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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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24 All 13 research articles in this special issue of Fisheries Research are the consequence  
25 of work undertaken within the 3-year EU 5<sup>th</sup> Framework project *Reproduction and Stock*  
26 *Evaluation for Recovery* (RASER; contract n<sup>o</sup>: Q5RS-2002-01825; <http://raser.imr.no>)  
27 with the following institutional partners: IMR, Bergen, Norway; AZTI-Tecnalia, Basque  
28 Country, Spain; IIM-CSIC, Vigo, Spain; and Cefas, Lowestoft, England (Fig. 1). In  
29 addition to these articles, two articles are in print elsewhere (Domínguez-Petit et al.,  
30 2008; Witthames et al., 2009) (Fig. 1), whilst parts of the Final Report to the EU  
31 Commission (Kjesbu et al., 2006) not featured in this monograph are work in progress.

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

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

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

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

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

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

70 Sea and the North Sea in the south (southern population) to the Barents Sea in the north  
71 (northern population), i.e., contrasting Irish Sea, North Sea and Northeast Arctic cod  
72 (Nash et al., 2010). Additional data on Icelandic cod was successfully accessed to further  
73 broaden the picture (Thorsen et al., 2010). However, only one stock of Atlantic herring is  
74 included in this monograph, i.e., the Norwegian spring-spawning herring (Ndjaula et al.,  
75 2010), one of the largest fish stock in the world. Subsequent research has been  
76 undertaken on North Sea herring, concluding that autumn and winter spawners, in theory,  
77 can switch reproductive strategy (produce high fecundity and small eggs and *vica verca*)  
78 (van Damme et al., 2009).

79 The SSB of southern cod populations as well as for the European hake are clearly  
80 below a sustainable level (although the Northern hake shows recent signs of recovery),  
81 while both the Northeast Arctic cod and the Norwegian spring-spawning herring today  
82 appear to be successfully managed in compliance with the agreed harvest control rules  
83 (for more details see the various ICES Expert Group reports; <http://www.ices.dk>).  
84 Spawning stock recovery plans are in place but the reproductive biology of these stocks  
85 (as well as for many others) is rather sparse to appreciate how stock size will likely  
86 impact on recruitment variability and recovery. Note here that ‘recovery’ refers to  
87 increase in biomass only but ‘rebuilding’ to restoring of the full diversity of population  
88 characteristics (including genetic and behavioural traits) (S. Murawski, National Marine  
89 Fisheries Service, Maryland, USA; Opening address, ICES UNCOVER Symposium  
90 2009, <http://www.uncover.eu> ) and thereby has a much longer time horizon (Enberg et  
91 al., 2009).

92 Although some field data on the reproductive investment of the presently evaluated  
93 species exist (e.g. herring: Óskarsson et al., 2002; hake: Murua and Motos, 2006; Murua  
94 et al., 2006), and more so for cod (see Thorsen et al., 2006), no thorough estimates have  
95 been made using an inter-calibrated method over a wide geographic range inhabited by  
96 each species. In 'lean species' like cod and hake, there is still uncertainty about how  
97 annual cycles in hepatic condition or past body growth relate to variability in realised  
98 fecundity in wild stocks. For hake growth analyses are complicated by inaccurate age  
99 reading (see Mellon-Duval et al., 2010 and references therein). However liver lipid  
100 energy has been shown to be an useful indicator of egg production in cod (Marshall et al.,  
101 1999) and so it may be possible to assess reproductive investment directly in this capital  
102 breeder from maternal or paternal weight or condition indices. Conversely, in the  
103 continuously feeding hake, other body constituents (proteins, lipids and glycogen) may  
104 give a better picture of spawning status in addition to measuring the standing stock of  
105 fecundity and spawning frequency. Said in another way, fish condition before spawning  
106 is expected to correlate better with egg production in capital breeders than in income  
107 breeders. Prior to this project there was sparse information for either of these two  
108 piscivorous species on the dynamics of either fecundity proliferation or down-regulation  
109 through follicular atresia with respect to body reserves or previous growth history. The  
110 same shortage of insight also applied to the zooplantivorous herring, where, however, the  
111 fat is stored in the muscle instead of in the liver, supporting the extreme long non-feeding  
112 period ('overwintering').

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

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

118 The relationship between SSB and recruitment generally is impaired at low stock size,  
119 which in turn makes the expected recovery from low SSB to be more difficult to predict  
120 (Shelton et al., 2006; Morgan et al., 2009). Consequently a key issue to any recovery plan  
121 is to understand the changes and the underlying mechanism of egg production dynamics  
122 of depleted stocks. In addition, SSB is often incorrectly assumed to be proportional to egg  
123 production (Marshall et al., 1998). SSB is used to set reference points to regulate fishing  
124 mortality and forecast stock recovery trajectories (Kell et al., 2005a; Hauge et al., 2007).  
125 The estimation of SSB, however, rarely includes stock demographic or reproductive  
126 biology information which have been demonstrate to affect the fecundity and spawning  
127 success of the population (Cardinale and Arrhenius, 2000; Marteinsdottir and  
128 Thorarinsson, 1998; Morgan et al., 2009). Moreover, recruitment of cod has been shown  
129 to be sensitive to climate change (Clark et al., 2003) and, therefore, the impact of climate  
130 change on the success of stock recovery or rebuilding needs to be considered (Kell et al.,  
131 2005a,b) using, for example, Management Strategy Evaluations (MSE) tools.

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## 133 **2. Advancement in studies on stock reproductive potential**

134

### 135 *2.1. Development of methods to assess fecundity and spawning rates*

136

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

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

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

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



182 2010), but 3-D reconstruction and examination of whole mounts indicated there was a  
183 rapid size reduction following ovulation (Korta et al., 2010c). More work needs to be  
184 done but this observation offers a more robust approach to age POFs. Unexpectedly,  
185 POFs were shown experimentally to persist after the cessation of spawning in a Barents  
186 Sea simulation for more than four months or in a north summer warming simulation for  
187 more than three months providing a useful tool to hindcast previous spawning activity  
188 (Witthames et al., 2010). The latter observation has important application to identify  
189 skipped spawning (Skjæraasen et al., 2009).

190

## 191 *2.2. Herring reproductive biology*

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

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

211

### 212 *2.3. Cod reproductive biology*

213

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

228 press) supported by field studies indicating the same pattern (Skjæraasen et al., in press).  
229 In the estimation of fecundity we found that the predictive power ( $r^2$ ) of explanatory  
230 variables (such as body weight) varied seasonally but peaked in late vitellogenesis  
231 (Kjesbu et al., in press), which to some degree deviates from results seen in Skjæraasen et  
232 al. (2006) pointing instead at early vitellogenesis. Complementary studies have  
233 demonstrated that these trends in predictive power are partly influenced by environmental  
234 temperature (Kjesbu et al., 2010b). To make a fully unbiased comparison of the potential  
235 fecundity in time and space we constructed models that included OD as one of the  
236 explanatory variables. Our results clearly indicated a north-south gradient with increasing  
237 relative fecundity towards the south indicating a true difference in reproductive  
238 investment (Thorsen et al., 2010). The higher investment of the fish in the south could  
239 only partly be explained by the noted higher condition. Furthermore, the maturation  
240 schedules of Irish, North Sea and Northeast Arctic cod were extensively analysed  
241 clarifying that i) there have been major drops in the age and length at maturity over time  
242 and ii) Irish Sea cod mature extremely early (typically at 2 years) followed by North Sea  
243 cod (typically at 3-4 years) and then, following a jump upwards, to Northeast Arctic cod  
244 (typically around 8 years) (Nash et al., 2010). The length-at-age-at-maturity trajectories  
245 differed between the three stocks indicating fundamental differences in phenotypic  
246 plasticity. Taken together, the various models developed for cod during the RASER  
247 project have in effect been run successfully to quantify the dynamics of oocyte  
248 recruitment and maturity reaction norms from basic information on fish length, condition,  
249 sampling time and origin (geographical area).  
250

251 2.4. *Hake reproductive biology*

252

253 Here the focus was devoted on how to obtain reliable estimates of realised fecundity,  
254 daily egg production rates and age at maturity in the Bay of Biscay and Galician Shelf  
255 populations (Korta et al., 2010a). Relative batch fecundity ( $BF_{rel}$ ) showed significant  
256 differences among years in both areas, indicating differences in productivity.  
257 Standardisation by month was required because  $BF_{rel}$  varied largely throughout the year.  
258 Both the intra- and inter-annual variation in  $BF_{rel}$  might be due to an overall fish  
259 condition effect (Kraus et al., 2000; Somarakis et al., 2005) but, more likely, an  
260 immediate response to variability in amount of prey at or near the spawning grounds in  
261 this income breeder (Domínguez-Petit and Saborido-Rey, 2010). A strong positive  
262 relationship appeared between  $BF_{rel}$  and gonadosomatic index (GSI). GSI as well as the  
263 total relative number of developing oocytes ( $NDO_{rel}$ ) followed the same trend as  $BF_{rel}$ ,  
264 although the overall pattern differed in the two areas of study (Korta et al., 2010a;  
265 Domínguez-Petit and Saborido-Rey, 2010).  $NDO_{rel}$  did not vary with maturity stage,  
266 corroborating that the fecundity of European hake is indeterminate (Murua and Motos,  
267 2006; Murua et al., 2006; Korta et al. 2010a). Based upon the results on spawning  
268 fraction, the European hake spawns once every 5-7 days during January and March in  
269 both areas but once every 10 days or more in the rest of the year (Korta et al., 2010a;).  
270 During the first quarter larger females showed higher spawning activity and shorter batch  
271 interval than smaller females (Mehault et al., 2010). Egg quality, in terms of diameter and  
272 dry mass, increased significantly with female size (Mehault et al., 2010).

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

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

284 Proteins, lipids, fatty acid, glycogen, water and ashes content were determined in liver,  
285 muscle and gonad (Domínguez-Petit and Saborido-Rey, 2010; Domínguez-Petit et al.,  
286 2010). Relationships between these components and somatic variables (length, weight,  
287 GSI, liver index and Fulton's K) were established but, although some significant  
288 relationships were established, their low explanatory power complicated meaningful  
289 biological interpretations.

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

293

294 *2.5. New findings of relevance to assessment and management advice*

295

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

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

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

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

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

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



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

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

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

391

### 392 **3. Concluding remarks**

393

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

410

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419

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## 589 FIGURE LEGEND

590

591 **Fig. 1.** Flow diagram showing interrelationship between various research topics  
592 addressed in the EU RASER project. Publications of this special issue are shown with  
593 numbers while previous publications of the same project are shown with letters. 1:  
594 Kjesbu et al. (2010a), 2: Ndjaula et al. (2010); 3: Witthames et al. (2010); 4: Korta et al.  
595 (2010c); 5: Thorsen et al. (2010); 6: Korta et al. (2010b); 7: Korta et al. (2010a); 8:  
596 Domínguez-Petit et al. (2010); 9: Domínguez-Petit and Saborido-Rey (2010); 10: Nash et  
597 al. (2010); 11: Murua et al. (2010b); 12: Mehault et al. (2010); 13: Murua et al. (2010a);  
598 a: Witthames et al. (2009); b: Dominguez et al. (2008); c: Kjesbu et al. (2006); d: consult  
599 ‘ICES Working Group on North Sea Cod and Plaice Egg Surveys in the North Sea’  
600 ([www.ices.dk/workinggroups](http://www.ices.dk/workinggroups)).

601

602 **Fig. 2.** Examples of different laboratory methodology outputs and sex cell types or  
603 structures considered within the EU RASER project consulting ovarian samples from  
604 Atlantic cod. (a): Whole mount preparation from an early maturing specimen (formalin-  
605 fixed material; stereo microscope) (photo: A. Thorsen, IMR); (b): a histologically  
606 sectioned vitellogenic oocyte (Technovit® as embedding medium and toluene blue as  
607 stain; light microscope) (photo: M. Fonn, IMR); (c) Electron micrograph of the internal  
608 structures of a vitellogenic ovary (SEM) (photo: H. Kryvi, University of Bergen and O.S,  
609 Kjesbu, IMR) and (d) Post-ovulatory follicles remaining in the ovary following egg  
610 ovulation (same histological protocol as for (b), light microscopy/DIC) (photo: M. Fonn,  
611 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): POF<sub>1</sub> : new post-ovulatory follicle  
615 with cubical granulosa cells (dark colour), POF<sub>2</sub> : older post-ovulatory follicle with  
616 disintegrated follicle cells. Magnification is indicated by horizontal bar.

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