

# WORKING GROUP ON ATLANTIC FISH LARVAE AND EGGS SURVEYS (WGALES; OUTPUTS FROM 2022 MEETING)

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## i Executive summary

The objectives of the Working Group on Atlantic Fish Eggs and-Larvae Surveys (WGALES) was to achieve an updated overview on recent findings of ichthyoplankton surveys under-taken for assessment purposes and to discuss upcoming issues such as e.g. increasing amounts of wind farms and other aspects related to egg and larvae, sampling and analyses. Additionally, recent findings in early life history ecology and distribution and survival dynamics in a warming ocean covered aspects particularly related to effects of a changing environment on the phenology and physiology of fish eggs and larvae. To complement understanding of ontogenetic drivers, new studies on maturation, migration and environmental cues for fish reproduction focused on developing methods and concepts to analyse fish fecundity, maturation strategies and spawning rates to provide important baselines feeding into the conceptualization and timing of ichthyoplankton surveys.

As an umbrella group for ichthyoplankton surveys and data organization, WGALES together with the ICES data center discussed the status and progress on the ICES Eggs and Larvae database and isolated issues to be addressed.

In order to increase cross- WG collaboration, particularly bridging the work of Working Group on Biological Parameters (WGBIOP), Working Group on SmartDots Governance (WGSMART) and WGALES, focus of the respective group was presented and subjects where input by WGALES is needed were pointed out.

Proceeding with the resolution regarding the implementation of modern ichthyoplankton processing tools, a comprehensive hands-on workshop on optical sample procedures and image data processing was included in the WGALES end-of-term meeting.

## ii Expert group information

<b>Expert group name</b>	Working Group on Atlantic Fish Larvae and Egg Surveys (WGALES)
<b>Expert group cycle</b>	Multiannual fixed term
<b>Year cycle started</b>	2019
<b>Reporting year in cycle</b>	4/4
<b>Chair(s)</b>	Patrick Polte, Germany Cristina Nunes, Portugal
<b>Meeting venue(s) and dates</b>	2019, By correspondence 19-21 October 2020, Online meeting (35 participants) 27-28 October 2021, Online meeting (22 participants) 17-21 October 2022, Boulogne-sur-Mer, France (29 participants)

# 1 Fish egg and larvae surveys throughout Atlantic regions (News, issues, methodology etc.)

For ichthyoplankton surveys, issues with sampling standardization and reference collections will be discussed (including high quality images for fish eggs and larvae, for reference collections and calibration procedures). This session will cover results of existing ichthyoplankton surveys as well as concepts for new surveys, proposals for adjustment of survey designs and demonstration of survey methodology. The objective is to provide a forum for discussion on the implementation and standardization of fish larvae and egg surveys and their contribution to ICES stock assessments.

## 1.1 The 2022 MIK-IBTS Herring larvae survey – A survey with many challenges

Authors: Bastian Huwer & Matthias Kloppmann (together with MIK survey participants from Ifremer, IMR, SLU, WUR and Marine Scotland )

During the International Bottom-trawl Survey in the first quarter (Q1 IBTS), night-time catches are conducted with the MIK net, a fine meshed (1600  $\mu\text{m}$ ) 2-m-midwater ringnet (ICES 2017) providing abundance estimates for large herring larvae (0-ringers) of the autumn spawning stock components. In addition, the Q1 IBTS also provides the time-series for the 1-ringer herring abundance index in the North Sea from GOV bottom trawl catches carried out during daytime. The total abundance of 0-ringers in the survey area is used as a recruitment index for the stock. Since 2017, this 0-ringer index (also called MIK index) time-series is calculated with a new algorithm, which excludes larvae of Downs origin more rigorously. This is done by excluding the smaller larvae – presumably of Downs origin – from the analyses in certain parts of the survey area. The index from the 2022 survey (corresponding to the 2021 year class) is 47.8. This is one of the lowest values in the time-series, with only 4 other year classes being even lower (2003, 2007, 2014 and 2016). The 2022 IBTS survey was faced with numerous challenges concerning the weather as well as technical and Covid-19 related issues, which also affected the MIK sampling. Only 433 depth-integrated hauls were completed with the MIK-net, which is only approximately 60% of the planned MIK stations. However, thanks to intensive coordination between participants during the survey and more decent weather in the final part of the survey period, at least 1 MIK haul (and mostly 2 or more) could be conducted in most ICES rectangles. Nevertheless, 24 rectangles were not covered at all by the MIK sampling, but these were mainly located in the northwestern parts of the survey area (Figure 1.1) which usually yield small numbers of herring larvae. Thus, the majority of the main herring larvae distribution area could be covered, and several data tests showed that the poor coverage had only a minor effect on the index. In summary, despite the encountered issues and low overall number of MIK hauls, it can be assumed that the 2022 MIK survey provides a representative 0-ringer index. As in previous years, sardine larvae were again found in the samples. Most sardine larvae occurred in the southern and south-eastern North Sea as well as in the Skagerrak. The 1-ringer recruitment estimate (IBTS-1 index) is based on GOV catches in the entire survey area. The index from the 2022 survey (corresponding to the 2020 year class) is 806. This is less than half of the long-term average of the time-series, and only 3 other year classes were even lower (1997, 2014 and 2016). As in previous years, the majority of the 1-ringers of the 2020 year class were found in the Kattegat/Skagerrak area,

however at much lesser abundance. In addition, 1-ringers were also found in the southeastern parts of the North Sea as well as in the Moray Firth. After a longer period where the trajectories of 1-ringer abundance and 0-ringer index seemed to be uncoupled (year classes 2003–2012), the two trajectories again corresponded well for the year classes 2013 – 2018 but weakened for the 2019 year class. The 0-ringer and 1-ringer data for the 2020 year class correspond better than for the 2019 year class, but the 1-ringer value seems rather low compared to the 0-ringer value. This leads to the question if there may be an issue with the 1-ringer index for the 2020 year class, which could e.g. be related to the various challenges during the 2022 survey. Due to these challenges, a total number of 33 ICES rectangles were not covered by GOV hauls in 2022. However, the uncovered rectangles are mainly located in the northwestern North Sea (Figure 1.1), which is an area that usually did not yield relevant catches of 1-ringers in previous years. Besides, the ICES rectangles in the northwestern areas that actually were covered did not yield relevant catches of 1-ringers during the 2022 survey (Figure 1.1), indicating that the unsampled rectangles would not have yielded any relevant catches either. Thus, the poor spatial coverage in these areas in 2022 does not seem to have an influence on the 1-ringer index, which is mainly driven by catches in Kattegat, Skagerrak and the German Bight, i.e. areas that were decently covered in 2022. However, the adverse weather conditions during much of the 2022 survey may have had a more general influence on the catchability of 1-ringers, e.g. by reducing the schooling effect due to low visibility in the water. Besides, it should be kept in mind that the 1-ringer index is based on hauls with a GOV Trawl, i.e. a bottom trawl which might not be ideal to catch pelagic species like herring. In summary, there is a possibility that the 1-ringer abundance from the 2022 survey, corresponding to the 2020 year class, may be underestimated.

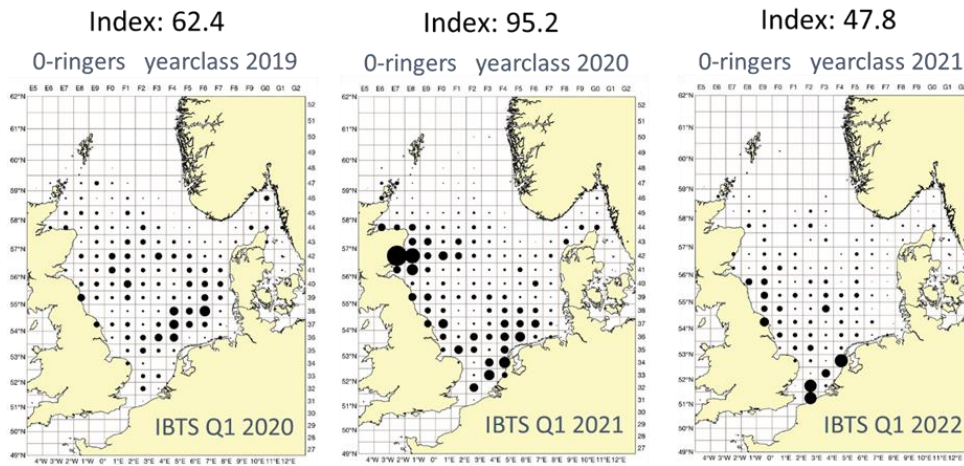


Figure 1.1. North Sea herring. Distribution of 0-ringer herring, year classes 2019–2021. Density estimates of 0-ringers within each statistical rectangle are based on MIK catches during Q1 IBTS in January/February 2020–2022. Areas of filled circles illustrate densities in  $\text{no m}^{-2}$ , the area of the largest circle represents a density of  $3.82 \text{ m}^{-2}$ . All circles are scaled to the same order of magnitude of the square root transformed densities.

## Discussion



The quarter MIK-IBTS survey in 2022 was confounded by many factors. These included the Covid-19 outbreak reducing the German vessel survey time with a loss of 15 days, a breakdown of the Scottish vessel, and exceptionally bad weather for all participating countries. The typical coverage of the MIK-IBTS 2022 included 433 hauls with 250 hauls less than typically undertaken. Especially the coverage of the central area of the North Sea was underrepresented with samples that included 24 ICES rectangles left unsampled. The most relevant rectangles based on historical observations of herring larvae were covered with at least 2 hauls due to an exceptional effort of all participating countries. However, the index calculation for 2022 remained questionable and an effort was made to investigate the reliability of the data produced. A re-calculation of the MIK time-series from 1992 to 2022 indicated a generally useful index for 2022. The outcome of the 2022 survey underlines the necessity of a minimum of 2 hauls per ICES rectangle given by the re-calculation of the indices' time-series.

The quarter-1 IBTS survey provides an additional 1-ringer index which was also investigated for 2022. A number of 33 sampling stations were left unsampled in 2022. A comparison of historical surveys indicates that only in 2018 the unsampled rectangles in 2022 included a distinct number of the 1-year age class. The uncertainties are, therefore, considered marginal for the index calculation in 2022.

The post-larval index (0-ringer index) and the 1-ringer index were compared for their respective year and resulted in a generally low correlation between both time-series. One reason was discussed which is the usefulness of a bottom-trawl survey to provide a good index for the 1-ringer index.

However, it was also concluded that a general ecosystem change may be part of the low correlation since indices before 1992 were in good agreement with 70 % matching general trends in both indices with a decrease of the agreement to about 25 % since 1992. The index estimates for the 0-ringer 2021 and 1-ringer 2020 were the lowest at all times and suggest a very low recruitment in 2022.

Unanswered questions remain (1) how to improve the 1-ringers sampling, or (2) if the sampling is suitable but mortality from the 0 to 1-age classes changed since 1992.

## **1.2 Rügen herring larvae survey- heaven and hell of small-scale sampling**

Author: Patrick Polte

Most important spawning grounds and nursery areas for spring-spawning herring in the western Baltic Sea are found around the island of Rügen, with the Greifswald Bay as the center of reproduction. The strong correlation of year classes with the overall abundance of recruits in the Western Baltic Sea indicates high prediction power of the N20 larval index for the annual year-class strength (Figure 1.2). In the framework of the Rügen Herring Larval Survey (RHLS). During the entire spawning season, i.e. from mid-February to late June, 35 stations are sampled at weekly intervals. Herring larvae are quantitatively captured with bongo nets (335 µm). Beyond larval sampling, hydrographic parameters such as water temperature, salinity, Secchi depth/water turbidity, and dissolved oxygen content are recorded. The highly resolved time-series of the RHLS dating back to 1992 provides a unique dataset for studying the dynamics of early stages in the herring life cycle. However, the program includes extreme efforts in ship time, lab processing and data handling. Therefore, it needs to be regularly evaluated due to the value of results. By the nature of the program (single-bay sampling), the spatial coverage on a Baltic Sea scale is extremely low as far as larval herring distribution is concerned. On the other hand, the weekly

survey covering the entire reproduction period, including hatching peaks and growth pattern is extremely useful (if not unique) to study bottlenecks of early life stage survival and delivers a highly resolved seasonal picture of early life stage dynamics including shifts in herring spawning phenology. As databases allowing for direct application of monitoring data for mechanistic studies are globally scarce. We conclude that surveys such as this are well justified and valued.

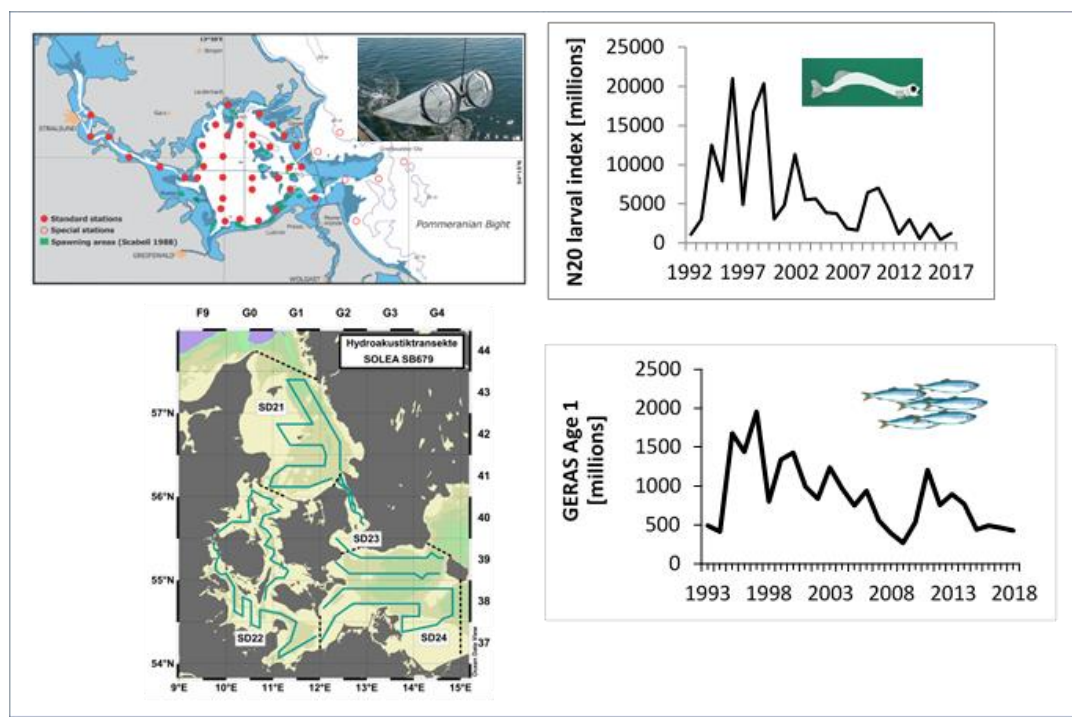


Figure 1.2. Upper panel: Station grid of the Rügen Herringlarvae survey (RHLS) and the N20 time-series, Lower panel: Age one juvenile abundance index from the German Acoustic Survey (GERAS).

## Discussion

To produce a recruitment index for the western Baltic herring stock component weekly surveys are conducted in Greifswald Bay that have proven to be a suitable index representative for the whole stock. The sampling is conducted in early spring covering the entire spawning season. The start of the survey was determined with the melting of surface ice cover but is recently not an issue anymore. The station grid covers a sampling of 36 Bongo tows which usually are sampled between February and June. In November a regular one-time check for spawning is conducted to monitor any autumn spawner activity. The number of stations range typically between 540 and 576 samples per year.

The time-series starting in 1992 indicates a distinct recruitment breakdown since 2004 with only the years 2008 and 2009 with just below average recruitment success. For the year 2020 the index was at its minimum suggesting a very low contribution to SSB. Given early life history stage indices the Rügen herring larval survey produces an index well correlated with the age 1 GERA survey of about 70 %. Recent low recruitment success seems to be affected by reduced periods

of ice cover (reduce of winter period) in that area and may be a result of ocean warming negatively affecting the survival of the early life stages of herring.

Yet, no other species that may either compete over food resources or an increase of predators are observed. A possible increase of jellyfish is suggested but the effect of jellyfish top-down control is yet to be investigated. Another possible effect of phenology shift may have increased a possible match–mismatch situation for larvae at first-feeding.

### 1.3 The missing eggs: The case of the mackerel and horse mackerel egg survey in 2022

Author: Cindy van Damme

The Atlantic mackerel and horse mackerel egg survey was carried out in 2022. The Netherlands surveyed in May and June east of Ireland, Celtic Sea and northern Bay of Biscay. The Dutch survey is carried out with a Gulf VII with a 280 µm mesh as the survey manual prescribes, with an internal and external flowmeter mounted on the sampler. During the surveys regular calibration of flowmeters is done. In good situations the internal flowmeter calibration is 1.2 times the external flowmeter. However, in some areas the internal flowmeter revolutions was 0.5 times the external flowmeter during a calibration without the codend. In that situation the external flowmeter was also somewhat lower compared to the normal situation. Clogging severity was estimated following ICES vocabulary. About 1/3 of all the stations sampled in both months showed major to severe clogging. A huge number of those samples were found in deep waters, off the continental shelf. In the past clogging sometimes occurred due to phytoplankton in the shallow coastal areas, but never in the deeper waters. The clogging in the deeper waters occurred to transparent slime in the water, but no jellyfish, salps or other animals could be recognized in the slime.

#### Discussion

MEGS is undertaken from January until July every 3 years with several coverages from the Bay of Biscay to the Northern North Sea until Iceland (in recent years). Flowmeters on the GULF sampler are used to measure the filtered volume during a plankton station. Flowmeters are normally attached in- and outside the mouth opening to monitor water flow during hauling. A typical ratio between the flowmeters is around 1.2 with 20 % higher flow rates due to the nose cone effect of the mouth. The flowmeters are regularly calibrated preferably ones per week on a MEG survey.

In spring 2022 during the Dutch survey, the ratio of the flowmeters indicated several sampling stations with a high degree of clogging seen by the decreased ratios between the inner and outer flowmeters. The clogging was a result of a seemingly organic layer of slime on the inside mesh during sampling. In total, only 55 % of sampling stations had a ratio of 1.2 while 45 % of the sampling stations were compromised with decreased filtering performance of the net. The clogging was variably affected with high clogging registered in the northern Celtic Sea and at some stations close the coast. These observations of clogging were confirmed in the June survey and the UK survey (Hannah Holah).

It seems to have affected several participating countries and warrants further investigations.

Questions raised by WGALES:

Should the organic layer responsible for the clogging be part of a further investigation, e.g. DNA analysis?

- A general agreement among participants was concluded but the question of financing was raised as well as if the observation is a unique and one-year observation or if MEGS will be affected in future surveys. If the total number of eggs sampled was affected cannot be answered with high confidence and warrants further investigations. The following points were suggested to deal with this new clogging issue:
  - The sampling may benefit from using larger mesh sizes reducing the possibility of clogging.
  - Future MEG survey participants are asked to perform comparative samplings with meshes of 280 and 500  $\mu\text{m}$ .
  - A general recommendation to the country participating members should include the shifting of 280 to 500  $\mu\text{m}$  to improve sampling performance.
  - Is anything done with the rest of the samples and is the change from 280 to 500  $\mu\text{m}$  then problematic? Is the changing feasible for all members?
  - Is the change to 500  $\mu\text{m}$  solving the clogging issue?
- The WG should include a guideline section in the MEGS manual to assure standardized procedures when clogging is registered during a survey.

Were there any changes registered in the depth of the thermocline?

- CTD data are so far not checked, and the question remains unanswered.

## **1.4 Five years of MIK pilot surveys during the Q3 IBTS – Potential for implementing a North Sea sprat recruitment index and future plans**

Authors: Bastian Huwer, Peter Munk, Mikael van Deurs, Matthias Kloppmann, Anne Sell

Sprat is a short-lived species, and the sprat stock in the North Sea is dominated by young fish. Thus, the size of the stock is to a large degree driven by the recruiting year class, and catches are mainly composed of 1 year old fish (up to ~80%). Sprat is an important forage fish and represents a major food source for many other fish species as well as seabirds and mammals. It is therefore a highly relevant species in multispecies approaches to fisheries management. An analytical assessment of sprat was established some years ago, however the availability and quality of data for the assessment are relatively poor and the assessment of and advice for the North Sea sprat stock need to be improved. There is currently no information available on young-of-the-year (0-group) sprat for possible use in short-term forecasts or for use in the stock assessment model. However, such information could be very useful, in particular because sprat is a short-lived species that matures early. The aim of the present study is - by conducting a series of pilot surveys - to evaluate the feasibility of establishing a sprat recruitment index based on larval sampling on the Q3 IBTS surveys and to contribute generally to a better understanding of the biology, ecology and distribution of the North Sea sprat stock (Figure 1.3). Thus, the basic idea is to follow similar procedures as the MIK herring larvae surveys during the Q1 IBTS. These surveys are targeting relatively large larvae (~ 2 to 3 cm) and the abundance of these has shown to relate to later recruitment to the stock, thus providing a recruitment index for autumn spawning herring in the North Sea. So far, a total of 5 pilot surveys (2018-2022) were conducted by DTU Aqua, partly supported by the Thünen Institute of Sea Fisheries in Bremerhaven, Germany. The sampling gear is a MIK net with a ring of 2 meter diameter, equipped with a depth sensor and a flowmeter, and is deployed in a double-oblique haul from the surface to 5 meter above the seabed at a speed of

3 knots through the water. Results indicated that a number of prerequisites for establishing a recruitment index were met, e.g. that sprat larvae can be caught in appropriate numbers and that the main spawning activity of sprat is finished before the time of the surveys. However, while the Danish IBTS seems to cover the most relevant areas of major sprat larvae occurrence, a better spatial coverage, in particular in the north, would be desirable. In all years, the sampled clupeid larvae not only contained sprat but also sardine larvae in high abundances, with sprat larvae mainly occurring in the northern part of the study area while sardine larvae were most abundant in the south (Figure 1.3). This shows that careful identification procedures to species level are mandatory. At the time of WGALES 2022, corresponding recruitment estimates from the stock assessment were available for the first 4 years of the pilot surveys (2018-2021). Preliminary analyses indicated similar trends between larval abundance and recruitment for the first 3 investigated year classes (2018-2020), while a weaker correspondence between larval abundance and recruitment was found for the 2021 year class. However, the recruitment estimate for this 2021 year class is so far only based on the age 1 sprat catches from the 2022 Q1 IBTS and therefore still very preliminary. Besides, due to extremely bad weather and other challenges during the 2022 Q1 IBTS, this age 1 estimate may be unreliable. Thus, it still requires more reliable recruitment estimates, a longer time-series, further analyses and probably also a more sophisticated recruitment index than a simple average abundance to make a final judgement if the larvae survey can provide an early recruitment index. Nevertheless, the 5 years of pilot surveys illustrate that this kind of larvae survey during night-time of the Q3 IBTS has the potential to provide larval abundance estimates and potentially a recruitment index for North Sea sprat. However, additional surveys will be necessary to provide further yearly observations and more data for the modelling of recruitment patterns. Based on the promising results from the first 5 years, DTU Aqua decided to continue the pilot survey in 2023 and to evaluate the suitability of the survey for assessment purposes at benchmark assessment for North Sea sprat, which is planned in autumn 2023. It is noteworthy that in addition to sprat and sardine, a number of larvae of other fish species were caught in the MIK. The more abundant species were mackerel, horse mackerel, sandeel, gurnards and lemon sole, scaldfish and several other flatfish, as well as several other, non-commercial species, e.g. gobies, crystal goby, rocklings, pipefish, dragonets and greater weever. In addition, a limited number of larger gadoid larvae and/or pelagic juveniles were caught. These other species showed relatively distinct, annually recurring distribution patterns. Mackerel larvae occurred e.g. mainly in the northern part of the sampling area, whereas horse mackerel dominated in the southern part (note: so far other larvae were only analysed for 2018-2020 due to lack of funding).

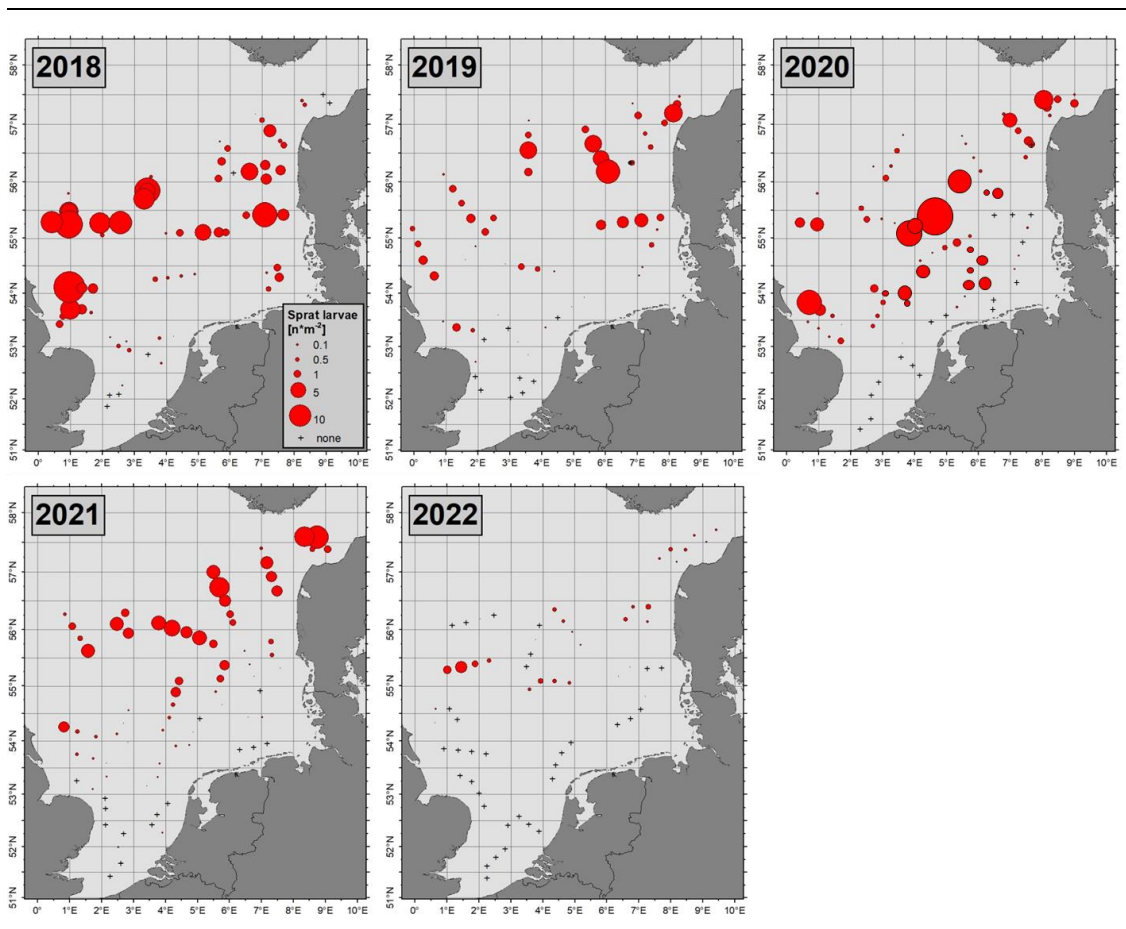


Figure 1.3. MIK pilot surveys during the Q3 IBTS. Abundance of sprat larvae per sampling station in 2018 - 2022 (preliminary data).

## Discussion

In the last five years of MIK sampling during Q3 IBTS, the potential for using sprat larval abundances to produce an annual sprat recruitment index is investigated. Yet, an age-1 recruitment index from Q1 IBTS is used but seems inadequate since a bottom-trawl survey is used to provide an index for a small pelagic species.

Two projects were funded to investigate potentials for a sprat recruitment index (BEBRIS and PELA) with the objective improving assessment models and the advice for sprat. Preliminary results of the first three years resulted in the decision to continue (without any third party funding) the sampling of sprat larvae in 2022 and 2023. This decision was based on the observation of reasonable amounts of sprat during IBTS Q3 and a good indication that sprat spawning has finished during Q3. There is a general recommendation that sampling with the MIK net should occur during the night for quantitative purposes which optimizes sampling effort and decreases the influence on daytime trawling for IBTS.

However, there were indications that some sprat larvae reached a size at which their abundance may not be a good indicator for recruitment and the coverage especially in the northern extent of sprat larval distribution may not be covered entirely. This leads to doubts that the data collected is sufficient for a robust recruitment index.

Another disadvantage is the dependence of plankton sampling on the location the daytime trawl sampling has stopped which determines the area of plankton stations during the night. However, with the additional night plankton sampling about 60 – 80 sampling stations can be added with a good coverage of the North Sea. Some larger areas may not be sampled which seems essentially be important in the northern sampling area, less the southern areas.

There are more possibilities to increase effort with ichthyoplankton sampling in Q3 IBTS since e.g. sardine larvae seems sufficiently sampled as well and becomes an increasing component in the North Sea in future. Some preliminary results indicate a spatial separation between sardine and sprat larvae with sardine larvae occurring dominantly in the south and sprat farther in the north with some transition areas of both species.

It is concluded that the number of three years for an index is yet not enough to make any recommendations to increase effort and if such an index is providing a robust and reliable addition to the assessment for sprat.

## **1.5 Integrating long-term plankton sampling with wind-energy monitoring**

Authors: Katey Marancik, H Walsh, C Orphanides, E Broughton

The US government has set goals of 30 GW of offshore wind by 2030 and 15 GW of floating offshore wind by 2035 while protecting biodiversity and promoting ocean co-use. This means that plankton sampling programs will need to coexist with offshore wind development. Large sections of the northeast US continental shelf may become unreachable using conventional gears and methods, and new ways to access and monitor these regions will be required. National Oceanic and Atmospheric Administration's Fisheries Service's Northeast Fisheries Science Center's Ecosystem Monitoring Program (EcoMon) samples the entire continental shelf from Cape Hatteras, North Carolina, to Canada 4-6 times a year since 1999 (Figure 1.4). The EcoMon Program fits into a system of 5 long-term projects that provide shelf-wide coverage for several seasons for nearly 50 years. These programs provide hydrographic, zooplankton and ichthyoplankton abundance and distributions, ocean chemistry, marine mammal, sea turtle, and seabird abundance data. The ichthyoplankton data are used in stock assessments, ecosystem assessments, and research. Offshore wind development will likely displace plankton sampling over large sections of the shelf, particularly effecting estuarine-dependent species data in the southern portion of the sampling area. Partnerships with researchers focused on protected species (namely the critically endangered North Atlantic right whale) have provided opportunities to sample where wind development will likely affect right whale feeding habitat. That research has focused primarily on zooplankton. However, limited directed studies have been started for ichthyoplankton. Ocean co-use could be an opportunity for innovation, but many questions about how to share the ocean with competing interests remain.

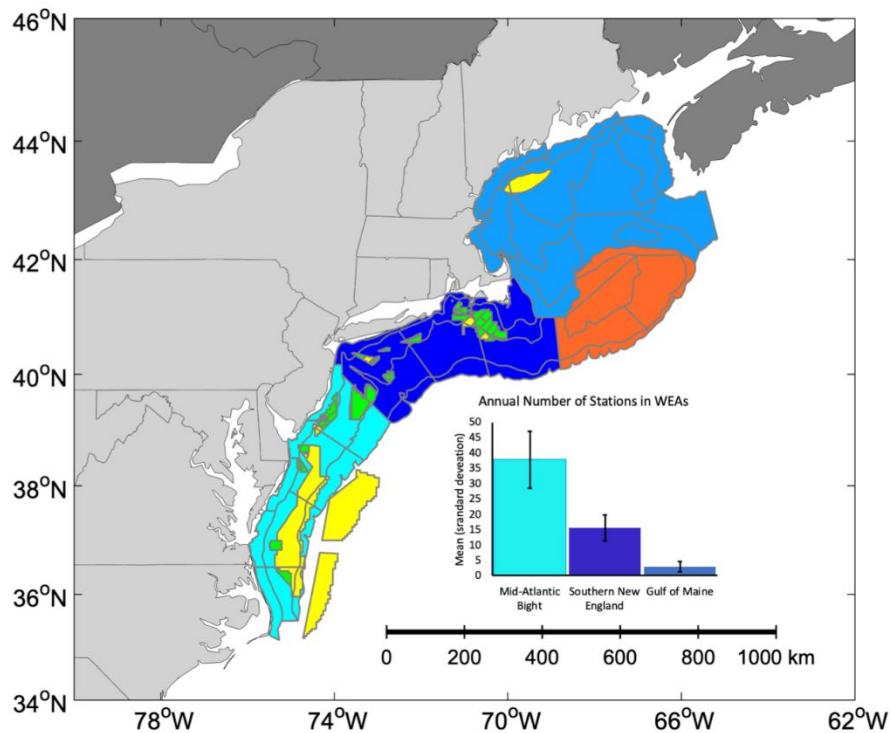


Figure 1.4. A map of the northeast United States with the NOAA long-term plankton sampling area in blues and orange. Pro-posed and active Wind Energy Areas are overlaid in yellow and green. The inset graph shows the number of stations sampled inside the proposed and active Wind Energy Areas each year by subregion (colours match the map).

## Discussion

The plans for offshore wind park establishments in the US are expanding and foresee a total of 30 GW offshore production in 2030. These expansions include areas along the Northeastern shelf areas in the US especially large areas in the Gulf of Maine and some smaller areas farther southward. Including these areas extending from North Carolina to Canada specifically since 1999 Bongo-61cm sampling was undertaken. Random stratified sampling is normally performed along 47 transects which include in average 120 plankton stations and about 35 CTD stations. These coverages are conducted 4-6 times per year with an entire coverage for spring and autumn while more scattered coverages in summer.

The leasing of new offshore wind parks will hinder maintaining the time-series that include important marine fish resource monitoring. About 34 % of the mid-shelf and another 17 % of the inner-shelf area will be inaccessible due to wind energy production which will affect on the time-series and the comparability of data points from the time-series. The wind farm expansion will hence lead to the question of how to maintain the 50-years time-series. Other methods except net sampling seems insufficient as a solid quantitative method to investigate the early life history stages of fish and the resulting recruitment success.



Another yet unresolved question arises if the effect of wind farms may impact (maybe negative) on the dispersal of eggs and larvae since flow patterns of currents may alter and are potentially able to affect early life stage survival. There is some indication for negative implications for menhaden and other species, but not enough data are available to formulate a sound recommendation.

A general conclusion is to investigate the area closure further and its effect on the larval time-series and the respective early life history stage indices. Replacements of the unsampled areas should be discussed and solutions investigated.

## 2 Early life history ecology: Insights on distribution and survival dynamics in a warming ocean

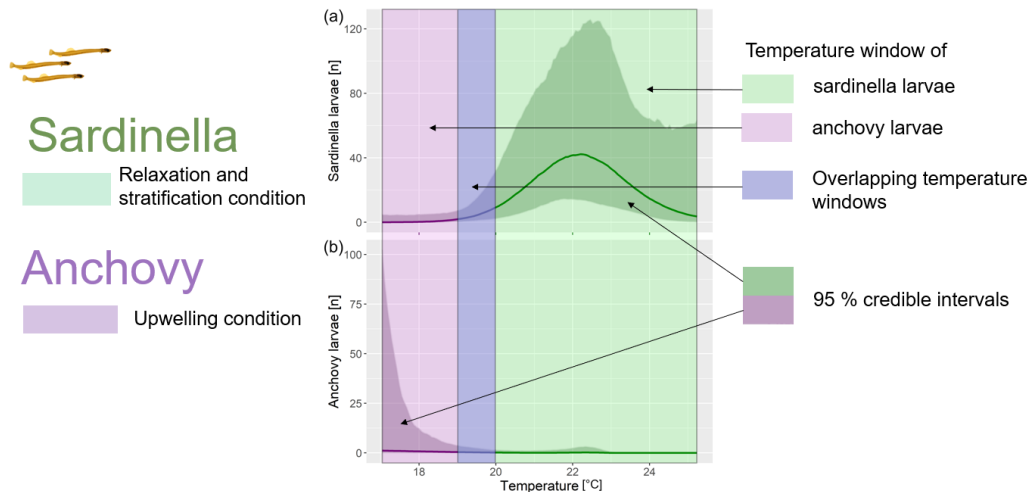
This section addresses all aspects of fish early life stage ecology with a special focus on direct (e.g. physiological) and indirect (trophic cascades) climate impacts on distribution and survival. It includes existing results and presents ways of using egg and larvae survey time-series to study environmental impacts and mechanisms of early life stage mortality.

### 2.1 Anchovy and sardinella reproduction are regulated by upwelling and relaxation conditions in the southern Canary Current ecosystem

Authors: Maik Tiedemann, Ismaïla Ndour, Fambaye Ngom Sow, Espen Bagøien, Jens-Otto Krakstad, Marek Ostrowski, Erling Kåre Stenevik, Tor Ensrud, Stamatina Isari

The anchovy *Engraulis encrasicolus* and the sardinellas *Sardinella aurita* and *Sardinella maderensis* are among the most dominant small pelagic fish species inhabiting the tropical and subtropical East Atlantic. There, they play a key role in the marine food chain and are targeted by artisanal and industrial fleets contributing to human food security. To manage these stocks sustainably the understanding of key life history parameters is crucial. One of these parameters is reproductive success. Hence, knowing when, where and under which circumstances reproduction occurs may help to understand stock dynamics. Therefore, an ichthyoplankton survey was conducted in May 2013 along the coast of Senegal, the Gambia, and Guinea Bissau within the framework of the EAF Nansen programme. The survey coincided with a strong environmental change from a cold upwelling to a warm upwelling relaxation event and triggered a shift in spawning activity from European anchovy *Engraulis encrasicolus* to round/flat sardinella *Sardinella* spp. To identify the main environmental drivers for the spawning shift, zero altered negative binomial regression models with a generalized additive structure were used to link early larval fish distribution patterns to the two different regimes. Only a slight overlap in the presence of both species was found (Figure 2.1) indicating species-specific spawning conditions. In the light of ocean warming, anchovy spawning opportunities may decrease, while sardinellas may have increased spawning opportunities in future, with implications for the pelagic fisheries conducted in these areas.

## Optimal larval (spawning) temperature regimes



**Figure 2.1.** European anchovy *Engraulis encrasicolus* and *Sardinella* spp. larvae along the coast of Senegal, the Gambia, and Guinea Bissau as encountered by the ichthyoplankton survey in May 2013 within the EAF Nansen programme. A strong environmental change from a cold upwelling to a warm upwelling relaxation event triggered a species shift in spawning activity.

## Discussion

Upwelling systems are 1-2% of the ocean surface but fuel 20-30% of fish production. May is the month when upwelling declines and anchovy and sardinella are present in large numbers. In June no upwelling occurs anymore. In this area eggs develop fast, within 1-2 days larvae hatch. Sampling is conducted in May. At the beginning of May there is still upwelling, in the second half of May no upwelling occurs anymore. In the upwelling centre, no sardinella larvae were found, but during relaxation of upwelling, sardinella larvae are found throughout the area. In contrast, anchovy larvae were found in the upwelling centre, also during the relaxation period they are in the area where the upwelling centre used to be.

A spatial autocorrelation occurs depending on distance between sampling stations. R-INLA does not use latitude and longitude, but uses distance.

Temperature is the driver for spawning of both species, but there is a clear spatial separation between the species. If an increase of 1 to 2 degrees would happen in future anchovy would probably not reproduce anymore in this area.

Temperature is a prominent parameter, but there are much more parameters behind it. Ways of including those were discussed as well as the potential for future predictions. Anchovy will probably find ways to adapt. More data are needed in to allow for further evaluation. For anchovy the area is not the major spawning ground.

For both species throughout the year a portion of the adult stock is always ready to spawn. Depending on the environmental clues some will spawn if conditions are right. However, the eggs will die and disappear immediately depending on the spatial distribution around the upwelling

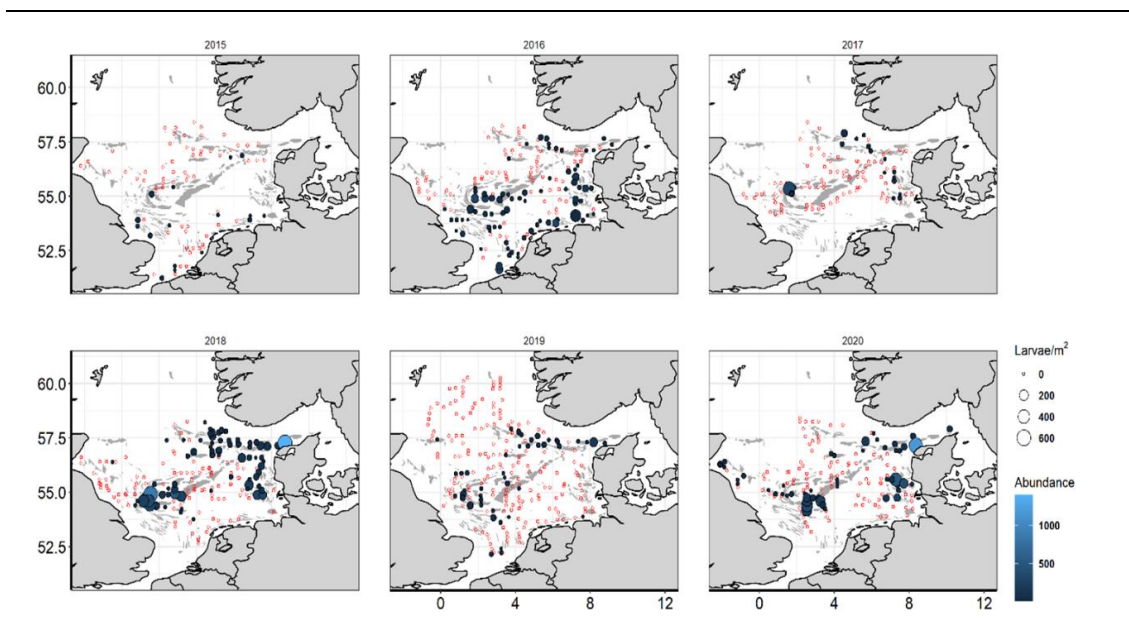
centre. It was further discussed if the spatial-correlation ( $w$ ) could be used to evaluate how far stations should be apart to avoid pseudo replication.

## 2.2 Identifying spawning and settlement areas of sandeel (*Ammodytes marinus*) in the North Sea based on the distribution of recently hatched larvae and drift modelling

Authors: Bastian Huwer, Mikael van Deurs, Ole Henriksen, Maria Makri, Tobias Mildenerger, Asbjørn Christensen, Matthias Kloppmann, Cindy van Damme, Christophe Loots, Richard Nash

Several sandeel species are found in the North Sea, with Raitt's sandeel (*Ammodytes marinus*) being by far the most common. Being a major prey for predatory fish, seabirds and mammals, this species is one of the most important forage fish in the North Sea and is also supporting one of the largest single species fisheries in that area. Due to its high ecological and economical importance, a relatively large body of research exists about the species, covering various aspects. However, there is only limited knowledge of the spawning ecology, which may be due to the unique life cycle. From late summer to autumn, the adults and newly recruited juveniles are burying into the sediment where they are overwintering for several months, utilizing specific areas with suitable sediment. Spawning is also taking place in these areas during winter, when the adults are briefly leaving the sediment to deposit demersal eggs on or in the sediment. Thus, the eggs are more or less impossible to sample, and knowledge of the occurrence and intensity of spawning activity in the different sandeel areas is largely lacking. The project PELA, which was conducted by DTU Aqua from 2019-2022, was aiming to close this knowledge gap by mapping the spatial distribution and abundance of recently hatched larvae as an indicator for spawning activity (Figure 2.2). The analyses made use of samples collected with a so called "MIKey M net", a small ringnet with a diameter of 20 cm and a mesh size of 335  $\mu\text{m}$ , which is attached to the larger MIK ring on the annual herring larvae surveys which are conducted at night-time during the Q1 IBTS. The use of this additional MIKey M net was introduced some years ago by ICES WGECCS2, with the aim to obtain information on the occurrence and distribution of cod and plaice eggs. However, it was noticed that the samples also contain very small sandeel larvae, which gave rise to the present study. MIKey M samples were analysed for a 6-year period from 2015-2020, which includes years with contrasting recruitment (2015 and 2017 very poor, 2016 exceptionally high, 2019 very good recruitment). The analyses were aiming to include all available samples from Denmark, Germany, Norway, the Netherlands and France, as these nations are covering the main sandeel habitats during their Q1 IBTS. Samples from Germany, Norway and the Netherlands were transported to DTU Aqua for analysis, larvae of sandeel and other fish species were sorted from the samples and counted, and sandeel larvae were scanned and length measurements conducted with an image analysis system. An exception are the French samples, which are anyhow regularly analysed by Ifremer via Zooscan, and for which the sandeel larvae data and images were provided to DTU Aqua. The majority of sandeel larvae in the samples were only about 5 to 6 mm. As the hatch size of *A. marinus* is approx. 5.5 mm, the sampled larvae can be considered to have hatched very recently, i.e. they have not drifted very far and should indeed provide an indication for spawning areas. Nevertheless, in order to further corroborate the spawning locations, a coupled hydrodynamic and individual based (IBM) model was used to back-drift the larvae from their catch to their hatch positions. The results of this back-tracking exercise showed that the larvae did indeed not drift a lot between hatch and catch positions and that the majority of larvae are so small that they can directly indicate the spawning locations. The spatial distribution of the larvae shows considerable larval abundances in the immediate vicinity of the known sandeel burying areas (Figure 2.2). However, there are also spatial

differences in the utilization of burying areas, as certain areas are frequently used for spawning while other areas are apparently not regularly used. In addition to the “raw” larval abundances, spatial GAMs were applied to identify spawning hot spots. The Dogger Bank area was identified as a major spawning area, as well as the Horns Reef area west of Denmark and an area north of Denmark along the Norwegian trench. In contrast, no or only few larvae were found the central area at “Elbow Spit”, which indicates that this is an area of minor importance for spawning. Furthermore, the data show clear differences in larval abundance between years. Furthermore, as the sandeel burying areas are located in different management areas (Figure 2.2), another aim was to gather more information if sandeel in a specific management area are also reproducing and recruiting in that area, or if they are migrating there from other management areas. This was investigated by forward drift projections in the coupled hydrodynamic and IBM model. Larvae produced in the Dogger Bank and Horns Reef areas experienced a relatively stable, directed drift in northeastern and northern directions, respectively, and a large part of these larvae settled on sandeel banks in the same management areas where they were spawned. In contrast, larvae produced in the northern area along the Norwegian trench experienced a much more dispersed drift and many of these larvae were lost as they were not able to find a suitable settling habitat. Larvae originating in Dogger bank settled mainly in the Elbow spit area that was apparently not used for spawning. This fits nicely to results from annual dredge surveys, that usually mainly find juveniles but no adults on Elbow spit. In combination with recent results from genetic analyses and a mark-recapture study, the results of the present study will be used to re-evaluate the borders of the sandeel management areas in the next benchmark assessment (planned in November 2022). Besides, by comparing the larvae/drift results with results from annual dredge surveys and commercial catches, it is planned to investigate if the sandeel are utilizing different areas for overwintering, spawning and foraging and how they are moving between different areas throughout the year. In addition, data on sediment samples from the dredge surveys may be used to analyse if the sandeel prefer different sediment types for overwintering and for spawning. Furthermore, it is planned to investigate if there is any connection between larval abundance and recruitment or stock size.



**Figure 2.2. Abundance of recently hatched sandeel larvae per sampling station in 2016 - 2020 from MIKey M net sampling during the Q1 IBTS (preliminary data).**

## Discussion

MIKeyM sampling was performed during the IBTS-MIK survey. MIKeyM samples contained recently hatched larvae. This allows to map and identify spawning areas and perform drift modelling on the results of the MIKeyM data. In winter adults are buried in the sediment but briefly leave the sediment to spawn in December-January. In spring they leave the sediment when food increases in the water column and stay there in summer. There are few areas in the North Sea with the right sediment for the adults to burry.

Species identification will be confirmed in the near future. A preliminary genetic study of the larvae confirmed it was all *Ammodytes marinus*. This is also underlined by the spawning time. Length of larvae in samples is 5.5 mm, thus recently hatched.

Samples are available from a 6 years time-series. Larvae distribution varies with year, but larvae are only found in the vicinity of the burying areas. Spatial GAM modelling is done to find hot spots. Doggerbank, Horn's reef and Norwegian trench are spawning hot spots. But Elbow's pit is not used for spawning at all. Dredge survey in autumn showed that on Elbow's pit only juveniles are found. It is yet unclear what happens with these juveniles. Genetic studies are carried out at the moment to investigate population structures.

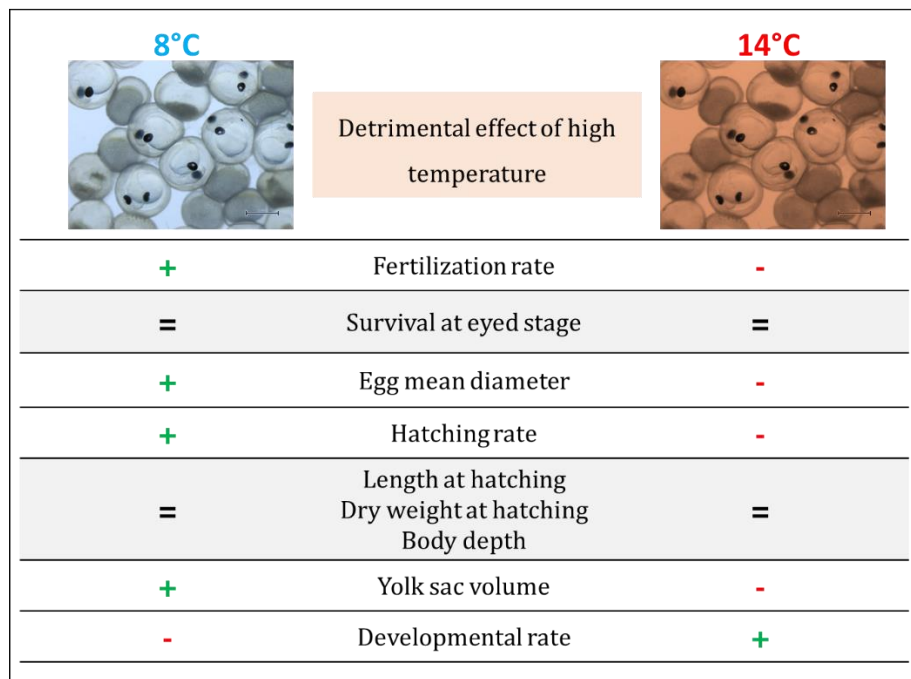
Back tracking shows that most larvae only drifted a short distance. Thus larvae distribution is considered to reflect the spawning distribution. Additionally, an IBM was coupled with a drift model. Larvae were starting at the known locations where they were caught. Modelling was performed until metamorphosis at 40mm occurred. If after 14 days no suitable settling habitat is found the larvae dies. Multiple drifts were simulated. Resulting drifts end in a small or large ellipse at the likely settling area. The size of the ellipse shows the difference in position between

the drift trajectories. For Doggerbank and Horn's reef the trajectory was stable and found a good settling area. The drift of the Norwegian trench showed high difference and larvae did not find a suitable settling area. This drift modelling used to review the present borders of the current sandeel management areas for the sandeel benchmark.

### **2.3 Impact of temperature on Downs herring embryonic stages and first insights on maternal effects**

Authors: Lola Toomey, Carolina Giraldo, Christophe Loots, Kelig Mahé, Paul Marchal, Kirsteen MacKenzie

Ocean warming is expected to be one of the major drivers of change in marine ecosystems, along with other stressors such as acidification and hypoxia. Fish species are particularly vulnerable during first-life stages, especially during embryogenesis. In this work, we evaluated the impact of temperature on embryonic stages of Atlantic herring (*Clupea harengus*), a species with a high socio-economic interest. We focused on the eastern English Channel winter-spawning component, Downs herring. In standardized controlled conditions, we evaluated key traits related to growth and development at three temperature regimes (8 °C, 10 °C and 14 °C), from fertilization to hatching. We demonstrated a global negative impact of temperature rise on fertilization rate, mean egg diameter at eyed stage, hatching rate and yolk-sac volume (Figure 2.3). We also highlighted a faster developmental rate and a change in development stage frequency of newly hatched larvae at higher temperature. Maternal effects (i.e. differentiation between progenies) were detected for four key traits (i.e. fertilization rate, eyed survival rate, mean egg diameter and hatching rate), suggesting a plasticity in the response to temperature rise. Finally, we tested whether relationships existed between female attributes and embryonic key traits in order to find potential proxies for embryonic quality. Age, traits linked to life history (i.e. asymptotic average length and Brody growth-rate coefficient), condition and length of females appeared as important predictors.



**Figure 2.3.** In a controlled experimental set-up, key traits related to growth and development of herring eggs were studied at differing temperature regimes from fertilization to hatching. A negative impact of higher temperatures on fertilization rate, mean egg diameter at eyed stage, hatching rate and yolk-sac volume was documented.

### Discussion

Thermal tolerance changes by life stage and the embryonic stage is more vulnerable to temperature rise. A herring larvae experiment in 8, 10 and 14 °C. The first batch taken on 7<sup>th</sup> Dec, second on 18<sup>th</sup> Dec. 1. Eggs were fertilized with a mix of sperm from 3 different males. Eggs of each female were divided over the three temperatures. 20 females were stripped at the start of the experiment, but when larvae were at the eye pigmentation -stage only eggs of 6 different females were left, eggs of the other females had died. Results of 8 and 14 °C were presented and showed no difference in survival toward the eyed stage. However, an effect on fertilization rate, egg diameters and hatching rate could be seen. Size at hatch showed no difference but yolk-sac volume was smaller in higher temperatures. The development rate was higher at higher temperatures. In the higher temperatures the larvae hatch earlier, but the hatching duration was the same as in colder waters. Thus, larvae have a more active metabolism in the warmer temperatures. This finding is considered in contrast to what is written in the literature. Additionally, there was a maternal effect on eyed stage survival, probably due to different fertilization rates. Maternal effects should be included when studying stressor influences. This experiment showed that bigger, older females provided better eggs. Paternal effects are going to be investigated in future experiments. Deformities in eggs and larvae could not be found in this experiment. The low fertilization success in this study could have been related to the freshness of the fish used for strip-spawning. Fish were received from the last haul of the day of a fishing vessel. Also, the sampling date (7<sup>th</sup> of December) has been relatively late in the spawning season.



## 2.4 Impact of global change on the development and survival of Atlantic herring (*Clupea harengus*): A multi-stressors approach

Author: Léa Joly

Ocean warming and acidification (OWA) are major threats to marine organisms. In addition to direct abiotic effects on the physiology of individuals, indirect biotic effects must be considered when addressing secondary consumers. Indeed, direct effects can affect different trophic levels and have cascading effects in the food chain. The combined effect of warming, acidification, and decreased prey quality was evaluated on the condition and fatty acid composition of herring. Larvae were reared from hatching to the last developmental stage in ambient and OWA treatments (+3°C, -0.4 pH), these treatments were crossed with two different feeding treatments. A difference of 2% docosahexaenoic acid (DHA) in prey was tested. The larvae were sampled at the end of the experiment and the results revealed a change in the structure of the herring cell membrane, but not in the level of DHA. Their condition was lower in OWA treatments for both food treatments. Most importantly, the fish/diet ratio results suggested a capacity for DHA bio-synthesis in herring larvae (Figure 2.4). This highlights the potential of herring larvae to be a buffer species for maintaining the level of essential fatty acid in the ecosystem.

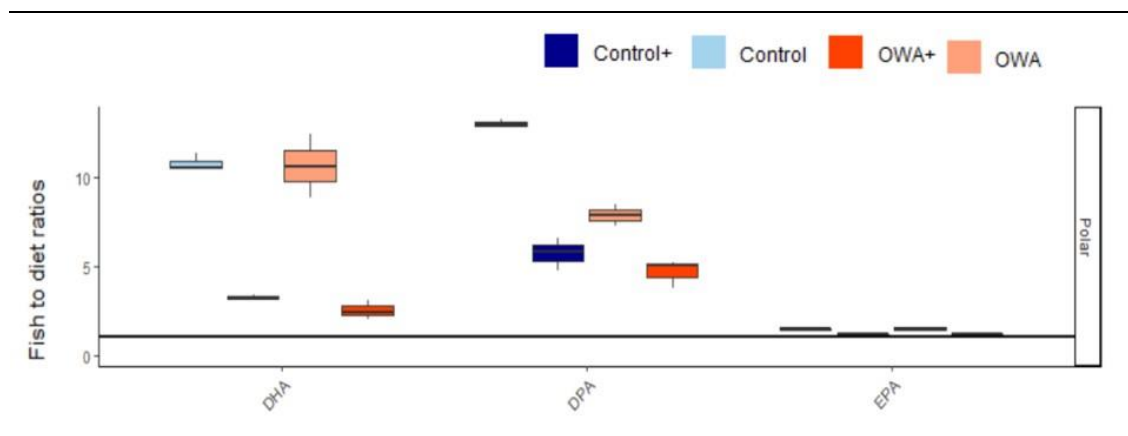


Figure 2.4. Polar lipid fish to diet ratios of herring larvae fed with different level of fatty acid through enriched artemia. Larvae in Control and Control+ were reared at 11°C and pH8.0. Larvae in OWA and OWA+ for « Ocean Warming and Acidification » were reared at 14°C and pH7.6. The « + » identifies treatments where the larvae were fed with artemia enriched in DHA (+2%). DHA: Docosahexaenoic acid, DPA: Docosapentaenoic acid, EPA: Eicosapentaenoic acid.

### Discussion

Down's herring eggs were all kept in the same environment. After hatching, larvae were put in the experimental environments. Worst case IPCC scenarios were used, thus with an increase of temperatures and an increase in acidification. The experimental design was: 11 °C with pH 8, and 14 °C and pH 7.6. Also, one treatment was added with DHA enriched food given (2% higher). Fatty acid structure of larvae in development stage 4 (post larvae) was investigated.

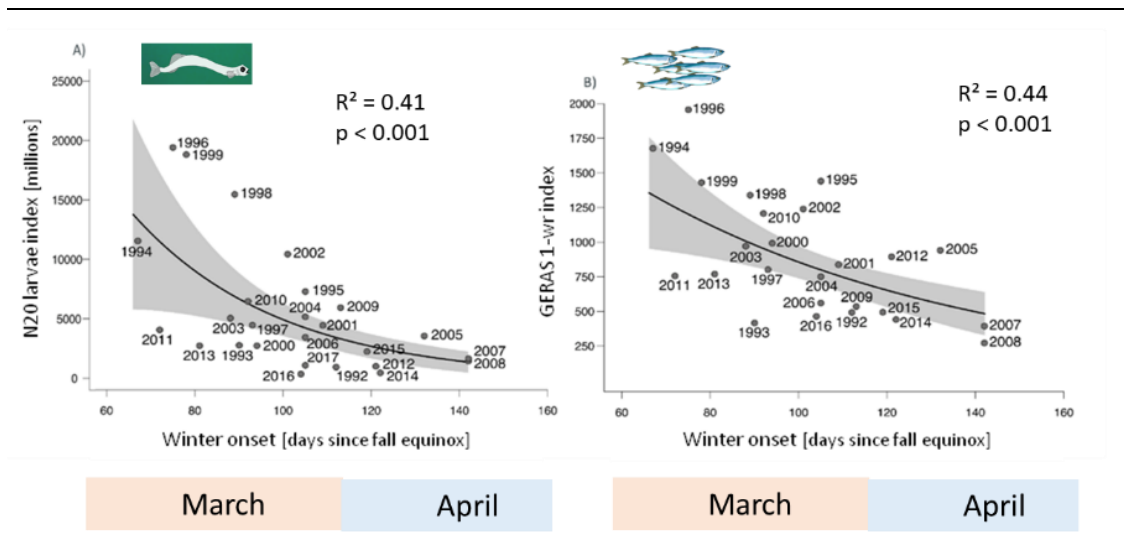
DHA was not different in the larvae of the different experimental environments. Maybe normal food has enough DHA. Fish to diet ratio was checked and it appeared that EPA and DPA in the non-enriched diet were higher compared to the DHA enriched food. DHA levels were similar in both feeding conditions. The EPA and DPA are pre-cursors of DHA. The diet of the females induced a change in cell membrane to deal with changes in the diet.

The presence of ARA is a sign of a stress response, in the increased temperatures ARA was higher. So, herring larvae were stressed at high temperatures. However, the larvae have the ability to survive the increase in temperatures. This is however in a situation where larvae were feed *ad libitum*. It is unknown how the survival would be in nature. A difference in metabolism or condition between the larvae was not found. Maybe the *ad libitum* food provision caused this effect.

## 2.5 Reduced reproductive success of Western Baltic herring (*Clupea harengus*) as a response to warming winters

Authors: Patrick Polte, Tomas Gröhsler, Paul Kotterba, Lena von Nordheim, Dorothee Moll, Juan Santos, Paco Rodriguez-Tress, Yury Zablotski, Christopher Zimmermann

The past decade saw a period of reduced production of larval herring (*Clupea harengus*) in a nursery area in the Western Baltic Sea which is considered a major contributor to overall population dynamics. Record low years of recruitment of spring-spawning herring population went together with exceptionally mild winter conditions. However, water temperatures during the main spawning and larval development phase generally remained below critical values for physiological temperature stress and direct effects of warming on survival of eggs and larvae are yet rather scarce. Besides direct effects on early life stage physiology and metabolism, climate driven shifts of the seasonal timing of reproduction processes (i.e. spawning-hatching-feeding) might significantly affect the year-class strength of recruits. We analysed a multidecadal time-series on larval herring abundance hypothesizing that according to changes in the climate regime the seasonal timing of major hatching events has shifted over time and that the timing of hatching is related to annual recruitment success. Results indicate a trend of earlier seasonal occurrence of maximum yolk-sac larvae abundance along a multi decadal time-series. The early onset and short duration of winter periods explained a significant part of the decreasing year-class strength (Figure 2.5) expressed as an annual index for larval production (N20) as well as abundance of 1-group individuals in the entire Western Baltic Sea as expressed by the German hydroacoustic survey (GERAS). Both indices are established proxies in the herring stock assessment. Along the entire reproduction period, phenology shifts might have affected initial cohorts of hatching larvae that potentially encountered lower densities of plankton prey since the critical period now occurs prior to spring plankton blooms. Together with severe eutrophication effects on later seasonal larval cohorts, climate change induced phenology shifts might presently structure herring populations in the Baltic Sea.



**Figure 2.5.** Effects of winter onset on (A) the N20 larvae index in the nursery area, and (B) abundance of 1-year-old juveniles (GERAS 1-wr) in the western Baltic Sea. The GERAS 1-wr index is lagged 1 year in relation to the descriptor.

## Discussion

In the shallow bay there are diurnal temperature high differences. In the past the trawl net fishery was conducted in the spawning aggregations at the mouth of the bay. There are environmental triggers for the spawners to move into the bay to start spawning. Recently there is a shift towards earlier spawning. Surveys were adjusted to find the first larvae in the bay. During recent years, the sampling started as soon as the ice disappeared. First spawning always was observed close to 4,5 °C. Phenology shifts were investigated by a winter descriptor model with spawning threshold at 4 °C (+/- 0.5). Limited (TAC driven) fishery data for the adult spawn timing. Hatching-peak dates and larvae data were used in the model. Results show that reproduction timing in recent years occurs earlier. And extension of the post-winter period extends hatching duration. The earlier the hatching peak the lower the N20 index, also longer hatching duration means earlier spawning and results in a lower N20.

There are yet no competitor species found to a changing biota in warming conditions. But there is also a phenology shift in jellyfish observed and they become more numerous in the last weeks of the survey. They could compete for food, currently the jellyfish are coming in when the larvae are rather large (> 20mm), so direct predation effects are rather unlikely. Sticklebacks are major predators on herring eggs. Their numbers are probably increased by nutrient loads and not so much by temperature but this needs future attention.

## 2.6 A staging system for *Clupea harengus* based on its skeletal development

Authors: Vivian Fischbach, Annegret Finke, Timo Moritz, Patrick Polte and Philipp Thieme

The Atlantic herring (*Clupea harengus*) is of high economic importance and plays a major role in temperate marine foodwebs. Herring stocks are often subject of monitoring programs and as recruitment dynamics directly translate into the adult stock biomass, its early life stage ecology has been thoroughly addressed in fisheries research. The management of herring stocks is often based on larval surveys which usually incorporate larval length measurements to estimate the recruitment dynamics. However, larval length data misses critical information on the larval stage and the coherent functional morphology. Also, larval lengths are strongly influenced by environmental factors such as temperature and food availability and the size range can vary significantly between populations, habitats, and ecotypes. An existing staging system from the 1970ies provides the most used guide to date for herring larval development, however it does not fully resolve important developmental stages. Therefore, we propose an improved staging system based on the skeletal development of the herring. Via the clearing and double staining technique bone and cartilage were made visible and development of postcranial elements could be traced (Figure 2.6). In combination with external features a staging system comprising 15 stages in five major developmental phases (yolk sac, dorsal fin development, caudal fin development, ventral fin development and juvenile phase) is proposed. The proposed stages are supposed to reflect developmental changes which can be linked to changes in the behavioural ecology and can therefore, can give evidence to potentially critical environmental conditions when applied to long-term dataseries.



**Figure 2.6. Herring staging system based on the skeletal development. By the clearing and double staining technique bone and cartilage were made visible and development of postcranial elements could be traced.**

## Discussion

Different larval development staging systems are available in the literature. For herring the available standard staging system is insufficient as it does not include important stages. A new staging system is developed based on skeletal development. Thus, fin development, shoulder girdle development and vertebrae development were used to identify the different stages. When compared to related species, development timing of the bone structure was found similar in clupeid species. It is considered important to identify the different stages to study effects of environment changes on larvae.

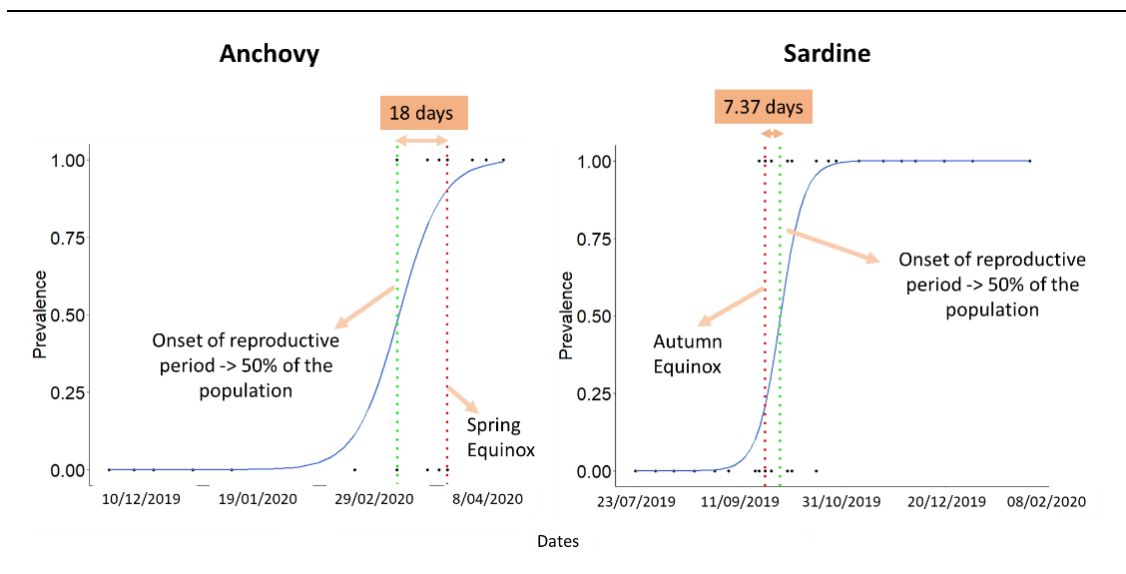
### 3 Maturation, migration and environmental cues for reproduction

This topic focuses on progress in developing methods and concepts to analyse fish fecundity, maturation strategies and spawning rates to provide important baselines feeding into the conceptualization and timing of ichthyoplankton surveys and promoting the research on the reproduction ecology capacity of fish populations. This is particularly important in respect to warming oceans and changing species phenology. Results include recent progresses in methodology on e.g. classification of fecundity strategies and estimation of spawning rates.

#### 3.1 Evidence of photoperiod induced effect on the onset of the seasonal reproductive activity in sardine and anchovy

Authors: Katerina Charitonidou, Emmanuel Panteris Kostas Ganiias

Photoperiod is an external factor that influences reproduction regulators, such as growth hormone, sex steroids, and thyroid hormone profiles. Several studies have shown that photoperiod triggers the onset of maturation in fish species. In this study, the relationship between photoperiod and the onset of seasonal reproductive activity in two indeterminate spawners with opposite spawning periods, the Mediterranean sardine, *Sardina pilchardus*, and the European anchovy, *Engraulis encrasicolus*, was investigated. Sardine has extended spawning periods mainly covering the colder months of the year (autumn to spring), whereas European anchovy reproduces during the warmer months of the year (spring to autumn). Samples were collected from the North Aegean Sea by commercial purse-seine in one-year monthly samplings (2019- 2020). Histological observations of ovarian samples were performed, as well as confocal laser scanning microscopy observations of whole mount samples. Specific characteristics of oocytes as they enter the secondary growth phase were used as markers for the commencement of the reproductive period. The presence of tiny oil droplets, cortical alveoli, and the disassembled Balbiani-body were used as markers for the secondary growth oocytes in sardine, and the presence of tiny cortical alveoli and the mitochondrial-rich ring in anchovy. The mean calendar dates for the reproductive period onset were estimated by analysing the prevalence of the respective markers as a factor of calendar date, using logistic regression. The reproductive period for 50% of the anchovy population started 18 days before the spring equinox, and in sardine, 7.37 days after the autumn equinox (Figure 3.1). This study provides an approach; however, more research including other environmental factors is needed.



**Figure 3.1.** The entry of 50% of the population into the reproductive period (green dotted line) relative to the photoperiod (spring and autumn equinox; red dotted line) on calendar dates, in anchovy (A), and sardine (B), respectively.

## Discussion

Sardine larvae in the North Sea (first quarter MIK, Jan-Feb) and also sardine in third quarter MIK (Aug-Sep), are roughly the same size. Therefore, both, spring and autumn equinox might affect the population in the same area. Probably also internal factors could be involved. In the Mediterranean Sea only one spawning peak is assumed. In this study, however, it looks like there are two spawning peaks since there are also a lot of sardine larvae caught in both MIK surveys. This could be a result of shifting spawning season of adults and low growth rates of larvae. Hence, more information on adults are needed. Larvae probably drift into the North Sea trough the Channel. In France (in the channel) they also observe two spawning peaks in October with plenty of eggs in the survey (could be the ones in the first quarter of the MIK) and then also a peak in spring (which could be the ones caught in the third MIK quarter), Scottish colleagues also found sardine in their survey (the sardine might also enter the North Sea trough there).

## 3.2 Interannual changes in the time of spawning of sprat in the Gotland Basin (Eastern Baltic Sea) in relation to the hydrographical conditions and the structure of spawning stock

Author: Andrei Makarchouk

Time of peak spawning of sprat (*Sprattus sprattus balticus* Schneider) in the central and southern parts of the Gotland Basin (Eastern Baltic Sea) has been determined for the period of years from 1973 to 2020 using the database of Institute "BIOR" on ichthyoplankton. Sprat has a very

extended spawning season, but maximum abundance of sprat eggs in this region was observed mainly in the middle of June. Time of spawning shifted from year-to-year, with the trend for earlier timing. In most recent years this shift became more pronounced, which could bring new problems with the application of daily egg production methods for the calculation of SSB. Ambient water temperatures increased in recent years, which led to shortening of the egg incubation period. Some significant correlations were found between the time of peak spawning and the temperatures of water at 10 m depth (Figure 3.2), which must be an indicator of how far the spawning progressed. Also, there was a strong influence of ambient water temperatures on the time of spawning. Colder temperatures led to later spawning. No influence of the age structure of the spawning stock of sprat on the time of spawning was found. It also looked like earlier spawning did not result in stronger year class.

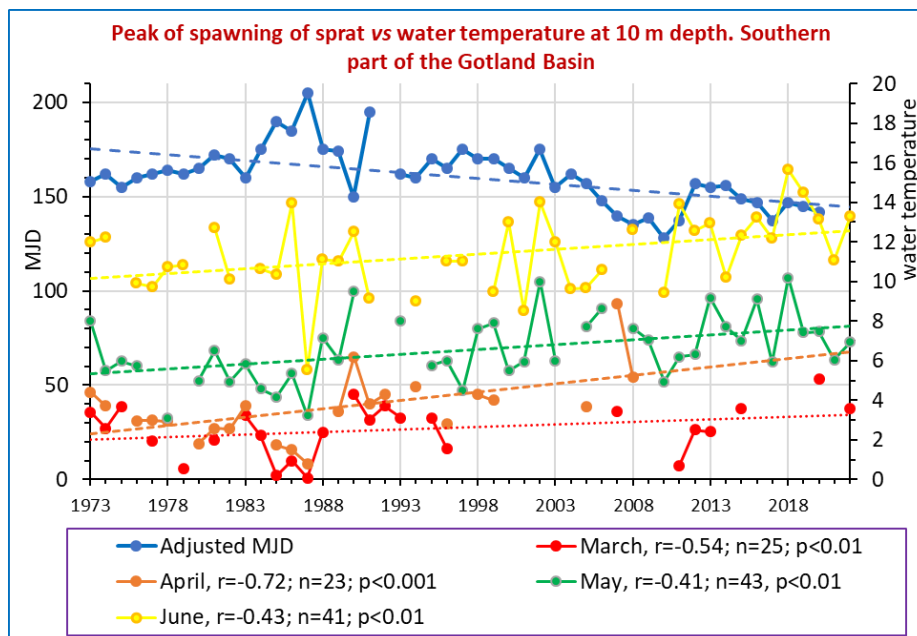


Figure 3.2. Sprat peak spawning was calculated as mean weighted Julian Day (MJD) of sprat eggs abundance from 3 – 6 ichthyoplankton surveys. Adjusted MJD was acquired by alternating the calculated date, considering other characteristics of spawning, such as changes in egg size, appearance of eggs in the surface water layer, and shares of eggs at different developmental stages. Water temperatures at 10 m depth were taken from hydrographic measurements at station 46 (56° 02'N, 19° 08'E) during the BIOR surveys.

## Discussion

The reasons for the observations that when adults overwinter in lower temperatures the peak spawning occurs earlier were discussed. Sprat is an indeterminate spawner which can spawn whenever environment conditions are suitable. Accordingly, when it is cold and suddenly temperatures increase it is a better environment with better conditions and then sprat start spawning.



### 3.3 Effects of global warming on the phenology of winter-spawning migrations

Author: Paul Marchal

We have investigated phenological shifts in winter-spawning migration of Downs herring (*Clupea harengus*) in relation to temperature and the availability of potential zooplanktonic prey (*Calanus finmarchicus*, *Calanus helgolandicus*, *Temora longicornis*). A two-tiered approach was developed to that purpose, which consisted of calculating phenological indicators based on estimated deviations from basic harmonic signals, and analysing their interannual variations through time-series analyses. Herring spawning peak timing was delayed by increased temperature and *Calanus finmarchicus* abundance, with a lag of up to two years, possibly reflecting some conservative behavior preventing herring to over-react to short-time environmental fluctuations. The combined effects of temperature and prey abundance also resulted in a rather complex spawning peak timing trajectory: slightly decreasing with large variations over 1985-1999, and steadily increasing with limited fluctuations thereafter. The duration of Downs herring spawning season was shortened by increased sea temperature. Additional covariates (including prey other than those examined in our study) could contribute to better explain the phenological drift in Downs herring spawning migrations.

#### Discussion

The proportion of Downs herring is seemingly increasing (more stable), the other populations are decreasing in stock size. Herring delays its migration and needs to spawn faster because perhaps the end of the spawning season has not changed and but the season has shorted because of the delayed arrival at spawning grounds. Environmental triggers inform fishes on when to migrate to spawning grounds (e.g. temperatures). Maybe colder temperatures come later and this is delaying the migration to spawning grounds. Another trigger maybe tells fish when the spawning period is over which has not changed, but in the Channel the migration is not as long. Future studies should couple maturity studies to spawning time, investigate change of temperatures on spawning grounds and look at potential temperature triggers. The ARIMAX Model includes two temperature values. It should be check whether they are correlated (two terms of SST). The shortening of the spawning season was analysed based on commercial fishery data. The same signals cannot be seen in the IHLS as the survey duration/timing does not cover the whole spawning season. It might be possible to see if the start of the spawning period has occurred earlier or later (because of yolk-sac larvae abundance). However, the end of the spawning season is missed (2018 survey ended in January). A mild winter maybe good for winter spawners? It could move the first-feeding period of larvae closer to the spring plankton blooms. There are indications for winter spawning stock is the "original" stock, however, parts shifted spawning towards spring and autumn. So maybe winter spawning was not always the best strategy. It is yet unclear if the different populations can adapt quickly enough to the changing conditions. Evidence from Downs herring shows that some individuals switched spawning time and started spawning in autumn (they probably make that switch once and then stay autumn spawners?).

## 4 Governance of ichthyoplankton studies

### 4.1 Eggs and Larvae Database (ToR e)

WGALES could be considered the closest to a governance group for the Eggs and Larvae database, and as such, ICES DC raised a few general issues regarding the information on the web page, the survey metadata and the process to include indexes calculations into TAF.

Some participants mentioned that maybe some of the more operational survey groups should be better able to act as governance group for the database, but it was also agreed that WGALES hosts members from most survey groups plus members with a wide variety of backgrounds related to eggs and larvae, which should be the best mixture of expertise required from a governance group.

WGALES feedback on the raised issues could be resumed as follows:

1. Current State of the database. Good progress with submissions in the new format.
2. Web page update, by end of 2022:
  - a) Add link to DATSU format
  - b) Add links to WGs webpages: WGALES, WGSINS, WGMEGS, WGACEGG, WGEELS
  - c) Add link to metadata catalogue with survey records. Reduce description on web landing.
  - d) Links to available SISPS, MEGS, MIK, others?
3. Metadata records should be updated by the survey coordinators. ICES DC to get in contact with them individually.
4. Index calculation: Probably needs to be discussed again in WGSINS (November 2022). Some feedback from WGALES on this:
  - a) WGMEGS calculates routinely at least 2 indices for assessments of WIDE.
  - b) MIK provides indices to HAWG. Calculation is in *github* but still to be confirmed, (WGSINS, November 2022,).
  - c) When any of the indices are a part of stock assessment the index calculations should be openly accessible for transparency reasons using the Transparent Assessment Framework (TAF). It is recommended to contact the survey coordinators directly for information.

### 4.2 Cross-WG collaboration (ToR d)

An overview of **WGBIOP** was presented by the Chair (Annelie Hilvarsson). The main objectives of WGBIOP are to review the status, issues, development and take care of the quality assurance of biological parameters used in assessment and management. In detail this includes:

- A repository with guidelines for age calibration (otoliths, scales) and maturity, workshops and exchanges of samples
- Development of quality scores, e.g. for age reading which indicate the quality for the reading (AQ1-AQ3)

A product of cooperation with WGALES can be to come up with common guidelines for identification and classification of larvae and eggs (similar to the guidelines in the aging manuals).

Potentially, quality scores can be adapted/developed for eggs and larvae work?

An overview of **WGSMART** and SmartDots was presented by the Chair (Karen Bekaert).

Smartdots is an online platform for facilitating exchanges, workshops and training events for biological readings based on images

- WGSMART = Governance group for Smartdots
- Development of software/tools for specific purposes, (online) workshops, Smartdots instruction videos on Youtube (<https://www.youtube.com/@icessmartdots2352>)
- Software for age reading available
- Smartdots for eggs and larvae (species ID and staging) as well as maturity, fecundity and atresia so far only web-based
- Official request by DGMARE and DEFRA UK to ICES to develop a larval module, egg module and maturity module for the software and to conduct user training
- Software modules for eggs, larvae and maturity are under development and planned to be implemented
- Training planned: 2-day online workshop for coordinators to introduce the 3 new modules and
  - Series of 3 online training modules for the users
  - Preliminary version of the larvae software module was presented
  - WGSMART needs input from WGALES concerning the development of the new egg and larvae modules, guidelines for eggs and larvae, format and design of training sessions and Youtube tutorials, quality scores for eggs and larvae, standardized result reports (if necessary/requested) as well as WGALES members as testers of the new egg and larvae modules to identify bugs etc.

The new software module for eggs and larvae looks very promising and much more user friendly than the web-based version available so far (used in previous egg and larvae ID workshops – WKIDCLUP2 and WKMACHIS).

WGALES members are interested to act as testers in January/February 2023: Cindy van Damme, Vivian Fischbach, Hannah Holah and Bastian Huwer.

User guidelines: Karen Bakaert presented user guidelines for age reading, which could serve as template for new egg and larvae guidelines.

SmartDots could be used as a reference collection/repository of egg and larvae images:

- SMARTDOTS can be used for training and for collecting images into a reference collection/ repository (there is a subgroup for a reference collection and to establish guidelines for that already)
- Do we need/want a reference collection/ repository for eggs and larvae, how to build it, and what will be the purpose?
- Reference collection could be nice for workshop organizers (as they would not need to take new pictures of everything every time)
- Could also be valuable for newcomers (as they could do some self-training before the actual workshops to become acquainted with the identification and the software)

There is a list of country coordinators and participants (including expertise level) for age reading on SMARTDOTS – would that also be helpful for eggs and larvae?

- The list can e.g. be used to sort results by country, experience level, role (e.g. country coordinator) etc. and can provide data on standard error of larval identifiers

- In contrast to age reading, where Smartdots is used to produce results directly used in assessment and management, Smartdots is so far only used for training purposes concerning eggs and larvae. Thus, such a user list may currently be of limited use for the egg and larvae work
- However, could become useful in future and could already be helpful to contact people working on the same subject and for workshop organizers to get a participant list including details such as experience level, affiliation etc.
- Do we also need/want country coordinators for egg and larvae work?

Discussion of quality score for egg and larvae pictures:

- We cannot directly adopt the quality scores for age reading but need specific quality scores for eggs and for larvae.
- The picture quality is mainly determined by the visibility of the features that are necessary to identify the species or stage.
- Thus, there are different types of quality issues: a picture can e.g. be of bad quality because it is generally blurred or out of focus, but also a perfectly focused picture can be of bad quality for the identification/staging if it is e.g. taken from an angle where relevant features (e.g. myomeres) are not visible.
- Guidelines/Manual on how to take good pictures of eggs and larvae may be useful

Short discussion with WGBIOP members on maturity/fecundity/histology

- Combine maturity studies with WGALES
- More histological studies

### 4.3 Group discussion on egg and larvae issues (ToR a, b, c)

Discussion: survey sampling processing/fixation standardization

- A table for the different sampling methods at different institutes was already started by Matthias Kloppmann during the WKCIUPID Workshop and Mackerel Egg Workshop (WKMACHIS) and is on the Mackerel Egg Workshop Sharepoint.
- Guidelines/Guidance on how to treat samples should include the reasons why some procedures are preferred and provide reasoning for the choices made according to sampling and sample storage in different environments.
- The above table should be filled in to develop the guidelines. Locate or start a list of country coordinators.
- Country coordinators are the contact persons regarding ichthyoplankton surveys within a country (within ICES)
- Formalin substitution analysis:
  - Netherlands: exercise last year, minimize the use of formaldehyde, someone from a forensic lab came and said they tried a lot of different things (just glycerol e.g. but not for small material) however, in the end it was concluded that someone always needs a little bit of formaldehyde otherwise you cannot store your samples for a long time
  - Histofix: solution for histological processing, you need to add a lot of alcohol to achieve the best preservation, was tried with gonads, and the samples with histofix where useless for histological samples, was not tried for ichthyoplankton, larvae pigmentation is lost with this fixation
  - The experts at ICES say formalin is best

- In France: Light version of formalin (less than 1% formalin concentration), add by-products (less poisonous than formalin) to ensure that no pigmentation is lost, quality of fixation dependent probably on sample size, recipe is in several reports and in one publication -> tested in the Netherlands and results by Matthias' Kloppmann indicate that samples cannot be stored for a long time.
- ADR: international agreement on the transportation of dangerous goods on the road
- Calibration of flowmeter: Frequencies of calibration:
- Netherlands: every week, flowmeters lose performance over time and there are different calibration factors.
- France: no calibration
- Germany/Baltic Sea: real-time data transfer. Data evaluation on board based on reference values per depth/towing speed
- Flowmeter usage also with Apstein nets
- Every sampling gear that has a nose cone attached is strongly affected by flowmeters

#### 4.4 Group discussion on future ToRs

Background description for ToR a: Include a section on environmental factors that are surveyed as well (CTD Data), include environmental data (such as hydrography, zooplankton). It was split the original ToR a into two parts. The first part on the standard ichthyoplankton surveys will be kept ToR a and including more environmental data and ecosystem studies will be added as a specific ToR on ecosystem studies.

ToR a: review on estimation methods and surveys + standard observations one should include in survey (hydrography, zooplankton), A new ToR b: ecosystem studies + also reference to environmental observations as background (additional samples for other surveys e.g. sandeel, plastics), also for new surveys that someone wants to start.

ToR a background: anthropogenic influences/changes (closure of sampling sites) and expanded ocean use (wind farms, aquaculture, shipping etc.).

New ToR b description: Identifying potential of the existing ichthyoplankton surveys to address additional research needs and knowledge gaps (Identify the needs of new surveys/studies) -> identify gaps in knowledge, evaluate if surveys could help close those gaps, reach out to people who could do the studies proposed by the groups/ find collaborators e.g. for fish maturity/ increase chances of anybody doing this research, identify the impact of anthropogenic influences on the environments, not only studies for fish species but also plankton organism such as jellyfish (including non-target species), link fish maturation analysis to fish reproduction timings

Background new ToR b: reasoning for why surveys need to be evaluated to see if they can be helpful to other environmental studies, e.g. to evaluate species phenology shifts due to climate change, shifts in physiology, spatial distribution and behavior, population resilience, relating fish ecology to ecosystem functioning/environmental drivers, exploring the relations between environmental drivers and fish reproductive success (thereby adults and larvae are linked)

Expected deliverables for ToR b: dedicated theme sessions on fish ecology, and potential new studies

Old ToR b: should be kept but it should be made clear that this is about methods: technical research, sampling process and data analysis.

ToR d: includes the collaboration with WGBIOP and WSMART but should be widened to include other assessment groups such as HAWG, WGWIDE, WGBFAS, WGHANSA (anchovy and sardine), WGNSSK

ToR c: Description should be restructured so that it focuses on timing of spawning and evaluating the timing of the surveys to target the correct species/ life stage. Effects of changing dynamics on current ichthyoplankton surveys and their design will be evaluated.

ToR c Background: how the dynamics may change

### 4.5 Workshop on optical ichthyoplankton processing methods and image analyses (ToRs a, b)

During the WGALES meeting in Oct 2022 in Boulogne Sur Mer, France, the hosts from Ifremer organized a sophisticated workshop held by an invited Ifremer expert Jean-Baptiste Romagnan on modern techniques of optical sample processing using quantitative imaging analyses by e.g. ZooScan, ZooCAM and Flowcam. In addition, the use of ECOTAXA as data processing tool and database for zooplankton, fish eggs and fish larvae images was presented in the workshop (Figure 4.1). These approaches provide access to honest taxonomy information as well as body size information at the individual, population and community levels. They are cost-effective, fast, and may represent the future of zooplankton, fish eggs and fish larvae data acquisition tools. We advocate for benchmark studies with more traditional methods as well as case studies to promote these approaches.

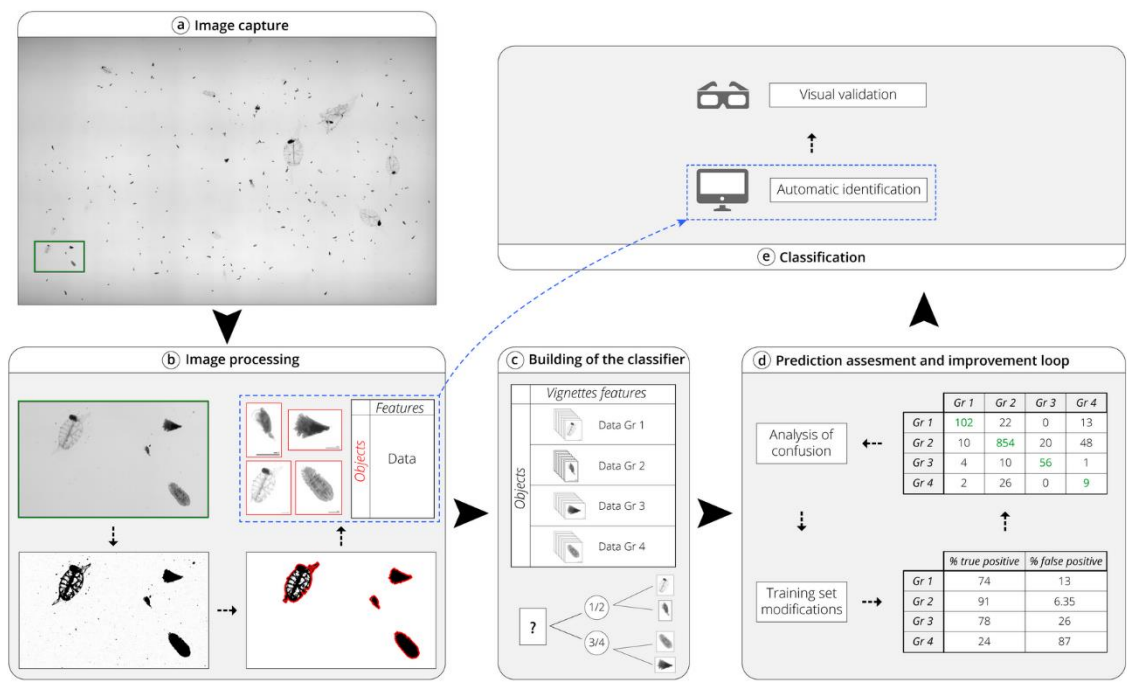


Figure 4.1. Sample acquisition using optical imaging devices (e.g. ZooScan and Zoocam) and processing with ECOTAXA.

## Annex 1: List of participants

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## Annex 2: Resolutions

**2018/2/EOSG16** The **Working Group on Atlantic Larval and Egg Surveys (WGALES)** chaired by Patrick Polte, Germany; and Cristina Nunes, Portugal; will work on ToRs and generate deliverables as listed in the Table below

	Meeting dates	Venue	Reporting details	Comments (change in Chair, etc.)
Year 2019		By correspondence		
Year 2020	19–21 October 2020	Online meeting	E-evaluation	Richard D.M. Nash replaced by Cristina Nunes
Year 2021	27-28 October	Online meeting	E-evaluation	
Year 2022	17-21 October	Ifremer, Boulogne-sur-Mer, France	Final report by 15 December	

### ToR descriptors

ToR	Description	Background	<a href="#">Science Plan codes</a>	Duration	Expected Deliverables
a	Review the current ichthyoplankton surveys in light of their original purposes, with respect to design, estimation methods and challenges and identify their potential for other purposes such as ecosystem surveys.	Ichthyoplankton surveys collect abundance data on early life history stages useful for estimating fish standing stock biomass (SSB) and recruitment of several fish stocks.	1.4, 2.2, 3.2	year 2, 4	
b	Survey scientist work together to evaluate and recommend methodologies and research needs for sampling, processing and data analyses for ichthyoplankton surveys, concerning the Early life history stages and the contributions from the adult components. WGALES also offers the possibility for data users to gain insights into the rationale, methodology and potential applications of fish early life stage ecology (and	Ichthyoplankton surveys need to keep pace with developing data needs and technological developments. The provision of a workshop/conference environment provides a forum for improvement, development of new ideas and innovative insights for these surveys.	1.4, 3.2, 4.4	year 2, 4	

	adult fish maturity) re- search.				
<b>c</b>	Present and report on re-productive dynamics and fish early life strategies relevant to ichthyoplankton surveys	Successful surveys are dependent on understanding the life-history dynamics of the target organisms and understanding how this may change with ecosystem variability and change.	1.7, 2.2, 3.2	year 2, 4	
<b>d</b>	To work together with ichthyoplankton data providers and experts to evaluate and improve surveys. This will include collaboration across members in several ICES groups including IBTSWG, WGACEGG, WGMEGS, WGSINS (WGEGBS2).	Specialist working groups need a forum with experts from other types of ichthyoplankton surveys and personnel working in different areas to seek guidance and advice.	2.3, 3.2, 3.4	year 1, 2, 3, 4	
<b>e</b>	Provide a standardized framework for ichthyoplankton databases and facilitate implementation of new survey data into the ICES egg and larvae database in collaboration with the ICES Data Center.	Ichthyoplankton data needs to be of high quality and centrally available for the assessment working groups and the science groups more generally to do their work and demonstrate transparent ways of working.	3.2, 4.2	year 1, 2, 3, 4	Updated dataset on the ICES egg and larval database

**Summary of the Work Plan**

<b>Year 1</b>	WGALES will communicate by correspondence to act upon urgent ToR's from ichthyoplankton survey groups (ToR d)
<b>Year 2</b>	WGALES will meet to address ToRs a, b, c, d, e, f
<b>Year 3</b>	WGALES will communicate by correspondence to act upon urgent ToR's from ichthyoplankton survey groups (ToR d)
<b>Year 4</b>	WGALES will meet to address ToRs a, b, c, d, f
<p>This Working Group meets every two years with a meeting format that covers general matters concerning ichthyoplankton surveys (ranging from new innovations in survey equipment and design through considering current ichthyoplankton surveys and their protocols) and also includes a specialised theme session or two on current and innovative relevant topics. The new topics are chosen at the end of each meeting to allow participants to work on them in the period between meetings. As such, new meeting ToRs can arise every two years to provide a focus for part of the biannual meeting.</p>	

**Supporting information**

<b>Priority</b>	The activities of WGALES are vital for the delivery of state-of-the-art ichthyoplankton surveys, ensuring high standards and incorporating new techniques and developments for the future. WGALES will lead to the cross fertilization of ideas, methodologies, developments and standardization of ichthyoplankton surveys in the ICES area. Hence providing a platform from which to improve the assessments based on the ichthyoplankton surveys.
<b>Resource requirements</b>	The research programmes which provide the main input to this group are already underway, and resources are already committed.
<b>Participants</b>	The Group will be attended by members of ICES groups, WGMEGS, WGEGBS2/WGSINS, IBTSWG, WGACEGG and guests carrying out ichthyoplankton surveys in the non-ICES areas. The Group is normally attended by some 25–30 members and guests.
<b>Secretariat facilities</b>	None.
<b>Financial</b>	No financial implications.
<b>Linkages to ACOM and group under ACOM</b>	There are linkages with ACOM through the individual ichthyoplankton surveys groups that are associated with WGALES and their assessment groups that use plankton data.
<b>Linkages to other committees or groups</b>	There is a close working relationship with the all the ICES expert groups of ichthyoplankton surveys, WGMEGS, WGEGBS2/WGSINS, IBTSWG, WGACEGG and their assessment groups, WGWIDE, HAWG, WGHANSA.
<b>Linkages to other organization</b>	No formal linkages.

## Annex 3: Recommendations to WGALES

**ID 98, 2021, WGWIDE recommends that all historic data from the North Sea Mackerel Egg surveys is compiled, quality checked and uploaded to the ICES Eggs and Larvae database.**

The methodology for the estimation of SSB in the North Sea area should be reviewed, documented and an updated time-series calculated.

This recommendation was discussed at the WGALES interim meeting Oct 2021. It was concluded that the subject can only be handled by WGMEGS and was therefore passed over.

**ID 106, 2021, WGSINS recommends the sprat larvae pilot survey conducted in the 3 quarter IBTS showed very promising results in the first four years (2018-2021). A potential recruitment index may be derived by the sprat survey. However, a more complete area coverage would be beneficial. WGSINS recommends to continue the survey and encourages additional participants to join the survey.**

Results of the first 5 years of the MIK pilot surveys for sprat larvae on the Q3 IBTS were presented at WGALES. These results indicate that a recruitment index for North Sea sprat may be developed from this survey, however a longer time-series is needed to come to a final conclusion. Besides, the survey could profit from a better spatial coverage. Thus, WGALES endorses the continuation of the survey and the improvement of the spatial coverage by additional participants. However, as was already pointed out by the IBTSWG (i.e. the other recipient of this recommendation), the decision to participate in the survey lies with the individual institutes participating in the Q3 IBT

**ID 111, 2021, WGBIOP recommends ongoing collaboration with WGALES in the development of larvae and eggs, fecundity and atresia modules in SmartDots. It is recommended that a member of WGALES joins the WGBIOP meeting, so that tasks can be allocated effectively between the two groups and progress can be monitored**

The recommendation was addressed by cross-presentations of group chairs, outlining the ToRs of the group, ongoing activities and potential for collaboration between groups. WGALES-Chair joined the 2022 meeting of WGBIOP (Oct 5<sup>th</sup> 22) and the WGBIOP chair vice versa joined the WGALES meeting on Oct 18<sup>th</sup> 22). It was concluded that WGALES will work on guidelines for image quality requirements for ichthyoplankton to be analysed with Smartdots. Cross group collaboration will be increased by identifying members participating in both groups.

**ID 107, 2021, WGSINS recommends: The area coverage of windfarms continues to expand in the WGSINS survey areas. Direct effects for the IHLS, IBTS-MIK, NINEL and NIMIK are that sampling stations have been dropped. Due to potential changes in drift patterns, changes in the ecosystem may occur. It is unclear at the moment what the effect of this is on the herring larvae indices. There are currently in the Netherlands developments to install solar panels in the windfarms that will float on the sea surface. This could have direct effects on the herring larvae, but also act indirectly through less plankton availability as food.**

WGSINS recommends that effects on the indices of this are investigated.

The recommendation was addressed by including wind farm related subjects in the outlines for the new ToR a (3.8) and the talk provided by Katey Marancik (1.5) and the following discussion (4.0).

**ID 113, WKIDCLUP2 In order to successfully organize and conduct ichthyoplankton identification workshops, the availability of a sufficient number (generally in the range of several hundreds) of good quality samples and/or images of the different target species at different developmental stages is crucial. A clear and precise instruction for collection, creation, and maintenance of such reference collections is needed.**

And

**ID 114, WKIDCLUP2 Conducting online workshops on fish larvae identification requires excellent quality images of the different development stages. A guideline for taking standardized, good quality micro-scopic images of fish larvae is required.**

Both recommendations were addressed by the cross-group collaboration with WGSMART (3.6). Concluding that guidelines for imaging of ichthyoplankton will be created by WGALES and group member will volunteer in testing the SmartDots application for ichthyoplankton identification.

**ID 116, WKIDCLUP2 Sampling of ichthyoplankton during internationally coordinated surveys is standardized. Sample work-up is still highly variable among survey participants and between surveys. (chapter 6 of the recent WKIDCLUP2 report) A higher grade of standardization is required.**

This recommendation was addressed by the group discussion on the subject (3.7.)

Recommendation	Adressed to
1. WGALES recommends to carry out comparative sampling for mackerel and horse mackerel eggs in the Atlantic with the 280 and 500 $\mu\text{m}$ . In order to investigate differences in catchability between the mesh sizes. If no differences are found, WGALES recommends to use the 500 $\mu\text{m}$ not only in the North Sea, but also the Atlantic, to prevent clogging issues.	WGMEGS

## Annex 4: Summary of the Work Plan

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Year 1	WGALES will communicate by correspondence to act upon urgent ToR's from ichthyoplankton survey groups (ToR d)
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Year 2	WGALES will meet to address ToRs a, b, c, d, e
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Year 3	WGALES will communicate by correspondence to act upon urgent ToR's from ichthyoplankton survey groups (ToR d)
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Year 4	WGALES will meet to address ToRs a, b, c, d
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This Working Group meets every two years with a meeting format that covers general matters concerning ichthyoplankton surveys (ranging from new innovations in survey equipment and design through considering current ichthyoplankton surveys and their protocols) and also includes a specialised theme session or two on current and innovative relevant topics. The new topics are chosen at the end of each meeting to allow participants to work on them in the period between meetings. As such, new meeting ToRs can arise every two years to provide a focus for part of the biannual meeting. Interim meetings in year 1 and 3 will be held online and address urgent matters on specific subjects.

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