species are prone to the growing anthropogenic effects threatening the coastal zone (e. g. constructional building, runoffs and over exploit of local populations). Additionally, stocks occupying coastal areas may be comprised of smaller populations with significant differences in genotypes and adaptations, especially in areas where eggs are retained by hydrographic forces and migration of adult individuals is small. The need for mapping of spawning and nursery areas in the coastal zone becomes increasingly important for a sustainable management of fish stocks and populations. For years the focus on mapping of spawning areas of the coastal cod (*Gadus morhua*) has been well researched, but the increasing focus on an ecosystem-based management emphasizes the need for more information on other "neglected" costal species, including flatfish. In the present study the following hypothesis were set: "Do any of the four species of flatfish (European flounder (*Platichthys flesus*), dab (*Limanda limanda*), long rough dab (*Hippoglossoides platessoides*) and plaice (*Pleuronectes platessa*)) spawn in coastal areas of Skagerrak.", "Can possible coastal spawning areas of flatfish indicate local population structure". These hypotheses were assessed by weekly sampling of eggs from January to July near the Institute of Marine Research, Flødevigen, served as reference for when the spawning of the species peaked. A temporal egg survey was conducted sampling eggs along the Norwegian Skagerrak coast, from this estimation of possible spawning areas were performed. The sampling of the pelagic eggs was conducted using a WP2 net hauled at a constant speed to sample vertically in the water column. Lastly, an ocean current model and particle model was applied to the estimated spawning areas and the particle drift from the location of discharge was estimated for 10, 20 and 30 days. Eggs retained within the spawning area can indicates self-recruitment at the area and possible genetic variation among costal populations. In the study, estimations of spawning areas were significant for European flounder (38 areas), dab (24 areas) and long rough dab (three areas). Plaice had no significant spawning areas and the presence of plaice eggs were scarce and was therefore not included in the particle drift analysis. From the ocean current model, retention areas were registered for the three species with European flounder, dab and three long rough dab, 17, 14, 3 areas, respectively. In conclusion, hydrographic forces retain eggs in spawning areas for three of the investigated species indicating important population structures and self-recruitment areas.

Table of contents

1 Introduction

Stocks and fisheries are primarily regulated through removal of adult fish from the population, but fishery regulation will not be enough in order to maintain a sustainable fish population. Szuwalski et al., (2015) suggests that the recruitment of offspring is not only affected by size of the spawning biomass but also by environmental factors in their habitat. Costal bound species and their spawning and nursery areas are prone to anthropogenic alterations in the coastal zone which can affect survival and recruitment (Espeland et al., 2013). According to McCauley et al. (2015) habitat loss will be a new large threat possibly causing extinction of marine fish stocks. The increase of mapping marine habitats which holds favourable environmental properties has become important for managing stocks and populations, considering regulation of fishing activities or limit marine protected areas (Schismenou et al., 2017). Recognition of these types of habitat and the location of these are therefore important for the life history traits and for initiating conservation and management methods (Wilson et al., 2010).

The complex life cycles of fish and their life stages (egg, larvae, juvenile and adult) face different challenges, especially considering that different life stages require various habitats (Rijnsdorp et al., 2009). Petitgas et al., (2013) also highlights that each life stage has different habitats and the recognition of these are important regarding habitat availability, habitat requirements and connectivity. For many marine species the dispersal (spawning) of pelagic larvae is key to understanding the effect of this stage on the population connectivity in marine systems (Cowen & Sponaugle, 2009). For species who spawn in open waters the transport or migration of larvae to nurseries are important (Grioche et al., 1997).

Spawning areas are important for the first life stage of marine species and the recruitment of new individuals. In biology spawning is defined as when the mature fish release gametes into the water column. Traditionally spawning areas has been deciding based on catch of mature spawning individuals. But, observation of mature fish does not exclusively mean that it is a spawning area because of other factor that may attract the fish, and spawning areas are not necessarily attractive to the mature fish (Espeland et al., 2013). The use of newly spawned eggs for estimating spawning areas and future recruitment have been addressed in several studies (Espeland & Sannæs, 2018; Espeland et al., 2015; Ciannelli et al., 2010). Espeland et al., (2018) also emphasizes that determination of the development stage of the sampled egg provides information about the place of origin of the sampled egg. Assuming that eggs that are less developed are more likely to have originated at the sampling area compared to farther developed eggs that may have drifted to the sampling area from afar. In addition to identifying the development stage of the eggs, particle drift and oceanic models has been utilized as a prediction method of egg drift from the spawning areas (Espeland et al, 2015).

Even if coastal areas are among some of the most productive and valuable areas, there are still great losses of these habitats, either directly (e.g., costal engineering) or indirectly by continuous stressors build-up (Costanza et al., 1998; Crain, et al., 2009). Anthropogenic alterations may destroy costal habitats, but in some cases, they may also provide new ones. For example, an estuary formed by a human made canal connecting a lake to the open sea provided new habitat for an adapted and specialized populations of herring (*Clupea harengus*) (Eggers, et al., 2015). Regarding adaptation and division of species populations along the coast, Knutsen et al., (2003) found that the costal cod (*Gadus mohua*) is comprised of many small populations which differs in genetic composition. Therefore, the local adaptations for the costal bound species increases the need for management at a local scale and protection of marine habitats.

Along the ~ 25 000 km Norwegian shelf coast research on flatfishes is limited, especially in regard to the multiple species residing there and their distribution patterns (Albert et al., 1998). For the last 30 years, commercially important species have been the priority regarding population restauration and therefore other species of less commercially importance have been neglected. But, the prominence of an Ecosystem Approach to Fisheries Management (EAFM) now direct attention to less commercially important species with the objectives saying that biodiversity and ecosystem function should be ensured (Gullestad et al., 2017). In 2018, the Ministry of Trade, Industry and Fisheries ordered an extensive investigation of the spawning areas of our costal species. Earlier, the program focused exclusively on costal cod (*Gadus morhua*) but has now decided to look to other species in order to conserve a larger part of our costal fish stocks (Statsbudsjettet 2018: Tildelingsbrev til Havforskningsinstituttet). As a part of this, our study will focus on four species of the family pleuronectidae where the spawning period is similar to the one of cod and the eggs of these four species is also possible to identify by visual identification which logistically matches with the yearly egg survey.

The four species of interest in this study are European flounder (*Platichthys flesus*), dab (*Limanda limanda*), long rough dab (*Hippoglossoides platessoides*) and plaice (*Pleuronectes platessa*). These species are of the family plauronectidae, (right-eyed flounder), also referred to as true flounders (Allaby, 2014). Flatfishes are usually recognized by having both eyes on the same side of the head and differentiations in colours (coloured on the eye-side and white on the blind side) (Brewster, 1987). The symmetrical larva of the flatfishes attains their characteristic asymmetric looks when undergoing metamorphose in the post-larva stage (Brewster, 1987). Following this the asymmetrical larva shifts from being pelagic to benthic. The flatfishes are usually found on the seafloor in different substrate bottoms (mud, sand, sand-shell and rocky/pebble) (Gibson, 2005). Common for these four species is that they spawn pelagic eggs and the spawning occur during the winter – summer halve year, with an average spawning period between March and May (Munk & Nielsen, 2005). Spawning strategy may vary between species, either spawning once or for a short period, or some species are so called batch spawning (Maddock & Burton, 1998; Ganias, et al., 2015). The batch spawning strategy has been thought to have certain advantages. By spawning in batches survival of offspring can increase because the temporal and/or spatial difference may increase the probability of avoiding predators and meeting favourable conditions that enhance growth and development. Fecundity may also increase allowing species with smaller body cavities to produce more eggs (McEvoy & McEvoy, 1992). In addition to their importance regarding both the ecology and economy, flatfish also function as an energy pathway from unexploited benthic production to us humans in the form of fishery (Link, et al., 2002).

European flounder (*P. flesus*) is a widely distributed species and is found in coastal areas through-out the north-eastern Atlantic Ocean and is versatile considering its tolerance to low salinity (Hemmer-Hansen et al., 2007). Usually it spawns pelagic eggs, but in the Baltic Sea they have adapted to spawn demersal eggs due to the low salinity. According to Nissling (2002), the adaption to spawn demersal eggs in low salinity habitats is the reason for the high abundance in the Baltic Sea. The spawning takes place from January until July and larvae usually exploit estuarine nurseries (Munk & Nielsen, 2005; Daverat et al., 2012).

Dab (*Limanda limanda*) is copious in the North Sea and stretches all the way from the French coast to the Barents Sea (Saborowski & Buchholz, 1997; Munk & Nielsen, 2005). Dab has a high fecundity, is a serial spawner, and the spawning period is normally long and located near the nurseries (Daan, 1990). Bolle et al. (1994) studied the nursery grounds of dab in the south North Sea, finding that the newly settled larvae do not exploit either coastal areas, estuaries or tidal zones, but enters them later after a stay in the deeper waters.

Long rough dab (*Hippoglossoides platessoides*) has never been exploited in the fisheries, even if it may occur in high numbers (Walsh, 1996). The species is found well distributed in the North Atlantic and is a batch spawner which spawns between January - May (Morgan, 2003; Maddock & Burton, 1998; Munk & Nielsen, 2005).

Plaice (*Pleuronectes platessa*), among the four species of interest it is considered as a commercially important species in Norway (Albert et al., 1998). The species is distributed from the White Sea to the Mediterranean and the North Sea plaice stock is highly researched (Munk & Nielsen, 2005; Gulland, 1968; Hufnagl et al., 2013). The spawning season stretches from December to April (Munk & Nielsen, 2005)

1.1 Objectives and hypothesis

To improve knowledge on spawning individuals and their use of the habitat in the coastal zone based on distribution of eggs, the following main questions investigated:

1. Do any of the four species of flatfish (*P. flesus*, *L. limanda*, *H. platessoides* and *P. platessa*) spawn in coastal areas of Skagerrak?
2. Can possible coastal spawning areas of flatfish indicate local population structure?

To assess these questions the timing of spawning (temporal) and the location of spawning area(s) (spatial) for four flatfish species (*P. flesus*, *L. limanda*, *H. platessoides* and *P. platessa*) were investigated based on egg sampling along the Skagerrak coast. Also, an ocean current model and particle model was applied to address the population structure and connectivity of the spawning areas. The three following objectives, three hypothesis and eight sub hypotheses were carried out in order to address the aim

Objective 1: Monitor the seasonal variation in egg abundance for all four species from January – June (six months), to serve as a reference of when spawning was initiated and when it peaked.

Hypothesis 1: Does the four species of flatfish have a spawning peak that corresponds with the time period were the egg survey was conducted.

> **Hypothesis 1.1**: Does the timing of the spawning peak for European flounder (*P. flesus*) correspond to the period as the egg survey was conducted.
>
> **Hypothesis 1.2**: Does the timing of the spawning peak for dab (*L. limanda*) correspond to the period as egg survey was conducted.
>
> **Hypothesis 1.3**: Does the timing of the spawning peak for long rough dab (*H. platessoides*) correspond to the period as the egg survey was conducted.
>
> **Hypothesis 1.4**: Does the timing of the spawning peak for plaice (*P. platessa*) correspond to the period as the egg survey was conducted.

Objective 2: Map possible spawning areas for all four species based on spatial sampled eggs and their variation in abundance.

Hypothesis 2: Can patterns in egg distribution and quantity indicate spawning areas for the four species of flatfish along the Skagerrak coast.

> **Hypothesis 2.1**: Can patterns in egg distribution and quantity indicate spawning areas for European flounder (*P. flesus*).
>
> **Hypothesis 2.2**: Can patterns in egg distribution and quantity indicate spawning areas for dab (*L. limanda*).
>
> **Hypothesis 2.3**: Can patterns in egg distribution and quantity indicate spawning areas for long rough dab (*H. platessoides*).

Hypothesis 2.4: Can patterns in egg distribution and quantity indicate spawning areas for plaice (*P. platessa*).

Objective 3: Estimate particle drift from spawning areas inferred by an ocean current model and a particle model and interpret the drift of particles to determine areas with retention or dispersal.

Hypothesis 3: Does hydrodynamic forces retain eggs within the potential spawning areas.

2 Materials and methods

Fieldwork was executed in collaboration with the Institute of Marine Research (IMR, Flødevigen, Norway), as a part of the national program "monitoring of spawning and nursery area for costal commercial species".

2.1 Small-scale temporal sampling

Five stations were sampled weekly over a period of approximately six months (January – June 2019). This resulted in 24 samples (one for each week) consisting of five stations. The five samples were merged into one sample because of the small geographical differences among the stations and since the purpose was to map the spawning peaked during in the sampling period. Even if it limited the possibilities regarding statistical analysis and variation.

Fieldwork was performed on days with minimum wind, based on the weather report. The time-range between the sampling varied from 3-11 days and an had an average of 7 days. The stations where visited by a small boat, (Arronet 23.5 SP, Sweden), equipped with a chart plotter with an integrated echo sounder (Garmin echomap 52DV, USA) and an electric hauler (hobbyfisher E150, Lithuania). The chart plotter was used to store the stations position coordinates and measure depth.

During the sampling the boat was placed at the sampling station. To collect the pelagic eggs in the water column a standard WP2 net with a mesh size of 500 µm and an opening diameter of 60 cm was used. The top of the net was connected to a rope and at bottom of the net had a detachable cup (made by Sebastian Bosgraaf at the IMR) and was connected to a weight (6 kg). The purpose of the weight was to keep the net vertical in the water column during the haul (elevation of the net from the sample depth to the surface). The rope attached at the top of the net was marked at 5, 10, 20, 25 and 30 meters, and controlled the depth immersion of the net. Sampling depth ranged between 20 -30m and was determined by the depth at the station (figure 1). To avoid contamination of sediments the samples were collected approximately four meters above the sea floor to avoid collision.

Because of the small mesh size of the net, the hauling speed had to be slow enough to allow the water to be filtrated. Too high hauling speed would cause the net to push the water in front of the opening instead of filtrating it and the eggs would have been pushed away instead of collected. A hauler was used, to ensure a constant speed during the haul. An optimal hauling speed is approximately 0.5 m/s (Barnes, 1949). When the net reached the surface, it was lifted back in the boat holding the bottom of the net as vertical stable as possible, this was done to keep the eggs at the bottom of the detachable cup. Next the cup was detached and with a spray bottle the sample was carefully flushed with sea water into a sample glass (100 ml). This procedure was repeated for all five stations and were treated as one sample during the identification.

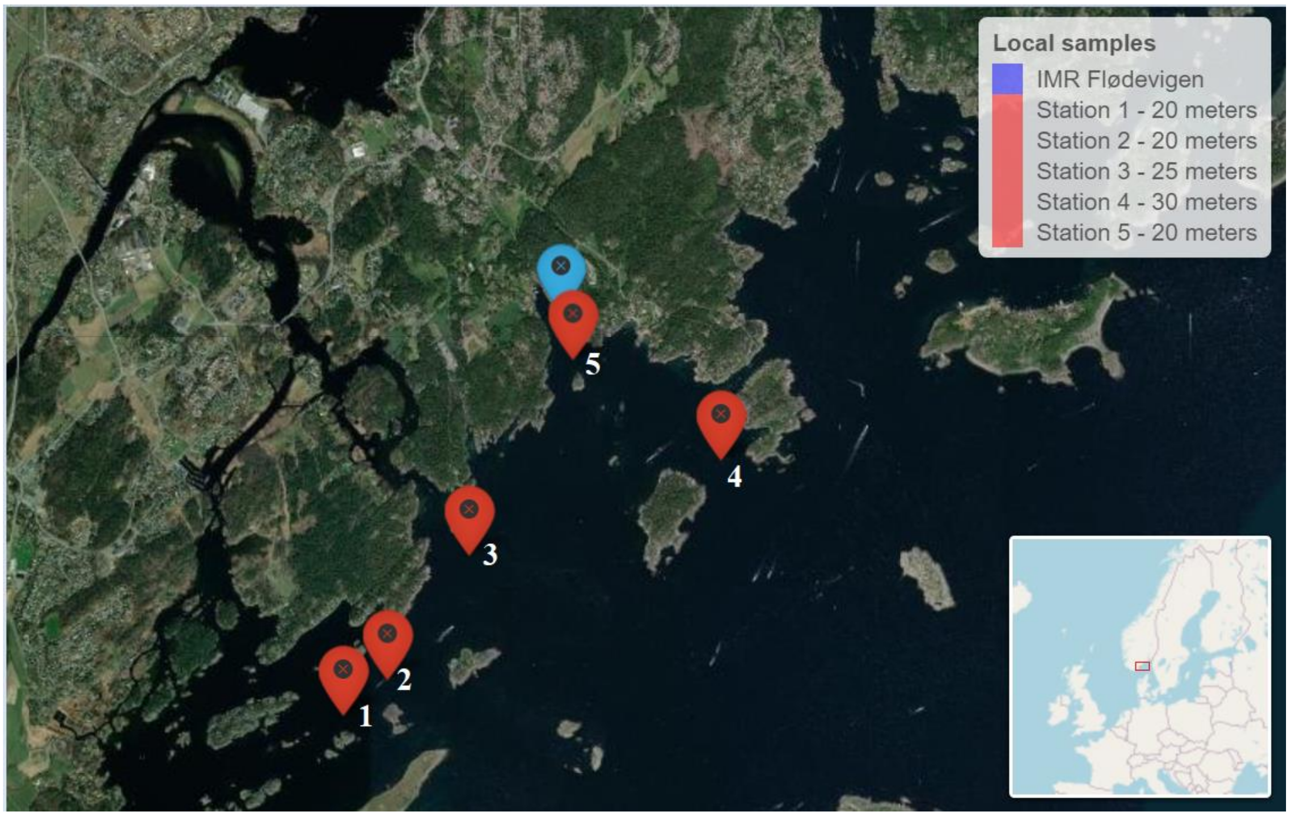

Figure 1. Map showing the five locations of the sampling (red) and the IMR, Flødevigen (blue). The sampling depth for each station in the insert.

2.2 Identification, photography and conservation

The egg samples were examined, and the species were identified in the zooplankton laboratory at the IMR, Flødevigen. The samples where kept in a refrigerator (4°C) until treatment. Next smaller portions of the egg samples were transferred into a counting chamber (made by Sebastian Bosgraaf, IMR). The samples usually had to be treated in smaller portions because of the capacity of the counting chamber, and in periods during the spring when the high concentration of zooplankton in the sample could interfere with the egg extraction precision (figure 2A). If the sampled contained a high concentration of zooplankton or fish larvae dilution with salt water was necessary. The counting chamber consisted of five wells and measured approximately 10 x 15 centimeters in total. The portion size that was transferred varied as the zooplankton concentration increased or decreased, and no exact volume was added to the counting chamber. The sample had to be diluted enough to easily see the eggs among the zooplankton and fish larva, but not extend the top of the counting chambers partitions that would have caused the eggs to float freely around. Dead fish eggs were removed from the sample. A stereo loupe (Leica MZ16 A, Germany) was used to extract the eggs with a pasteur pipette (7 ml). The light settings of the stereo loupe were usually sat to high over light and low under light during the egg extraction (figure 2B).

Figure 2 A – B. A) Picture illustrating the density of zooplankton in the egg samples during the spring, before diluting with seawater. The picture shows one of the wells in the counting chamber. B) The process of extracting the eggs from the sample. Credit: Anders Jakobsen (IMR).

After the extraction the eggs were categorized into four groups: small eggs (< 1 mm), large eggs (> 1 mm), small eggs with oil globule (< 1 mm) and large eggs with oil globule (> 1 mm). Size and presence/no presence of oil globule are two of the traits that can easily give an impression of which specie it is (Munk & Nielsen, 2005). The literature used for the identification of the species was Munk & Nielsen (2005) and Russell (1976). Next, the eggs were measured to the exact diameter, which usually narrows the alternatives of the species further (figure 3 & 4). Following size measurement traits like oil globule, segmentation of the yolk, pigmentation, color of pigmentation and shape of the pigments helped identifying the egg (figure 3 & 4).

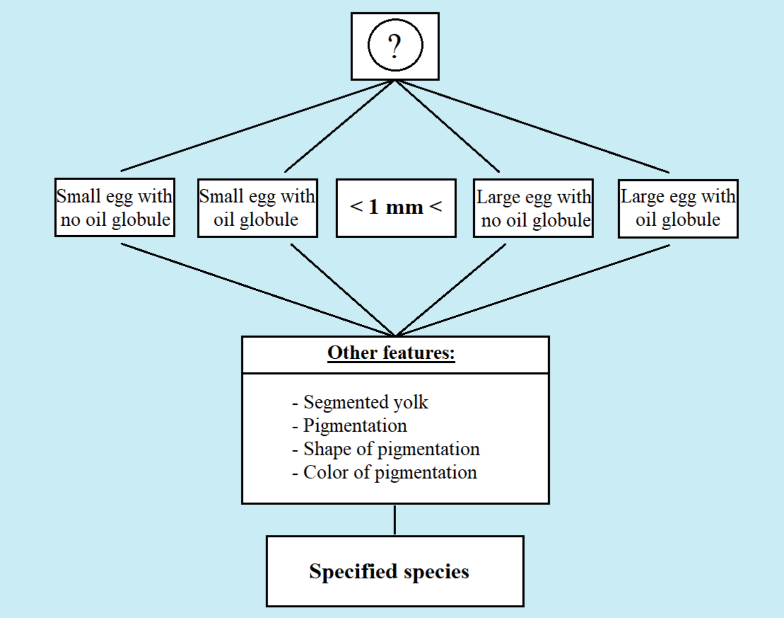

Figure 3. Illustration of the procedure of identifying the eggs.

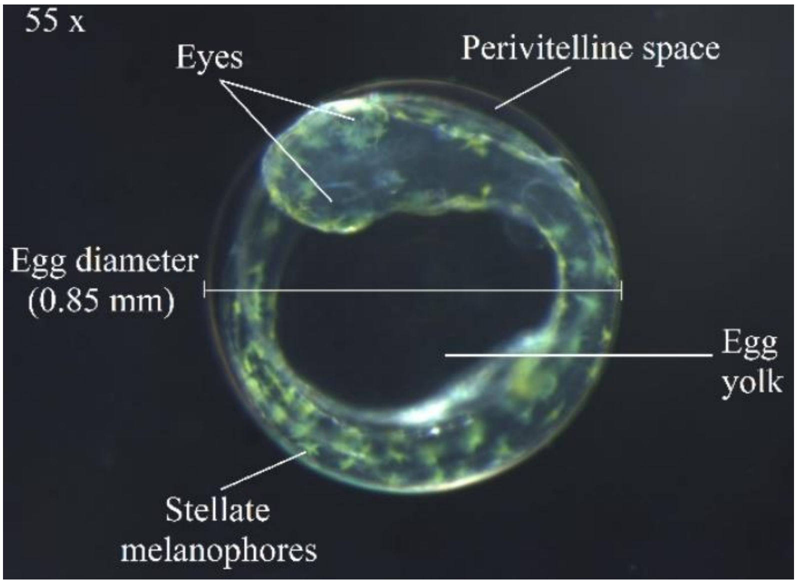

Figure 4. Photo of a common dab (*Limanda limanda*) egg. Typical features and structures contributing to identifying the species are marked on the embryo and the surroundings (private photo).

Typical characteristics of the flatfishes is the abundance of bright yellow pigments (melanophores). The pigments appear in the late development of the larvae and vary in intensity and yellow tone. Dab (*L. limanda*) eggs varies between 0.66 – 0.98 mm in diameter (with a mean diameter just above 0.8 mm) and has bright lemon yellow melanophores (figure 5A). European flounder (*P. flesus*) eggs varies between 0.82 – 1.13 mm in diameter (with a mean diameter just below 1 mm) and has characteristic chrome yellow melanophores in the late stage of the embryo (figure 5B), (Munk & Nielsen, 2005). European flounder and dab eggs may be hard to distinguish because of the similar diameter and before the appearance of the characteristic yellow melanophores. A limit of 0.8 mm was set to differ the two, where the eggs measuring 0.6 - 0.8 mm in diameter set to be dab and eggs measuring 0.8 – 1 mm in diameter set to be European flounder, if the characteristics of the larvae was lacking. Long rough dab (*H. platessoides*) and plaice (*P. platessa*) have larger eggs than the European flounder and dab. The long rough dab eggs vary between 1.38 – 3.50 mm in diameter (mean 2.50 mm), (figure 5C). and plaice eggs vary between 1.66 – 2.17 mm in diameter (mean 1.90 mm), (figure 5D). These two species were distinguished because of the large perivitelline space of the long rough dab and both species have yellow pigments on the developed larvae (Munk & Nielsen, 2005).

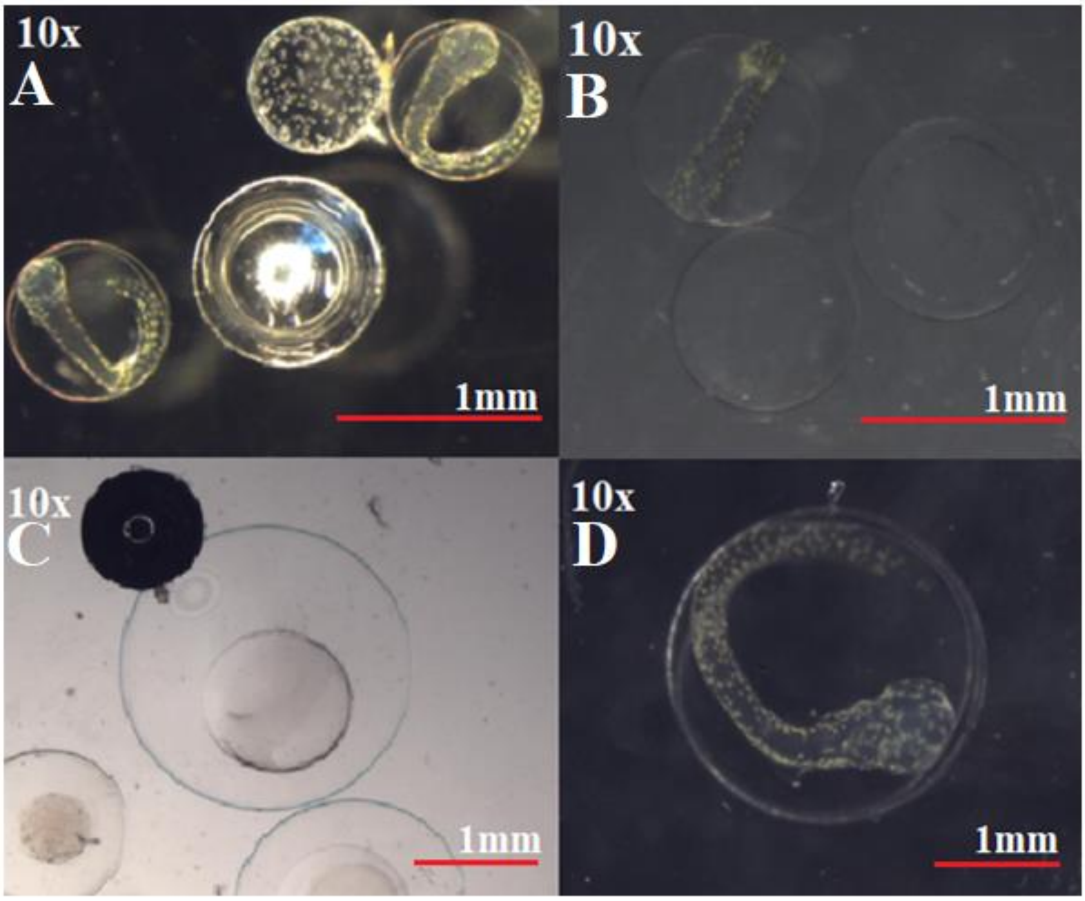

Figure 5 A – D. A) Dab *(L. Limanda)* eggs with characteristic yellow pigments on the larvae. The egg with all the small scattered oil globules is the egg of solenette (*Buglossidium luteum*). B) European flounder (*P. Flesus*) eggs, one egg contains a developed larva with chrome yellow pigments and two eggs are newly spawned and in the blastula stage. C) Long rough dab (*H. Platessoides*) egg with the large perivitelline space, the larva and pigments have not developed yet. D) Plaice (*P. platessa*) with developed larvae and yellow pigments. The reason for the difference in the 1 mm reference is due to editing of photographs.

After identification, the eggs of the different species were photographed with a camera (Leica IS 1000 10 OMP, Germany) attached to the stereo microscope. All the photos were taken at ten times magnitude and every sample picture contained a sphere measuring exactly one millimeter as a size reference. The photographs were used as a back-up in case of sample or data loss and during correction of counts or identification.

After image acquisition, the eggs were transferred to individual glass vials (15 ml), divided into flatfish eggs (all flatfish species found in the sample), other eggs, flatfish larvae, and other larvae. Samples were placed in the vial with waterproof paper containing date, name, preservative, species content and number. Lastly the vials were filled with 96% ethanol for conservation of the eggs.

2.3 Large-scale spatial sampling

The cruise took place from 19.03.2019 to 03.04.2019, reaching all the way from Risør, Aust-Agder to Siragrunnene, Rogaland. The purpose of the cruise was to sample spatial data and investigate the possibility of spawning areas for the target species. Research vessel G. M. Dannevig were used during the cruise. In total 389 out of 421 stations were sampled during the cruise in Skagerrak. During the cruise 32 stations were cut. Reasons for this was because of ice covering sampling areas and too shallow waters for G.M Dannevig to reach. Some stations were cut at Siragrunnen, Rogaland since the stations were outside the area which was to be covered in 2019 (Agder county). The stations that were sampled at Siragrunnen was set to be sampled if time allowed it. Also, 83 of the stations egg samples had to be identified after the cruise was conducted (figure 6). This was executed by using the back-up photos taken of each sample. On an average 20-30 stations were sampled per day.

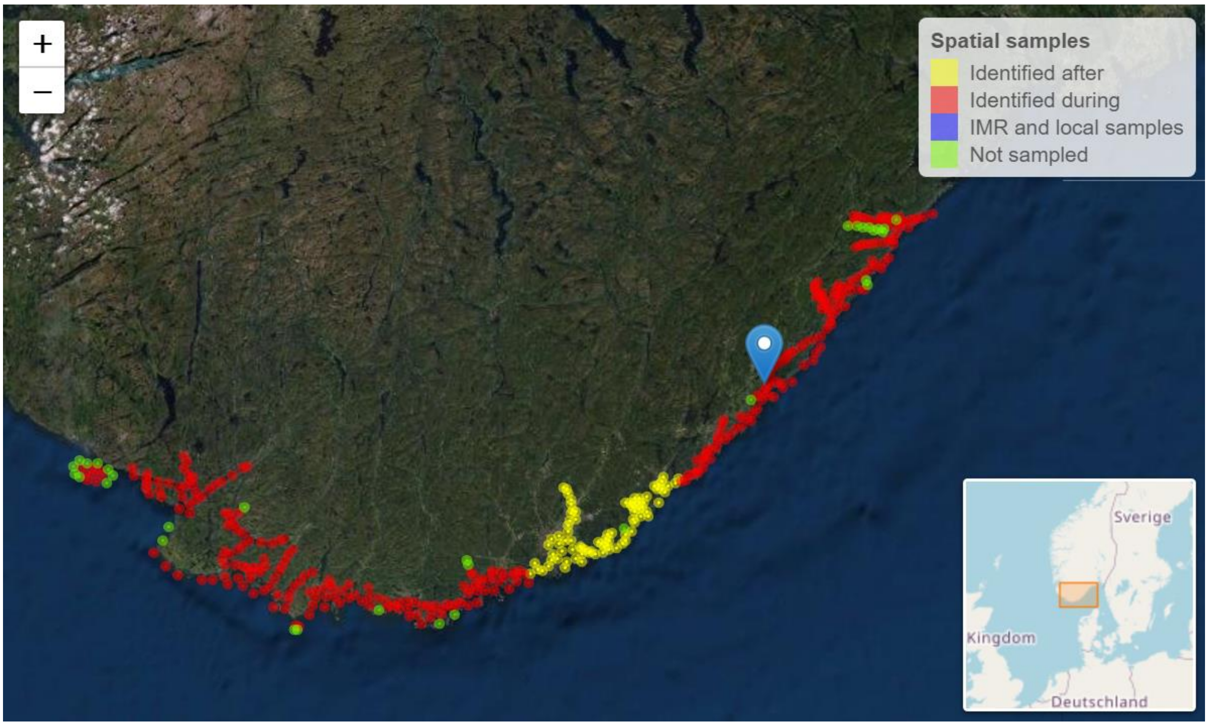

Figure 6. Map showing all the stations along the southern coast of Norway. The sampling started in Risør, Agder and continued to Siragrunnen, Rogaland, covering the entire coast of Agder county. 32 stations were cut during the cruise and are marked as green. 83 of the stations had their egg-samples identified after the cruise was conducted (yellow) and 306 of stations were sampled and the eggs were identified during the cruise (red). The blue marker indicates the position of the IMR, Flødevigen and the local temporal sampling.

The same equipment as described in 2.1 were used during the cruise, except that the hauler was replaced by a larger crane equipped on the boat and a CTD (conductivity, temperature and density) devise (Cast-Away, USA) was connected to the bottom of the WP2-net (figure

7). The procedure for the egg sampling were as following. The vessel was equipped with a crane with settings controlling the haul to raise the egg net at a constant speed of 0.5 m/s. The egg net was connected to the wire of the crane at the top and with solders attached to the bottom (figure 7). Standard depth of sampling was set to 50 m, but in shallower areas the net was lowered to four meters above the seafloor as a standard procedure. When arriving at the surface the net was lifted out of the water and flushed with a saltwater to flush down all eggs that were stuck in the net wall. The cup containing the sample was detached and brought inside to the lab onboard. Information about the sample was noted (station number, depth at the station, sampling depth, time and date) and followed the sample during treatment.

In the lab the sample was flushed out of the eggcup and into a sample glass (100 ml) with the sample note. Samples were kept in a refrigerator (4°C) until treatment to keep the eggs alive. Next the eggs and fish larvae were separated from the sample. In areas with high abundance of copepods and other zooplankton the sample was diluted with saltwater in order to make it easier to extract the eggs. Eggs and larvae were separated into two containers and taxonomists from the IMR identified the species. Identification of the species were done the same way as described above in 2.2. The eggs were then put into glass vials with 96% ethanol with the sample note and which species it contained.

Figure 7. Picture of the egg net used during the cruise. On the bottom of the net is the detachable cup and the Cast-Away CTD.

2.4 Determining spawning area

Data from the large-scale spatial sampling was analyzed for potential spawning areas and the stations that was not sampled was extracted from the dataset. To find which hauls where performed in a spawning area, groups of stations which are thought to be representative for the underlying density of eggs were picked. These groups will have a low mean of observed eggs and a minimum of stations (usually 10% of the sampled stations), (Espeland et al., 2013). First, all sampled stations were divided into geographical groups. the sampling area was divided into ten groups (A-J, respectively) covering about 10 % of the total samples each. The area was divided regarding the structure of the coast and contained both sheltered and exposed stations (figure 8). Since the cruise extended over a period of 16 days the areas varied between 1-2 days of sampling and consisted of 39 stations on an average.

Figure 8. Spawning areas groups that were set in order determine the possibility of spawning areas along the Skagerrak coast. The areas are divided into groups from A-J covering all sampled stations.

2.5 Ocean current modelling

After the sampling of eggs and identification of spawning areas it is important to connect this to where the eggs drift, both for understanding the population structure and how the spawned eggs may spread with hydrodynamic forces in the ocean. An oceanographic model was created for a total of 421 stations (all intended stations). For each station 50 particles drifted in the model resulting in a total of 21 050 individual observations of particle drift (figure 9). Variation in time and the physical environment was compensated by performing the model for seven different days, with two days apart, covering the variation for a 14 days period (20.03-01.04.2019) which gives a total of 147 350 drift observations. The drift models of interest will be the ones where high densities of eggs are registered. Particles are released into the model at the same locations as they were found during the cruise sampling at various depths. From there, the movement of the particles in the model can be monitored and for this study registered at 10, 20 and 30 days. Particles that in the nature would have left the modelled area will instead be trapped at the limits of the model. When calculating the mean the these values would have to be excluded, while looking at the distribution and drift pattern these particles still explain the direction an partially the distance of the drift.

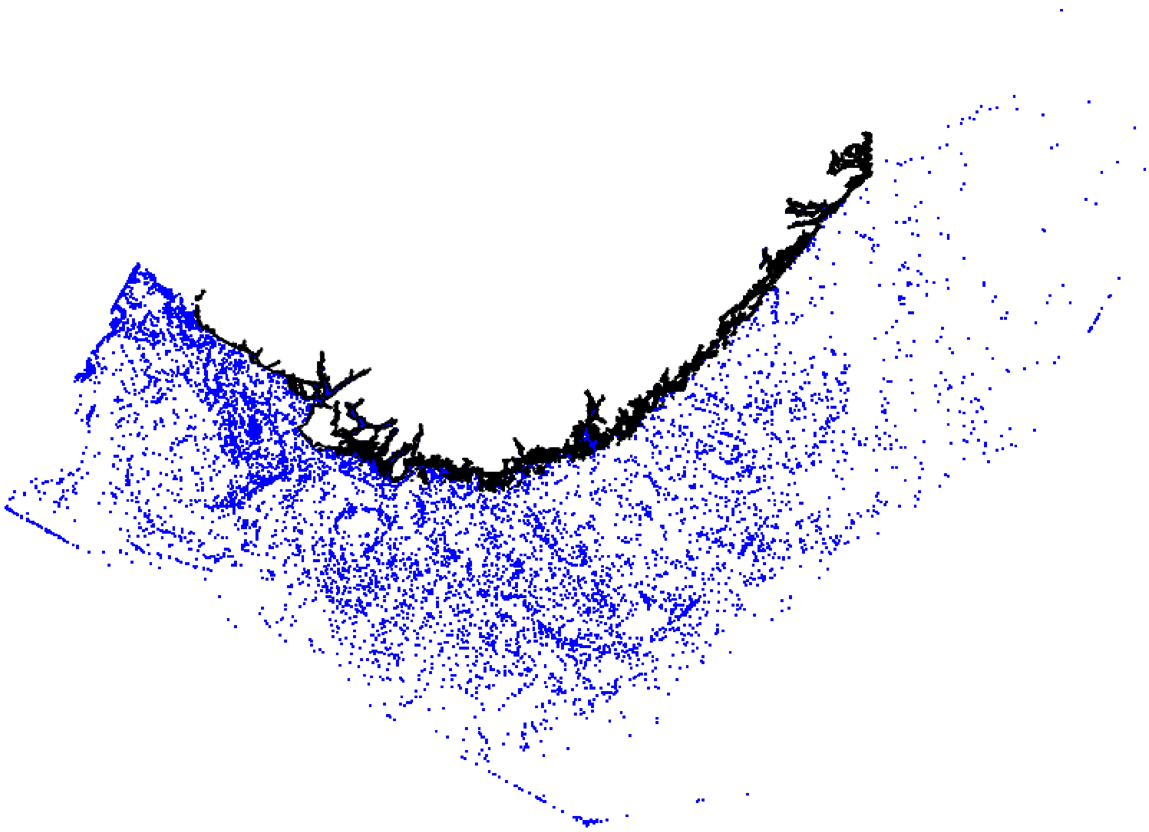

Figure 9. The start position of each sampled station, and registration points at 10, 20 and 30 days of egg particle drift results in 21 050 observations in the model on the 20th of March 2019. The figure shows the amount of registered particle drift for one day. Each registration point is represented by 21 050 observations, therefore in this figure there are 84 200 particles showing the distribution and magnitude of one day of particle drift. Instead of drifting further the eggs that leave the currents model will gather at the limit were the model stops.

The oceanic model used was the Regional Ocean Modelling System (ROMS) and the modelling was performed by Jon Albretsen (IMR). The following text is a summary of Espeland et al, 2013 explaining the mechanisms behind ROMS (see, Shchepetkin & McWilliams, 2005, Haidvogel et al., 2008, for more details) and LADIM (see Ådlandsvik & Sundby, 1994, for more details), that were used to study the drift of the eggs.
The modelling is executed by two steps, first ROMS gives a prediction of the oceans physical processes (currents) in a pre-defined area. Second the LADIM model use the modelled currents information to predict the particle drift.

Hydrodynamic models can describe currents, turbulence, water levels and hydrography for a predefined ocean area for a given time period. Simply explained ROMS estimates direction and speed of currents in a grid cube by including all relevant physical processes. The advantages of using ROMS is that one can choose from a large register of numerical solutions or schemes where many of these are highly accurate. ROMS is also used internationally increasing the rate of development and it is adapted to most high-performance computers. ROMS uses terrain following vertical coordinates which has proved to be fitted for shelf areas and shallow oceans. Important for the modelling is availability of information affecting the physical processes such as atmospheric variables, tide, output from rivers, currents, hydrography and water level. What determines if the oceanographic model is good is how well it recreates reality, and validation is an important step to reveal weakness or strength in the model. This has been executed by including a CTD devise at each egg sampling.

LADIM (Lagrangian Advection and DIffusion Model) base the particle drift on currents estimations by ROMS. To compensate for the loss of natural variation in the currents model a calculated random addition is added to the speed vector of the model. This does not affect the particle drift dramatically but allow the particles to perform a “random walk”. The particles are only allowed to drift in pre-defined depth levels. There is also a possibility of either allowing the egg to move along a set isopycnal (levels based on density, and in that case each particle would need their own density value) or allowing vertical drift. Vertical drift based on the egg density would be the most realistic since the buoyancy of the egg determines the vertical position. The problem with this type of particle drift is that small inaccuracies in both estimation of egg and water density can lead to large errors in the vertical position of the particle and the consequential error will be wrong estimation of drift. Because of these insecurities in the model the drift simulation should be set to a pre-defined depth as in this study.

The hydrodynamic models are constrained in the way that the large dataset requires high-performance computing. Given the large dataset this is a slow process and for the fjord modelling the area must be limited. By limiting the modelling area, eggs may drift outside the model and information on the final destination of the eggs are lost. For now, the eggs that drift out of the model is compensated by providing enough particles so that the statistical foundation is enough even if some of the eggs are lost.

2.6 Statistical analysis

All statistical analyses were performed using the statistical software Rstudio version 1.2.5033, working in R Markdown.

The maps were created using the Leaflet package (version 2.0.3) in R Markdown. The coordinates used for the plotting of spatial points were WGS 84.

The spatial egg data was divided into ten groups (see 2.4). To distinguish samples taken in a background density of eggs and samples taken in a spawning area following analysis was performed. The number of eggs sampled at the stations will represent the underlying density of eggs in the area. One can therefore say that each haul will be random occurrences producing whole numbers which has a density given by the mean number of eggs sampled and for a number of samples. Best to describe this information is the Poisson distribution. The background density of each group (A-J) was therefore calculated for each species and used to create a probability density function of the Poisson distribution. From here the p-value was set to be 0.5 and the upper 95 % confidence interval was calculated. The upper confidence interval of the function will give a number explaining that observations exceeding this value will have a low proverbiality of occurring, indicating a spawning area (Espeland et al., 2013).

From the ocean currents model and particle drift model all significant spawning areas were plotted in the leaflet map to display the distribution and drift for 10, 20 and 30 days.

Particles that left the ocean current model was excluded when calculating mean drift distance and the coordinates were transformed to Universal Transverse Mercator (UTM) for estimating drift distance and direction vectors. For each station with significant number of eggs 50 particles were modelled for seven different days of discharge (seven days of observations per station). The distance between the point of discharge and 30 days of drift was calculated for all 50 modelled particles per day. Next the mean drift distance was calculated for each day, and an additional mean of these seven days were calculated to describe the mean drift distance. Median, standard deviation max and min distance was also noted (table 2, 3 & 4).

Vectors explaining the mean direction the drift were estimated from the mean of the UTM coordinates per day (50 particle drift models) which resulted in seven vectors. Here the particles leaving the models were included since they still explain the direction of the drift, even if the length of the vector will be slightly longer than without the particles. Plaice was not included in the ocean current modelling due to the lack of station with significant numbers of eggs.

Finally retention areas was determined based on the pattern of simulated particles in combination with the vectors and mean drifting distance.

3 Results

3.1 Small-scale temporal sampling

Five local stations were sampled every week from January to July resulting in 24 pooled samples in total. In total, 2487 eggs where sampled and identified to 22 different species, where 709 eggs (28.5 %) represented the four/ flatfish species of interest. The eggs were divided into six groups; European flounder, dab, long rough dab, plaice, other flatfish and other species, to show the variation in egg abundance over time, (figure 10.)

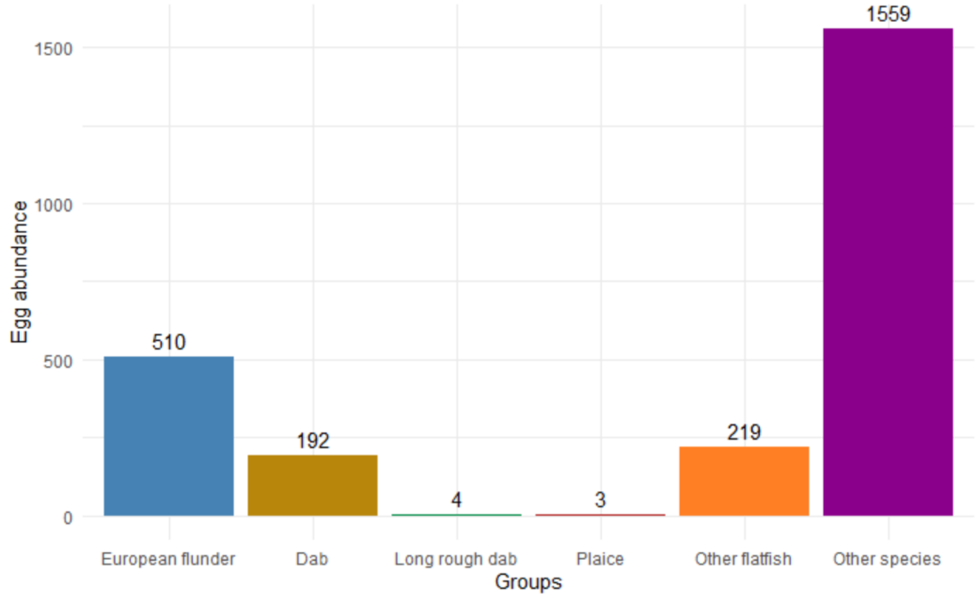

Figure 10. Variation in abundance between the target species and the two other groups. The y-axis shows the number of eggs within each group. X-axis shows each species/category group. Each bar is represented by 24 observations and over the period from Jan – Jun 2019.

European flounder and dab were the two most abundant species. The European flounder had the longest temporal distribution from the middle of January until the end of sampling (Figure 11). The egg abundance peaked early March and had a second smaller peak early May. Range of eggs per week for European flounder was 0 – 141. The first registration of dab was in the middle of February and ranged between 0 – 46 observed eggs per week. Dab did not peak until early May, almost two months later than the European flounder (Figure 12). On the contrary, for plaice and long rough dab, there were only 3 and 4 eggs, respectively, in total (Figure 13 & 14). Both species appeared early in March and were registered for 2-3 consecutive weeks. Range of number of eggs for plaice was 0 – 1 and for long rough dab 0 – 2.

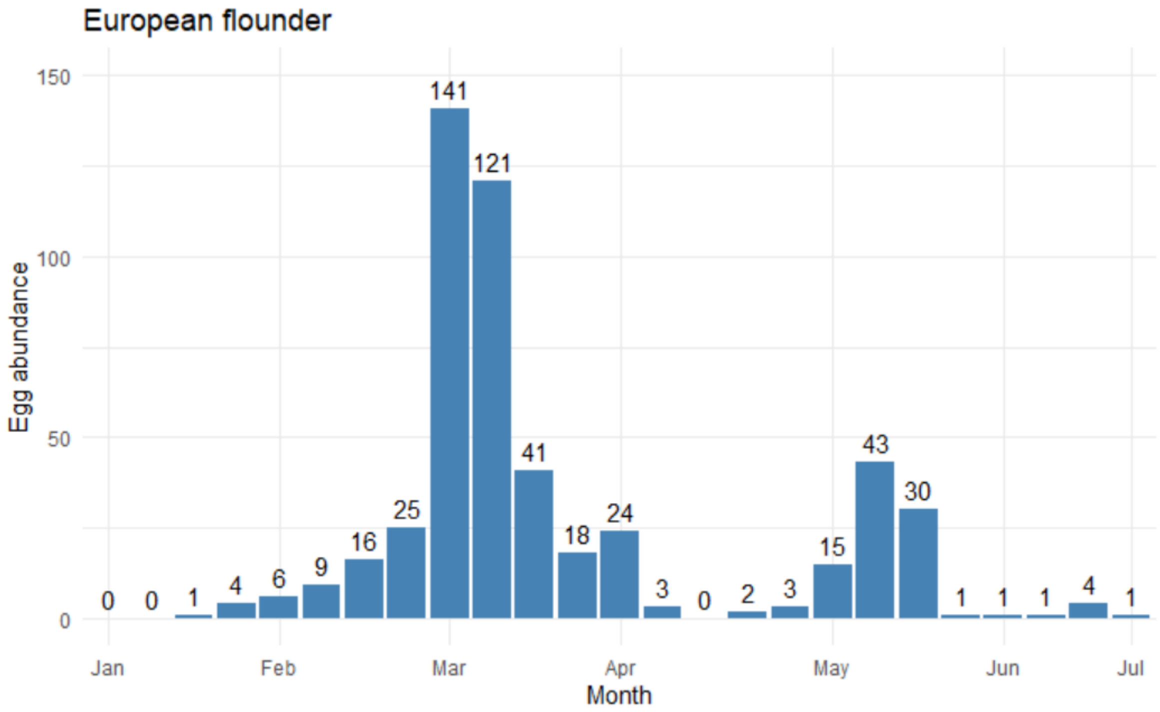

Figure 11. Seasonal distribution of European flounder (*P. flesus*). Spawning peaks in early March and has the highest numbers of eggs registered compared to the three other species. The y-axis shows number of eggs and the x-axis shows the temporal change. Each bar and the number above, represents the weekly sample and the specific number of eggs, respectively.

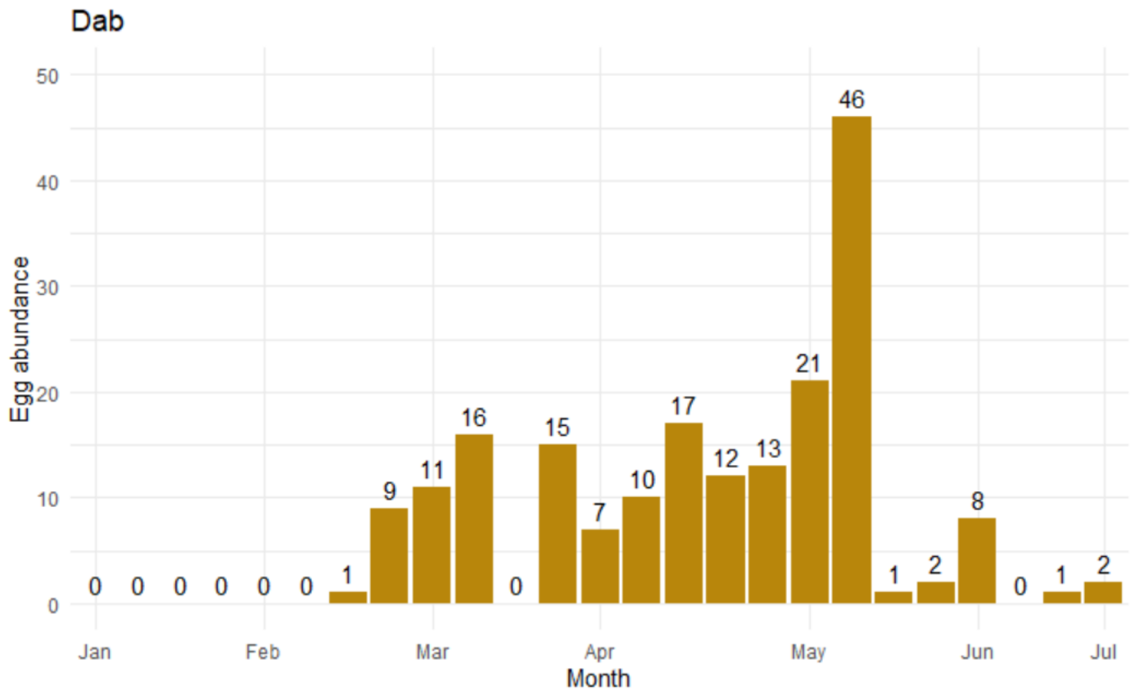

Figure 12. Seasonal distribution of dab (*L. limanda*). The y-axis shows number of egg and the x-axis shows the temporal change. Each bar and the number above, represents the weekly sample of eggs and the specific number of eggs, respectively.

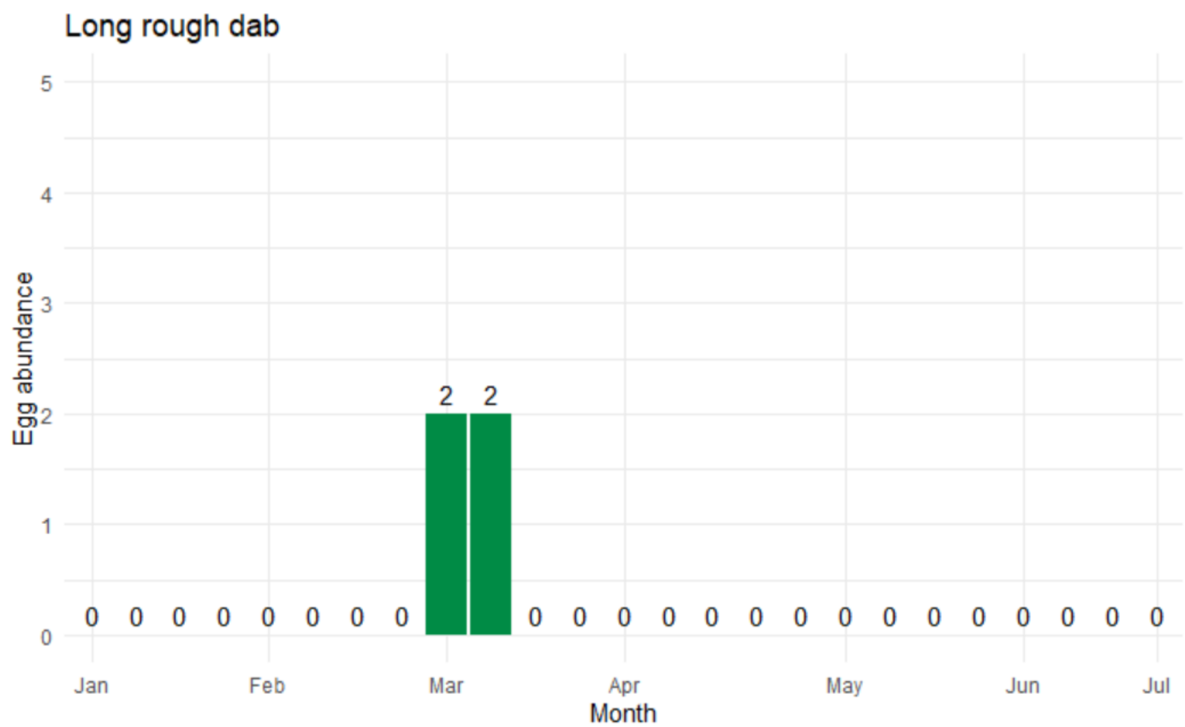

Figure 13. Seasonal distribution of long rough dab (*H. platessoides*). The y-axis shows number of egg and the x-axis shows the temporal change. Each bar and the number above, represents the weekly sample of eggs and the specific number of eggs, respectively.

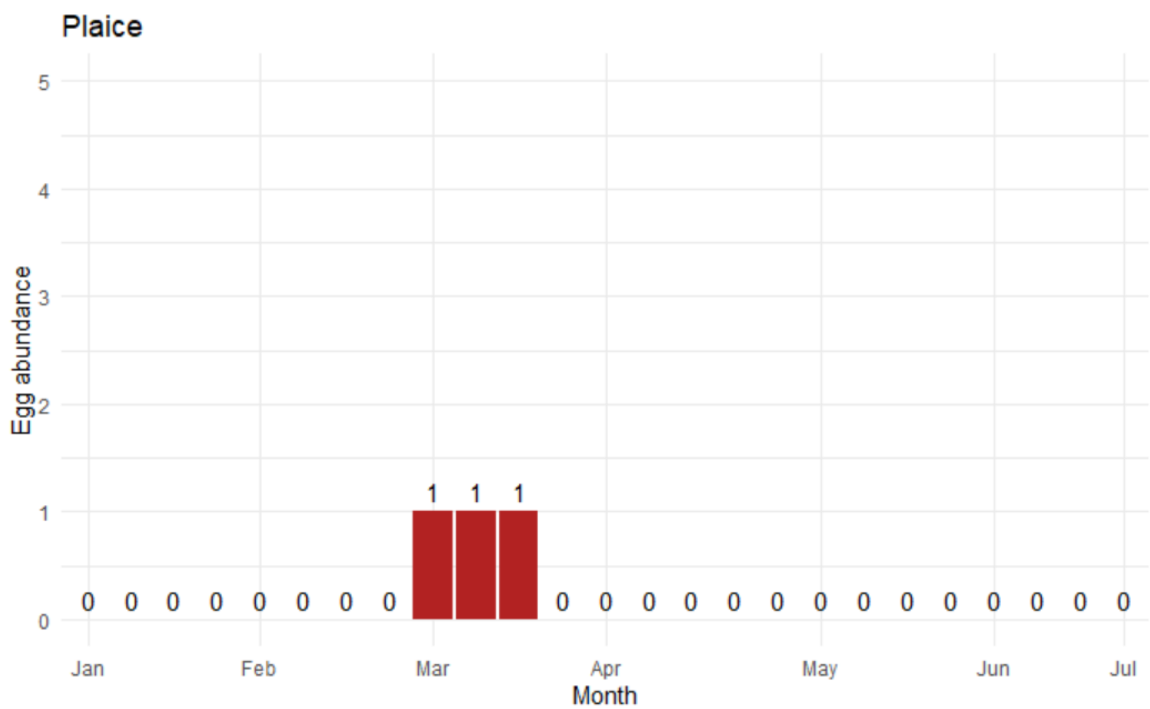

Figure 14. Seasonal distribution of plaice (*P. platessa*). The y-axis shows number of egg and the x-axis shows the temporal change. Each bar and the number above, represents the weekly sample of eggs and the specific number of eggs, respectively.

3.2 Large scale spatial sampling

The spatial sampling resulted in a total of 6630 eggs, where 2363 eggs (35.6%) represented the species of interest. The eggs were divided into the same groups as in section 3.1 to show the variation in egg abundance between the species and remaining groups. European flounder was the most abundant species of interest both in the number of observations and in abundance, followed by dab, long rough dab, and plaice (figure 15).

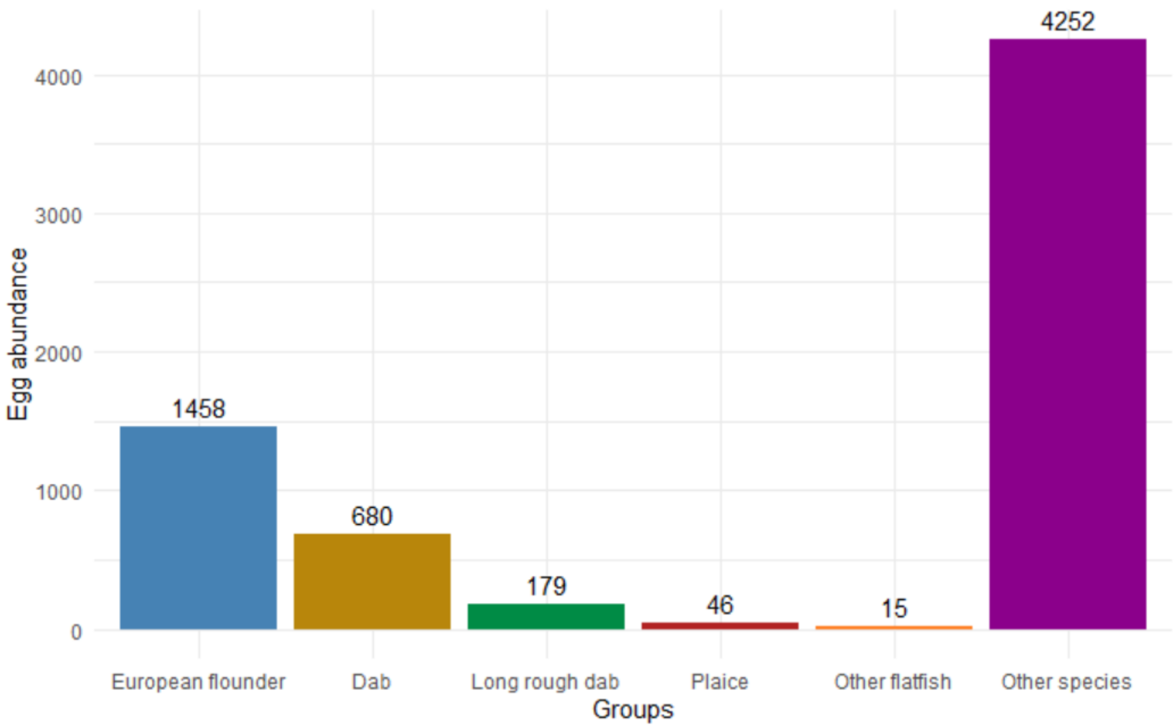

Figure 15. Variation in abundance between the target species and the remaining groups. The y-axis shows the number of eggs within each group and the x-axis shows each species/category group. Each bar represents all sampled eggs for the species/groups.

The temporal and spatial group composition were compared (figure 16). The difference in abundance among the groups ranged between 0.5 % - 2.6 %, except the group “other flatfish” which differed 8.6 %.

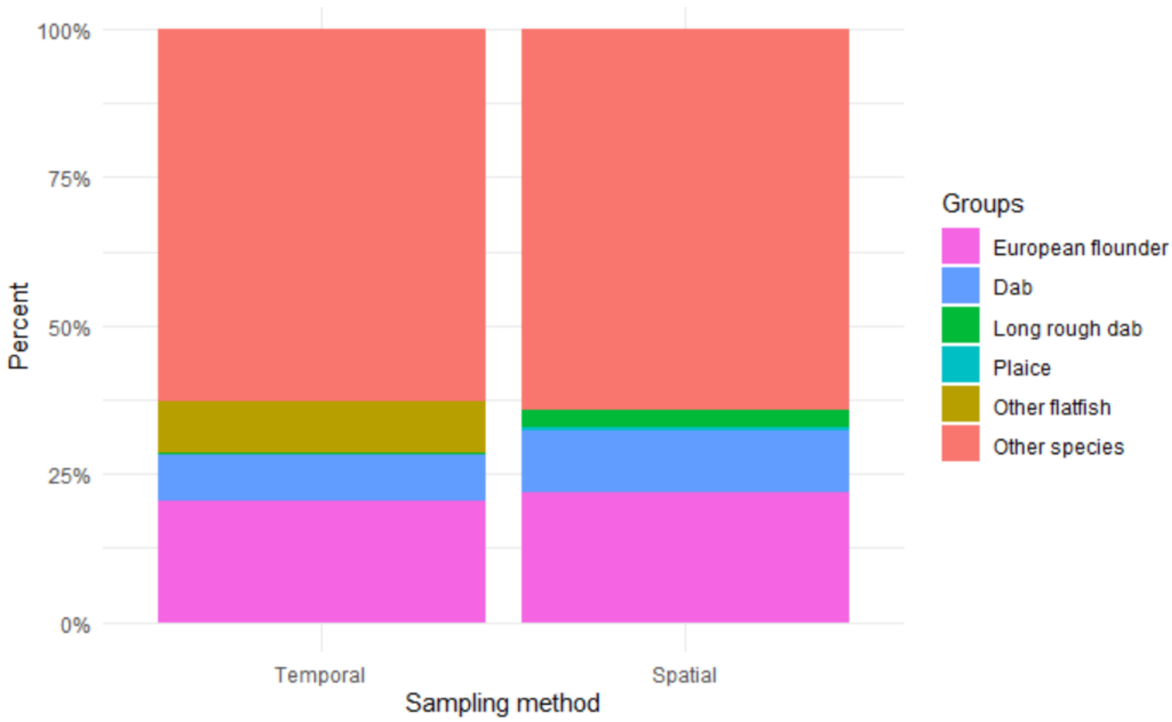

Figure 16. Percental comparison of the temporal sampled data and the spatial sampled data. The y-axis describes the percentage and the x-axis describes the groups, where the T and S after the group name indicates whether it is from the temporal or spatial sampling.

For the spatial distribution, European flounder, dab and long rough dab appeared more or less through out the investigated area (figure 17, 18 & 19), while the appearance of plaice fluctuated more and had larger spatial breaks between each observation compared to the other groups (figure 20).

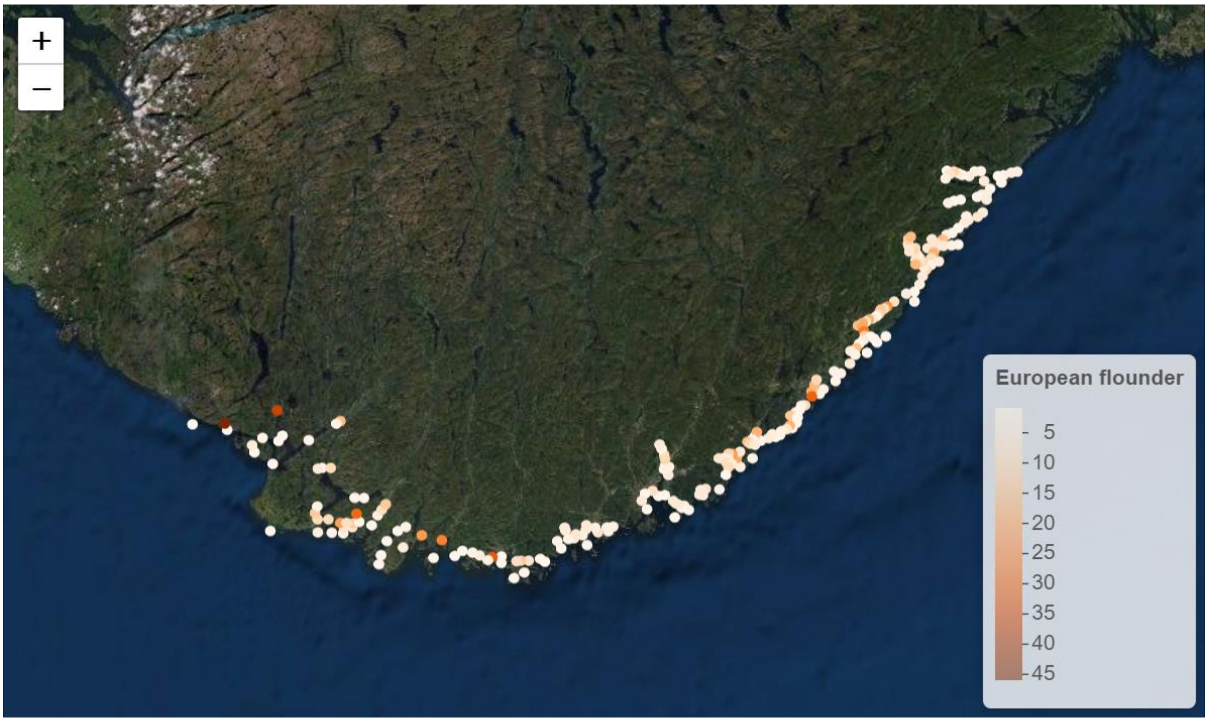

Figure 17. Spatial distribution of European flounder along the Norwegian Skagerrak coast. Colour gradient depicts the number of eggs sampled per station (min – max; 1-46)

Figure 18. Spatial distribution of dab along the Norwegian Skagerrak coast. Colour gradient depicts the number of eggs sampled per station (min – max; 1-41)

Figure 19. Spatial distribution of long rough dab along the Norwegian Skagerrak coast. Colour gradient depicts the number of eggs sampled per station (min – max; 1-9).

Figure 20. Spatial distribution of plaice along the Norwegian Skagerrak coast. Colour gradient depicts the number of eggs sampled per station (min – max; 1-3).

3.3 Spawning area analysis

The spawning area analysis gave a varying number of spawning areas when comparing between the species. Plaice (*P. platessa*) did not have any statistically significant areas and is therefore not included further in this section nor in section 3.4.

3.3.1 European flounder (*P. flesus)*

The species with the highest number of statistically significant spawning areas was European flounder, which had spawning areas in all ten groups. In total 39 stations were registered as spawning areas according to the statistical analysis (figure 21). Number of significant station observations in the groups varied between 1 – 7. Also, the number of eggs for the registered spawning areas varied and max – min where 8 – 46 eggs per station (figure 22 & 23). All the spawning areas were found in sheltered areas or fjord areas.

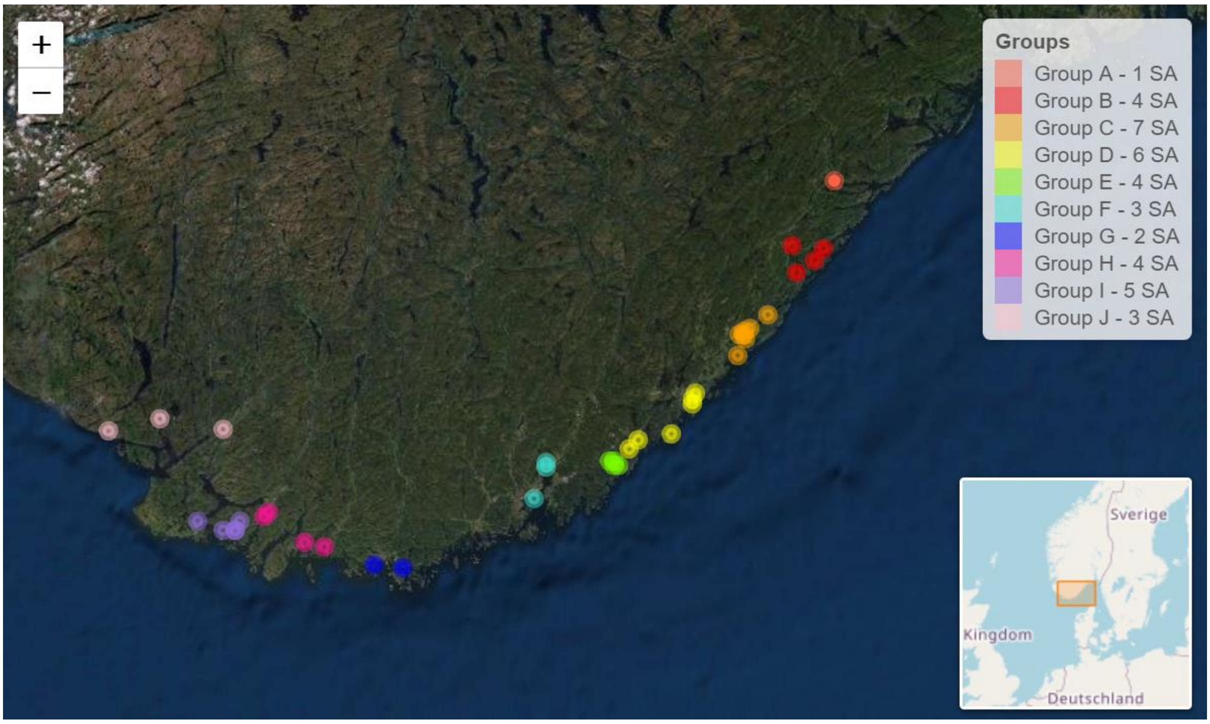

Figure 21. Spatial distribution of statistically significant spawning areas for European flounder per group. The colours of the markers indicate which geographic group they belong to. The legend shows the groups and the number of spawning areas (SA) they contain.

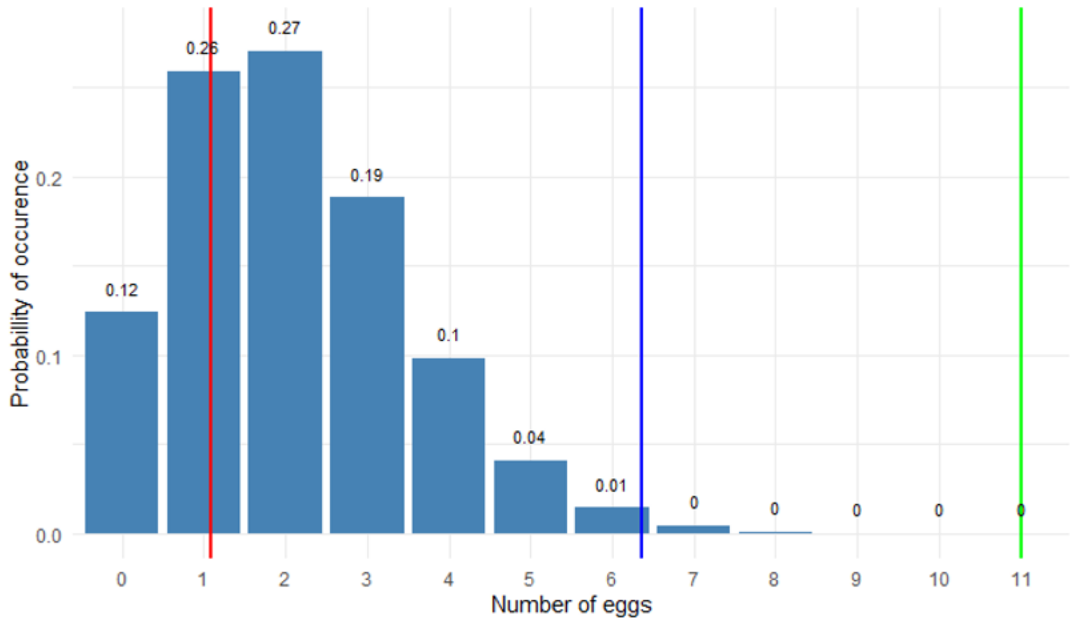

Figure 22. Poisson probability density function for European flounder group A with the lowest registration of spawning areas. The y-axis shows the probability of occurrence and the x-axis shows the number of eggs. The red line shows the mean of the observations and the blue line shows the upper limit of the 95 % confidence interval. The green line shows the number of eggs sampled which exceeds the 95% confidence interval.

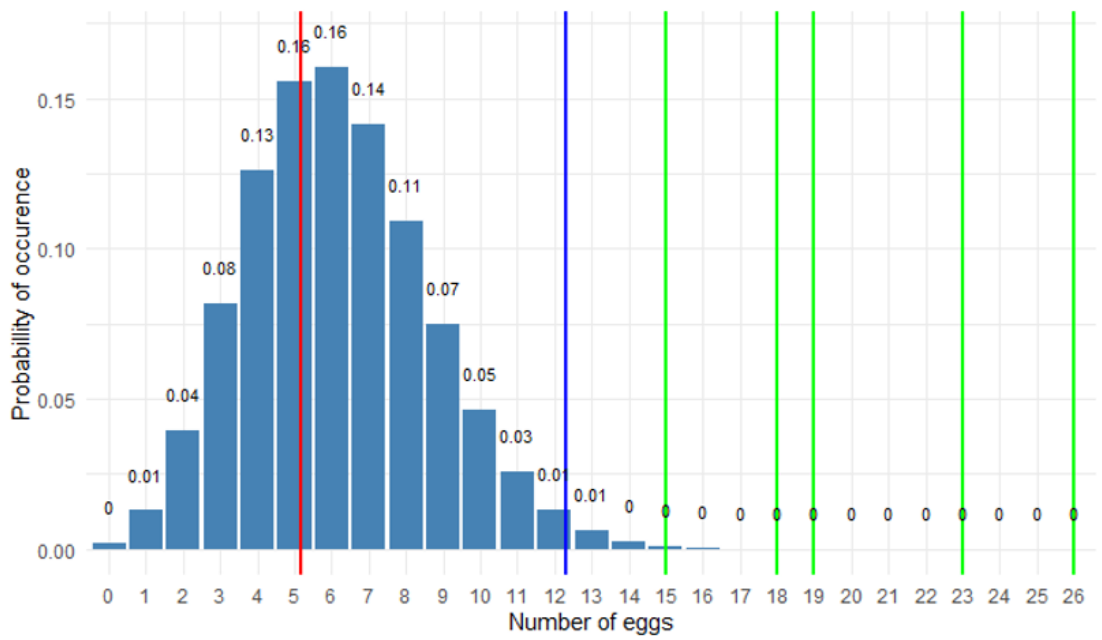

Figure 23. Poisson probability density function for European flounder group C with the highest registration of spawning areas. The y-axis shows the probability of occurrence and the x-axis shows the number of eggs. The red line shows the mean of the observations and the blue line shows the upper limit of the 95 % confidence interval. The green lines show the number of eggs sampled which exceeds the 95% confidence interval. There are five green lines on the plot, but 15 and 18 eggs appeared two times in group C and is only represented by one line in the plot.

3.3.2 Dab (*L. limanda*)

Dab had spawning areas in eight out of ten groups, and a total of 23 stations were registered as spawning areas according to the statistical analysis (figure 24). Number of significant station observations in the groups varied between 1 – 5. Also, the number of eggs for the registered spawning areas varied and max – min where 6 – 41 eggs per station (figure 25 & 26). All the spawning areas were found in sheltered areas or fjord areas except for one station.

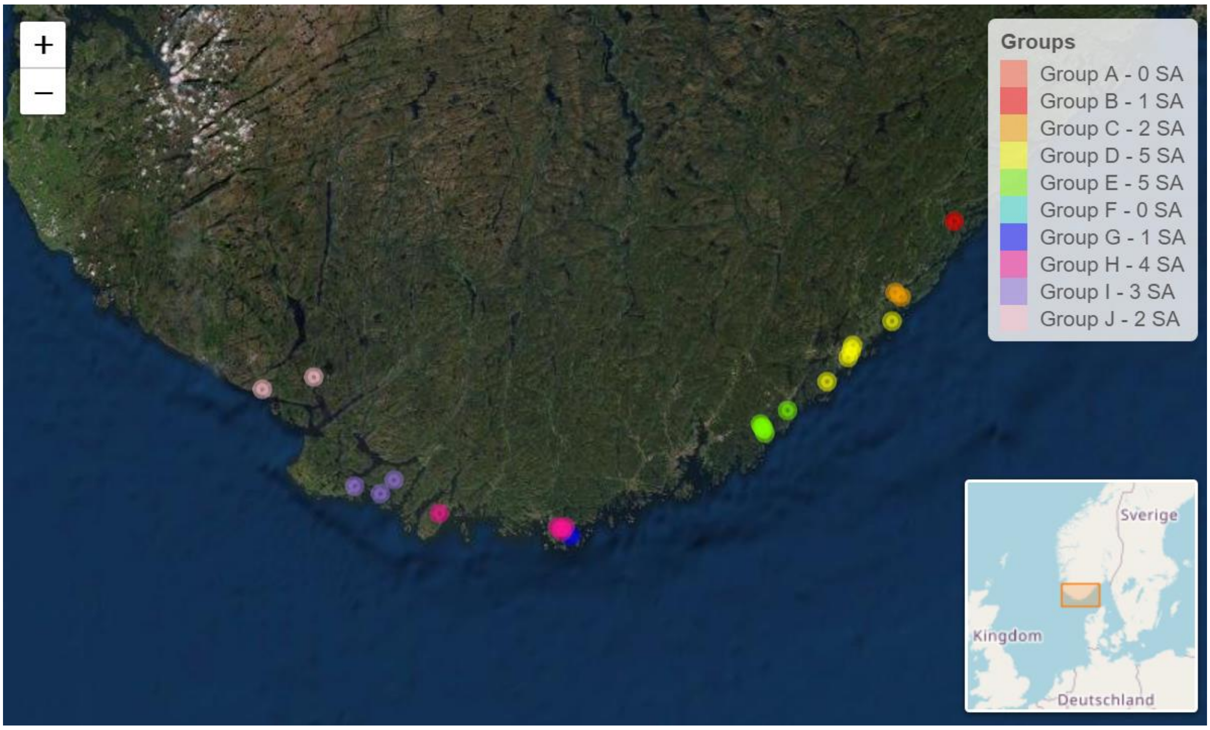

Figure 24. Spatial distribution of statistically significant spawning areas for dab per group. The colours of the markers indicate which geographic group they belong to. The legend shows the groups and the number of spawning areas (SA) they contain.

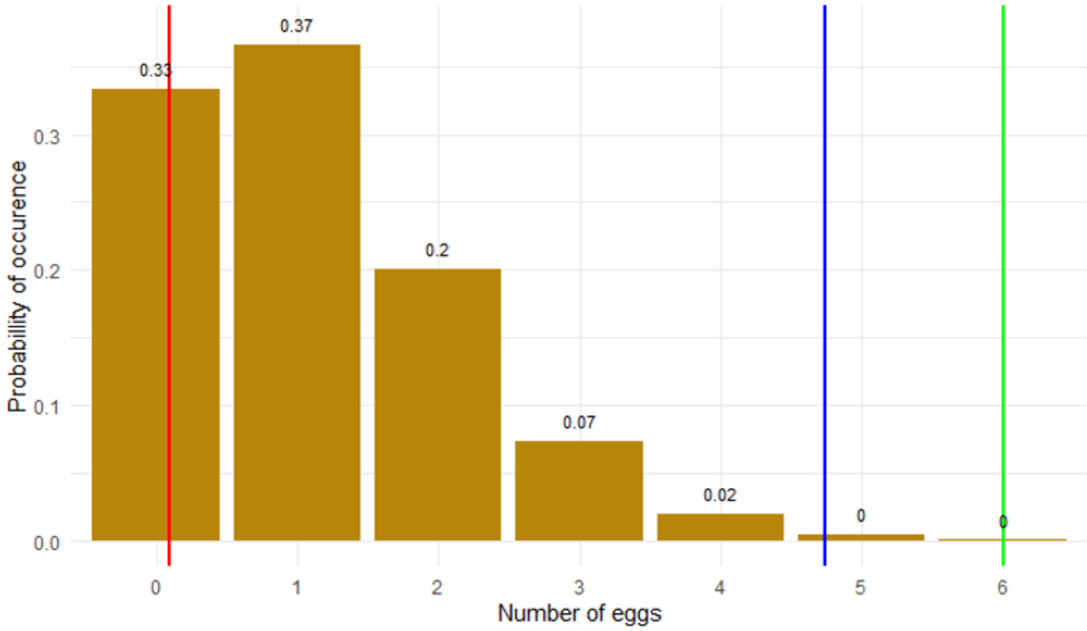

Figure 25. Probability density function of the Poisson distribution for dab group B with the lowest registration of spawning areas. The y-axis shows the probability of occurrence and the x-axis shows the number of eggs. The red line shows the mean of the observations and the blue line shows the upper limit of the 95 % confidence interval. The green line shows the number of eggs sampled which exceeds the 95% confidence interval.

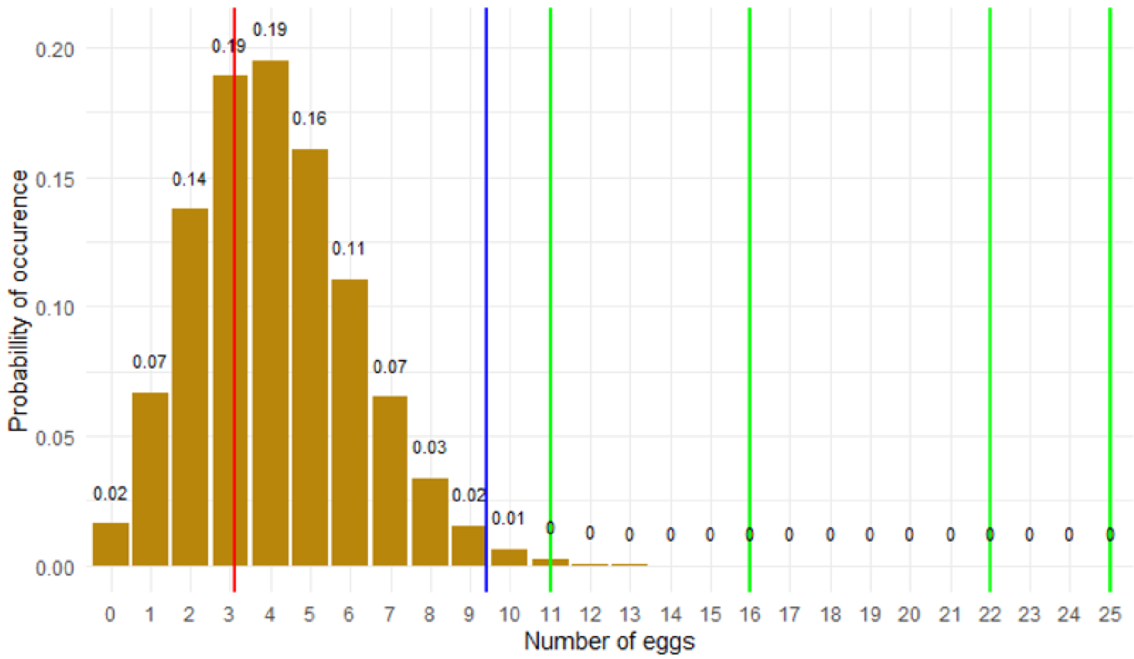

Figure 26. Probability density function of the Poisson distribution for dab group E with the highest registration of spawning areas. The y-axis shows the probability of occurrence and the x-axis shows the number of eggs. The red line shows the mean of the observations and the blue line shows the upper limit of the 95 % confidence interval. The green lines show the number of eggs sampled which exceeds the 95% confidence interval. There are four green lines on the plot, but 16 eggs appeared two times in group E and is only represented by one line in the plot.

3.3.3 Long rough dab (*H. platessoides*)

Long rough dab had spawning areas in two out of ten groups, and a total of 3 stations were registered as spawning areas according to the statistical analysis (figure 27). Number of significant station observations in the groups varied between 1 – 2. Also, the number of eggs for the registered spawning areas varied and max – min where 7 – 9 eggs per station (figure 28 & 29). All three spawning areas were found in fjord areas.

Figure 27. Spatial distribution of statistically significant spawning areas for long rough dab per group. The colours of the markers indicate which geographic group they belong to. The legend shows the groups and the number of spawning areas (SA) they contain.

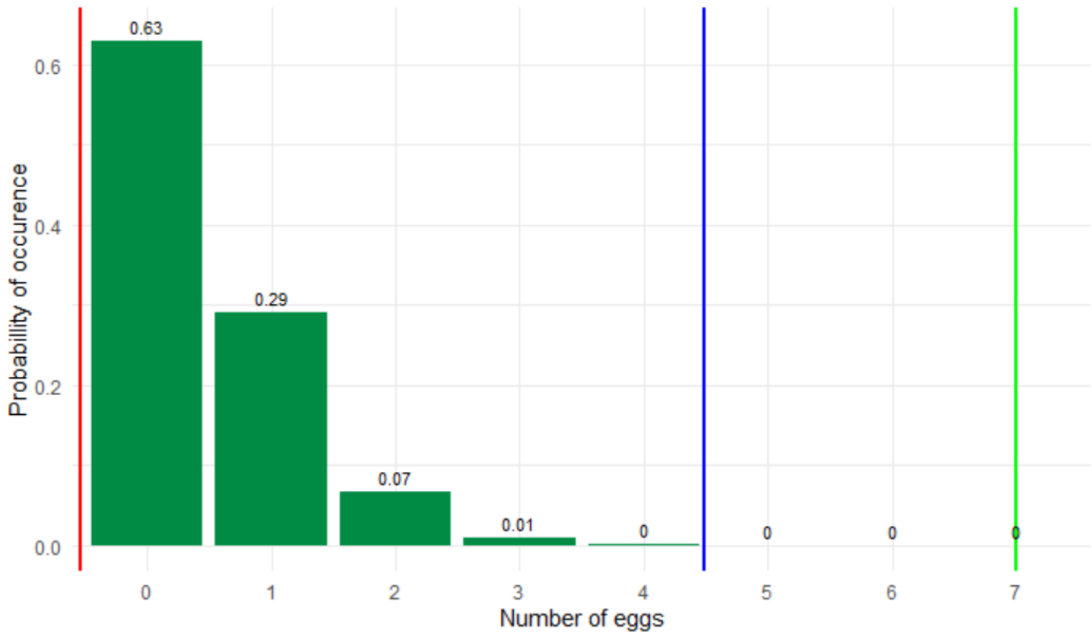

Figure 28. Probability density function of the Poisson distribution for long rough dab group B with the highest registration of spawning areas. The y-axis shows the probability of occurrence and the x-axis shows the number of eggs. The red line shows the mean of the observations and the blue line shows the upper limit of the 95 % confidence interval. The green line shows the number of eggs sampled which exceeds the 95% confidence interval. Here, the green line represents two observations of seven eggs.

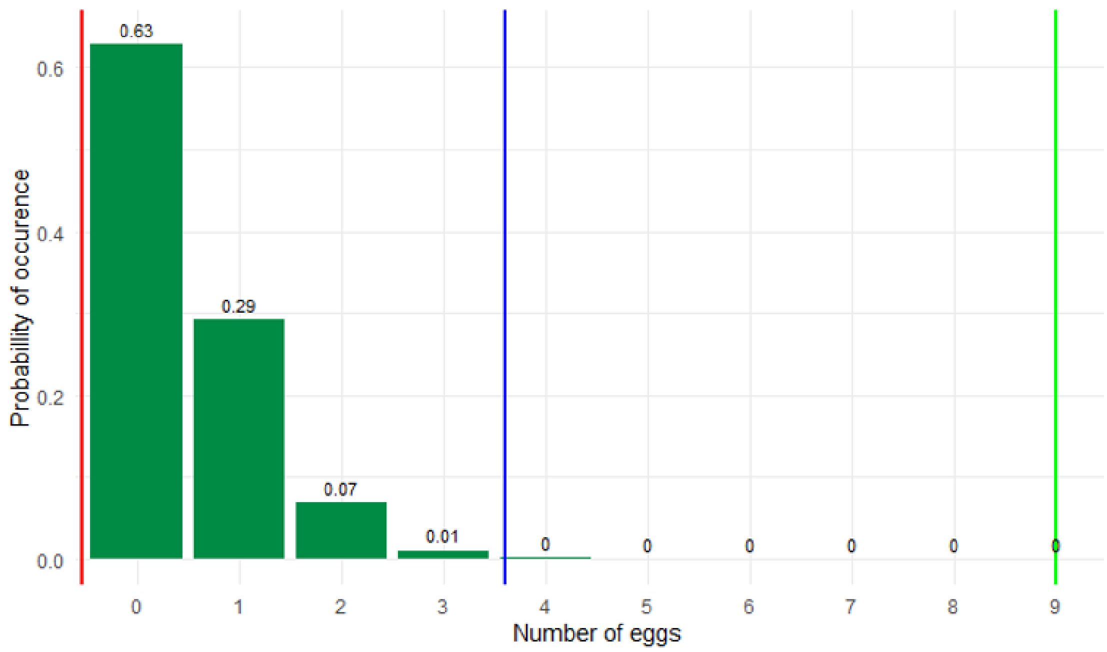

Figure 29. Probability density function of the Poisson distribution for long rough dab group I with the lowest registration of spawning areas. The y-axis shows the probability of occurrence and the x-axis shows the number of eggs. The red line shows the mean of the observations and the blue line shows the upper limit of the 95 % confidence interval. The green line shows the number of eggs sampled which exceeds the 95% confidence interval.

3.4 Ocean current modelling

The number of particles which were modelled was 21 050 per modelling day (50 particles per intended sampling station and 47 350 particles in total). For the species of interest, the modelled drift was applied to all stations with significant numbers of eggs (table 1). Plaice (*P.* platessa) was not included in this process due to the lack of stations with significant numbers of eggs. The number of particles leaving the model varied between 1818 – 3899 particles (8.6 - 18.5%) and were not included in the estimation of mean drift distance (table, 2, 3 & 4) as the length value would cause over estimation of the total drift distance. The particles were included considering retention/dispersion at the significant stations. The position of the particle at the limit of the model will still give information on the drift pattern of the particle.

Table1. Overview of the input (species, groups, significant stations) and output (Number of particles per day, number of modelling days and total number of particles modelled). Input also included a number of particles (50) per day in the ocean current and particle model. Only stations with significant numbers of eggs were tested in the model.

Species	Groups	Significant stations	Particles per day	Number of modelling days	Particles in total
European flounder	A – J	39	1950	7	13650
Dab	B – E & G – J	23	1150	7	8050
Long rough dab	B & I	3	150	7	1050

3.4.1 European flounder (*P. flesus*)

The average particle drift for European flounder varied between 700 – 76165m (0.7 – 76,1 km). The number of retention areas was 17 while the number of stations with dispersal was 21 (table 2). Out of the 17 stations with retention station number 81, 83 (figure 30), 164, 166, 167, 168 (figure 31) and 382, 390, 404 (figure 32) were some of the areas with several significant stations and retention located in common fjord systems. Table 2 summarises all results from the particle drift model for the estimated spawning areas for European flounder.

Table 2. Summarised results for the particle drift of European flounder with groups, station number and number if egg, respectively. Mean distance is calculated from the mean of each modelling day. The same goes for median and standard deviation, and the minimum and maximum distance. Retention is answered by yes and no, yes meaning retention and no meaning dispersion. Directional is answered by yes and no, yes meaning directional and no meaning random.

Group	**Station number**	**Number of eggs**	**Mean distance 0 - 30d (m)**	**Median distance 0 - 30d (m)**	**Standard deviation (m)**	**Min. distance 0 - 30d (m)**	**Max. distance 0 - 30d (m)**	**Retention (Yes/No)**	**Directional (Yes/No)**
A	17	11	32359	29514	17330	24694	40055	No	No
B	56	15	72422	63509	23463	43033	105578	No	Yes
B	65	17	57848	50536	49572	4492	145755	No	No
B	81	15	754	725	172	576	1105.0	Yes	No
B	83	16	1876	700	2848	351	8229.8	Yes	Yes
C	95	23	10278	8711	5088	5283	20960	Yes	
C	102	18	22560	19952	12483	8386	47858	No	No
C	104	18	33087	36534	19721	4120	52884	No	No
C	105	15	5411	4767	3568	1914	11335	Yes	No
C	106	19	50422	47059	17126	26308	73593	No	No
C	107	26	64229	63234	23617	32232	96278	No	No
C	115	15	68416	76165	18150	45013	92847	No	No
D	130	18	68953	71115	12058	43387	80038	No	Yes
D	131	16	41833	37475	25795	2852	76181	No	No
D	134	30	40772	41269	8885	25720	50187	No	Yes
D	145	17	54858	55477	17679	21170	72738	No	Yes
D	154	17	9688	9553	1569	7610	11916	No	Yes
D	156	15	8106	5241	6864	1483	19978	No	No
E	164	18	4913	3858	3239	2029	11519	Yes	No
E	166	19	4818	1965	4632	941	12077	Yes	No
E	167	21	2287	2134	1359	742	4324	Yes	No
E	168	11	3186	2415	2157	1210	6641	Yes	No
F	218	11	9854	8578	2403	7079	13543	Yes	No
F	219	9	8559	8377	1973	6285	11995	Yes	No
F	223	8	26009	27677	4806	17480	30496	No	No
G	273	13	14540	15148	6930	4628	26535	No	No
G	281	32	21325	15571	15210	8998	52034	No	No
H	295	25	15247	15786	5031	9122	24076	No	No
H	300	21	24940	21981	11808	8493	42743	No	Yes
H	328	8	6910	6418	3182	2215	11498	Yes	Yes
H	329	13	7480	7218	1800	5446	10906	Yes	No

I	335	13	12811	11989	4926	8285	21343	No	No
I	337	29	8574	8741	4310	4350	16039	Yes	No
I	341	20	12342	13507	8857	2556	27843	No	No
I	344	13	2089	841	3432	364	9852	Yes	No
J	382	15	16643	16927	2540	11848	19737	Yes	Yes
J	390	36	6065	5750	2300	3067	9142	Yes	No
J	404	46	5838	5488	2179	3507	9408	Yes	Yes

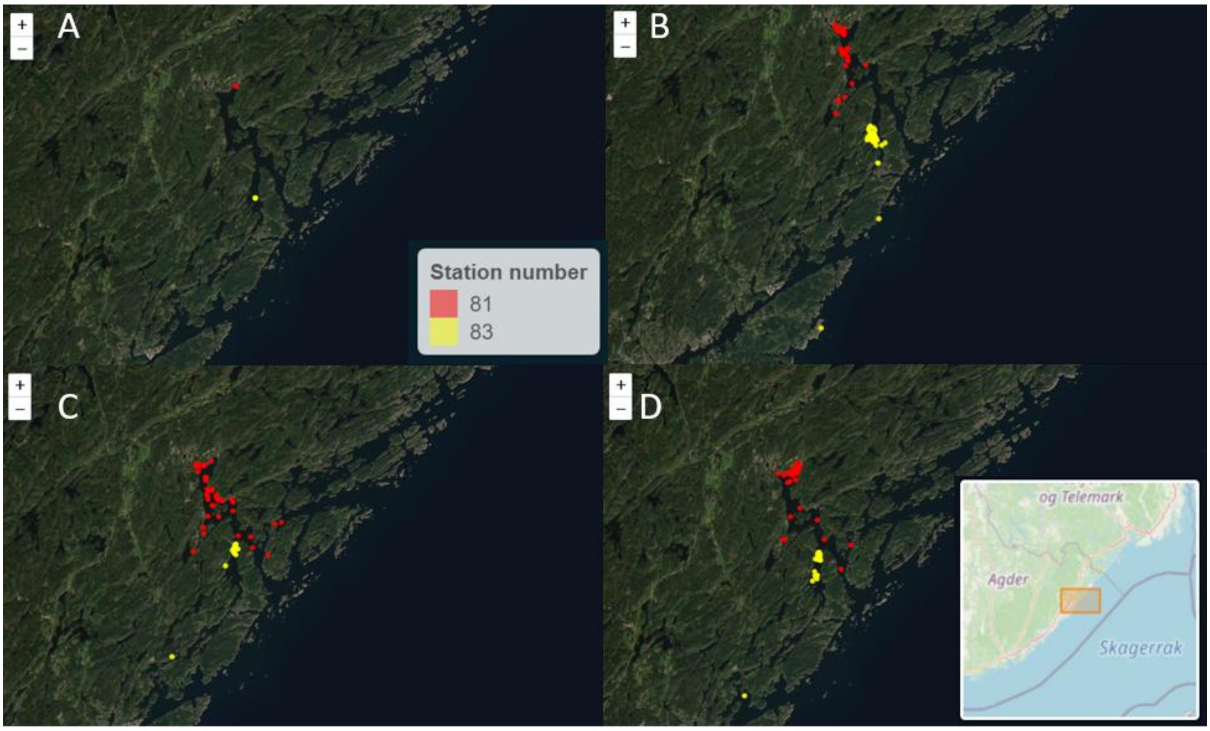

Figure 30 A – D. 30 days of particle drift for European flounder on the 20th of March at station number 164, 166, 167, 168 (50 modelled particles per station). A) Discharge point set to the location where the station with significant egg numbers were sampled. B) Drift after 10 days, 2 eggs are outside the figure frame. C) Drift after 20 days, 3 eggs are outside the figure frame & D) drift after 30 days, 3 eggs are outside the figure frame.

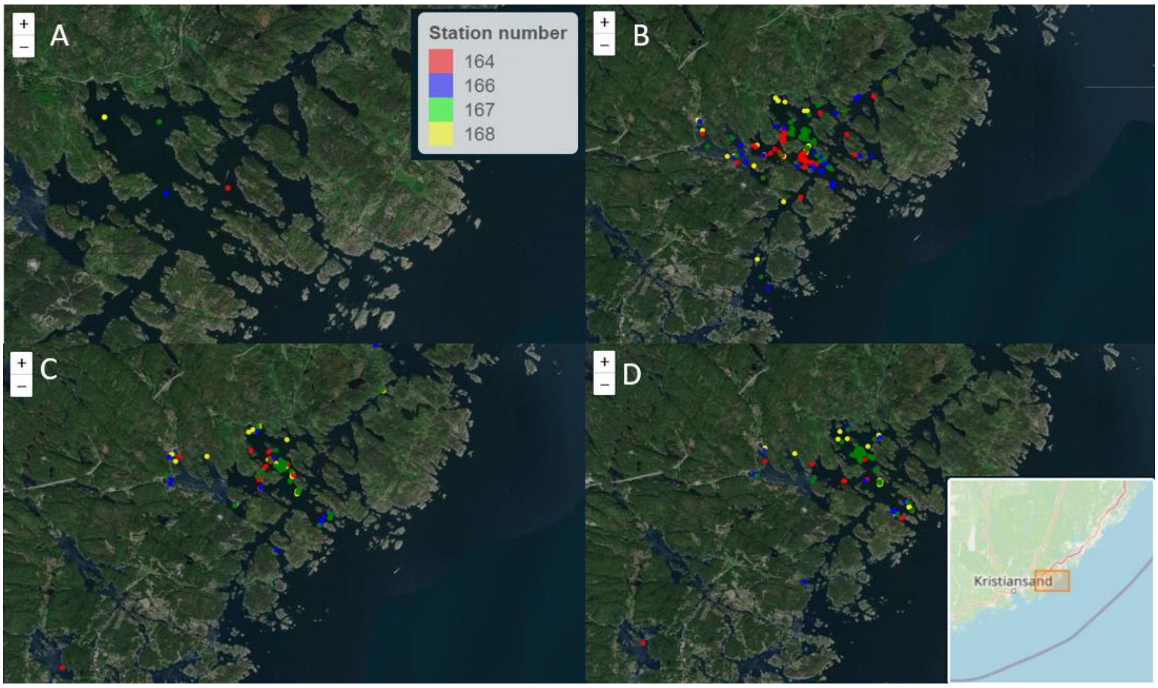

Figure 31 A – D 30 days of particle drift for European flounder on the 20th of March at station number 164, 166, 167, 168 (50 modelled particles per station). A) Discharge point set to the location where the station with significant egg numbers were sampled. B) Drift after 10 days, 8 eggs are outside the figure frame. C) Drift after 20 days, 14 eggs are outside the figure frame & D) drift after 30 days, 16 eggs are outside the figure frame.

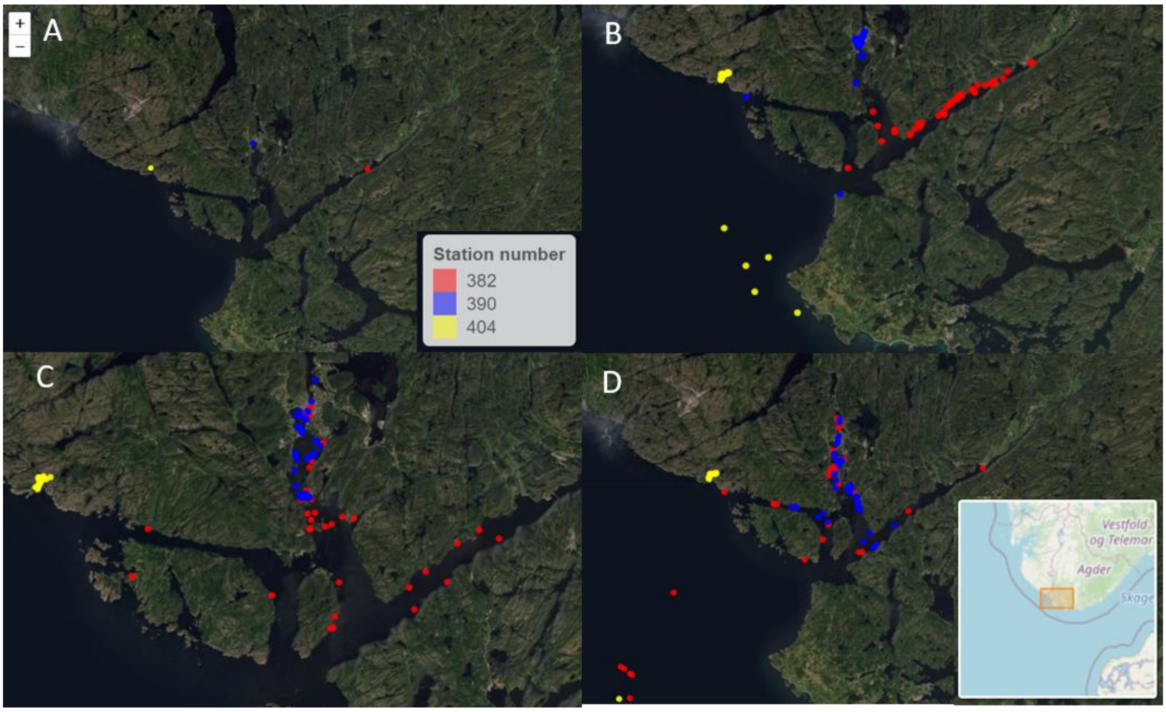

Figure 32 A – D. 30 days of particle drift for European flounder on the 20th of March at station number 382, 390, 404. (50 modelled particles per station). A) Discharge point set to the location where the station with significant egg numbers were sampled. B) Drift after 10 days, 8 eggs are outside the figure frame. C) Drift after 20 days, 17 eggs are outside the figure frame & D) drift after 30 days, 17 eggs are outside the figure frame.

3.4.2 Dab (*L. limanda*)

The average particle drift for dab varied between 275 – 100777m (0.2 – 100,7 km) (table 3). The number of retention areas was 10 while the number of stations with dispersal was 14 (table 3). Out of the 14 stations with retention station number 170, 172, 173 (figure 33) and 337, 343 (figure 34) were some of the areas with several significant stations and retention located in common fjord systems. Table 3 summarises all results from the particle drift model for the estimated spawning areas for dab.

Table 3. Summarised results for the particle drift of dab with groups, station number and number if egg, respectively. Mean distance is calculated from the mean of each modelling day. The same goes for median and standard deviation, and the minimum and maximum distance. Retention is answered by yes and no, yes meaning retention and no meaning dispersion. Directional is answered by yes and no, yes meaning directional and no meaning random.

Group	Station number	Number of eggs	Mean distance 0 - 30ds (m)	Median distance 0 - 30d (m)	Standard deviation (m)	Min. distance 0 - 30d (m)	Max. distance 0 - 30d (m)	Retention (Yes/No)	Directional (Yes/No)
B	73	6	7168	4198	6965	1783	21747	No	No
C	81	25	64229	63234	23617	32232	96278	No	No
C	105	18	5411	4767	3568	1914	11335	Yes	No
C	107	15	64229	63234	23617	32232	96278	No	No
D	120	15	82856	87886	15994	52843	100777	No	Yes
D	130	13	68953	71115	12058	43387	80038	No	Yes
D	131	14	41833	37475	25795	2852	76181	No	No
D	132	18	2733	2656	1682	1166	5858	Yes	No
D	140	11	75627	74502	11370	61042	96333	No	No
E	157	16	6219	6891	4432	1308	12241	Yes	No
E	170	16	2083	2442	1022	286	3198	Yes	No
E	172	22	5012	2698	5208	1230	14209	Yes	No
E	173	25	2322	1483	2774	361	8320	Yes	No
E	175	11	12946	13222	5997	5069	22391	No	No
G	268	9	30881	30580	11287	18031	52780	No	No
H	271	14	27959	24170	16512	5991	58687	No	No
H	272	10	10805	6457	9428	4958	31361	No	No
H	273	41	14540	15148	6931	4628	26535	No	No
H	306	24	13441	11683	5626	9091	25858	No	No
I	333	8	9668	8253	3213	6568	14483	No	No

I	337	15	8574	8741	4310	4350	16039	Yes	No
I	343	8	3821	3679	2779	275	7938	Yes	No
J	390	9	6065	5750	2300	3067	9142	Yes	No
J	404	9	5838	5488	2179	3507	9408	Yes	Yes

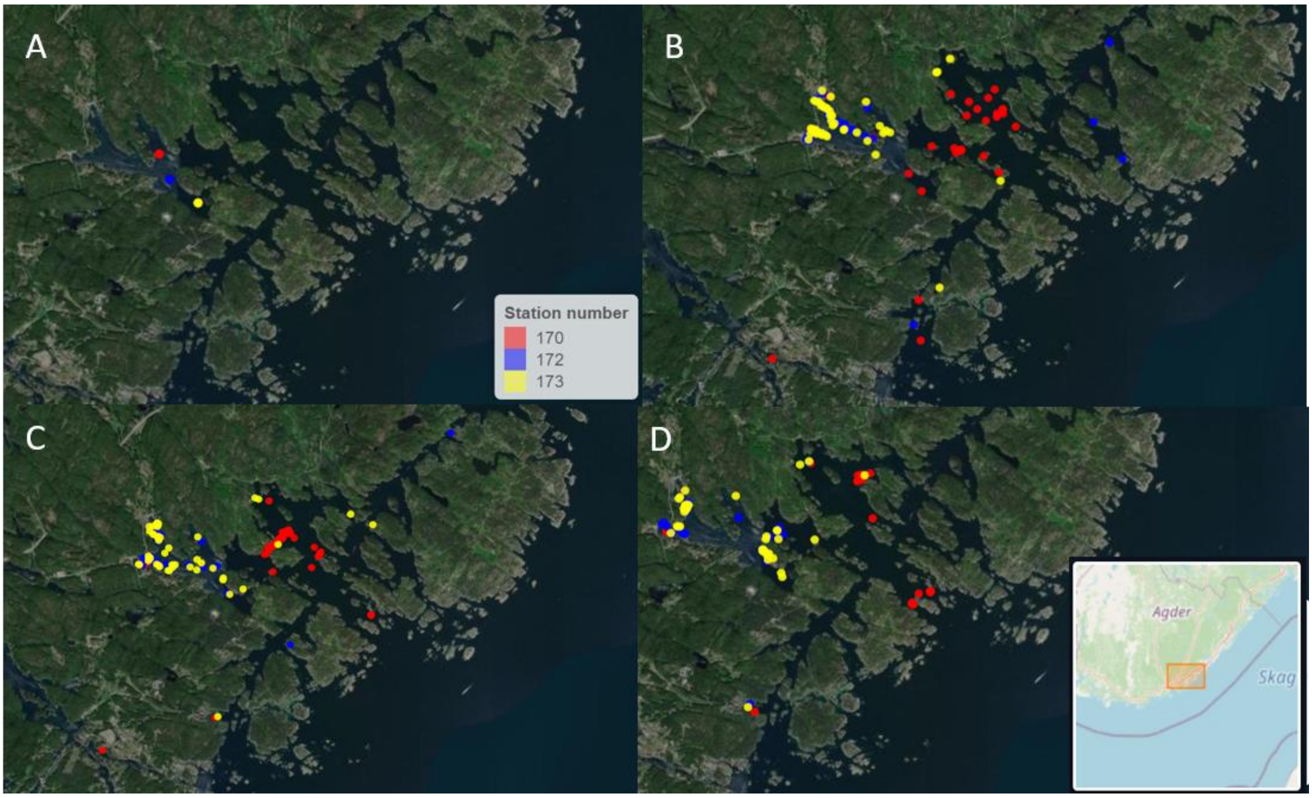

Figure 33 A – D. 30 days of particle drift for dab on the 20th of March at station number 170, 172, 173. (50 modelled particles per station). A) Discharge point set to the location where the station with significant egg numbers were sampled. B) Drift after 10 days, 8 eggs are outside the figure frame. C) Drift after 20 days, 11 eggs are outside the figure frame & D) drift after 30 days, 13 eggs are outside the figure frame.

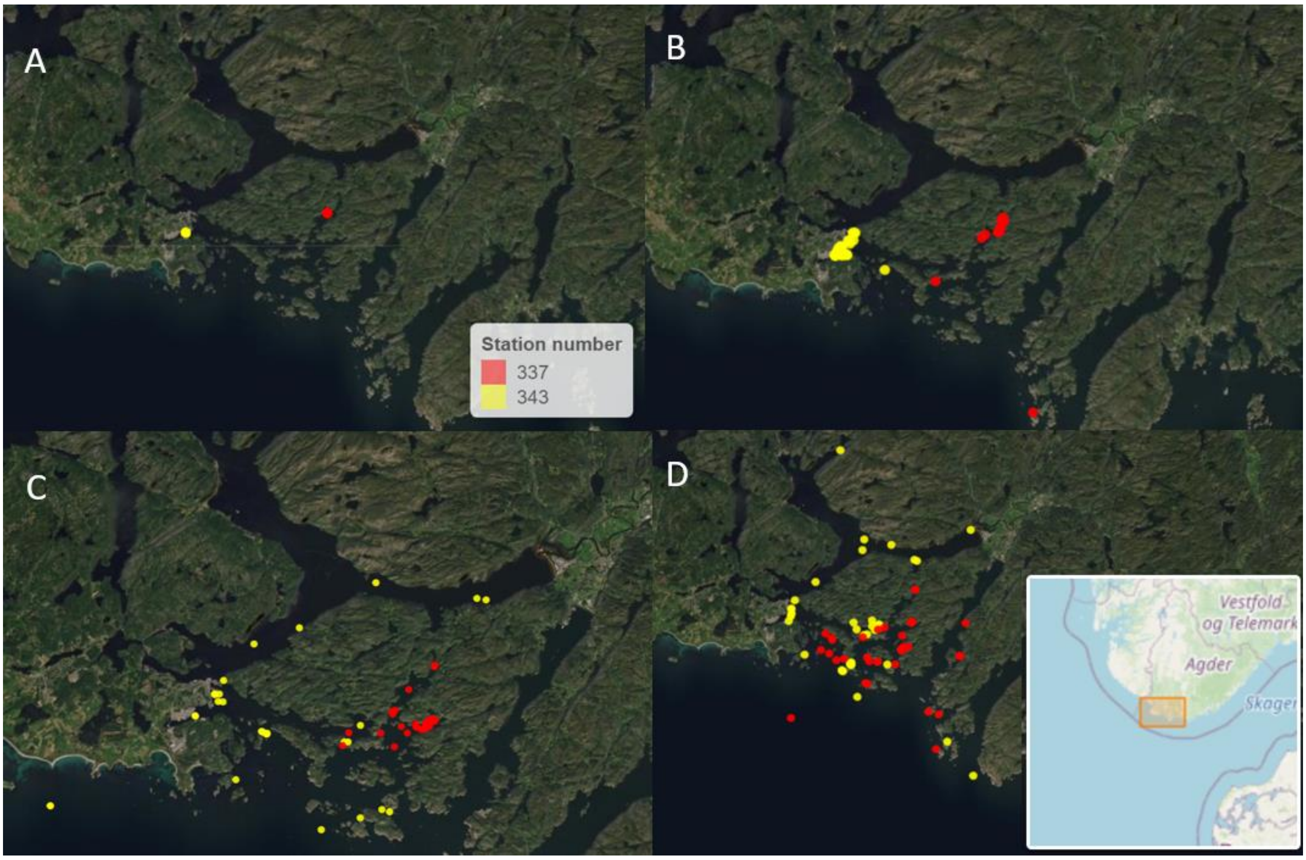

Figure 34 A – D. 30 days of particle dab from the 20th of March at station number 337 and 343 (50 modelled particles per station). A) Discharge point set to the location where the station with significant egg numbers were sampled. B) Drift after 10 days,8 eggs are outside the figure frame C) Drift after 20 days, 13 eggs are outside the figure frame & D) drift after 30 days, 13 eggs are outside the figure frame.

3.4.3 Long rough dab (*H. platessoides*)

The average particle drift for dab varied between 639 – 34420m (0.6 – 34,4 km) (table 3). The number of retention areas was 10 while the number of stations with dispersal was 14 (table 4). Out of the 14 stations with retention station number 37, 39 (figure 35) and 357 (figure 36) were some of the areas with significant stations and retention located in common fjord systems. Table 4 summarises all results from the particle drift model for the estimated spawning areas for long rough dab.

Table 4. Summarised results for the particle drift of long rough dab with groups, station number and number if egg, respectively. Mean distance is calculated from the mean of each modelling day. The same goes for median and standard deviation, and the minimum and maximum distance. Retention is answered by yes and no, yes meaning retention and no meaning dispersion. Directional is answered by yes and no, yes meaning directional and no meaning random.

Group	Station number	Number of eggs	Mean distance 0 - 30d (m)	Median distance 0 - 30d (m)	Standard deviation (m)	Min. distance 0 - 30d (m)	Max. distance 0 - 30d (m)	Retention (Yes/No)	Directional/ (Yes/No)
B	37	7	17926	18236	12820	2764	34420	Yes	No
B	39	7	7649	8743	4595	639	13362	Yes	No
I	357	9	2463	2106	926	1835	4369	Yes	No

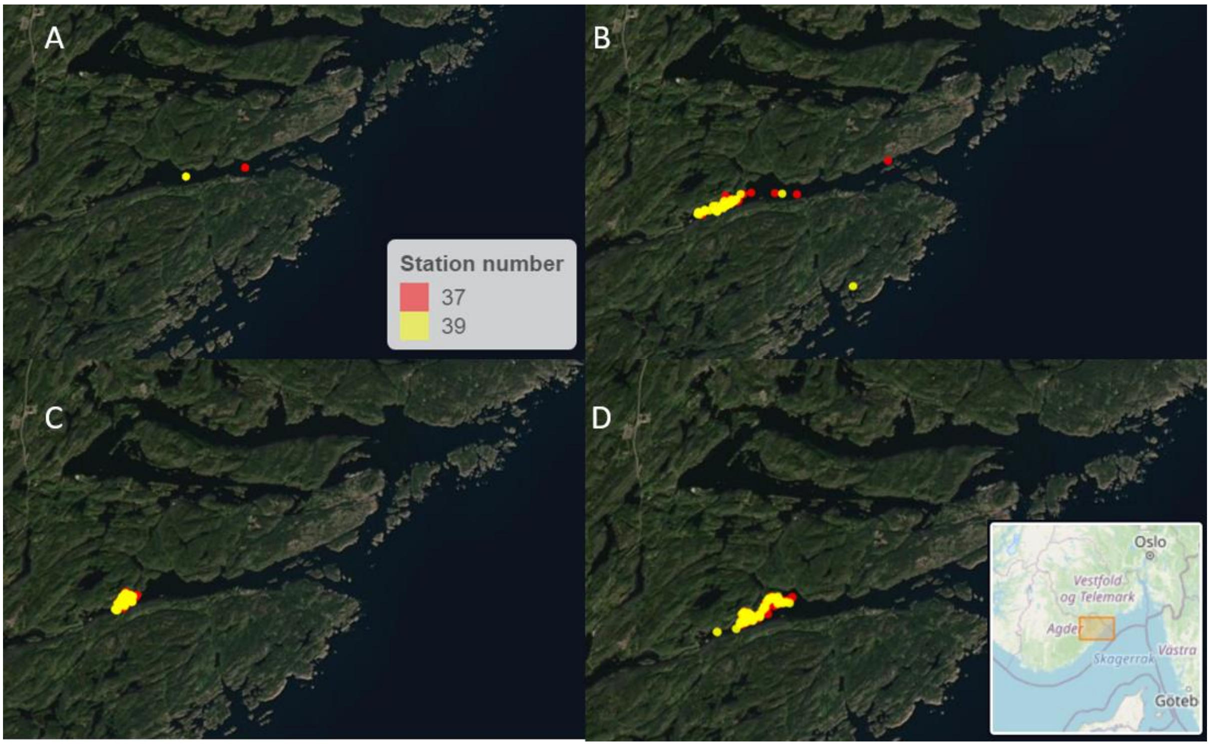

Figure 35 A – D. 30 days of particle drift for long rough dab from the 20th of March for station number 37 and 39 (50 modelled particles per station). A) Discharge point set to the location where the station with significant egg numbers were sampled. B) Drift after 10 days, 15 eggs are outside the figure frame C) Drift after 20 days, 17 eggs are outside the figure frame & D) drift after 30 days, 18 eggs are outside the figure frame.

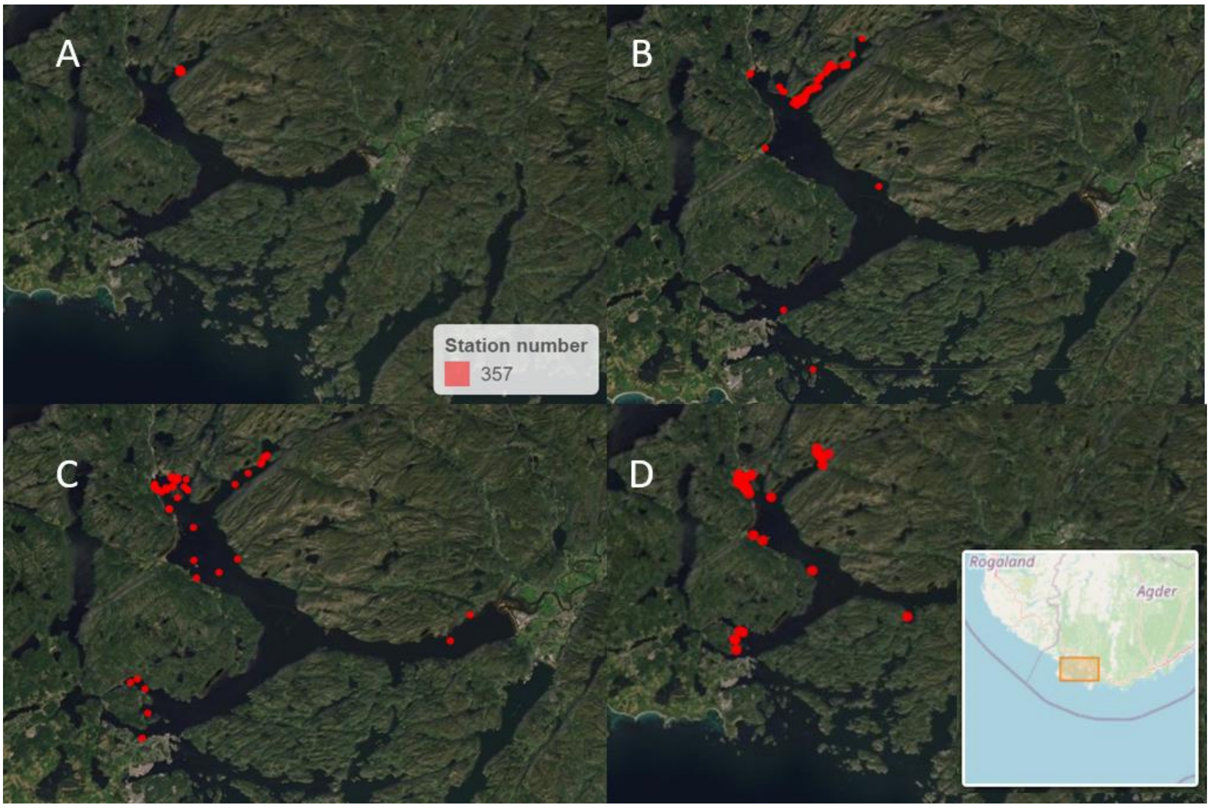

Figure 36 A – D. 30 days of particle drift for long rough dab from the 20th of March at station number 357 (50 modelled particles). A) Discharge point set to the location where the station with significant egg numbers were sampled. B) Drift after 10 days. C) Drift after 20 days & D) drift after 30 days. All eggs stayed in the sheltered fjords and the same pattern was for all seven modelling days.

4 Discussion

4.1 Small-scale temporal sampling

The temporal sampling showed a variation in abundance among the four species of interest. European flounder was the most abundant species with a bimodal distribution. One reason for this could be that many river outlets are located among the sampling stations (Hemmer-Hansen et al., 2007). The spawning peaked two weeks before the spatial sampling and even though significant number of observations were found during the cruise it could still be that the spatial sampling took place while the spawning frequency decreased. Especially considering that the maximum value of observed eggs for the temporal sampling was 141 and the maximum observation of eggs from the spatial sampling was 46. From the start to the end of the cruise, the number of egg observations had shrunk to 41, 18 and 24 egg observations per week (compared to 121 eggs the week before), which is similar to the significant egg samples found during the cruise.

Dab were frequently observed during the temporal sampling and the peak of the spawning was in the middle of May (41 eggs), almost two months after the spatial sampling took place. Maximum observation for dab during the spatial sampling was also 41 eggs as in the temporal sampling, but much earlier than the temporal sampling. Still, spawning activity occurred at the same time as the egg survey.

Both plaice and long rough dab had few observations during the temporal sampling. Even if the numbers of eggs are few, the timing of spawning during the temporal sampling overlaps with the spatial sampling. Long rough dab is usually found in deeper waters and there is usually little spatial difference in spawning area and adult habitat. Since the sampling stations varied between 25 – 40 m in depth (too shallow), it could be that the stations are in habitats or abiotic factors that is not preferred by long rough dab (Swain et al., 1998).

Since the egg’s development stage were not identified it is difficult to say if the sampled eggs were spawned in the sampling area, or if they were transported there with currents. This could affect the results of the spawning peak considering the wish to map newly spawned eggs, and not eggs that were spawned up to weeks ago. Still, all species showed spawning activity during the timing of the spatial egg survey.

4.2 Large-scale spatial sampling and spawning areas

From the large spatial sampling European flounder, dab and long rough dab was scattered relatively continuous along the coast varying in abundance. Variation in pattern and abundance resulted in potential spawning areas for European flounder, dab and long rough dab along the Norwegian Skagerrak coast. Plaice only occurred sporadically and in lower concentrations compared to the other species and the number of sampled eggs were not enough to estimate spawning area.

The stages of development for the flatfish eggs were not registered, meaning that the spawning areas may consist of either newly spawned eggs, far developed eggs or both. As mentioned well developed eggs could have been transported with currents to the sampling area, meaning that areas with high concentrations of these eggs may not be a spawning area, but an aggregation area. Martinho et al., (2013) found that the estimated duration of the pelagic stage of European flounder in Sørfjorden (60°) was, on average, 24 days. A hatching and spawning experiment with long rough dab showed that hatching time of eggs varied between 16 – 35 days in an incubator holding 6°C ± 2°C (Nagler et al., 1999). This means that the eggs could have had enough time to travel from other spawning areas to the sampling stations that we based our spawning area analysis on. The stations indicating spawning areas should therefore be interpreted with caution until identification of the development stage of the eggs has been investigated. Still, the stations with significant numbers of eggs represent an area with an unlikely abundance of eggs.

4.3 Drift and retention of spawning areas

From the analysis of the ocean current modelling and drift pattern retention areas was observed were for the three species European flounder, dab and long rough dab. In Espeland et al., (2013) a kernel density distribution was applied to the particle drift of the spawning area to interpret the probability of observing 50, 75 and 95 % of the particles in relation to distance. From this determination of a spawning area depended on three factors: modality of the distribution, distance to the spawning area and the size of the area. This procedure was not conducted in this study and which may weaken the determination of retention and dispersal areas since they are only based on observed distributions for the modelled particle drift due to time limitations. Still, the modelled areas will provide a pattern of particle densities and explain the geographical distribution. The particle drift model shows that most of the eggs are retained within the fjord of the spawning area, but even if most of the eggs circulates in the same area the presence of only a few far drifting particles will have a larger impact on the mean. Based on the discussion above the assumption of retention area in this study should be interpreted with cautious.

5 Conclusion

In this study we have shown that the spawning of the four different species takes place during the time of the egg survey, although the spawning peak varies among them (H_1). European flounder showed temporal spawning activity corresponding to the timing of the spatial sampling survey and peaked right before the survey was conducted ($H_{1.2}$). Spawning peak for dab was registered almost two months later than the egg survey, but spawning activity was registered during the survey (H_3). Long rough dab and Plaice both had spawning activity during the egg survey, although the numbers of eggs were low ($H_{2.3}$ & $H_{2.4}$)

For the investigation of spawning (H_2) the pattern in egg density did indicate spawning areas, but not for all the species. Plaice had no stations with a significant number of eggs that indicated spawning areas ($H_{2.4}$), while for the rest of the species indications of spawning areas were found ($H_{2.1}$, $H_{2.2}$, $H_{2.3}$). However, because of lacking data on the development stage of the egg it is difficult to say if the identified spawning areas are comprised of newly spawned eggs, or if the sampling station is a location where eggs from other areas have aggregated, carried with the currents.

The study also showed that the hydrodynamic forces retained eggs within the potential spawning area (H_3). Retention areas were found for all the three species, European flounder, dab and long rough dab, that were included in the ocean current model. However, the estimations carry some uncertainty regarding the lack of a kernel density distribution.

In conclusion, indication of spawning areas for European flounder, dab and long rough dab were found along the coast of Skagerrak and the hydrodynamic forces contributes to retaining the eggs in the estimated spawning area.

6 Future studies

The sampled eggs for all the four species were only identified on a species level. If the eggs were also categorized to which development stage, they belong to it would be easier to determine if the eggs had been drifting for a short or long period in the water column. Hence making the estimation of spawning area more accurate by selecting eggs which were newly spawned compared to in this study.

The time of the spatial sampling was set to match the spawning period of cod. This spawning period matches with the species of this study, but by postponing the spatial sampling a difference in species composition an abundance may be cover. Especially considering that the temporal sampling showed a difference in species composition compared to the spatial sampling.

In this study DNA-analysis were not included. For future studies it would be favourable to use DNA-analysis to distinguish European flounder and dab eggs, which can be confused, often at early development stages of the eggs. Also, DNA-analysis could also be used to examine possible difference in the genetic between the spawning areas with high retention to establish differences in the population structure.

Hatching and weighing of the eggs for the four species. Estimating the time of hatching from spawning at different temperatures will give a better understanding of at which time larvae can be expected to shift from a pelagic to a benthic habitat and from a passive to an active swimmer. In combination with the ocean current model and the particle model estimation of the position were the larva will shift to the benthic community. A weighing of the eggs and estimating their buoyancy will give an estimation of where the eggs are found in the water column.

Finally, revisit the significant spawning areas with high retention and estimate abundance of group-0 individuals and evaluate if the retention of egg is within the benthic nursery area.

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