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3 **Method Development and Evaluation of Stock Reproductive Potential** 4 **of Marine Fish**

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22 **1. Introduction**

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All 13 research articles in this special issue of Fisheries Research are the consequence of work undertaken within the 3-year EU 5th Framework project *Reproduction and Stock Evaluation for Recovery* (RASER; contract n^o: Q5RS-2002-01825; <http://raser.imr.no>) with the following institutional partners: IMR, Bergen, Norway; AZTI-Tecnalia, Basque Country, Spain; IIM-CSIC, Vigo, Spain; and Cefas, Lowestoft, England (Fig. 1). In addition to these articles, two articles are in print elsewhere (Domínguez-Petit et al., 2008; Witthames et al., 2009) (Fig. 1), whilst parts of the Final Report to the EU Commission (Kjesbu et al., 2006) not featured in this monograph are work in progress.

The overall objective of the RASER project was to introduce more effective tools for studies of individual reproductive investment in marine fish for less uncertainty in estimating Stock Reproductive Potential (SRP) to be used either in recruitment studies or in egg production methods. More specifically, the three main topic areas were:

1. Develop methodology to reduce costs of analysis and increase the accuracy and precision in estimating realised fecundity, i.e., by automation through image analysis and less reliance on histology;
2. Assessment of reproductive investment across latitudinal range;
3. Synopsis, including:
 - a. Assess the geographic variation in size and age at maturity, fecundity and spawning activity in relation to the observed variation in environmental conditions including fishing pressure;
 - b. Incorporate new experimentally proven data on atretic and post-ovulatory follicle (POF) duration into previous, relevant International Council for the Exploration of the Sea (ICES) egg production based stock assessment to

determine the potential bias in the quantification of fecundity and, thus, in the final calculation of spawning stock biomass (SSB);

c. Evaluate the impact on assessment and management of including models of more realistic variations in SRP.

The key candidate species for evaluation were the two gadoids Atlantic cod (*Gadus morhua*) and European hake (*Merluccius merluccius*). Supplementary studies were undertaken on the clupeoid Atlantic herring (*Clupea harengus*). Thus, this monograph deals with three highly important species, both commercially and ecologically. In combination these species demonstrate a broad range in reproductive strategies leading to different levels of complexity (and thereby challenge) in terms of fecundity quantification: from the ‘simple’ herring, via the ‘semi-intricate’ cod to the ‘highly intricate hake’ (Table 1). Here the concept of ‘indeterminacy’ refers to continuous recruitment of developing follicles (oocytes) during the spawning season while ‘determinacy’ refers to no further recruitment well before the start of spawning (Murua et al., 2003; Kjesbu, 2009). Hence, oocyte counts prior to spawning give a good impression of the maximum fecundity value subject to further down-regulation by atresia (oocyte resorption) in determinate spawners but represent a clear underestimate in indeterminate spawners.

Studies on stock-specific differences in reproductive traits stood out as most central in the project. For hake this was based on a comparison between Northern (Bay of Biscay) and Southern hake (off Galicia), or, more formally speaking, hake stocks in this region separated geographically by the Cape Breton Canyon (Korta et al., 2010a). In the case of cod this evaluation was achieved by spreading the sampling programme from the Irish

Sea and the North Sea in the south (southern population) to the Barents Sea in the north (northern population), i.e., contrasting Irish Sea, North Sea and Northeast Arctic cod (Nash et al., 2010). Additional data on Icelandic cod was successfully accessed to further broaden the picture (Thorsen et al., 2010). However, only one stock of Atlantic herring is included in this monograph, i.e., the Norwegian spring-spawning herring (Ndjaula et al., 2010), one of the largest fish stock in the world. Subsequent research has been undertaken on North Sea herring, concluding that autumn and winter spawners, in theory, can switch reproductive strategy (produce high fecundity and small eggs and *vica versa*) (van Damme et al., 2009).

The SSB of southern cod populations as well as for the European hake are clearly below a sustainable level (although the Northern hake shows recent signs of recovery), while both the Northeast Arctic cod and the Norwegian spring-spawning herring today appear to be successfully managed in compliance with the agreed harvest control rules (for more details see the various ICES Expert Group reports; <http://www.ices.dk>). Spawning stock recovery plans are in place but the reproductive biology of these stocks (as well as for many others) is rather sparse to appreciate how stock size will likely impact on recruitment variability and recovery. Note here that ‘recovery’ refers to increase in biomass only but ‘rebuilding’ to restoring of the full diversity of population characteristics (including genetic and behavioural traits) (S. Murawski, National Marine Fisheries Service, Maryland, USA; Opening address, ICES UNCOVER Symposium 2009, <http://www.uncover.eu>) and thereby has a much longer time horizon (Enberg et al., 2009).

Although some field data on the reproductive investment of the presently evaluated species exist (e.g. herring: Óskarsson et al., 2002; hake: Murua and Motos, 2006; Murua et al., 2006), and more so for cod (see Thorsen et al., 2006), no thorough estimates have been made using an inter-calibrated method over a wide geographic range inhabited by each species. In ‘lean species’ like cod and hake, there is still uncertainty about how annual cycles in hepatic condition or past body growth relate to variability in realised fecundity in wild stocks. For hake growth analyses are complicated by inaccurate age reading (see Mellon-Duval et al., 2010 and references therein). However liver lipid energy has been shown to be an useful indicator of egg production in cod (Marshall et al., 1999) and so it may be possible to assess reproductive investment directly in this capital breeder from maternal or paternal weight or condition indices. Conversely, in the continuously feeding hake, other body constituents (proteins, lipids and glycogen) may give a better picture of spawning status in addition to measuring the standing stock of fecundity and spawning frequency. Said in another way, fish condition before spawning is expected to correlate better with egg production in capital breeders than in income breeders. Prior to this project there was sparse information for either of these two piscivorous species on the dynamics of either fecundity proliferation or down-regulation through follicular atresia with respect to body reserves or previous growth history. The same shortage of insight also applied to the zooplantivorous herring, where, however, the fat is stored in the muscle instead of in the liver, supporting the extreme long non-feeding period (‘overwintering’).

Several methods to estimate fecundity and spawning fraction (and thereby spawning frequency) already exist in the primary literature (Hunter and Macewicz, 1985; Hunter et

al., 1989; Murua et al., 2003), but they are labour intensive. The high costs of these methods have not been conducive to assessing reproductive potential in populations where high numbers must be analysed to account for natural variation.

The relationship between SSB and recruitment generally is impaired at low stock size, which in turn makes the expected recovery from low SSB to be more difficult to predict (Shelton et al., 2006; Morgan et al., 2009). Consequently a key issue to any recovery plan is to understand the changes and the underlying mechanism of egg production dynamics of depleted stocks. In addition, SSB is often incorrectly assumed to be proportional to egg production (Marshall et al., 1998). SSB is used to set reference points to regulate fishing mortality and forecast stock recovery trajectories (Kell et al., 2005a; Hauge et al., 2007). The estimation of SSB, however, rarely includes stock demographic or reproductive biology information which have been demonstrate to affect the fecundity and spawning success of the population (Cardinale and Arrhenius, 2000; Marteinsdottir and Thorarinsson, 1998; Morgan et al., 2009). Moreover, recruitment of cod has been shown to be sensitive to climate change (Clark et al., 2003) and, therefore, the impact of climate change on the success of stock recovery or rebuilding needs to be considered (Kell et al., 2005a,b) using, for example, Management Strategy Evaluations (MSE) tools.

2. Advancement in studies on stock reproductive potential

2.1. Development of methods to assess fecundity and spawning rates

A range of different laboratory methods were applied, producing different ways to interpret the basic unit of fecundity, exemplified in Fig. 2. This section concerns further developments of these methods or others for use in quantification of fecundity and spawning activity. PAS (Periodic acid-Schiff reaction) and Rose Bengal stains were applied to improve automatic fecundity assessment in whole mounts so that image analysis can be applied to a range of species (Witthames et al., 2009). We were able to demonstrate that the relationship between oocyte packing density (OPD; number of oocytes per gram ovary) and mean oocyte diameter (OD) was not significantly different in cod, herring, mackerel (*Scomber scombrus*), redfish (*Sebastes marinus* and *S. mentella*) and plaice (*Pleuronectes platessa*) so that data from each partner's laboratory fell on the same trend line. The latter provides the basis for a multi-species calibration to apply the auto-diametric method (Thorsen and Kjesbu, 2001) to any species where the fecundity is determinate and minimum oocyte size is larger than 185 μm (Witthames et al., 2009). Hake was somewhat problematic in this respect and more evaluation was needed for this species. However, the combination of stereological methods (to estimate volume fractions) and advanced OPD theory made it possible to successfully 'tighten up' the hake OPD-OD relationship (Korta et al., 2010b) based on oocyte stage-specific refinements of algorithms developed by Kurita and Kjesbu (2009). It is foreseen that the next generation of these types of OPD formulae can make possible predictions of the total annual fecundity production in indeterminate spawners as done today for determinate spawners. If so, the practical use of this type of new basic data should be considered. Nonetheless, Korta et al. (2010b) estimated that there are in the order of 500 000 early

previtellogenic oocytes per gram of hake ovary, reflecting an extreme baseline production.

A original method to collect fecundity samples from the ovary was also developed based on a solid displacement pipette using very small quantities of fixative (< 1ml) to preserve the sample (Witthames et al., 2009). This provides important benefits by reducing the environmental impact of fecundity sampling, exposure to carcinogenic chemicals (formaldehyde) and compatible for use on commercial vessels or for sending replicates by courier overseas. A related goal was to develop recognition criteria so that dispersed oocytes in a petri dish, taken in a gravimetric sample, can be used to identify and quantify atretic oocytes and POFs without the need to use expensive histology. Furthermore, shortcomings of the traditional histological profile method when used to quantify atresia were addressed (Kjesbu et al., 2010a). As a consequence of this unbiased calibration technique, labeled the stereo-profile method, reliable data on atretic intensities can now be quickly collated. Also, information from image analysis of oocyte distribution along with measurements of body reserves of proteins and lipids were used to develop statistical conclusion of maturity of indeterminate species (hake) using principle component analysis (Domínguez-Petit et al., 2010).

Experimental studies on ‘biopsied’ (ovarian catheterised) cod at different temperatures made it possible to present figures on the accuracy of the duration of the so-called alpha stage, i.e., vitellogenic oocytes undergoing resorption, although the high variance was somewhat disappointing (Witthames et al., 2010). These results emphasized once more that the atretic cells break down quickly, i.e., within a week or so. POFs required some assumptions to link age following spawning to size or morphology (Witthames et al.,

2010), but 3-D reconstruction and examination of whole mounts indicated there was a rapid size reduction following ovulation (Korta et al., 2010c). More work needs to be done but this observation offers a more robust approach to age POFs. Unexpectedly, POFs were shown experimentally to persist after the cessation of spawning in a Barents Sea simulation for more than four months or in a north summer warming simulation for more than three months providing a useful tool to hindcast previous spawning activity (Witthames et al., 2010). The latter observation has important application to identify skipped spawning (Skjæraasen et al., 2009).

2.2. *Herring reproductive biology*

The included study on Norwegian-spring spawning herring investigated how best we can model and predict Total Egg Production (TEP) with respect to available information on population abundance, population structure and individual's condition factor using extensive time series (1935 to 2005) (Ndjaula et al., 2010). The population dynamics of Norwegian spring-spawning herring are well documented but little has been done on what role these could have played on the productivity of the stock. This study used fecundity models from both relatively recent and historical data and found that fish in different conditions of body mass to body length result in different TEP which, in turn, alter the perception of stock productivity. The results indicate that in general, the time-series of TEP estimated using the recent fecundity length and weight model agreed well with the available published historical data, using annually varying fecundities over the period 1951 to 1983. Deviations from a linear relationship between the different TEPs

occurred more at times of high TEP production, which corresponds to times of high stock abundance. The relationships between TEP and larvae production estimate suggests a disproportionately higher larvae production with an increase in TEP. The observed agreement between TEP estimate and those of historical annual estimates shows the importance of the population structure and condition factor dynamics in the assessment for SRP.

2.3. Cod reproductive biology

These works concentrated on reproductive traits of the individual female cod based on sampling programmes undertaken in the Irish Sea, North Sea and Barents Sea, as well as tank experiments. Several new approaches were taken when addressing the different methodological problems. This included accurate standardisation of maturity stage by using oocyte diameter (OD) data from image analysis (Thorsen et al., 2010), and the Disector method, i.e., stereology, to accurately report atresia levels (Kjesbu et al., 2010a). For North Sea, Icelandic and Northeast Arctic cod we demonstrated a significant reduction in relative potential fecundity as the fish approached spawning, i.e., when OD increased. This could not be properly tested on Irish Sea cod due to a narrow OD range. In the case of Northeast Arctic cod we found levels of atresia that could fully comply with this decrease in fecundity (Kjesbu et al., 2010a). This down-regulation was preceded by an accumulation of developing oocytes, forming all together a dome-shaped fecundity production curve over time, following tracking of the stereometric and histomorphic development of different oocyte stages and phases in captive specimens (Kjesbu et al., in

press) supported by field studies indicating the same pattern (Skjæraasen et al., in press). In the estimation of fecundity we found that the predictive power (r^2) of explanatory variables (such as body weight) varied seasonally but peaked in late vitellogenesis (Kjesbu et al., in press), which to some degree deviates from results seen in Skjæraasen et al. (2006) pointing instead at early vitellogenesis. Complementary studies have demonstrated that these trends in predictive power are partly influenced by environmental temperature (Kjesbu et al., 2010b). To make a fully unbiased comparison of the potential fecundity in time and space we constructed models that included OD as one of the explanatory variables. Our results clearly indicated a north-south gradient with increasing relative fecundity towards the south indicating a true difference in reproductive investment (Thorsen et al., 2010). The higher investment of the fish in the south could only partly be explained by the noted higher condition. Furthermore, the maturation schedules of Irish, North Sea and Northeast Arctic cod were extensively analysed clarifying that i) there have been major drops in the age and length at maturity over time and ii) Irish Sea cod mature extremely early (typically at 2 years) followed by North Sea cod (typically at 3-4 years) and then, following a jump upwards, to Northeast Arctic cod (typically around 8 years) (Nash et al., 2010). The length-at-age-at-maturity trajectories differed between the three stocks indicating fundamental differences in phenotypic plasticity. Taken together, the various models developed for cod during the RASER project have in effect been run successfully to quantify the dynamics of oocyte recruitment and maturity reaction norms from basic information on fish length, condition, sampling time and origin (geographical area).

2.4. Hake reproductive biology

Here the focus was devoted on how to obtain reliable estimates of realised fecundity, daily egg production rates and age at maturity in the Bay of Biscay and Galician Shelf populations (Korta et al., 2010a). Relative batch fecundity (BF_{rel}) showed significant differences among years in both areas, indicating differences in productivity. Standardisation by month was required because BF_{rel} varied largely throughout the year. Both the intra- and inter-annual variation in BF_{rel} might be due to an overall fish condition effect (Kraus et al., 2000; Somarakis et al., 2005) but, more likely, an immediate response to variability in amount of prey at or near the spawning grounds in this income breeder (Domínguez-Petit and Saborido-Rey, 2010). A strong positive relationship appeared between BF_{rel} and gonadosomatic index (GSI). GSI as well as the total relative number of developing oocytes (NDO_{rel}) followed the same trend as BF_{rel} , although the overall pattern differed in the two areas of study (Korta et al., 2010a; Domínguez-Petit and Saborido-Rey, 2010). NDO_{rel} did not vary with maturity stage, corroborating that the fecundity of European hake is indeterminate (Murua and Motos, 2006; Murua et al., 2006; Korta et al. 2010a). Based upon the results on spawning fraction, the European hake spawns once every 5-7 days during January and March in both areas but once every 10 days or more in the rest of the year (Korta et al., 2010a;). During the first quarter larger females showed higher spawning activity and shorter batch interval than smaller females (Mehault et al., 2010). Egg quality, in terms of diameter and dry mass, increased significantly with female size (Mehault et al., 2010).

Relative batch fecundities in conjunction with spawning fractions were used to calculate population estimates of relative daily egg production. In both areas the maximum production appeared in January-March and was mainly driven by high fecund fish spawning frequently. Likewise, the subsequent decrease in daily egg production was due to a simultaneous drop in both factors. These findings were in agreement with ichthyoplankton data; the peak of pelagic egg abundance occurs in March in the Bay of Biscay (Álvarez et al., 2001).

Total Egg Production (TEP) for southern stock showed a clear decreasing trend in the period 1982-2007 influenced by a fall in mature female population biomass (FSSB). Simultaneously, the TEP/FSSB ratio also dropped related to a higher proportion of younger females (Mehault et al., 2010).

Proteins, lipids, fatty acid, glycogen, water and ashes content were determined in liver, muscle and gonad (Domínguez-Petit and Saborido-Rey, 2010; Domínguez-Petit et al., 2010). Relationships between these components and somatic variables (length, weight, GSI, liver index and Fulton's K) were established but, although some significant relationships were established, their low explanatory power complicated meaningful biological interpretations.

In summary, hake show a protracted spawning season; spawning females were detected all the year around. Obvious differences in the level of egg production, depending both upon the month of the year and the year itself, were found.

2.5. New findings of relevance to assessment and management advice

Whilst stock-recruitment relationships were originally developed using population fecundity to recruitment (Ricker, 1954; Beverton and Holt, 1957; Shepherd, 1982), SSB has generally been used as a proxy for population fecundity or total egg production. However, as said before, various demographic and reproductive biology characteristics indicate the assumption of direct proportionality between SSB and total egg production may be not correct. In this context, we (Kjesbu et al., 2006; Domínguez et al, 2008; Mehault et al., 2010; Murua et al., 2010a; Rijnsdorp et al., in press) examined the physical patterns affecting egg production, and relevant covariates to suggest causal mechanisms, and considered the potential impact of incorporating more realistic reproductive characteristics in the egg production estimation in the ICES working group assessment; where SSB is generally estimated based on fixed maturity ogives without including sex ratios or variation in fecundity. The following text gives a brief overview of the findings in the above-mentioned RASER final report to the EC and the resulting, relevant articles published so far.

Microscopic maturation and egg production estimates were obtained for the species of interest using a range of available data. These approaches are expected to provide more precise analyses of spawning condition and reproduction than the macroscopic analyses usually carried out. Variations in reproductive parameters were assessed for cod and hake stocks. Standardised North Sea cod data indicated a north-westward shift in spawning population from the 1970s to the present. This shift was significantly correlated with the reduction in spawning stock biomass. For hake, batch fecundity did not differ significantly between stocks and for both stocks spawning fraction was highest around February (see above).

In the project, differences, among others, arose from the impacts of improved information on trends and variation in maturity ogives, particularly where ICES working groups used constant ogives over time. Since management advice is largely based upon the relative values of spawning stock estimates and limit/precautionary biomass reference points (BRP), it is expected that this will directly affect the assessment results and, thus, this area requires further investigation. As such, the inclusion of improved biology and fecundity information is expected to improve the fit of stock-recruit models to the data. However, in the case of Northern hake there were no clear differences in the S/R model fit when alternative reproductive potential indices were used showing that alternative indices did not significantly improve the S/R relationships. Those findings are similar to those of Marshall et al. (2006) and Morgan (2008), whilst are not in agreement with Kraus et al. (2002) and Murawski et al. (2001) who demonstrated an improvement in the S/R relationship when more biological information was included.

For Northeast Arctic cod, deviations from the ICES working group fit were notable at high spawning stock biomass levels, in part due to the impact of increased egg production at higher SSB levels. However, stock-recruitment relationships did not differ significantly and improvements were marginal. As a result of the similarity between the ICES working group and egg production based Ricker models, reference points were generally comparable – certainly the values were close enough for differences to be lost in the noise of assessment uncertainties. The exception was F_{MSY} , which was notably higher for all stocks where fecundity was taken into account. While equilibrium SSB/yield curves were robust to the stock-recruitment models used, the position of BRP on these curves varied considerably. The inclusion of improved biological information changed the perceived

productivity of the stock in all cases. Where increased biological knowledge was included, a reference point level equated to higher SSB levels, implying lower relative yields than the ICES working group case for a given fishing mortality. Current reference points may therefore be optimistic in their assumption of the resulting impact of exploitation. Similarity in fishing mortality reference point values implies they are robust to the underlying biology at equilibrium. However, ICES advice does not explicitly incorporate important dynamic changes in biological processes that may affect limit reference points, such as decreases in maturity-at-age or age structure, as TEP of hake was shown to decrease with the rejuvenation of the SSB. Nor does it explicitly consider carrying capacity and productivity. As recovery occurs, reference points will change, which is not acknowledged within the current assessment and management process. Examination of stock recovery impacts for North Sea cod indicated that the perception of the ICES working group ('standard' SSB-based Ricker stock-recruitment curve assumed), and dynamics of the underlying population (recruitment based upon female-only spawning stock biomass and including fecundity considerations) differed, but only slightly. The perception of the ICES working group lagged behind the actual situation in the underlying population during recovery, by approximately one year. Actual and perceived fishing mortality was also comparable, with the actual level being slightly lower than that perceived by the ICES working group.

In order to account for those changes, improved time-series estimates of female reproductive parameters and egg production were developed using biological information available from surveys for the stocks of interest. In the case of Northern hake, inclusion of improved biological and fecundity information led to different perceptions of the

absolute level of spawning stock biomass, but the overall trend in SSB was comparable between approaches. Moreover, the inclusion of more biological information affected BRP as well as the population situation in relation to those BRP. Similarly, the management performance, i.e. the capacity of maintaining the population above biological reference points, was different between the SSB estimated in the working group and in the population when including realistic reproductive characteristics. For example, Murua et al. (2010a) concluded that the probability of a wrong perception, i.e., the working group population (using SSB) perception in relation to BRP was contrary to the perception using alternative reproductive indices in all cases analysed. Moreover, an effect was seen to result from the harvest control rule implied by EU regulations, where constraints on the rate of change of fishing mortality limited the responsiveness of management. Simulations for Northern hake examined the differences in perception and the underlying population in the historical component, as well as examining the impact of results in the medium term future. This led to a greater difference between the underlying population and ICES working group perception. While perceived and actual trends were similar, actual values varied depending upon the comparability between true and assumed maturity ogives. Indeed, the ICES working group, which assumes constant maturity, may not identify actual decreases in total egg production.

The findings in relation to egg production and POFs resorption processes have allowed us to fine-tune the work on hake and cod egg production methods. For example, the data gathered through RASER in conjunction with the ICES Triennial Egg Survey were for the first time used on Northern hake in the Daily Egg Production Method (DEPM) (Murua et al., 2010b). Despite the limitations in the estimation of various parameters, the

DEPM can be potentially applied to European hake. However, as European hake is not a target species of the Triennial Egg Research Surveys, the egg sampling strategy at sea should be adapted to the spawning behaviour of this species.

3. Concluding remarks

The RASER project resulted in a high number of publication, which has significantly broaden the insight from the cellular to the population level within applied fisheries reproductive biology of clear interest to many marine fish laboratories. This ‘scaling up’ is considered necessary to understand the complex dynamics of reproductive styles and investments, which requires the establishment of well-functioning research consortia such as seen in this EU RASER project. Our work has largely been methodological with a cost-effective, modern focus. The new, quicker methods should be taken as an encouragement to intensify the field sampling programmes to improve assessment and management routines. In top of that, the results of RASER has allowed to cross the generally lost bridge between fishery biology and assessment, using the available tools to incorporate the results of reproductive biology into the assessment and management system through, for example, Management Strategy Evaluation simulation tool. This is important since the balance between fishery assessment experts and fishery biologists is sometimes biased and, thus, initiatives like RASER bring together related disciplines to work together in a multidisciplinary way to better address current issues of incorporating more biological realism into the assessment and management process.

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FIGURE LEGEND

Fig. 1. Flow diagram showing interrelationship between various research topics addressed in the EU RASER project. Publications of this special issue are shown with numbers while previous publications of the same project are shown with letters. 1: Kjesbu et al. (2010a), 2: Ndjaula et al. (2010); 3: Witthames et al. (2010); 4: Korta et al. (2010c); 5: Thorsen et al. (2010); 6: Korta et al. (2010b); 7: Korta et al. (2010a); 8: Domínguez-Petit et al. (2010); 9: Domínguez-Petit and Saborido-Rey (2010); 10: Nash et al. (2010); 11: Murua et al. (2010b); 12: Mehault et al. (2010); 13: Murua et al. (2010a); a: Witthames et al. (2009); b: Dominguez et al. (2008); c: Kjesbu et al. (2006); d: consult 'ICES Working Group on North Sea Cod and Plaice Egg Surveys in the North Sea' (www.ices.dk/workinggroups).

Fig. 2. Examples of different laboratory methodology outputs and sex cell types or structures considered within the EU RASER project consulting ovarian samples from Atlantic cod. (a): Whole mount preparation from an early maturing specimen (formalin-fixed material; stereo microscope) (photo: A. Thorsen, IMR); (b): a histologically sectioned vitellogenic oocyte (Technovit® as embedding medium and toluidine blue as stain; light microscope) (photo: M. Fonn, IMR); (c) Electron micrograph of the internal structures of a vitellogenic ovary (SEM) (photo: H. Kryvi, University of Bergen and O.S., Kjesbu, IMR) and (d) Post-ovulatory follicles remaining in the ovary following egg ovulation (same histological protocol as for (b), light microscopy/DIC) (photo: M. Fonn, IMR). Abbreviations: in (a): EV = early vitellogenic oocyte, PVO = previtellogenic

612 oocytes; in (b): CA = cortical alveoli, N = nucleus, YG = yolk granules; in (c): CT =
613 connective tissue, OG: nest of oogonia, VTO: vitellogenic oocyte (note the high number
614 of blood capillaries in the surface (theca) layer); (d): POF₁ : new post-ovulatory follicle
615 with cubical granulosa cells (dark colour), POF₂ : older post-ovulatory follicle with
616 disintegrated follicle cells. Magnification is indicated by horizontal bar.

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