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ECOSYSTEM OBSERVATION STEERING GROUP

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Report of the Working Group on Atlantic Fish Larvae and Eggs Surveys (WGALES)

22-26 October

Lyngby, Denmark

ICES
International Council for the Exploration of the Sea

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Contents

Executive summary

The Working Group on Atlantic Fish Larvae and Egg Surveys (WGALES) met at DTUAqua in Copenhagen, Lyngby, 22–26 October 2016.

The meeting was attended by 28 participants from 8 countries (representing 10 different institutes).

The agenda included general WGALES business and three thematic sessions which were carried out in plenary. Session 1. "*General presentations concerning egg and larvae surveys*" addressed general aspects related to surveys and the main issues concerned with egg and larvae, sampling and analyses, Session 2. "*Fish eggs and larval development and early life strategies in boreal and tropical regions.*" covered aspects related to egg and larvae physiology, ecology and abundance and production estimation and Session 3. "Adv*ances in adult reproductive parameters focusing on spawning frequency estimations*", focused on issues related to the adult fraction of the populations in particular with the development of a new approach for the estimation of the spawning fraction an important parameter for SSB estimation using EPMs.

During session one eleven presentations were delivered. The discussions considered diverse aspects related to surveys and sampling and debate on the opportunity/feasibility for using ichthyoplankton surveys to gather extra information on the pelagic ecosystem in particular on the zooplankton communities for ecology and trophic structure studies but also to address the MSFD requirements. An update on the status of the ICES Database for eggs and larvae was also presented. Session two included six presentations. Studies were presented focusing on ichthyoplankton spatio-temporal variability and also on egg and larvae physiological aspects relevant to its condition and survival. In session three, four presentations were provided. Research results on reproductive behaviour and fecundity estimations were reported. There was also a comprehensive presentation on the collaborative work, developed within this WG and involving several institutes, was delivered to show the progresses on the new methodological approach for improving spawning frequency estimation for different species.

Besides the plenary sessions the participants took part in subgroups discussions to address specific questions related to: (1) Egg and larvae issues: Breakout Group A – Baltic larvae surveys; Breakout Group C – Flowmeter considerations; Breakout Group D – Other issues and (2) Adult parameter issues: Breakout Group B – Laboratorial methods and new approach for spawning fraction estimation

Breakout Group A reviewed and discussed the Baltic larvae surveys, a thorough presentation on the surveys was delivered pointing out its pros and cons. The WG considered that given the financial constraints the survey was being conducted in the most efficient way possible. The current practices do not appear to involve undue loss of precision or be introducing any undue uncertainty to the data (indices) presented to the assessment group(s).

Breakout Group C discussed flowmeter utilization with particular attention to issues related to its location in the samplers and recommendations on calibration procedures and its frequency.

Breakout Group D discussed other aspects related to egg and larvae surveying including: the relevance of vertically discrete sampling, net colours and potential avoidance issues, the use of molecular techniques for egg and larvae identification and staging and for substock unit allocation, the use of image analysis techniques to speed up the

egg identification and staging and also to get extra data on the zooplankton communities and finally a summary on the current egg and larvae preservation solutions in use in the different laboratories was providedBreakout Group B addressed adult reproductive aspects and the adequate methodologies to correctly estimate the parameters needed for EPMs. Further discussions on the joint work on spawning frequency estimation were conducted. The results achieved allowed testing of the method for spawning frequency estimation at the individual level for a few species and it was agreed to continue the collaborative work in order to progress to the estimation of the spawning fraction of the population using this new approach which will permit in the future the use of DEPM for all species regardless of their fecundity type being determinate or indeterminate.

1 Administrative details

Working Group name

The Working Group on Atlantic Fish Larvae and Egg Surveys (WGALES)

Year of Appointment within the current six-year cycle

Last year

Reporting year concluding the current six-year cycle

Chair(s)

Cindy van Damme, The Netherlands (2013-2014)

Maria Manuel Angélico, Portugal (2013-2018)

Richard Nash, Norway (2015-2019)

Meeting venue(s) and dates

1-5th December 2014, San Sebastian, Spain, (27 participants)

17-21st October 2016, Thessaloniki, Greece, (22 participants)

22-26th October 2018, Copenhagen/Lyngby, Denmark, (28 participants)

2 Terms of reference, work plan and agenda

2.1 Terms of reference

ToR	Description	Background	Science Plan topics addressed	Duration	Expected Deliverables
a	Present current ichthyoplankton surveys in the light of their original purposes, with respect to design, estimation methods and challenges (including their potential as ecosystem surveys);	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. WGALES fits into the ICES science plan sections 5.1 and 5.2.	5.1, 5.2	Years 1, 2, 3 (2014, 2016, 2018)	Report in 2014, 2016, 2018
b	Present current understanding and future research needs of natural mortality of fish eggs and larvae in order to improve accuracy and precision of egg production and larvae abundance estimates of the ichthyoplankton surveys;	Use of natural mortality in egg production and larvae abundance estimates is limited. Current developments and use of natural mortality estimates to improve accuracy and precision of ichtyoplankton survey estimates.	5.1, 5.2	Year 1 (2014)	Report with review of developments and needs for future research of natural mortality of fish eggs and larvae. Suggestions on how natural mortality can be incorporated in egg production and larvae abundance estimates of the ichthyoplankton surveys.
c	Prepare a template for the ICES ichthyoplankton survey protocols	A new publication series of survey protocols on ICES surveys has been		Year 1 (2014)	Survey protocol template for ICES ichthyoplankton

		initiated. No template exists for the ichthyoplankton survey protocols.			surveys.
d	Receive and act upon ToRs from Working Groups within the umbrella of ichthyoplankton surveys e.g. IBTSWG, WGACEGG, WGIPS, WGMEGS, WGEGGS2.	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.	5.1, 5.2	Years 1, 2, 3).If necessary WGALES can react by correspondence on urgent ToR's from other ichthyoplankton surveys groups in 2013, 2015 and 2017. During the meeting in 2014 ToR's from ichthyoplankton survey groups from 2013 and 2014 will be addressed and reported on.	Report in 2014, 2016, 2018. Responses to specific requests from the ichthyoplankton Working Groups.
e	Present current understanding and future research needs for integrating ichthyoplankton data and methodologies into methodologies for assessing population sizes. Undertake investigations on streamlining and cost-effective surveys and methodologies for the implementation of Egg Production Methods for estimating Spawning-stock biomass	Ichthyoplankton surveys are often part of a larger and more encompassing set of studies which are aimed at determining population size e.g. Egg Production Methodologies. There is a need to review the various aspects of such research, as a complete study, to ensure the correct data are collected and there is a general understanding of how all the parts fit together to ensure complete and as accurate and precise as possible estimates of stock size are realized.	5.1, 5.2	Year 2, 3 (2016, 2018)	Report with review of methodologies and potential 'pitfalls' for undertaking multidisciplinary estimations of stock size which involve the use of ichthyoplankton surveys, and needs for future research for a better integration of fish egg and larvae data.
f.	Present and report on fish eggs and larval development and early life strategies in	The timing and type of ichthyoplankton survey needs to be tailored over spatial	5.1, 5.2	Year 3 (2018)	Report in 2018 on the life history strategies of

	boreal and tropical regions.	and temporal scales. Consideration also needs to be made for the taxonomy, egg and larvae mortality and development, stage duration (e.g. in relation to temperature, salinity, start of spawning season etc.). Other basic information needs to be also considered such as strategies of foraging larvae to cope with ambient conditions in different regions of the marine environment. Some of this information can also be obtained from the surveys providing an appropriate survey design is implemented.	fish eggs and larvae over a range of ecosystems. The report will suggest optimal sampling strategies for determining abundance, mortality, distribution and ecology of the early life history stages of fish.

2.2 Adoption of the agenda

The adopted agenda addressed all terms of reference and can be found in Annex 2.

3 Summaries of thematic sessions (ToRs a, e and f)

3.1 General presentations concerning egg and larvae surveys

This session consisted of a series of 11 diverse presentations. The abstracts for these talks are given in Annex 3 and a summary of the discussions concerning each of the presentations are given in Annex 4.

3.2 Fish eggs and larval development and early life strategies in boreal and tropical regions

This session consisted of a series of 6 varied presentations. The abstracts for these talks are given in Annex 3 and a summary of the discussions concerning each of the presentations are given in Annex 4.

3.3 Advances in adult reproductive parameters focusing on spawning frequency estimations

This session consisted of a series of 4 presentations including one extended presentation with several parts presented by different speakers. The abstracts for these talks are given in Annex 3 and a summary of the discussions concerning each of the presentations are given in Annex 4.

3.4 Subgroup discussion and egg and larvae issues

Breakout Group A – Baltic larvae surveys

The Working Group was asked to evaluate if the Baltic Ichthyoplankton Surveys are conducted in a sound manner and provide reliable results for input to the stock assessments. In particular, WGALES was requested to evaluate:

1. *If the present Baltic ichthyoplankton surveys are applying a sound, suitable and standardized methodology to determine Eastern Baltic cod egg and larval abundance.*
2. *If the survey coverage is sufficient to cover the current main spawning activity of Eastern Baltic cod in time and space*
3. *If the surveys are suitable to provide reliable estimates of daily and/or annual egg production.*
4. *If the surveys allow investigation of processes impacting on early life stage development and reproductive success.*
5. *If there are any suggestions for improvements to the surveys or data submitted to the assessment Working Group(s)*

And lastly - the outcome of the evaluation and potential suggestions for improvements should be summarized in the WGALES 2018 report.

Fritz Köster and Bastian Huwer (DTU-Aqua) provided a Working Document (Evaluation of the Baltic Ichthyoplankton Surveys. Working Document - ICES WGALES 2018, DTU Aqua, October 22-26, 2018) and a comprehensive presentation of the ichthyoplankton surveys (supplementing the presentation S1_5 which was given on the Monday afternoon). The present surveys were originally implemented in 1986 by the Institute of Marine Science (now GEOMAR) in Kiel, Germany and are currently conducted by 6 cooperating institutes giving sampling through the majority of the year i.e. covering the majority of the spawning season and occurrence of early life history stages of the principal target species, cod and sprat. The responses to the specific questions and recommendations from WGALES are outlined below:

1. Are the present Baltic ichthyoplankton surveys applying a sound, suitable and standardized methodology to determine Eastern Baltic cod egg and larval abundance?

The current survey has been standardized since 1986 with minor updates to sampling procedures, equipment and sample processing as developments occurred. In general, there has been an effort to ensure that any new developments are done in parallel with the older techniques to provide continuity in data output. In our opinion the survey is well thought out, using the appropriate equipment to quantitatively collect the eggs and larvae and there is sufficient temporal coverage (sampling trips per year) to capture the annual production of eggs and larvae.

2. Is the survey coverage sufficient to cover the current main spawning activity of Eastern Baltic cod in time and space?

The survey coverage at present is sufficient to describe the spawning, egg production and larvae abundances in the main spawning area (Bornholm Basin). The survey coverage has been increased over the years as more participants contributed to the survey. The coverage was also increased to ensure that the majority of the egg and larvae distribution was sampled and areas where there may be 'leakage' out of the survey area may occur and could be monitored. Surveys in adjacent areas (e.g. Gdańsk Deep and Gotland Basin) are also considered as far as available, and provide additional information on connectivity between management areas. In regard to the temporal coverage, 7 to 8 surveys per year are conducted since 2008, covering the majority of the spawning activity. Prior to 2008, the seasonal coverage is somewhat variable, ranging between 3 and 15 surveys per year. However, most years were covered by at least 6 surveys, and it was possible to maintain a continuous time-series in spring (April/May) and summer (July/August) throughout all years. Furthermore, it should be noted that in the early part of the time-series there has been a gradual change in peak spawning time, shifting from May to July. Thus, although survey coverage during summer was more limited in this early part of the time-series, peak spawning was still adequately covered. Overall, the continuity of the surveys since 1986 means that there is a reliable time-series of data which can describe the spawning activity of the Eastern Baltic cod stock.

3. Are the surveys suitable for providing reliable estimates of daily and/or annual egg production.

The survey design includes both sampling the adults for adult parameters (e.g. sex ratio, size and fecundity) and the plankton for egg densities. There has been an ongoing research programme on factors affecting fecundity along with continued evaluation of methods for determining fecundity in individuals and at the population level. The WG was satisfied that the most up to date methodologies were being used and the researchers have the wherewithal to implement any new developments as and when they occur. In regard to the egg abundances, the sampling techniques are 'state-of-the-art' and include additional sampling to give extra ecological information on processes that may be affecting e.g. mortality rates. There is a suitable sampling programme for sampling environmental factors so egg stages (ages) can be combined with development times to estimate the egg production over a whole spawning period. The sampling regime is suitable for undertaking both the DEPM and the AEPM.

4. Do the surveys allow investigation of processes impacting on early life stage development and reproductive success?

As mentioned above, the surveys also incorporate a number of additional sampling programmes. The detailed standard sampling of the physical environment and the egg

and larvae abundance provides the basic data for the Production or Abundance information. The addition of acoustic data and sampling of the larger organisms e.g. predators provides information on potential mortality over both temporal and spatial scales. The stomach content data of the main ichthyoplankton predators provide insights into potential scales of mortality and selectivity for certain size fractions of ichthyoplankton. The zooplankton sampling provides insight into variability in prey levels for e.g. larvae, again over spatial and temporal (both annual and interannual) scales. The physical data also provide insights into habitat suitability, both spatially and with respect to variability over annual and interannual scales. In addition, related biological and ecological studies, run through scientific and collaborative links provide vital information on the early life history of the target species. The surveys are imbedded in to a research programme that is more wide-reaching than simply the delivery of egg production or a recruitment index from the larvae abundance.

5. Are there any suggestions for improvements to the surveys or data submitted to the assessment Working Group(s).

Overall the WG was satisfied that given the financial constraints the survey was being conducted in the most efficient way possible. The WG recognized the logistical problems of procuring shiptime, personnel for manning the cruises and personnel for working up the samples (over multiple institutions and a number of different countries) in a timely manner. There is always a need for greater standardization of equipment and protocols between vessels and institutions, however, the current practices do not appear to involve and undue loss of precision or be introducing any undue uncertainty to the data (indices) presented to the assessment group(s).

Breakout Group C – Flowmeter considerations

A series of bullet points were presented by the chair, the following is a summary of discussions by the group on specific points raised by the chair and from within the group.

1. Recommendation that flowmeters are used on all plankton equipment used for quantitative estimates of fish eggs and larvae.

a. Appropriate flowmeters to be used e.g. non-reversing on e.g. vertically deployed equipment. The recommendation is to use flowmeters on any cruise if possible. If it is a vertical net, we need non-reversing flowmeters. There is general concern about how the flowmeters are mounted. It should be checked on every cruise that the flowmeters cannot flip to make sure that no filtered volume measurements are taken at deployment without filtering the water column.

2. Regularly calibrated

a. Methods for calibration

Three methods are recommended, either during the cruise, in a flow-tank on land (under controlled conditions on land) or sent back to manufacturer, as manufacturer guarantees correct filtered volume measurements. Land-based calibration is probably the best method, but also expensive. An important note is to clean and check the general flowmeter from time to time. Concerns, especially for the GULF nosecone mouths openings, are mentioned according to the distance from the nose opening to the part, where the flowmeter inside the nosecone is mounted, which may produce different water volume calculations (differences appear between institutes and gears). Considerations are in place to standardize this issue.

b. How often should flowmeters be calibrated?

Once a year would be a desirable option and is the general recommendation. A problem is the funds that are different according to the institutes. Concern: During a cruise, the longer the cruise is, the flowmeter performance changes. Hence, there is recommendation to calibrate flowmeters more than ones during a cruise. Depending on which gear is in use, this can make low or reasonable changes in abundance estimates (e.g. GULF versus MIK).

c. How to use the calibration data e.g. more than one calibration per cruise

As long as there are no noticeable changes in flowmeter measurement performances these measurements should be used primarily (under low wind conditions). If there is clear indication of flowmeter rotation during gear deployments outside the water, it is recommended to calculate the distance of gear deployments and compare the water volume filtered from flowmeter calculations. If there are noticeable differences the distance measurements should be used. With the general recommendation of calibrating flowmeters once a year, and not several times during a cruise, deciding how to use the various calibrations is not an issue. General recommendation is to keep records of calibration coefficients from each cruise so there is a history for each flowmeter. Depending on which flowmeter, people should be aware that some propellers move towards the impeller leading to changes in flowmeter performance.

3. Use of mechanical versus electronic flowmeters. Include limitations e.g. vessel capabilities, costs etc.

a. In-haul monitoring capabilities etc

It is generally recommended to use electronic flowmeters due to real-time observation possibilities. This enhances monitoring of net clogging and of real-time water volume filter performance during stratified samplings with multinets. One main advantage is to be able to store any flowmeter performances for each haul. So, that allows an afterward monitoring of flowmeter problems also after gear deployment or even after a cruise. But, if there is no chance of using electronic flowmeters, mechanical flowmeters are recommended to be used instead of no flowmeter.

4. Use of external flowmeters, clogging efficiency etc

a. As above re in-haul monitoring

It is recommended to use external flowmeters whenever possible. It should be noted that the external flowmeters should be attached to any gear at a position where is it not influenced by other attachments to the gear in use. The monitoring of the ratio between out and in flowmeter is essential to clogging issues.

b. Additional through the water log

It should be noted that using external flowmeters also provide another chance to back calculate the distance of a gear deployment.

5. Location of flowmeters re sampling equipment

a. Parallel openings e.g. ringnets, Bongos etc

Depending of the gear it is important where to mount internal and external flowmeters. Gear manuals should have this information and they also should provide information of parallel mounting or other options.

b. Nose cones

Plankton manuals should provide the information where to mount flowmeters for those gears that have nose cones as mouth openings.

c. External

The position and mounting of the external flowmeters should also be in the plankton manuals or/and in the gear manuals.

6. Question of maintenance, potential failures, damage etc.

- All flowmeters should be kept well maintained and preferably along with a log on where and when they were used and when they were calibrated.
- It was noted that it can be difficult to replace flowmeters during cruises
- On a separate issue it was noted that water volume measurements are problematic with PUPNETs and it would be useful to have some guidance on volume calculations for this sampler.

Breakout Group D – Eggs and larvae, other issues

A series of bullet points were presented by the chair, the following is a summary of discussions by the group on specific points raised by the chair and from within the group.

1. The use of vertically discrete sampling

The usefulness of vertically discrete sampling during ichthyoplankton surveys was discussed. Though it was considered by most as time consuming when added to a standard sampling program, vertically resolved ichthyoplankton sampling was considered as delivering valuable information. The information could be utilized to check the assumptions on vertical distribution of the target species on which survey and sampling strategies are based: e.g. if the depth sampling range is too small or too large for the species vertical distribution or if depth distribution is similar over the whole survey range. Information about depth distribution of a species can also be used as valuable input to various models, e.g. drift models or egg production models. The group, therefore, recommends that survey planning groups should consider and encourage the utilization of vertically resolved sampling during their survey. Specific objective for these kinds of programs should, however, be elaborated.

2. Colours of plankton nets

Particularly in the MIK survey, there exists a range of colours of the netting material. While the standard was set as black, blue and green nets may be found in the equipment of the survey participants. The group, therefore, discussed whether the colour of the net would be an issue in ichthyoplankton surveys. It was pointed out that colour in ichthyoplankton nets should be selected with care and with respect to the visibility of the nets to the target species. Colour vision appears to be well developed in fish larvae, since cones are the first receptors that develop in fish larvae. However, as Marta Moyano of IMF in Hamburg pointed out, in experiments, fish larvae did not seem to be susceptible to red light when kept in darkness. Rods, that would allow fish larvae to view in a low light environment develop later in the larval life. However, in the size range of the MIK herring larvae, rods should be already in place. Therefore, when sampling at night, nets should be at least of dark colour (dark blue or green) at best, however, black. It was highlighted, that a once chosen colour should be kept in a survey time-series. The group also recommended that colour should be added as a field in the

fish egg and larvae database. A low range of standard colours should be allowed only for entry: white, black, red, blue, green and others

3. Use of genetic techniques and/or related molecular techniques as routine in egg and larvae surveys. Pros, cons, pitfalls, objectives etc.

There were a number of presentations during this meeting, where molecular/genetic methods were applied to identify fish eggs and/or larvae. The group discussed, therefore, whether it should be recommended that these methods were utilized more regularly during surveys. Considering that at current the application is not only still expensive but also requiring much more additional work during the surveys, the group decided that at the moment it would not be practical to apply genetics on a regular basis and should only be implemented when necessary. The development of these kind of methods is, however, fast and could, therefore, become compulsory particularly in egg surveys in the near future.

4. The use of genetic techniques for apportioning eggs and larvae to substocks in ichthyoplankton surveys

Related to the above topic, the group also discussed whether genetics could be useful to apportion fish egg and larval abundances to substocks. A presentation by Bastian Huwer of DTU-Aqua suggested that this was possible in herring. However, as he pointed out during the discussion, this separation only worked between Downs and the other components. It was impossible to separate Orkney/Shetlands larvae from those originating from Buchan of Banks. It was also pointed out by Richard Nash (IMR, Norway) that mixing occurs between West of Scotland herring stocks and those of Orkney/Shetlands. Therefore, it was considered that genetic separation of (sub)-stocks should be extremely difficult for some stocks, if not impossible.

5. The use of image analysis.

Image analysis is currently in use at several institutes to aid processing of fish egg and larvae samples. E.g. ImageJ and ObjectJ routines are used to measure and stage fish eggs from pictures taken with either commercial SLR or microscope cameras. With these systems, 50 – 150 fish eggs can be processed from one picture. The ZooScan system is used to measure herring larvae. In France at IFREMER, the ZooCAM system (Colas et al. 2018) is used to separate fish eggs from the rest of the plankton as well as to stage and measure them simultaneously. ZooCAM appears to be the currently most developed system for processing zooplankton samples, which is able to deliver almost real time data on fish egg distribution when used during surveys. WGALES is confident that with increasing computing power, the use of image analysis systems will spur ichthyoplankton field studies in the future.

6. Options for preservation of samples.

Some participants raised the question whether alternatives to formaldehyde as the recommended preservation fluid for fish eggs and larvae are currently available. This was in particular of interest since formaldehyde preserved samples cannot be used for genetic and other molecular analysis. Ethanol, which would enable a later genetic processing, has several disadvantages for visual identification of egg and larvae samples. Not only does it dehydrate the specimens, it also turns fish eggs completely opaque so that staging and identification becomes impossible. Therefore, 4 % buffered formaldehyde solution is still considered as the best preservative for fish egg and larvae samples.

A solution with a very low formaldehyde content (< 1 %) would be the Battaglia solution (Lelièvre et al. 2010). This solution should also allow genetic analysis of the samples if specimens are not kept longer the 2 weeks therein.

References

Colas, F.; Tardivela, M.; Perchoc, J.; Lunven, M.; Foresta, B.; Guyaderc, G.; Danielou, M.M.; Le Mestre, S.; Bourriau, P.; Antajan, E.; Sourisseau, M.; Huret, M.; Petitgas, P.; Romagnan, J.B. 2018: The ZooCAM, a new in-flow imaging system for fast onboard counting, sizing and classification of fish eggs and metazooplankton. Prog. Oceanogr. 166, 54 – 65.

Lelièvre, S.; Verrez-Bagnis, V.; Jérôme, M. et al. 2010: PCR-RFLP analyses of formalin fixed fish eggs for the mapping of spawning areas in the Eastern Channel and Southern North Sea. J. Plankton Res., 32, 1527–1539.

3.5 Subgroup discussion on adult parameter estimation issues

Breakout Group B – Adult parameters

(i) visit to the histology lab at DTU Aqua and discussion on laboratorial analyses

The subgroup dealing with adult parameters met to discuss methodologies applied to access in particular batch fecundity and spawning interval related to DEPM and AEPM. A histology workshop was held 24th October 9-12.30, including a visit to the preparation, histology and image analysis labs, where methodologies were discussed in detail. The methodologies applied in the lab at DTU for embedding, sectioning, staining and mounting was demonstrated and for each step methodologies used in the various lab's for various species were discussed. At the end pro's and con's using different methods were discussed illustrated by digital images related to an interim analyses performed by AUTH, IMR and DTU. This discussion was followed up on the 25th October 9-10.30. Here, focus was on comparing methodologies, quality of output for different purposes, sources of error, and potential for improvement.

24th October - Practical workshop.

Introduction – Olav Kjesbu and Kostas Ganias

Program of - Jonna and Inger

- Sampling and sample preparation – embedding in paraffin and historesin
- Sectioning, staining – hematoxylin-eosin and toluidine blue and slide preparation
- Image analysis – viewing some sections prepared and stained by different methods
- Tissue characteristics

Initial comparison of methodological as intro to the next day's discussion

25th October - Follow-up discussion and evaluation

Interpretation – Kostas Ganias and Olav Kjesbu

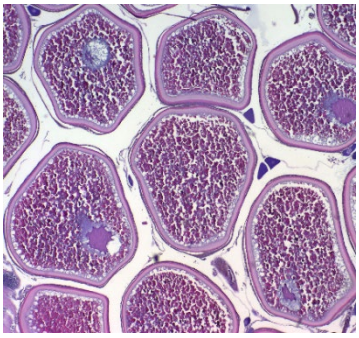
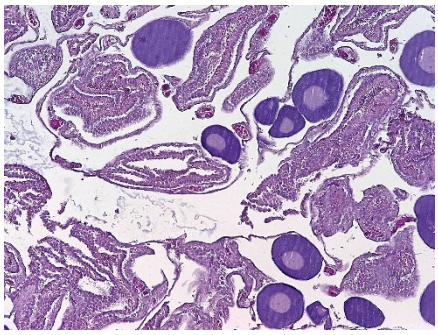
Comparison of methodologies

- Staging of previtellogenic oocytes

- Staging of post ovulatory follicles (POF)
- Staging of atresia

Discussion of pro's and con's, and the potential to improve methodologies and quality of resulting data and estimates.

The conclusion was that methodologies can be substantially improved by coordination among partners in relation to protocols used in the lab for different purposes and species. This ranged from consumables like sampling containers (BioSafe, http://biopsafe.com/ http://www.axlab.dk/), materials (historesinvs.Technovit, different paraffin products), to temperature effects of tissue preparation and staining techniques (H&E, toluidine blue and Schiff/PAS).

The subgroup concludes that collaborative work can significantly enhance protocols and harmonize outcome, thereby creating consistency in interpretation and increased quality of data for DEPM and AEPM. The strategy to reach this goal would be include collaboration on:

- Methodology -
- Image analysis -
- Reproduction strategies –

Here, inter-laboratory exchange of species-specific protocols and exchange of staff will play an important role, as evidenced by the workshop experience at this WG-meeting.

(ii) work plans for the continuation of the collaborative work on spawning frequency analyses

A discussion on the achievements and plans on the collaborative work was undertaken by the participants of the subgroup. The results attained and presented during plenary session 3 allowed testing of the methodology proposed for spawning frequency estimation at the individual level for a few species. It was then agreed to continue the collaborative work in order to progress to the estimation of the spawning fraction of the populations using this new approach which will permit in the future the use of DEPM for all species regardless of their fecundity type being determinate or indeterminate.

4 Cooperation

Cooperation with other WG

WGMEGS (Egg sampling protocols and fecundity issues), WGEGGS2 (MIKeyM sampling and incorporation of other surveys e.g. IHLS and raising of a replacement WG (WGSINS) for all North Sea area surveys), WGIPS (IHLS), HAWG (MIK survey)

Cooperation with Advisory structures

Providing a forum for evaluating ichthyoplankton surveys e.g. Baltic gadoid survey and WBSS Rügen herring recruitment survey.

Cooperation with other IGOs

5 Next meetings

Next meeting of WGALES will be held at Ifremer in Bologne-sur-mer, France, on 19–23 October 2020.

If necessary WGALES will meet by correspondence during 2019 to act upon urgent requests from ichthyoplankton survey groups (ToR d).

The 2020 meeting will consist of four sessions:

Session 1

General topics on surveys

Session 2

Egg and larvae issues

Session 3

Adult fish reproductive issues

Session 4 (specialist topic)

The use and development of image analyses and molecular techniques for ichthyoplankton surveys

The Working Group will also develop a proposal for a theme session for the ICES ASC in 2021, which will be submitted to SCICOM in September2020 with the following working title:

Linking fish reproductive dynamics and early life history with recruitment.

6 Summary of working Group self-evaluation and conclusions

A copy of the full Working Group self-evaluation should be included in the report as Annex 6.

Annex 1: Agenda

Meeting of the ICES Working Group on Atlantic Larvae and Egg Surveys (WGALES)

Location: DTU AQUA - National Institute of Aquatic Resources, Section for Marine Living Resources, Technical University of Denmark, Kemitorvet, 2800 Kgs. Lyngby, Denmark, Meeting Centre in Building 202.

Dates and times: 1400h 22 October 2016 – 1200h 26 October 2018

Date	Time		Speaker	Title
22.10.2018	14:00	***Introduction to WGALES***	Richard D.M. Nash Maria Manuel Angélico	
Monday	14:15	***Local arrangements***	Bastian Huwer	
	14:30	***Discussion on ToRs for the 2018 Meeting***		
	15:00	Coffee		
	15:30	***Session 1 - selection of rapporteur***		
	15:30	S1_1	Andrei Makar-chouk	Changes in the size structure of spawning cod in the Baltic Sea could have caused de-pensation.
	16:00	S1_2	Bastian Huwer	A pilot survey on the feasibility of establish-ing a sprat recruit-ment index based on larval sampling dur-ing Q3 IBTS surveys
	16:30	S1_3	Cindy J.G. van Damme	Improving North Sea Herring assessment through new re-cruitment survey
	17:00	S1_4	Alejandro Mateos-Rivera	Assessment of the spawning time for the major North Sea fish stocks
	17:30	S1_5	Fritz Köster	Application of ich-thyoplankton sur-veys to estimate stock trends, spawn-ing-stock biomass and recruitment of Eastern Baltic cod
	18:00	End		
23.10.2018	**09:00**	***Session 1 continuation - selection of rappor-teur***		
Tuesday	09:00	S1_6	Bastian Huwer	When (How?) things go right: Separating MIK-IBTS herring larvae by genetic testing presents a new method to improve stock abundance estimates
	09:30	S1_7	Richard D.M. Nash	Considerations in the use of

				Multinet samplers for fish larvae.
	10:00	S1_8	Maria Manuel Angélico	Added value of regular egg and acoustics surveys for pelagic ecosystem monitoring.
	10:30	Coffee		
	11:00	S1_9	Carlos Pinto	The ICES Egg and Larvae Database and recent developments in Databases at ICES
	11:30	S1_10	Cindy J.G. van Damme	Importance of flow measurements and calibrations in ichthyoplankton surveys
	12:00	S1_11	Richard D.M. Nash	Internal to external flow (efficiency) characteristics for MIK and Gulf VII samplers.
	12:30	Lunch		
	14:00	***Session 3 - selection of rapporteur***		
	14:00	S3_1	Kjesbu/Charitonidou/ Santos Schmidt /Ganias	A joint effort across marine laboratories for improving spawning frequency estimations in fish.
	15:30	Coffee		
	16:00	S3_2	Cindy J.G. van Damme	Improving egg production methods with new fecundity estimation methods
	16:30	S3_3	Thassya C. dos Santos Schmidt	Is Northeast Atlantic mackerel spawning activity extending northwards?
	17:00	S3_4	Cristina Nunes	DEPM surveys and reproductive behaviour of horse mackerel (*Trachurus trachurus*) from the southern stock (Atlantic Iberia, ICES 9a)
	17:30	End		
24.10.2018	09:00			
Wednesday	09:00	***Breakout: A - Baltic larvae surveys, B – Adult parameters***		
	10:30	Coffee		
	11:00	***Breakout: C - Flowmeter considerations, B – Adult parameters***		
	12:30	Lunch		
	14:00	***Session 2 - selection of rapporteur***		
	14:00	S2_7	Patrick Polte	Effects of phenology shifts on herring (*Clupea harengus*) recruitment in the Western Baltic Sea
	14:30	S2_8	Marta Moyano	Using short-term physiological measurements to explore long-term trends in recruitment variability: a case study using Atlantic herring
	15:00	Coffee		
	15:30	S2_2	Matthias Kloppmann	Identification of Fish Eggs Using Proteomics

	16:00	S2_3	Carolina Giraldo	Lipids components as proxy of plaice larval condition (*Pleuronectes platessa*) in the North Sea and English Channel
	16:30	S2_4	Julien Di Pane	Spatial and temporal variations of larval condition during the critical period of three fish species in the Eastern English Channel: an histological-based approach
	17:00	S2_5	Christophe Loots	Spatio-temporal variability of winter larval assemblages in the eastern English Channel and North Sea
	17:30	End		
25.10.2018	09:00	***Breakout: D - Egg and larvae, other issues, B - Adult parameters***		
Thursday	10:30	Coffee		
	11:00	S2_1	Kjesbu/ Tiedemann/ Stiasny/ Nash	Recruitment dynamics of Norwegian spring-spawning herring: tracing the fate of early developing oocytes to the 0-group stage.
	12:30	Lunch		
	14:00	S2_6	Andrei Makarchouk	Influence of global warming on vertical distribution of sprat eggs in the Eastern Baltic and its possible impact on recruitment success.
	14:30	S2_9	Hannes Höffle	The death of Sebastes: Modelling natural mortality in the early life of an ovoviviparous fish
	15:00	Coffee		
	15:30	***Selection of rapporteur***		
	15:30	**General discussion on future topics, structuring of the WG, Theme session suggestions for future ASCs**		
	16:30	**Report writing update, ToRs, new chairs**		
	17:00	End		
26.10.2018	09:00	***Report drafting***		
Friday	10:30	Coffee		
	11:00	***Finalize future ToRs and new chairs***		
	12:00	End		

Annex 2: List of participants

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Annex 3: Abstracts of presentations

Abstract number	Authors	Title and Abstract
S1_1	Andrei Makarchouk (speaker), Ivo Šics, Tatjana Baranova	**Changes in the size structure of spawning cod in the Baltic Sea could have caused depensation.** Mean size of cod in the Baltic Sea decreased during last decades, though inter-annual fluctuations sometimes were substantial. The greatest decrease happened in the group of the largest fish. We have analysed data from scientific surveys starting from 1978 and commercial hauls from 1998 in order to follow alterations in the dynamic of spawning. Our study has showed that the sex ratios at the beginning and in the end of the spawning season became much skewed. Proportion of females among spawners in the start of spawning season was often less than 20 %, but exceeded 70-80 % in the end of the season. It could be caused by gradual extinction of large size females that used to start spawning first and large size males that used to dominate in the end of spawning season. This could lead to loss in the effectiveness of fertilization and production of viable eggs in the start and the end of spawning season.
S1_2	Bastian Huwer (Speaker), Peter Munk	**A pilot survey on the feasibility of establishing a sprat recruitment index based on larval sampling during Q3 IBTS surveys.** There is only limited information on early life stages of North Sea sprat. However, such information, e.g. on the extent of recruitment variability, would be important for short-term forecasts and stock assessment. Therefore, a pilot survey targeting sprat larvae with a MIK net was conducted during the Danish Q3 IBTS in 2018 to investigate if a similar concept as the Q1 herring larvae surveys, which provide an important recruitment estimate for stock assessment, could be implemented for sprat. Specific aims were to investigate (1) if sprat larvae of relevant sizes could be caught during the Q3 IBTS, (2) to which degree the relevant areas of main larval abundance could be covered by night-time sampling and (3) if spawning had finished well before the survey, thus a reasonable amount

		of larvae would be of "late larval" sizes. To target potentially occurring smaller larvae and eggs, an additional, fine-meshed MIKey net was used. 81 hauls could be conducted, including day/night comparisons of catchability. Preliminary results are discussed regarding possibilities for future sampling programs and establishment of recruitment forecasts which could improve the assessment of North Sea sprat.
S1_3	Cindy J.G. van Damme (speaker), Niels Hintzen, Frank Kleissen, Loes J. Bolle Matthias Kloppmann, Richard D.M. Nash	**Improving North Sea Herring assessment through new recruitment survey.** Recruitment is one of the major drivers of fish stock dynamics. Getting a correct perception of recruitment is therefore essential for successful fisheries management. Predicting the level of recruitment is still is one of the most difficult aspects in fish stock dynamics, even if dedicated surveys are in place to sample larvae or juveniles. In the case of North Sea herring (*Clupea harengus*) there is a larvae survey (IBTS-MIK) for estimating recruitment. Once considered as a very good predictor of herring recruitment, in recent years the MIK index has become a poorer forecaster because the samples collected do not cover the increasing spawning component in the English Channel. A new larvae survey has been setup and carried out in April 2018, to collect information on the recruitment of this winter spawning component. The temporal and spatial coverage of this new survey was based on larval drift modelling. This survey is being implemented without increasing the survey budget available for the herring larvae surveys.
S1_4	Mateos-Rivera, A. (speaker), Mozfar, B. (speaker), Skern-Mauritzen, R., Dahle, G., Thorsen, A., Sundby, S., Glover, K., Kleppe, L., Wehde, H., Krafft, B.A.	**Assessment of the spawning time for the major North Sea fish stocks.** Increased and updated knowledge of reproductive strategies of fish is crucial to optimize the planning of seismic surveys to reduce its potential negative ecosystems impacts. The main goal of this study is to describe the current spawning times and locations for the major North Sea fish stocks. Molecular and traditional visual taxonomic analyses were performed in parallel on eggs and larvae collected weekly (WP2) at three locations along a south –

		north axis in the North Sea. For the molecular barcode analysis, the samples were kept in ethanol and Sanger sequenced targeting the COI gene, whilst the visual taxonomic classification were determined using stereomicroscope on samples fixated on formalin. During this ongoing project we have analysed samples from August 2017 to July 2018 and 21 different taxa has so far been identified. The results confirm the previous findings and both analysis methods show the same pattern. The presentation will highlight the findings and elaborate the information on differences in analytical methodologies used.
S1_5	Fritz Köster (Speaker), Bastian Huwer, Gerd Kraus, Rabea Diekmann, Margit Eero, Andrei Makarchouk, Serra Orey, Jan Dierking, Piotr Margonski, Jens Peter Herrmann, Jonna Tomkiewicz, Holger Haslob, Daniel Oesterwind, Paul Kotterba, Rüdiger Voss	**Application of ichthyoplankton surveys to estimate stock trends, spawning-stock biomass and recruitment of Eastern Baltic cod.** During recent years, uncertainties related to growth and natural mortality have prevented quantitative assessment of Eastern Baltic cod, which has therefore been treated as data poor stock since 2014. Furthermore, the standard assessment has only been able to determine recruitment-at-age 2 based on bottom trawl surveys (BITS). Therefore, fishery-independent methods to estimate stock trends, stock sizes and recruitment, e.g. based on ichthyoplankton surveys and egg production methods, are urgently needed. Results from ichthyoplankton surveys conducted since the mid 1980's, as well as accompanying trawl catches to determine adult parameters such as individual spawning frequency, relative fecundity and sex ratios, were used to apply the annual and daily egg production methods (EPMs). Both methods provide comparable results, following largely SSB trends derived from BITS surveys, but with less year-to-year variations. However, EPMs yielded consistently lower SSB's, which can be explained by different processes and at least some of these can be taken into account in the EPMs. Furthermore, possibilities to use larval abundance as recruitment index are explored, which would allow to estimate year-class strength already at age 0 instead of age 2 as previously.

S1_6	Bastian Huwer (Speaker), Dorte Bekkevold, Cindy van Damme, Matthias Kloppmann	**When (How?) things go right: Separating MIK-IBTS herring larvae by genetic testing presents a new method to improve stock abundance estimates.** At previous WGALES meetings, Matthias Kloppmann gave presentations entitled "When (How?) things go wrong - How larval drift can distort stock abundance estimates", focusing on recent challenges to estimate a reliable recruitment index for North Sea herring from the MIK surveys. The main issue was that in some years larvae of the Downs component had increased in number and were drifting further north than usually, resulting in difficulties how to exclude these larvae from the index calculation. In the meantime, a new algorithm to calculate the recruitment index was developed, using larval sizes to exclude the Downs larvae. Furthermore, recent advances in molecular methods do now allow to detect genetic differences between the different herring components in the North Sea. In the present analysis, larvae from the Danish MIK-IBTS surveys were analysed using 96 SNP markers selected to maximize stock identification, to determine if they originated from the Downs or from other spawning areas. The results show specific patterns of origin related to larval size, and highlight that genetics may help to improve the procedure for excluding the Downs larvae from the index and thereby improve stock abundance estimates.
S1_7	Richard D.M. Nash (speaker)	**Considerations in the use of Multinet samplers for fish larvae.** Field studies on fish eggs and larvae are often focused on obtain abundance estimators and when considered for assessment purposes are often presented as abundance through the water column. More process orientated studies are interested in finer scale sampling, both over horizontal and vertical scales through the water column. Modelling studies are now requesting information on relatively detailed information on vertical and horizontal distributions and how these change with physical water structure and over diel and seasonal time periods. Whist multiple opening and

		closing nets have been available for a long time, in recent years Hydrobios (Germany) has successfully marketed a series of Multi-net samplers ranging from the min ($0.125m^2$) to the Mammoth ($1m^2$). These samples have the option for 'onboard' CTD and pitch and roll sensors. In this presentation I will examine some of the options available for sampling strategies using this equipment, types of data which can be made available and some of the potential problems we have encountered.
S1_8	Maria Manuel Angélico (speaker) *et al*	**Added value of regular egg and acoustics surveys for pelagic ecosystem monitoring.** Egg and acoustics surveys are being conducted regularlly for more than three decades. Over this period many technological and methodological improvements have been introduced which have revamped the monitoring processes and outcomes of surveying allowing for more detailed observations of the systems and the inclusion of complementary sampling other than the traditionally undertaken for egg methods estimates. At present, it is possible, making use of up-to-date equipments and processing techniques (e.g.. multifrequencies echosounders, semi-automated sample collectors, image analyses processing, genetics probing) to have a much more complete characterization of the ecosystems. Modern gear and analyses also permit the recovery of past data by reanalysing archieved plankton samples. The actual demand for integrated ecosystem assessment and the commitements for compliance with EU legislation (namely, for the MSFD) require more effective sampling/processing methods which may serve simultaneusly several objectives. Such challenges are discussed considering egg survey primary objectives versus extra ecological information acquisition and its use for the main survey goals.
S1_9	Carlos Pinto (speaker), Anna Osypchuk	**Eggs and Larvae, checking and uploading files to the database.** Eggs and Larvae database was developed to accommodate ichthyoplankton surveys. Until now, each survey had distinct formats, and data were delivered to ICES Data Centre and exported to the Eggs and Larvae Database based on their native format. This creates some inconsistency, puts a burden on ICES Data Centre to interpret and accomodate each format, and opens a

		window for errors in the datasets. This also negatively affects data analysis and comparison. In 2015, WKALES proposed a common data submission format for the Eggs and Larvae Database, followed by slight revision by WGMEGS in 2017. Based on these recommendations, ICES Data Centre has established an automated data submission routine for the ichthyoplankton surveys, including a common data format, online data submission and re-submission that are automatically uploaded to the database, and the revised data output. The new approach makes it easier to accommodate the new data submissions in the database, and brings all the data to the same standards.
S1_10	Cindy J.G. van Damme (speaker), Richard D.M. Nash	**Importance of flow measurements and calibrations in ichthyoplankton surveys.** Ichthyoplankton surveys are an important tool to provide information for management of stocks and marine areas. However, this requires reliable data to be collected in the ichthyoplankton surveys. To estimate the amount of eggs and larvae in the water most plankton gears use a flowmeter to measure the volume of water filtered during the haul. The position of the flowmeter in the opening of the gear will influence the performance of the flowmeter. Especially in gears using an angled nosecone, since the flow pattern varies over the gear opening. Modern flowmeters are robust and there is little lapse in the performance of the flowmeter. Nevertheless, it is important to calibrate the flowmeters each survey. This presentation will show the impact of positioning and calibration of flowmeters on the flowmeter measurements.
S1_11	Richard D.M. Nash (speaker)	**Internal to external flow (efficiency) characteristics for MIK and Gulf VII samplers.** The efficiency of plankton nets is often ignored when sampling for fish eggs and larvae. Flow rates throughout a tow is important especially when considering the abundance of larvae. Net efficiencies are also an important factor when considering size composition in the water column whether it is across a larval community or within a single species. Net efficiencies can be estimated using at least two flowmeters, one measuring the flow through the net

		and at least one which is exposed to free flow. Ideally, data should be available from the whole tow and stored electronically so that the time and location of any net clogging is recorded. Informed decisions can then be made about the tow and/or subsequent sampling. If the electronic option is not available then estimates of efficiency can be made using e.g. mechanical flowmeters. The location of external flowmeters and how the subsequent data are calculated and used needs careful consideration. External flow rates also provide additional information on some of the samplers through the water characteristics.
S2_1	Olav Sigurd Kjesbu (speaker), Maik Tiedemann (speaker), Martina Stiasny (speaker), Bridie J.M. Allan, Valeriya Komyakova, Jessica L. Rey, Arild Folkvord, Erling Kåre Stenevik, Aril Slotte, Richard D.M. Nash	**Recruitment dynamics of Norwegian spring-spawning herring: tracing the fate of early developing oocytes to the 0-group stage.** The RECNOR project, *Recruitment dynamics of commercially important NE Atlantic fish species in changing ecosystems*, focuses on why there has not been a significantly large recruitment of NSS herring, one of the keystone species in the Norwegian Sea ecosystem, since 2004 (according to official statements based on the 2017 stock evaluation). To address this complex problem, we examine both the adults and their performance (including factors affecting reproductive output), offspring survival (including predation by Atlantic mackerel), growth and origin (the latter from otolith microchemistry). So, in addition to using data from previous studies and surveys undertaken by IMR, this research utilizes a number of new techniques. Methodologically we use approaches ranging from laboratory studies on the responses of herring larval behaviour to different temperatures, molecular examinations of mackerel stomachs to identify predation rates on herring larvae, extensive field sampling on spawning grounds (both north and south along the Norwegian coast) for herring reproductive traits, and dedicated larvae and postlarvae field studies over the last two years to examine predation rates, distribution patterns and provide samples for studies on predation and spawning origins of larvae and recruits plus nursery origins of recruits.
S2_2	Matthias Kloppmann (speaker), Thomas Knebelsberger, Andrea Barco	**Identification of Fish Eggs Using Proteomics.**

		Fish egg are notoriously difficult to identify, particularly when they are in an early developmental stage, which is why in many species genetic methods have to be used. DNA sequencing, however is extremely expensive and also requires tedious processing of the egg samples. Protein mass spectrometry, which is widely used in clinical diagnostics for e.g. separation of bacterial species, has only been recently applied to also discriminate between metazoan taxa/species and is here for the first time used to identify fish eggs. The eggs were collected during the 2018 first quarter IBTS and individually preserved in ethanol after being staged and measured fresh from the samples. A subsample of 298 eggs were at first identified to species level using DNA barcoding and then analysed by protein mass spectrometry in order to build a reference database. A remainder of 804 egg were then successfully identified using protein mass spectrometry alone. The experiment showed that species identification in fish eggs is possible using protein mass spectrometry. The method is not only much cheaper than barcoding but also delivers reliable results much faster.
S2_3	Joly, L., Tavernier, E., Loots, C., Di Pane, J., Giraldo, C. (speaker)	**Lipids components as proxy of plaice larval condition (*Pleuronectes platessa*) in the North Sea and English Channel.** Mortality during early life stages is one of the causes of variability in the recruitment of fish and can be linked to critical periods like the shift between endogenous and exogenous nutrition. In this study, lipids were used to quantify the energy reserves and condition of plaice larvae. The total amount of lipids was linearly related to the quantity of structural phospholipids, suggesting that growth is favoured over lipid storage. The lipid condition index triacylglycerol: cholestérol ratio showed interannual variability on the condition, probably related to prey quality or quantity. Larvae with low levels of triacylglycerol: cholestérol could be either growing or under starvation. Phospholipids class analysis - which can also be reduced during starvation - allowed the identification of larvae truly in poor condition. Our results indicate that larvae were globally in good condition but suggest that individuals in 2017 had lower potential to withstand environmental stressors than larvae in 2018. Lipid

		components appeared as a useful tool to monitor interannual nutritional condition and can have a widespread applicability for other larval fish species.
S2_4	Di Pane, J. (speaker), Gendrot, F., Loots, C., Koubb,i P	Spatial and temporal variations of larval condition during the critical period of three fish species in the Eastern English Channel: an histological-based approach Spatio-temporal variations of the larval condition of three spring-spawning species (sole, whiting and sprat) during the critical period were studied using a histological–based approach. Fish larvae were sampled during three ichtyoplanktonic surveys in the eastern English Channel in 2017 and the larval condition was assessed at the larval and tissular scale by visual observation of the main digestive organs (guts, liver, pancreas). Larger numbers of larvae in good condition or at beginning of starvation were found compared to ones in degraded state. Sole and sprat larvae showed a coastal-offshore segregation of the condition. Larvae associated with high planktonic productivity of the coast were in better condition than those offshore. Whiting larvae were in good condition while sole and sprat 's condition improved along with the season. Since, previous results for sole from 1995 showed an inverse relationship with the season, potential factors of interannual variations such as prey concentrations in the field have now to be investigated.
S2_5	Loots, C. (speaker), Pernak, M., Giraldo, C., Damme, C., Huwer, B., Klopmann, M., Nash, R., Ritchie, L.	**Spatio-temporal variability of winter larval assemblages in the eastern English Channel and North Sea.** Spatio-temporal variability of winter larval assemblages in the English Channel and North Sea in relationship with environmental parameters was studied between 2012 and 2017. Fish larvae other than herring coming from the IBTS-MRN sampling in January-March were used. Among the 32 species found, eight taxa (crystal goby, transparent goby, other goby, sandeels, plaice, lemon-sole, pilchard and dragonets) were the most frequent. Three larval assemblages were found and distributed following a diversity gradient from North to South. The first one located in the northern North Sea was represented by crystal goby and *Pomatoschistus* sp., the second one in the middle North Sea by lesser sandeel, lemon-sole and dragonets and the last one

		in the southern North Sea and English Channel by plaice, pilchard, other goby and sandeels. Mapping of recurrent, occasional, rare and unfavourable areas showed a clear spatial distribution with a high interannual variability. The OMI (Outlying Mean Index) and WitOMI (Within Outlying Mean Index) analyses suggested the relevance of several environmental parameters (temperature, salinity, fluorescence, chlorophyll *a* and nutrients concentrations) to explain the variability.
S2_6	Andrei Makarchouk (speaker)	**Influence of global warming on vertical distribution of sprat eggs in the Eastern Baltic and its possible impact on recruitment success.** Specific gravity of sprat eggs decreases during spawning season, and starting from April or May part of eggs occurs on the water surface, where mortality is much higher than in the deep water layers. We have compared vertical distribution of sprat eggs with the hydrographical conditions in the period of years from 1973 to 2016, paying special attention to the alterations in density of water on the surface in May-August. Interannual changes of the water density were considerable, but the general trend was decrease. This decrease was in the range of 0.6-1.1 $kg*m^{-3}$ in different months. It is difficult to forecast changes in the salinity of water in the Baltic Sea, but it is expected that the surface water temperature would increase in coming years due to global warming. We have made forecasts for different ranges of temperature and calculated approximate decrease in the share of sprat eggs that would avoid unfavourable conditions in the upper layer as a result of global warming.
S2_7	Patrick Polte (speaker), Paul Kotterba, Dorothee Moll, Nielja Knecht, Paco Rodriguez-Tress, Lena von Nordheim, Juan Santos	**Effects of phenology shifts on herring (*Clupea harengus*) recruitment in the Western Baltic Sea.** 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 the spring-spawning herring population went together with exceptionally mild winter conditions. Besides immediate 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 spawning has shifted over time and that the timing of larval hatching is related to annual recruitment success. Results indicate a trend of earlier seasonal occurrence of maximum yolk-sac larvae abundance. Early hatching explained much of the variability of the year-class strength expressed as an annual index (N20), which is an established proxy in stock assessment. Along the entire reproduction period, phenology shifts might have affected initial cohorts of hatching larvae. They potentially encountered lower densities of plankton prey since the critical period now occurs prior to the spring plankton blooms. Together with severe eutrophication effects on larval cohorts later in the season, climate change induced phenology shifts might presently structure herring populations in the Baltic Sea.
S2_8	Marta Moyano (speaker), Björn Illing, Patrick Polte, Paul Kotterba, Myron A. Peck	**Using short-term physiological measurements to explore long-term trends in recruitment variability: a case study using Baltic herring.** Climate variability and change is challenging the life of ectothermic organisms in marine systems, leading to changes in abundance, distribution and phenology. Warming is one of the most important factors behind those changes. However, thermal sensitivity changes through an organism's life and knowledge on the life stage-specific thermal windows and endpoints is still limited. Here we first estimated the optimal temperature (T_{AB}) and other thermal endpoints (e.g. temperatures at which arrhythmias start) of the cardiac function in larvae of Western Baltic Spring-spawning (WBSS) herring reared at 7, 11 and 15 °C. Then, we compiled thermal limits and ranges for growth and survival of Baltic herring through ontogeny estimated via laboratory and field studies. Finally, we tested the hypothesis that suboptimal warm temperatures (beyond T_{AB}) lead (directly or indirectly) to decreased larval abundance (and thus recruitment) in the field an available 20yr-time-series. The challenges of integrating lab physiological data and a high time and space resolution time-series are also discussed.

S2_9	Hannes Höffle (speaker), Benjamin Planque, Alf Harbitz, Tone Vollen	**The death of Sebastes: Modelling natural mortality in the early life of an ovoviviparous fish.** Natural mortality is an uncertainty in stock assessment as it is difficult to determine when clear predator-prey relationships are missing. Therefore, expert judgement is a common source for natural mortality rates, including the rate of 0.05 for beaked redfish (*Sebastes mentella).* A recent re-evaluation, using a range of estimators from the literature showed that most estimates were below 0.1, with the mode at 0.058. It also revealed outlying results up to 0.6 and that results based on unrelated taxa or different climate zones can be nonsensical. Whilst these estimates refer to the entire life cycle, mortality in the early life stages is particularly high. *S. mentella* is an ovoviviparous species that releases larvae after they have hatched. This may impart an advantage to the larvae which have at least limited control on their depth and therefore the current they are in. Here we attempt to construct a model, governed by body size, that describes natural mortality in the early life of beaked redfish and compare the results with studies on oviparous fish.
S3_1	Ganias, K. (speaker), Charitonidou, K. (speaker), dos Santos Schmidt, T. (speaker), Angélico, M.M., Costa, A.-M., Domínguez-Petit, R., Garabana, D., Korta, M., Krüger-Johnsen, M., Nunes, C., Santos, M., Alix, M. (speaker), van Damme, C.J.G., Kjesbu, O.S. (speaker)	**A joint effort across marine laboratories for improving spawning frequency estimations in fish.** During the last ICES-WGALES meeting held in Thessaloniki (Oct. 2016) an initiative was taken by the participants of the Plenary Session on Adults for the development of a new methods for estimating spawning frequency. These new methods should be applicable to any kind of spawners/species and are anticipated to improve accuracy and reduce cost and effort in applications of Egg Production Methods. This ambition led to a collaborative mission headed by AUTH and IMR with the participation of AZTI-Tecnalia, Wageningen Marine Research, IEO, IPMA and DTU-Aqua. The material for the method development was bases on ovarian samples of four species with distinct fecundity types (sardine, mackerel, horse mackerel and cod), collected through national sampling programmes. A large amount of samples (n~450) has already been sent to AUTH and IMR for histological analysis and oocyte size fre-

		quency distribution (OSFD) analysis, respectively. At present, all samples have been processed for OSFD, all mackerel and most sardine samples have been processed histologically (paraffin, haematoxylin/eosin) while an amount of sardine and mackerel samples (n~70) has been further processed in resin (DTU) and scanned in high quality photomicrographs (at IMR). The analysis of this material is currently in progress and focuses on investigating how spawning rhythm is reflected in ovarian dynamics, drawing evidence from sardine and mackerel as two fishes with highly contrasting OSFDs and spawning frequencies (and thereby POF degeneration patterns). During the ICES-WGALES meeting the two co-coordinators and the early-career researchers who are mainly involved will present the results and advances that have been achieved so far during this collaborative work.
S3_2	Cindy J.G. van Damme[1] (speaker), Olav S. Kjesbu, Rosario Domínguez-Petit, Dolores Garabana, Maria Korta, Maria Santos, Maria Kruger-Johnsen, Anders Thorsen, Thassya Dos Santos Schmidt, Maria Manuel Angélico, Kostas Ganias	**Improving egg production methods with new fecundity estimation methods.** Egg Production Methods (EPM) are a relatively simple way to estimate Spawning-stock biomass (SSB), estimate the number of eggs spawned and divide this with the number of eggs produced by a female (fecundity) and this give the female SSB. However, a correct egg production and estimation of the adult parameters is not so easy for many species. The estimation of fecundity involves many steps, with different analyses involved. Recent method developments have improved knowledge on reproductive biology and estimation of adult parameters. With Northeast Atlantic mackerel as a study case, this presentation aims to bring these together and show the impact for EPM.
S3_3	Thassya C. dos Santos Schmidt (speaker), Aril Slotte, Anders Thorsen, Olav Sigurd Kjesbu	**Is Northeast Atlantic mackerel spawning activity extending northwards?** Over the last 10 years, mackerel has extended the feeding migration north- and westwards, being widely distributed in the Nordic Sea during the feeding season. North- and westwards expansion of eggs density has been also recorded (north of 60°N and west of 15°W). Likewise, presence of young mackerel (age-classes 1 and 2) along the Norwegian coast has been recorded. The CLIMRATES project (Climate

		and vital rates of marine stocks) aims to investigate whether mackerel is spawning northerly of the main spawning grounds, also after the spawning season. To achieve this goal gonads have been collected in 2018 and this sampling program will continue in 2019, when the Triennial Mackerel Egg and Larvae Survey will take place. Therefore, the entire maturation cycle will be studied. The oocyte packing density theory will be applied to estimate fecundity, this technique has provided good results for both determinate and indeterminate spawners fish. Preliminary results have demonstrated increased presence of fish in spawning and spent condition from May to July (since 1984), suggesting that the northern areas are becoming more important as spawning areas under ocean warming.
S3_4	Cristina Nunes (speaker), Ana Maria Costa, Maria Manuel Angélico	**DEPM surveys from IPMA in ICES Division 9a – current methodology for daily fecundity estimation.** Daily Egg Production Method (DEPM) surveys are carried out by the Portuguese Institute (IPIMAR, IPMA) since the late 80´s. The methodology was first applied for SSB estimation of the Atlanto-Iberian Southern stock sardine (*Sardina pilchardus*), the area between the Northern Spain-Portugal border and Cape Gibraltar being surveyed triennially targeting this species, close to its peak spawning period (January, February). Subsequently, and following growing evidences that horse mackerel (*Trachurus trachurus*) in Atlantic Iberian waters (Southern stock) exhibit an indeterminate fecundity, the DEPM started also being applied to this resource in 2007, a triennial dedicated survey being conducted who covers the area from Cape Finisterra to Cape Gibraltar, also close to the species peak spawning period (January, February). This presentation aims at briefly presenting the methodology currently being applied at IPMA in terms of the DEPM adults "component", including survey design, samples laboratory processing, statistical analysis and daily fecundity estimation, taking into account the several developments already achieved and the challenges still to be addressed, aiming at improving the accuracy and precision of the estimates.

Annex 4: Sessions report

Notes on the presentations

Session 1

S1_1. Changes in the size structure of spawning cod in the Baltic Sea could have caused depensation - Andrei Makarchouk

Large female cod used to start spawning first and large males used to dominate the end of the spawning season. Large fish are removed by fisheries which must have led to skewed sex ratios.

Was shift only caused by fisheries or could there be other reasons. Some studies show that hydrological changes have also occurred.

Around 2000 in the Bornholm and Gdańsk also a change in the spawning time was observed, like in the Gotland basin. But what is the reason for the shift? There is probably a complex of environmental changes and fisheries. Baltic is getting lower salinity and this may cause a problem in producing fatty acids needed to finish the development of the eggs.

How quickly would changes in spawning period occur to be able to still survey the spawning season. When do you need to change the survey timing? Changes are gradual, but it is was a regime shift, so rather quickly. Cod spawning in the Baltic occurs over a large spawning season. This is a relative small area that can be sampled in a few days. In the Baltic various surveys are carried out over the spawning season that would pick up changes in the spawning season.

S1_2. Pilot survey on feasibility of establishing a sprat recruitment index by Bastian Huwer

Sprat stock size is driven by the recruiting year class, catches are mainly 1-year olds. Sprat is also an important food fish. Increase in recent years in sprat recruitment. Carry out a pilot in 2018 and 2019 to sample sprat larvae during the 3rd quarter IBTS using MIK and MIKeyM. Large numbers of sprat caught from small larvae to juveniles. Day versus night catches, 2 larvae at 18:30 when still light to 900 at 23:00 when it is dark.

Is the towing speed high enough for catching eggs with the MIKeyM? That should not be a problem as is shown during IBTS-MIK.

Is it possible to check GOV trawl catches for other clupeids to give information on possible other clupeid larvae? GOV is a bottom trawl, thus not a good sampling of pelagics. However, clupeid larvae will be identified to species.

Can the data be used for other species for recruitment index? Funding is always the problem for getting the data on other species.

S1_3. Improving herring assessment through new recruitment survey - Cindy van Damme

Current herring recruitment index only includes data on 3 of the 4 spawning component. The idea is to move one IHLS survey to April to create a Downs Recruitment Survey. This will provide recruitment information on the 4th spawning component.

There is material for line for rods that is not visible in water. Maybe this material could be used for the net.

How problematic is it that not the whole distribution area is covered. We do not know what we are missing, but the results seem to reflect the results from the other indices.

S1_5a. Application of ichthyoplankton survey to estimate stock trends of Eastern Baltic cod - Fritz Köster

Recent years there are problems with the Baltic cod assessment, and there has been no analytical assessment. Idea is to use the ichthyoplankton survey carried out in the Bornholm basin carried out in the last decades.

Eggs are also outside the correct water body. The question is what is the egg production, so the question is if the non-surviving eggs are also in the samples. The survival of the eggs is not a problem, it is the total egg production that is important. For recruitment the survival is a problem.

Relative fecundity seems to be high and might be a problem. Did the Örey study sample just prior to spawning or earlier when down regulation is still happening. This needs to be checked. The other problem is that the fish are very skinny, thus increasing the relative fecundity.

S1_5b. Identification of critical life stages of Eastern Baltic cod - Fritz Köster

Paulik-style analyses to estimate recruitment. There seems to be a relation between larval abundance and recruitment.

Egg production was averaged over two data points (surveys) to get early and later egg production. Was this compared to the supposed peak spawning period. Peak spawning the last years was in either June or July. Before it was May or even April.

Is there a solution to the ageing problem of Baltic cod? Daly increments and microchemistry is used for confirmation up to age 3. Tagging study is currently being carried out. Growth and natural mortality is necessary for a reliable assessment to validate the models.

S1_4. Assessment of the spawning time for the major North Sea fish stocks - Alejandro Mateos-Rivera and Bahar Mozfar.

Alejandro. Presented the IMR KINO2 project. In the first section he covered the molecular and taxonomic studies. Sampling is undertaken by the oil industry support vessels at three sites, close to oil platforms. Eggs and larvae are collected with WP2 nets with 500 micron, using vertical hauls. Samples split in two, formalin, alcohol. Currently they have 72 samples. Fewer samples in the northern area.

Molecular samples, PCR amplification, Sanger sequencing. Total 1457 eggs and larvae. So far 22 different species. Southern thirteen species, middle 17 spp, north 13spp. 290 not assigned. Of the larvae they have a total of 138.

Bahar – description of the taxonomic methods. 890 eggs and larvae. Northern part very few. Only fish larvae identified initially. 162 larvae, mainly *Limanda limanda* and *Scomber scombrus*. 208 eggs processed thus far. Pointed out the potential problems of undertaking visual identifications. Showed *M. merluccius* spawning in September. Covers some of the challenges etc. originally one sample per site per week, then asked for 2 samples per site per week. Weather conditions pose a problem for full sample coverage at all sites and each month. Discussed the pros and cons of the visual versus molecular methods.

S1_6. When (How?) things go right: Separating MIK-IBTS herring larvae by genetic testing presents a new method to improve stock abundance estimates – Bastian Huwer.

During the MIK survey the area close to Denmark was sampled by the Germans and the Danes a number of weeks apart. The size structure and abundance of larvae changes considerably between the two samplings. In the past otolith microstructure has been used to distinguish the Downs component in mixed catches of herring. Now there is a new genetic method that can distinguish between larvae originating from the Downs component of the North Sea Autumn spawning herring stock and the rest. This new technique indicated intermediate sized larvae were of the Downs component.

S1_9. Eggs and Larvae, checking and uploading files to the database – Carlos Pinto.

A presentation on the value of the egg and larvae database at ICES and improvements to the system. There was a discussion on the responsibilities for uploading data and data maintenance within the database.

S1_8. Added value of regular egg and acoustics surveys for pelagic ecosystem monitoring - MM Angelico

First describes the pelagic ecosystem surveys at IPMA:

- 2 egg production surveys (1 sardine, since 1988, not regular, every 2-3 years; 1 horse-mackerel, since 1995, every 3 years)

- acoustic surveys: spring (annual, 1995- present) + autumn (1997-2008 for recruitment, since 2013 back after sharp decrease in sardine recruitment but smaller coverage). Next week discussions will happen between IPMA and IEO to develop surveys for anchovy and sardine recruitment

All cruises done with RV Noruega, rather small vessel, rolls a lot sometimes which makes working difficult

There are things to be happy and unhappy about these IPMA pelagic ecosystem surveys:

- HAPPY: measurements on acoustic energy recording, pelagic and bottom trawling, CUFES, plankton hauls (calVET, Bongo, WP2, neuston), CTDF profiles, census birds/mammals, vessel activity register, litter monitoring

- UNHAPPY: acoustics of just 2 species; not all plankton sizes are monitored and phyto and zooplankton analysis are not regularly done; ID is only done to the main pelagic ichthyoplankton species; water current measurements not available; chemistry analysis are rare

There is a willingness to contribute towards ecosystem-integrated surveying, in the frame of the MSFD, WFD… For example, the survey contributes to several GES descriptors, e.g. D1 biological diversity. Maintain biodiversity through surveillance of ecological indicators (McQuatters-Gollop et al 2017), which tell us something about community structure and functioning. For this there are several methodologies available, they use imaging techniques. Last year they tested the Ifremer flow-through ZooScan system.

But there are some challenges to implement this surveys to comply with the MSFD:

- How to increase number of variables to sample in more cost-effective manner

- How to develop and implement new methodologies for sampling and processing/analysing

Specifically, there are some issues related to adding extra-sampling/new methodologies to regular egg and larval surveys. Maria mentions that getting money from MSFD in Portugal not easy, she asks how people are dealing with this in other countries.

As a conclusion, there are some pros and cons of this implementation of the survey:

- Pros: 1) improved and comprehensive set of data. 2) Some new variables may be useful in interpreting eggs and larval results and estimates

- Cons: 1) extra sampling very likely increases time and need of resources (at sea and lab). 2) need for adapting to complimentary methodological routines. 3) couldn't compromise some surveys primary objectives

Round of questions:

- Richard comments on the importance of adding new variables to the routine surveys, this issue has been discussed over and over again

- Matthias K points out ichthyoplankton sampling is already complementary to other surveys (e.g. MIK sampling during IBTS Q1). He emphasizes the need of highlighting the importance of doing ichtyo samples.

- Richard says that there are 4 ichthyo surveys added to other regular monitoring programs. He thinks that the MIK sampling is not an added extra to IBTS.

- Marta explains how they are selecting a priority set of stations during the regular surveys to take extra samples. Then Cindy highlights the problem is not getting the samples, but having the manpower to analyse them. Problem is MSFD is not providing funding

- Chris Loots mentions in France the MSFD funds technicians and support to do this "extra-work".

- Matthias says one can get funding in Germany, but then vessels are a problem. He explains that they wanted to get a spring survey in the German bight, and that it was not possible.

- Maria mentions that ideally surveys will be reviewed by the end of the year to see whether they have being improved. Then she suggests that at the same time that new surveys are planned, there is the need of making the most of the surveys they already have (with the stored samples). Idea of incorporating these discussion to the WGALES report, lots of information is available and all is needed for understanding the big picture. Richard mentions that there needs to be a clear plan: which variables we need and for which purpose!

S_10. Importance of flow measurements and calibrations in ichthyoplankton surveys - Cindy van Damme

Cindy explains the difference between the inflow of water inside a circular net (e.g. Bongo)vs.a nose-cone net (e.g. Gulf). Water speed in the nose cone center is higher, reduced inside in the outer section ◎ Importance of where you set your flowmeter. There are also differences in water speed to the distance from the entrance in gulf samplers: Nackthaivs.dutch Gulf samples. The flowmeter itself has an influence on the speed.

The advice is to put the flowmeter 1/3 to the top of the cone. Clogging is also influencing water speed inside the net.

Most of the people have an internal and external flowmeter. In Gulf VII net, external flowmeter set up in an external pipe for measuring clogging.

Conclusions:

- Modern flowmeters are robust, but position matters for the actual volume filtered
- Depending of flowmeter position, efficiency will vary
- Advice for gulf types, set your flowmeter 1/3 to top of cone, aligned with nosecone opening
- Position of flowmeters should be noted for international standards
- Calibrate your flowmeters regularly

Round of questions:

- Patrick asks whether anyone has experience with electronic Hydrobios propeller, as they have had several problems with it during the Baltic surveys.
- Cindy and Matthias say that they use it and are happy with it. They offer a real-time volume, but their electrical system (e.g. cables) are sensitive and need to be checked regularly.
- Matthias K says that they use a similar type in the Nackthai (as the ones you used in the Multinet), and that they can be very sensitive to disturbances

S1_11. Internal to external flow (efficiency) characteristics for MIK and Gulf VII samplers - Richard Nash

Introduction to the WISHINS8 (aka MIKey) net and how they came up on the idea on implementing the IBTS Q1 survey to sample eggs. Every country did it they own way: Ifremer, WMR, IMR. IMR uses only one of the nets, the other one is set without net, but with flowmeter (used as external flowmeter).

Dataset from July 2018 is shown: there is a variation in MIK "efficiency" (from 90 to 115%), e.g. lower values related to abudance of jellys. These are mechanical flowmeters, therefore there is no possibility to see whether the net is clogging. In January 2018, efficiency is ca. 95%, not as much variability as in July. He suggests that one should consider including an external flowmeter even for MIK nets.

Then data from the Gulf VII sampler (April/May 2018, norther NSea) are shown. Sometimes there are very big problems with clogging (efficiency ranging from 80% down to 20%), due to the spring blooms (phyto and zooplankton causing problems). With electronic flowmeters, one could get a vertical profile and see where problems start (again, these are mechanical flowmeters).

Take-home message:

- External flowmeters are important
- Even MIK samples need an indication of variability in efficiency (gear performance)

This will be discussed in the break-up groups. Therefore there are only a couple of comments:

- Richard: Vertical zooplankton nets done generally without any depth meters or flowmeters.

- Maria says they have experience with Calvet (double) nets in which one flowmeter has one reading, and the other one another.

S1_7. Considerations in the use of Multinet samplers for fish larvae - Richard Nash

Multinets can be used in horizontal or vertical mode, and they are easy to use. There are different sizes available (mini, midi, maxi, mammoth – first two with 5 nets, the other two with 9 nets). Works with zips. Nine nets, can be changed in 5-10min. Hard-core vs. soft-core ends. Can be used in oblique tows, or horizontal strata or vertical strata.

Multinets can provide fine-scale distributions: e.g. vertical variation in fish larvae or eggs, extremely relevant for particle tracking modelling. Example of vertical distribution day Vs night with Multinet. in "process stations" during Norwegian surveys (36h). Example of Differences across regions/water masses.

How can one compare filtered water volumes in Multinets and Gulf net? Filtration volumes of Multinet midi and Gulfs are shown: multinet 400m3, gulf 240 m3 in Shetland Process station, 300m3 Vs 133m3 in South Central process station (efficiencies lower in this station) ◎ avoidances possible in Gulfs compared to Multinet

Flow rates inside the Multinets are very stable. Example of flow ratio in Multinet mammoth ◎ more variable at the beginning when the net is not open going down. Once it opens, flow rates are very stable.

Open question: should we undertake more vertically stratified sampling?

Round of questions:

- Maik T. points out that vertically stratified sampling produces larger samples to check (more fish), for assessment suggests it is a lot of work. Multinet midi undersamples larvae in comparison with gulf net. Matthias K. says that depends on the larval size you want to address. He says that the Midi is easy to handle, but that the multinet tends to undersample upper layers. Richard says that the way they deal with this is by bringing the net open to the surface, get it out of the water, then close the net and bring it to ship

- Richard mentions that another issue is the height of the net for deploying and retrieving. Hazards for small vessels and high winds. Maik: also need to deploy from the back, but Richard says it can be towed from the side too.

- Andrei asks for a clarification on how the Multinet can be towed. Richard answer that it is generally used for oblique tows, but that it can also been used in horizontal tows

Session 2

S2_7. Effects of phenology shifts on herring (*Clupea harengus*) recruitment in the western Baltic Sea - Patrick Potte

This presentation spawning timing and climate change have been affecting herring recruitment. He started with a background section showing us the decline in the recruitment and spawning-stock biomass of Baltic herring, including ICES catch advice (zero

catch in 2019). Also, a background about the life cycle and annual migration pattern of Baltic herring. The objective was to investigate the effects of temperature (climate change) on larval physiology and metabolism. It was also showed that when the temperature reaches 4°C, the spawning activity starts to take place in the area. Winter period seemed to be the best proxy to estimate the recruitment success. In years, when the winter periods were longer, i.e. before the sea surface temperature reaches 4°C there were a better recruitment rate, and the inverse took place. Over the last years, the winter periods have been shorter, i.e. sea surface temperature has reached 4°C earlier, which may justify the lower recruitment of herring during this period. At the same time a strong mismatch effect has been observed since 2013; the density of Calanus has been low during the critical period for herring larvae. In conclusion, temperature triggers the initial spawning activity, and climate change can be correlated to the recruitment failure.

S2_8. Using short-term physiological measurements to explore long-term trends in recruitment variability: a case study Atlantic herring - Marta Moyano

During this presentation it was showed how laboratory experiments can be applicable in field studies to understand the larvae physiological responses to changes in temperature. The most important point was to determinate the optimum temperature for herring larvae. Herring egg and larvae stages have a narrower thermal tolerance window than juveniles and adults. During the laboratory experiment (cardiac performance), herring larvae were exposed to a constant increase of temperature to find the optimum temperature. The optimum temperature was at 16.4°C. It was also demonstrated that the optimum temperature was similar in different reared temperature. She also highlighted that this laboratory experiment is a quick process. After find the optimum temperature, the laboratory results were applied in field data. It was evaluated the effect of temperature on growth rate. The thermal tolerance window favorable for growth is between 5 and 17 °C. Above this temperature, larvae show signals of stress. The last point on this presentation was that over the last 5 years, the temperature being around 16°C recorded for 8 years consecutives has been earlier than before.

S2_2. Identification of fish eggs using proteomics - Matthias Kloppmann

A new method to identify fish egg larvae using molecular analysis was presented. The traditional method to identify larvae has many biases. The main problem related to the identification is the overlapping in morphological features and between egg size among different fish species. On the other hand, genetic identification, by using DNA barcoding is expensive to be applied. The method introduced was MALDI-TOF and it analyses the proteome; i.e. the protein present in the tissue. This method, already used in different organisms (e.g. Bacteria and metazoan), showed to be cheaper and faster than DNA barcoding. A subsample of winter spawners eggs (n = 298) were used in this method, and a library recorded was created for 6 species. The results showed that the success rate in identify species using this method was 99.6%. It was suggested that eggs of different species, especially spring spawners, could be sent to be identified using this method, so a better library records could be created and therefore, improving the egg identification. The disadvantages of this method are that eggs need to be preserved in ethanol 96%, therefore the eggs need to be staged fresh and pictures should be taken for future measurements. The advantages as mentioned before are that it is cheaper and faster method than DNA barcoding, and the high identification success rate achieved in the pilot study.

S2_3. Lipids components as proxy of plaice larval condition (*Pleuronectes platessa*) in the North Sea and English Channel - Carolina Giraldo.

The question about how the condition of the larvae can be said to be poor, the answer is that it is only a hypothesis, but suspects it is related to prey-abundance or prey quality. The criterium for staging the sole and place larvae is based on Christophe`s expertise. Stage 3 is categorized as post-flexion and stage 4 is based on fin development.
Questions about the how long time it takes before the zooplankton is reflected in the larvae (lipid), and the answer a few days.
The aim of adding the study of stable isotopes is to get a better understanding of the whole ecosystem. Mentioning other studies looking at cod and larvae, and the effect of acidification. The study showed that the stressed larvae got fat. This might be an idea to look at other factors in addition to lipids, like histology.

S2_4. Spatial and temporal variations of larval condition during the critical period of three fish species in the Eastern English Channel: an histological-based approach. - Julien Di Pane.

Comment: highlights the question that if you do a survey and collect a lot of samples: what does these numbers tell us? It is important what we do with this information. This study is an example of that.
The question of how long an analyse takes, and the answer is 6 hours, including many individuals at once. With training, 15 larvae in a day.
The pros of this method compared to other methods: less observatioer bias with this method (grade 1-6).

S2_5. Spatio-temporal variability of winter larval assemblages in the eastern English Channel and North Sea - Christophe Loots.

Unfavorable area sardine: earlier studies from 2017 show the sardine are there. This raise the question about the meaning of unfavorable area and definition of the term. Another question regarding the substrate and if the substrate differs from the areas. This might influence the flatfish found.
Discussing nursery ground, mentioning plaice and the stage (stage 5) of the fish found in the nursery ground.
MIK sampling mentioned: 55mm flatfish captured in the winter. Which is surprising.

S2_1. Recruitment dynamics of Norwegian spring-spawning herring: tracing the fate of early developing oocytes to the 0-group stage – Olav S. Kjesbu, Maik Tiedemann and Martina Stiasny.

Presentation:

The authors presented the undergoing project, RECNOR, which aims at studying the recruitment dynamics of NSS herring in climate changing environment, especially because recruitment has been systematically poor in the last years. A Paulik diagram was shown for the NSS herring, presenting the adults and early life production, and the different sources of information for the different life stages (surveys).

For fecundity, we have to include time-lag effects (y, y-3), there are 3 categories of PVO oocytes (S, M, L), with different characteristics, and if there is a lower PVO production

3 years before, it may have an influence in the fecundity 3 years later. Not considering the lag effect could lead to egg production overestimations. The formula to estimate fecundity should consider also the slope of the time variation of the condition K factor.

Important to consider not only the present fish condition but also to check the fish condition in the past and during the whole process, as a fish could start with a high oocyte production, and then with atresia, reduce fecundity.

There is an interannual variation in the time when the fish start developing oocytes, and in the last years herring are starting earlier. Another project will consider a 4-y experiment with herring in captivity with 2 groups of fish, with 2 different light cycles.

Martina will analyse a large set of data in terms of length and maturity for herring.

Norwecom.e2e is an environmental model, to be coupled to individual based models for fish and for zooplankton.

The whole area around Norway is used as spawning ground, but there are 3 main spawning grounds for NSSS herring. Juveniles stay for 2-3 y in the Kola area, then move with other adults to the feeding grounds, and then to the spawning grounds when they mature.

For many years, a positive correlation between SSB and temperature existed in Kola section, but no longer in the last years (increase SST but declining trend of SSB). Hypothesis: competition between herring and mackerel in the feeding grounds, due to temperature increase, mackerel having become more important in the area; and mackerel feeding on herring larvae in Norwegian waters.

In Møre, which is an important spawning ground, no larvae were observed in April.

During the survey, when moving south, larvae were much less abundant in MIK, but with the krill trawl, very large numbers of larvae were collected in the south. Acoustic transects: large number of small herring in the north, and mackerel present mostly in the south, which overlap with the herring larvae in the south in space but low overlap in time. Larvae in the 2nd spawning ground maybe come from a lens of oceanic water.

Again different selectivity in terms of larvae size between MIK and krill trawl, which one should be used for each life stage? Depending on the stations, there's a spatial variability in the size range of larvae, the same depending on the depth.

Fundamental studies to carry out:

- Predation of mackerel on herring postlarvae:

No visual observations of herring larvae in mackerel stomachs: effect of daytime, or larvae very quickly digested? More amount of herring gene copies in mackerel stomachs in the southern stations

- Effect of temperature on larval behavior and prey availability: experiments will be carried out

- a field drifting device with a camera and a GPS will be used to monitor how larvae orient in the field

Comments:

How was the experience with the krill trawls? It did not work very well, the crew did not work very well with it.

Predation by krill would be a more stable component, macrovertebrates predation may be more variable.

Any information on egg predation in the area? Not included in the objectives of the survey, but there is literature on the subject (haddock is one of the most predating fish on herring larvae).

When estimating biomass, no large differences exist when different stages are considered, so egg production was not an issue. NSSH is what is expected from egg production, not for the autumn spawning herring.

Specifications of the trawl used for the larger larvae: krill trawl designed primarily to catch krill, needs 1h30-2h to make a tow, less stations in the south, 44 m^2 opening but with wings that influence the number of larvae caught, as well as the mesh size.

Information for juveniles often biased because no specific device was built for them, the plankton and adult fishing equipment are both inefficient for juveniles, so for the larger sizes/later stages, we still need specific efficient devices.

Vertical distribution of the larvae: results seem not coherent, but data still need to be carefully analysed, because small larvae graphs were obtained with MIK, and large larvae graphs were obtained with the krill trawl.

Small scale spatial variability was investigated because the objective was to compare the larvae inside mackerel stomachs and the larvae found in the surrounding area. But we do not know from how far the mackerel are coming, and how long digestion takes place.

S2_6. Influence of global warming on vertical distribution of sprat eggs in the Eastern Baltic and its possible impact on recruitment success - Andrei Makarchouk.

The vertical distribution of sprat eggs in the water column is not uniform in the Baltic. The location of the eggs is determined by the buoyancy of the eggs and the density structure of the water column. Under climate change the density structure will change and this will have a consequence for the sprat eggs and their vertical location. There has been a change in the vertical distribution over the time period of sampling which has both been due to interannual variations but also a trend. Estimates of the changing proportions of eggs that would be in unfavourable conditions in the upper layers over time with global warming have been undertake, However, whether we have adequate buoyancy data for Baltic sprat eggs or even buoyancy data taken from the field is questioned.

S2_9. The death of Sebastes: Modelling natural mortality in the early life of an ovoviviparous fish - Hannes Höffle.

Natural mortality is an uncertainty in stock assessment as it is difficult to determine when clear predator-prey relationships are missing. Often the level is determined by expert judgement. In the case beaked redfish (*Sebastes mentella*) a level of 0.05 is used. Often the values refer to the whole life cycle yet we know natural mortality in the early life history stages is usually much higher than in adults. *S. mentella* is an ovoviviparous species that releases larvae after they have hatched. This may impart an advantage to the larvae and thus the natural mortality rate may not be as high as for the larval stages of other species An Andersen type trait-based model was explored, based on body size, to determine plausible level of natural mortality in the early life of beaked redfish. The results were compared to values estimated for other oviparous fish.

Session 3

S3_1. A joint effort across marine laboratories for improving spawning frequency estimations in fishes

Ganias K., Charitonidou K., dos Santos Schmidt T., Alix M., Mouchlianitis F. A., *Angélico M.M., Costa A. M., Domínguez-Petit R., Garabana D., Korta M., Krüger-Johnsen M., Nunes C., Santos M., van Damme C.J.G.,* ***Kjesbu O.S.***

Kjesbu/Introduction

This study is a large collaborative work on spawning dynamics originating from the 2016 WGALES meeting, when it was decided to develop more accurate as well as cost- and labour-efficient methods for the estimation of egg production. The work is based on ca. 600 samples of four species, sardine (*Sardina pilchardus*), mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*) and cod (*Gadus morhua*), with distinct fecundity types.

The markers can be either preceding or immediate. Whilst postovulatory follicles (POFs) are definite indicators of preceding spawning they are cost-intensive and are resorbed over time. Final oocyte maturation (FOM) provides immediate markers which are easy to see but, especially in small pelagics, are only available for a short time as duration of the final stages in egg development is low. The oocyte size frequency distribution (OSFD) is a marker for the spawning interval on the individual level and by extension for the spawning fraction in the population. However, as seen from experiments with cod, spawning intervals might be irregular.

Mouchlianitis/Making use of primary and early secondary oocytes in oocyte recruitment studies exemplified in Alosine spp.

Whilst early secondary growth (SG) oocytes afford an accurate estimation of total fecundity, primary growth (PG) oocytes are often ignored. However, in late or spent spawners their proliferation could give an indication of total fecundity in the next spawning season and therefore reduce sampling effort. Both, PGOs and early SGOs are small and highly translucent, requiring stereology and, often time-consuming, staining with dyes such as Eosin, PAS, Rose of Bengal or Toluidine blue.

The presented method has two innovations; i) separation with an ultrasonic pen rather than a stirrer and ii) the use of Harris hematoxylin which gives effective staining with a few drops, works quicker than other stains and requires no rinsing.

Estimates for number and size of PGOs as well as SGOs are improved using the modified method. Additionally, hematoxylin helps to identify the two modes in the size frequency distribution of PGOs for active spawners. Separation using the ultrasound device is improved but still not perfect.

Alix/Spawning dynamics in a determinate spawner, the cod

The formerly depleted stock of North East Arctic (NEA) cod recovered quickly. This was helped by increasing temperatures in its habitat, +1.5°C on the Kola transect between 1970 and 2012. However, spawners are still younger than they previously were

and these younger fish spawn later. Hence, the study aims to elucidate the demographic structure of spawning NEA cod in the Lofoten archipelago as well as gonadal development and spawning dynamics.

Fish were sampled from four commercial vessels and were on average around 10 years old. The fecundity of female fish was determined using the autodiametric method. They then were ranked into four categories based on the ratio (OR) between previtellogenic (PVOs) and mature oocytes:

OR<1	multiple cohorts
1<=OR<=3	2 cohorts
3<=OR<=15	1 cohort
15<OR	Late spawning or spent fish, few PVOs and no bell-shaped cohorts

As 18 of 76 females were in final oocyte maturation the spawning fraction (S) was calculated as 0.24 and the spawning interval (SI) as 4.2 days. Fulton's K exhibited a significant decline over the spawning season. An advantage of the oocyte ratio categories (ORCs) is that the spawning dynamics can be estimated from a single sampling.

dos Santos Schmidt/How can the combined use of whole-mount techniques and histology improve assessment of oocyte size modalities

This study on mackerel set out to create OSFDs based on whole-mounts and estimate thresholds between PVOs and vitellogenic oocytes (VTGs) on the indeterminate spawners sardine and mackerel. The R-packages mixtools and Shazam were used to separate cohorts and determine the thresholds between PVOs and VTGs, respectively. Thresholds were 170 μm for sardine and 196 μm for mackerel. Mixtools offers the option to manually assign cohorts and gives results that fit well with those from histology. However, with many cohorts some of them can overlap. Shazam results fit well with literature values but is time consuming and needs selection of the correct statistical distribution to work.

Charitonidou/A stereological validation of the post-ovulatory follicles method in species with different values of spawning frequency.

As a population parameter spawning frequency can be determined as the %age of mature females with daily cohorts of postovulatory follicles using the POF method. The method requires the occurrence of one single daily POFs-cohort and is therefore difficult to apply to a species with high spawning frequency and slow resorption rate. In this study, sardine with low spawning frequency serves as control, whilst high-frequency spawning mackerel was assessed for the co-occurring cohorts. Stereology was done using a photo mosaic in Image J.
Results indicate that POFs in sardine first increase in size, then shrink. In mackerel, it is the opposite. Resorption in sardine is finished before the end of the spawning interval, whilst in mackerel POFs-cohorts overlap. This implies that for sardine spawning frequency can be established as population parameter through S and for mackerel as individual parameter through SI.

Ganias/How spawning rhythm is reflected to ovarian dynamics? Drawing evidence from two fishes with contrasting spawning frequencies.

Sardine is a clear batch spawner with distinct modes in the OSFD and the spawning batch can be distinguished by the mid-vitellogenic stage. In mackerel, it is only noticeable at the very end of the oocyte development. Batch fecundity for mackerel can thus only be determined for the rare individuals that exhibit a clear hiatus in the OSFD. The fecundity ratio (R_{FEC}) as the ratio between advanced oocytes and SG oocytes is around 5 for sardine and around 34 for mackerel. Whilst the 4-5 batches in sardine can be clearly distinguished, the >30 batches in mackerel can no longer be distinguished and produce a continuous size spectrum. Spawning interval can be calculated as the ratio between oocyte growth period (OGP) and R_{FEC}. SI for sardine is around 10 days and for mackerel 4-5, resulting in S of 0.1 and 0.23, respectively.

Concluding the whole talk series, it is shown that the POF-method is useful for some species but problematic for others and is in all cases cost- and labour-intensive. Imminent spawning markers appear very useful for some species like cod but limited for small pelagics. Finally, estimating the individual based SI rather than the population based S offers advantages for high-frequency spawning species.

S3_2. Improving egg production methods with new fecundity estimation methods – Cindy J.G. van Damme

The mackerel assessment is partially based on the triennial mackerel egg survey, which provides SSB derived from total egg production calculated with the annual egg production method (AEPM) as a relative index. The assessment group aims to compare AEPM with the daily egg production method (DEPM). The latter requires the daily egg production, the spawning area, batch fecundity and spawning fraction as parameters. Of these some are problematic in the context of Atlantic mackerel. The large spawning area makes a full coverage difficult, although the main area is covered. The ongoing studies on spawning frequency revealed that estimating batch fecundity from early oocyte stages may be less accurate than assumed. The same studies brought an improvement in estimating spawning fraction though. Another trial for DEPM will be conducted in 2019.

S3_3. Is Northeastern Atlantic mackerel spawning activity extending northwards? – Thassya C. dos Santos Schmidt

This study is sourcing data from historical biological data (1984-2017), triennial egg and larvae surveys (2001, 2007-2016) and fecundity data collected from May 2018 to June 2019. Atlantic mackerel has been shown to conduct increasingly more northward feeding migrations in recent years, with current peak catches in the northern Norwegian Sea. Similarly, the spawning area has extended further north and west but with low density frequencies there. Mackerel has been shown to experience reduced growth at high population densities and a large herring stock. Examination of the fecundity samples taken up to October 2018 show peak spawning in May, fitting the long-term trend. Whilst there is strong indication for some spawning in the North, using only maturity stages is too biased for a clear conclusion. Therefore, future work will include histology and whole-mount analysis and application of the OPD (oocyte packing density) method that provides fecundity estimates for each oocyte stage from the previtellogenic to the end of the maturation cycle.

S3_4. DEPM surveys and reproductive behavior of horse mackerel (*Trachurus trachurus*) from the southern stock (Atlantic Iberia, ICES 9a) – Cristina Nunes

Part of the assessment of the southern stock of horse mackerel, reaching from cape Trafalgar to cape Finisterre, is a triennial DEPM survey, conducted since 2007. Horse mackerel is a pelagic, indeterminate batch spawner, maturing at ages 2 and 3 and primarily reproducing in the first semester. This study was to examine the reproductive behaviour of horse mackerel in the context of DEPM, focusing on temporal and spatial patterns based on 14 surveys from 1998-2017 and histological data from adult fish from the three most recent DEPM surveys and monthly market sampling in 2014.

Results indicate that daily spawning is centred between 17:00 and 19:00 which is well correlated with a build-up of hydrated follicles during the day and an increase in sex ratio up to around 15:00. Seasonally, main spawning occurs from Dec./Jan. to May/Jun. but active spawners can be found throughout the year. All reproductive parameters decreased significantly between the surveys in 2013 and 2016. Potentially this is due to the large proportion (~25%) of immature females in 2016 and a poor correlation between survey and spawning activity. Interannually, abundance as well as spatial distribution of eggs exhibit large variability which is reflected in the wide range of temperatures at which eggs are found (12.5-18.5°C). Concerning depth, spawning mostly occurs over the outer shelf and the shelf break with a median bottom depth of 105 m. Active spawners exhibit a wider depth range than inactive ones and immature fish are deeper in the water column. However, there was no apparent relationship between sex ratio and bottom depth.

Annex 5: New draft for WGALES terms of reference

Working group meeting draft resolution for multi-annual ToRs (Category 2)

The **Working Group on Atlantic Larval and Egg Surveys (WGALES)** chaired by Patrick Polte, Germany, Richard D.M. Nash, Norway (to be replaced in 2020), and Cristina Nunes, Portugal (from 2020) 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	19–23 October 2020	Boulogne, France	Interim report by 15 December 2019	Richard D.M. Nash replaced by Cristina Nunes
Year 2021		By correspondence		
Year 2022	October	TBD	Final report by 15 December	

ToR descriptors[1]

ToR	Description	Background	Science plan codes	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	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 in-	1.4, 3.2, 4.4	year 2, 4	

[1] Avoid generic terms such as "Discuss" or "Consider". Aim at drafting specific and clear ToR, the delivery of which can be assessed

	for data users to gain insights into the rationale, methodology and potential applications of fish early life stage ecology (and adult fish maturity) research.	sights for these surveys.			
c	Present and report on reproductive dynamics and fish early life strategies relevant for ichthyoplankton surveys	Successful surveys are dependent on understanding the life-history dynamics of the target organisms and understanding how this may change with ecosystem vaiability and change.	1.7, 2.2, 3.2	year 2, 4	
d	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 (WGEGGS2).	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	
e	Provide a standardized framework for ichthyoplankton data bases and facilitate implementation of new survey data into the ICES egg and larvae data base 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

Year 1	**WGALES will communicate by correspondence to act upon urgent ToR's from ichthyoplankton survey groups (ToR d)**
Year 2	WGALES will meet to address ToRs a, b, c, d, e, f
Year 3	WGALES will communicate by correspondence to act upon urgent ToR's from ichthyoplankton survey groups (ToR d)
Year 4	WGALES will meet to address ToRs a, b, c, d, f
	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.

Supporting information

Priority	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. WGALES fits into the ICES science plan sections ??
Resource requirements	The research programmes which provide the main input to this group are already underway, and resources are already committed.
Participants	The Group will be attended by members of ICES groups, WGMEGS, WGEGGS2/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.
Secretariat facilities	None
Financial	No financial implications.
Linkages to ACOM and groups under ACOM	There are linkages with the advisory committees through the individual ichthyoplankton surveys groups which are associated with WGALES and their assessment groups that use plankton data. Through the review and standardization of the ichthyoplankton surveys the quality of the data for the assessments is ensured.
Linkages to other committees or groups	There is a close working relationship with the all the ICES expert groups of ichthyoplankton surveys, WGMEGS, WGEGGS2/WGSINS, IBTSWG, WGACEGG and their assessment groups, WGWIDE, HAWG, WGHANSA.
Linkages to other organizations	No formal linkages.

Annex 6: Copy of Working Group self-evaluation

Working Group on Atlantic Larvae and Egg Surveys (WGALES)

2013.

Current Chairs: Maria Manuel Angélico and Richard D.M. Nash.

Venues, dates and number of participants per meeting.

- 1-5th December 2014, San Sebastian, Spain, (27 participants)
- 17-21st October 2016, Thessaloniki, Greece, (22 participants)
- 22-26th October 2018, Copenhagen/Lyngby, Denmark, (28 participants)

WG Evaluation

If applicable, please indicate the research priorities (and sub priorities) of the Science Plan to which the WG make a significant contribution.

Integrated Ecosystem Observation and Monitoring Programme (EOSG)

In bullet form, list the main outcomes and achievements of the WG since their last evaluation. Outcomes including publications, advisory products, modelling outputs, methodological developments, etc. *

- As an umbrella WG much of the output is also listed under the associated WGs e.g. WGMEGS, WGEGGS2/WGSINS, IBTSWG, WGACEGG and their assessment groups, WGWIDE, HAWG, WGHANSA.
- Numerous posters and presentations to a wide range of meetings including ICES ASCs, Annual Larval Fish Conference of the AFS, International Flatfish Symposia on ichthyoplankton ecology, survey designs etc.
- A number of scientific papers e.g. Höffle et al. 2018 on North Sea fish egg distributions (Can. J. Fish Aquat. Sci 75(3): 357–374), stemming from PGEGGS/WGEGGS/WGEGGS2
- Collaborative work on spawning frequency as a methodological development, first results presented in 2018, ongoing work

Has the WG contributed to Advisory needs? If so, please list when, to whom, and what was the essence of the advice.

Yes, Through the various ichthyoplankton WGs e.g. IBTS (MIK to HAWG), WGIPS (IHLS to HAWG) etc.

Please list any specific outreach activities of the WG outside the ICES network (unless listed in question 6). For example, EC projects directly emanating from the WG discussions, representation of the WG in meetings of outside organizations, contributions to other agencies' activities.

See e.g. WGEGGS2/WGSINS.

Please indicate what difficulties, if any, have been encountered in achieving the workplan.

Funding for participants, especially experts, when specialist information is needed for discussions.

Future plans

The group thinks that there is a need for a continuation of the WG beyond its current term. This is because this WG provides an umbrella and a common forum for all the ICES WGs which undertake ichthyoplankton surveys. This WG provides a platform for discussion of general aspects of ichthyoplankton groups so that the more specialised WGs can discuss and learn from the experiences gained in other surveys. The WG also provides a forum for evaluating and providing advice to Assessment Working Groups on the efficacy of the ichthyoplankton surveys as input for assessments.

The Working Group is generally able to draw on the expertise of scientists working on ichthyoplankton surveys and more general research from within the European ICES area. It would useful to be able to bring in expertise from the US and Canada specifically but also to encourage more involvement from countries further afield e.g. Australasia.

The conclusions/or knowledge acquired by the WG that should be used in the Advisory process are:

1. Refined survey protocols and additional survey information for accreditation of surveys (validity of data).

Variability in early life history dynamics. The contribution of environmental variability versus exploitation and the effects on stock productivity.