HV 2019-32
ISSN 2298-9137

# HAF- OG VATNARANNSÓKNIR

*MARINE AND FRESHWATER RESEARCH IN ICELAND*

# Verification of macroscopic maturity staging in Iceland East Greenland Jan Mayen capelin (Mallotus villosus)

*Sigurvin Bjarnason, Birkir Bárðarson, Svanhildur Egilsdóttir og Guðmundur J. Óskarsson*

REYKJAVÍK MAÍ 2019

# Verification of macroscopic maturity staging in Iceland East Greenland Jan Mayen capelin (Mallotus villosus)

*Sigurvin Bjarnason, Birkir Bárðarson, Svanhildur Egilsdóttir og Guðmundur J. Óskarsson*

Haf- og vatnarannsóknir

Marine and Freshwater Research in Iceland

HAFRANNSÓKNASTOFNUN
Rannsókna- og ráðgjafarstofnun hafs og vatna

# Upplýsingablað

| | | |
|---|---|---|
| **Titill:** Verification of macroscopic maturity staging in Iceland-East Greenland-Jan Mayen capelin (*Mallotus villosus*) | | |
| **Höfundar:** Sigurvin Bjarnason, Birkir Bárðarson, Svanhildur Egilsdóttir og Guðmundur J. Óskarsson | | |
| **Skýrsla nr:**<br>HV 2019-32 | **Verkefnisstjóri:**<br>Sigurvin Bjarnason | **Verknúmer:**<br>9909 / 9123 |
| **ISSN**<br>2298-9137 | **Fjöldi síðna:**<br>14 | **Útgáfudagur:**<br>23. maí 2019 |
| **Unnið fyrir:**<br>*Hafrannsóknastofnun* | **Dreifing:**<br>Opin | **Yfirfarið af:**<br>Jón Sólmundsson |

**Abstract**

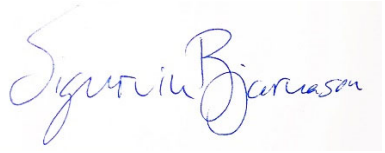
Capelin (*Mallotus villosus*) is a short-lived pelagic fish species of major commercial importance. Maturity determinations are important for assessment and management of the Icelandic capelin stock. Temporal- and spatial shift in the capelin's feeding migration since around 2000 called for moving the assessment survey by more than a month forward, which created challenges in capelin maturity staging. Macroscopic maturity assignment of capelin ovaries and testes were therefore verified with histological examinations to assess if and where improvements were required. The analyses indicate that the highest error occurred when distinguishing between maturity stage III and IV (35 %). Error in determining immature (stages I and II) and early maturation (stage III), which is the most critical staging for the assessment, was however low (<15%). Estimates of maturity ratio and length at maturity, did not differ between macroscopic and microscopic maturity staging. The results endorse the routinely used macroscopic determination of maturity stage in the stock assessment surveys. The main difference observed in the maturity staging between the two methods was related to developmental stage of mature gonads. A 6-point maturity scale for Icelandic capelin would yield more consistent maturity staging data.

**Ágrip**

Loðna (*Mallotus villosus*) er skammlíf og efnahagslega mikilvæg uppsjávarfisktegund hér við land. Mat á stærð veiðstofns og veiðráðgjöf einskorðast við kynþroska loðnu og rétt ákvörðun

á kynþroska er því mikilvæg. Haustmælingar á loðnu sem áður voru í nóvember-desember hafa færst fram í september vegna breytinga á fæðuslóðum og fari hennar. Við þessa tilfærslu hefur reynst erfiðara að greina hvort loðna muni hrygna á komandi vetri eða ekki. Í þessari rannsókn voru hefðbundnar kynþroskagreiningar (macroscopic) því sannprófaðar með vefjafræðiskoðun (microscopic) kynkirtla hænga og hrygna og ákvarðað hvort og þá hvar væri þörf á endurbótum. Niðurstöður sýndu að greiningarskekkjan var mest á milli kynþroskastiga III og IV (35%). Greiningarskekkjan milli ókynþroska (stig I og II) og snemmkynþroska (stig III) loðnu, sem er mikilvægust þegar kemur að stofnmati, var hinsvegar lág (<15%). Lítill munur var á mati á kynþroskahlutfalli og lengd við kynþroska þegar niðurstöður vefjasýnagreininga voru borin saman við hefðbundna kynþroskamatið. Niðurstöðurnar renna stoðum undir áframhaldandi notkun hefðbundinna kynþroskagreininga á loðnu í haustleiðangri. Mikil skekkja er þó á seinni kynþroskastigum loðnunnar og við teljum að nýr skali með færri kynþroskastigum (6) myndi auka samræmi gagna.

**Lykilorð:** *loðna, Mallotus villosus, capelin, kynþroski, kynþroskagreining, vefjafræði, kynþroskahlutfall, stofnmat*

| **Undirskrift verkefnisstjóra:** | **Undirskrift forstöðumanns sviðs:** |
| --- | --- |
|  |  |

# Table of contents



# Tables



# Figures

# Introduction

Traditionally, determination of sexual maturity in fish research has been based on visual inspection (macroscopic) of whole gonads (West, 1990). Macroscopic maturity estimation can however be inaccurate since it is generally restricted to unclear and often subjective measures of gonad size, shape or colour that do not necessarily correspond with cellular development (Vitale et al., 2006; Costa, 2009; Ferreri et al., 2009; McPherson et al., 2011; Midway and Scharf, 2012). Despite its limitations, macroscopic staging remains the most popular approach for assigning maturity status since it is the fastest method available and, perhaps more importantly, inexpensive. Alternatively, histological (microscopic) examination of structures within oocyte or testes is often considered to be the most accurate method for determining the maturity stage of fish (West, 1990). Histological staging provides information at a cellular level and a high degree of accuracy in distinguishing between immature and mature individuals (West, 1990; Vitale et al., 2006). The major drawback to histology is that it is labour- and resource intensive, requiring considerable time and expense for each sample processed.

Capelin (*Mallotus villosus*), a pelagic fish species of major commercial importance, is semelparous, meaning that most of it dies after spawning for the first time, particularly the males (Vilhjálmsson, 1994). Maturity determinations for capelin are of special relevance for stock assessment and management purpose. The Iceland-East Greenland-Jan Mayen capelin stock, often referred to as Icelandic capelin, migrates in autumn from its feeding grounds in Iceland Sea (along East Greenland shelf) to overwintering grounds north and northwest of Iceland. Parts of the stock may overwinter farther west in the Denmark Strait and on the Greenland Shelf. In January-March capelin undertakes spawning migration to the spawning sites in shallower waters of the south and west coast of Iceland (Hjálmar Vilhjálmsson, 1994; Olafsdottir and Rose, 2012; Carscadden *et al.*, 2013). Forberg (1983) estimated that the earliest time to establish if capelin is mature would be 1–2 months before this spawning migration.

The Marine and Freshwater Research Institute (MFRI) conducts an annual autumn survey with the aim to estimate the amount of mature and immature capelin. This estimate provides an initial advice of total allowable catch (TAC) for the upcoming winter fishery. In 2010, this survey was moved from November/December to early September because of a north- and westward shift in the capelin's feeding areas towards the east coast of Greenland and weather conditions in the area at that time of year (sea ice and bad weather). The earlier timing of

maturity estimation in September can be more challenging as macroscopic distinction of early maturation stages is less obvious than later in the season.

Estimating maturity stages in Icelandic capelin is a key component in calculating the size of spawning stock biomass (SSB). The fishery management of the stock is based on its spawning potential, which rely on assessments on stock number, the capelin's maturity and condition collected in the surveys (Hafrannsóknastofnun, 2018). Only the mature part of the stock undergoes spawning migration into Icelandic waters and is subject to the fisheries. Incorrect assignment of maturity stages in the autumn will result in inaccurate estimation of the SSB, which is reflected to the TAC advice. Thus, the objective of this study is to examine the accuracy of macroscopic maturity assignment in capelin around Iceland and assess if and where improvements are required.

## Methods

### *Sampling of gonads*

Gonads were sampled onboard the research vessels Bjarni Sæmundsson and Árni Friðriksson in the autumn and winter surveys from 2015–2018. At each station, a sample of 100 randomly sampled capelin were collected. For each fish, otoliths were extracted for age determination, total length (to the nearest 0.5 cm; from the tip of the snout to the upper lobe of the pinched caudal fin), total weight (0.1 g) and gonad weight (0.1 g; females only) were recorded, and the sex and maturity was estimated using a maturity scale (Forberg, 1982). A subsample of gonads from five fish was randomly taken at each station, weighed and photographed together with the fish prior to preserving it in a 4% buffered formaldehyde solution for histological embedding. The maturity scale used includes 8 maturity stages (Forberg, 1982), while in this study the focus was primarily on the first four stages, which were dominant (>98% of gathered samples) in autumn and winter surveys. Maturity stage I and II are considered immature and stages >III are mature (Table 1). Table 2 lists the number of gonad samples analysed per year and macroscopic maturity stage.

### *Histological analysis and classification*

The sampled ovaries and testes were analysed histologically in a laboratory. A transect from the middle of the gonad sample was extracted and embedded in paraffin, sectioned at 3 µm and stained using H&E (Hematoxylin and Eosin stain). The gonad sections were viewed with a compound microscope (CX41 Olympus and Leica M165 C) at 100–400× magnification. Digital images were taken with a DP72 Olympus video camera attached to the microscope. Specific histological characteristics were used to classify stages of gonadal development during the

reproductive cycle. The main characteristics used to identify female maturity development were based on oocyte components such as yolk accumulation and the formation of cortical alveoli as described by Forberg (1982, 1983). The microscopic criteria on maturity stage of male gonads represents the amount and growth of spermatogonia, spermatocytes and spermatozoa (Flynn and Burton, 2003). The microscopic and macroscopic description of each of the four stages of interest are listed in Table 1 and visually presented in Figure 1 and 2.

**Table 1**. Macroscopic (Forberg, 1982, 1983) and microscopic (Forberg, 1982; Flynn and Burton, 2003; Hagstrøm Bucholtz et al., 2008) description of capelin maturity stages 1-4 (stages 5-8 disregarded). For visual description see Figure 1 and Figure 2.

| MATURITY STAGE | MACROSCOPIC APPEARANCE | HISTOLOGICAL APPEARANCE |
|---|---|---|
| **I. IMMATURE A** | Juvenile fish. Gonads are very thin (<1 mm), translucent, and without colour. The sex is difficult to determine at this stage. | Only first growth phase (FGP) oocytes are present. The oocytes appear transparent and the nucleus is easily seen.<br><br>Males show large cyst cells containing only primary spermatogonia. |
| **II. IMMATURE B** | Ovaries are thicker (more volume), early stage are transparent and without colour and later stage are opaque with a hint of colour. Visible 'ripples' under the ovarian wall when the ovary is stretched. No eggs present.<br><br>Male testes are thicker (more volume), transparent and without colour or with a hint of colour. Testes are smooth when stretched, i.e. no 'ripples' visible. Relatively easy to determine sex. | Bigger oocytes with secondary growth phase (SGP) present in the cortical alveoli stage. The cortical alveoli oocytes are enlarged and the ratio nucleus to oocyte area has decreased.<br><br>Males are identified by secondary spermatogonia, appearing as relatively large cells. |
| **III. MATURE-RIPENING A** | Ovaries are bigger and occupy up to half the body cavity. Opaque with visible yellow/white specks (eggs). Blood vessels visible.<br><br>Male testes are opaque white or with white dots, firm and still of limited volume. Blood vessels visible. | Secondary growth oocytes containing yolk granules. The oocyte diameter increases dramatically. Yolk granules appear around nucleus.<br><br>Maturing males were identified by the occurrence of meiotic stages e.g. spermatocytes in cysts, accompanied by a reduction in cell size. Sperm starts to accumulate. |
| **IV. MATURE-RIPENING B** | Ovaries are bigger (more volume), colour yellow or white, and occupy up to 2/3 of the body cavity (related to somatic body conditions of the individual). Eggs (oocytes) distinct and grainy. Eggs, in the front end of the ovaries, becoming hydrated (turning transparent). Blood vessels visible.<br><br>Male testes are bigger (more volume), colour light grey or white, and milt has high viscosity (thick liquid). Blood vessels visible. | Secondary growth oocytes in the nuclear migration stage. Oocytes are filled with yolk; cortical alveoli are pressed against cell membrane and nucleus begins migration towards micropyle.<br><br>For males, as cyst cell mature, their walls begin to break down and pools of sperm are present. |

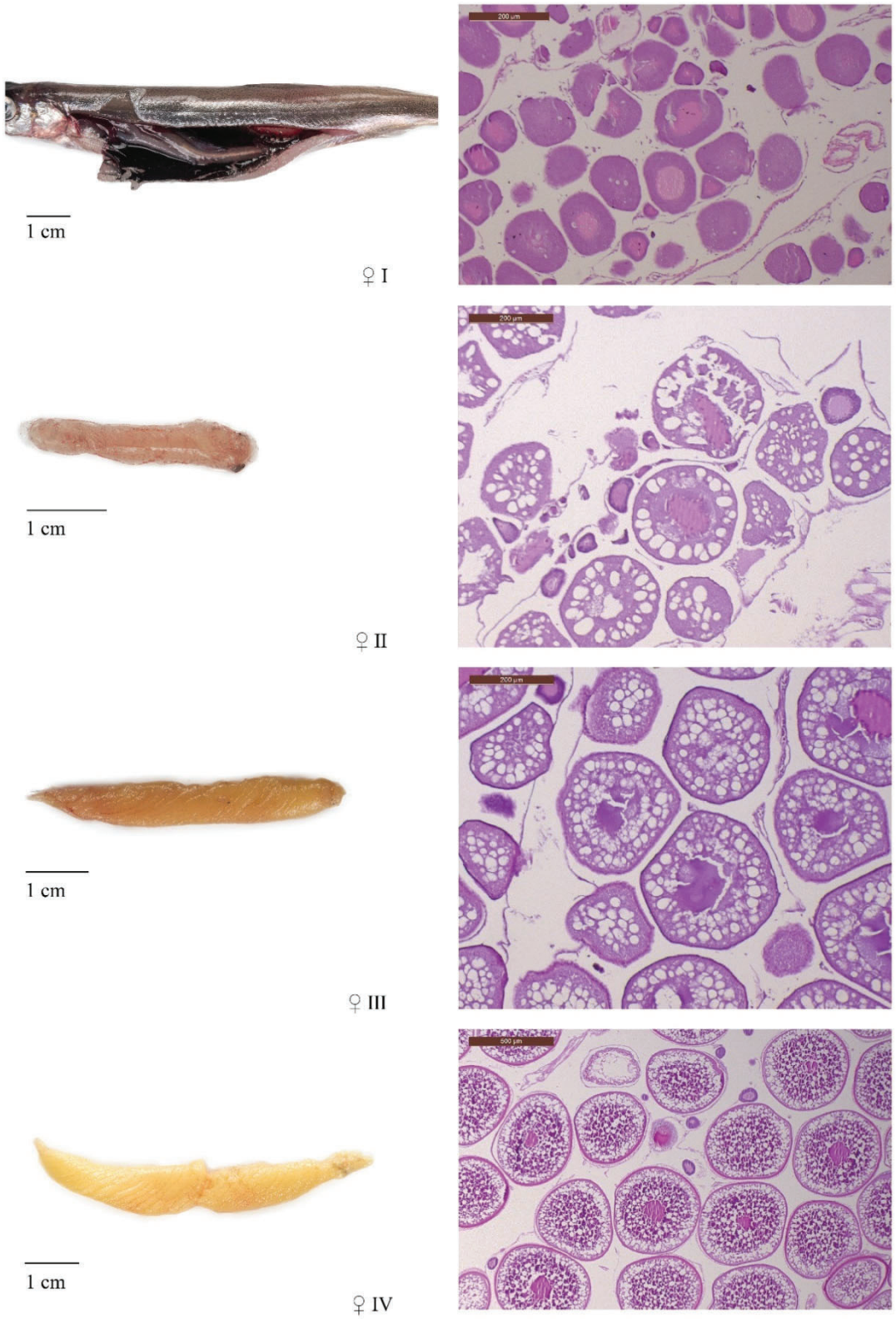



**Figure 1**. Macroscopic (left) and microscopic (right) images of capelin ovaries stages I-IV**.**

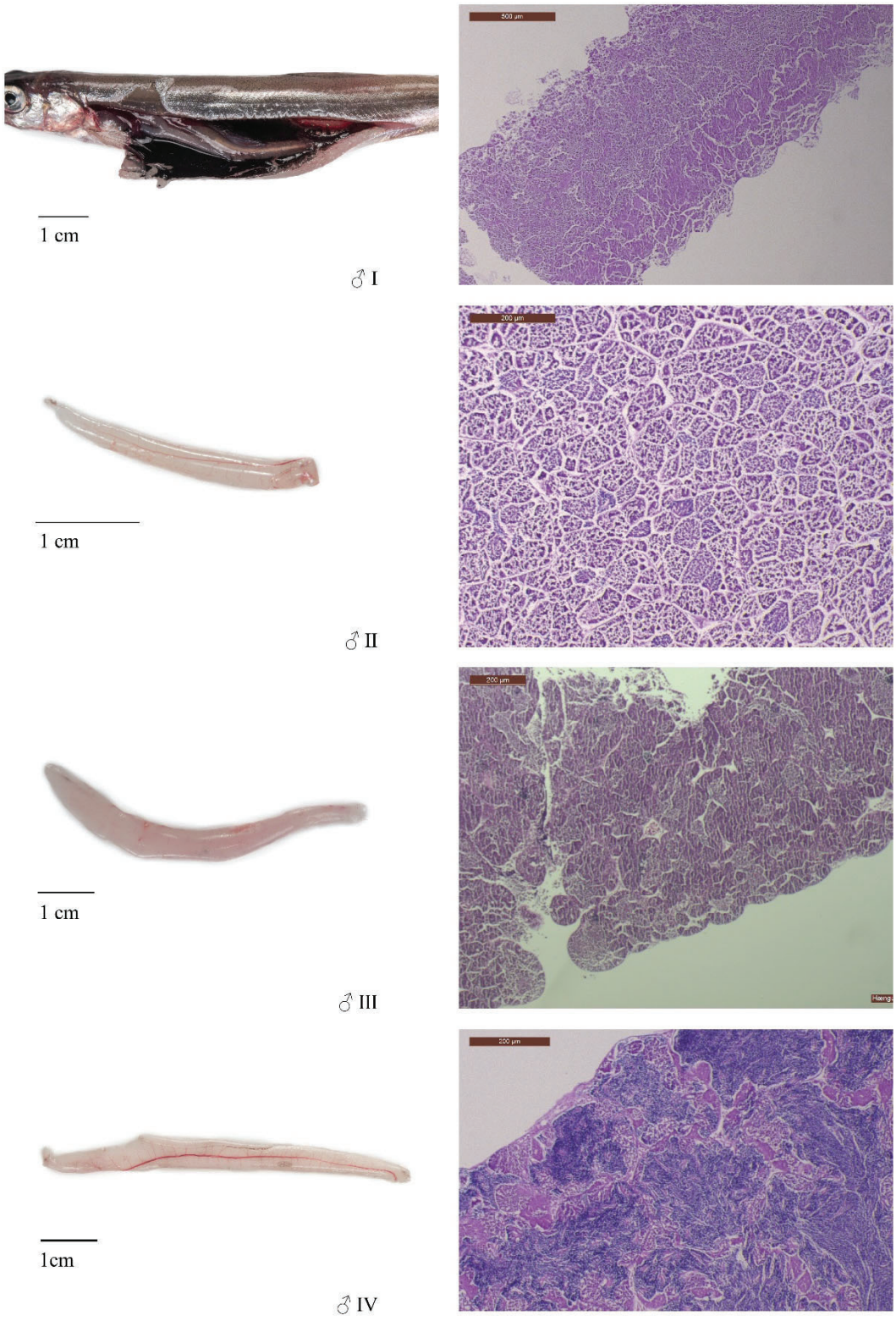



**Figure 2**. Macroscopic (left) and microscopic (right) images of capelin testes stages I-IV.

**Table 2**. Number of macroscopic analyses per year and per maturity stage. Samples are gathered in September and January cruises.

| YEAR | I | II | III | IV | TOTAL |
|---|---|---|---|---|---|
| **2015** | 1 | 29 | 43 | 1 | 74 |
| **2016** | 0 | 10 | 12 | 0 | 22 |
| **2017** | 0 | 6 | 11 | 10 | 27 |
| **2018** | 13 | 13 | 32 | 15 | 73 |
| **TOTAL** | 14 | 58 | 98 | 26 | 196 |

### *Statistical analysis*

The percentage error in macroscopic determination was calculated based on deviations from the microscopic determination. The error distribution among maturity stages was analysed by maturity stage, between season, sex and by fish length.

Length at sexual maturity was estimated and defined by the size at which 50% of the sampled fish was mature (L50). The data were fitted using a binomial logistic regression analysis (Magallanes, 2016)

$$Y = {}^{1}/_{(1 + exp^{-(a+b*L)})}$$

where *Y* is the percentage of mature individuals, a is intercept, b is slope and *L* is total length. We applied chi-squared test of homogeneity, which is commonly used to determine whether two or more independent samples differ in their distributions on a single variable of interest. The formula for calculating the test statistic is:

$$x^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i},$$

where n is the number of cells in the table, $O_i$ is the number of observation of type *i*, $E_i$ is the expected count of type *i*. The obtained test statistic is compared against a critical value from the chi-square distribution with $(r - 1)$ $(c - 1)$ degrees of freedom (Franke et al., 2012).
G-test of independence was applied using the formula:

$$G = 2 \sum_{i}^{n} O_i \, ln\left(\frac{O_i}{E_i}\right)$$

where *n* is the total number of observations, $O_i$ is observed frequency for each value and $E_i$ is the expected frequency for each given value under the null hypothesis (Campbell *et al.*, 1970).

Gonadosomatic index (GSI) was computed as the ratio between gonad weight (G) and ungutted weight (W) for female capelin as follows:

$$GSI = \left(\frac{G}{W}\right) \times 100$$

GSI for maturity stages II, III and IV were compared using a one-way ANOVA to see whether it can be used as an indicator for maturity staging in female capelin. To analyse which stages differed, a post hoc comparisons was carried out, using a Tukey HSD test.

## Results

Results of the comparison between microscopic and macroscopic stage assessment show that the highest error (35%) occurred when distinguishing between stages III and IV (Table 3). Maturity stages I–III were most often (> 84%) correctly identified. The incidence of error in stage II (immature) and III (maturing) was 14.6% and 11.6%, respectively. Stage III is the most abundant in the samples and shows the highest matching proportion between micro- and macroscopic classifications (88.4%) (Table 3).

It should be noted that the percentages for stage I are only based on 15 individuals, and for this reason their representativeness is proportionally low. Also, when a capelin is identified as stage I, there is no clear way of identifying its sex. Therefore, researchers often assign it as female or skip the sex identification.

**Table 3**. Distribution of the macroscopic identification error for capelin gonads. Emboldened is the percentage of correspondence with microscopic maturity assignment. In brackets is the number of samples.

| | **MACROSCOPIC IDENTIFICATION (%)** | | | | |
|---|---|---|---|---|---|
| | **Maturity stage** | | | | |
| **MICROSCOPIC IDENTIFICATION** | **I** | **II** | **III** | **IV** | **Total % (n)** |
| **I** | **86.7** (13) | 13.3 (2) | - | - | 100 (15) |
| **II** | 1.8 (1) | **83.6** (46) | 14.6 (8) | - | 100 (55) |
| **III** | - | 11.6 (10) | **88.4** (76) | - | 100 (86) |
| **IV** | - | - | 35.0 (14) | **65.0** (26) | 100 (40) |

The accuracy in maturity staging varied between sexes (Figure 3). Females had most maturity staging error at stage I and gradually reduced error with increasing stage. Males had the far

highest maturity staging error at stage IV and the lowest at stage III. No males were identified at stage I as sex determination is difficult at that stage. Hence, at maturity stage I only females had sex specific identification error and for stage IV the bulk of the error was for males (12 of 14 incorrectly assigned). Females were more often incorrectly staged at stages II and III.

Statistical analysis of homogeneity shows there is a significant difference (p-value = 0.014) between microscopic identification stages, implying a difference in error percentage between maturity stages.

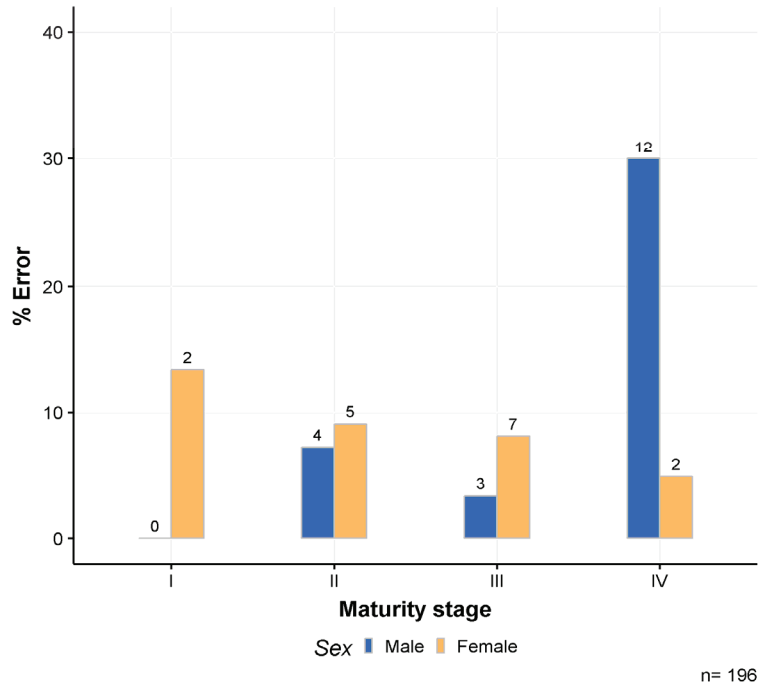



**Figure 3**. Percentage error in macroscopic identification of capelin gonads by microscopic maturity stage and sex.

There was a slightly higher error rate in the winter survey (21.4%) than the autumn survey (17.4%) and the source of the errors differed. In the autumn survey, 5.2% of immature capelin were macroscopically identified as mature, and 6.46 of those already mature were considered immature (together a 11.6% of total 17.4%). Most of the error in the winter survey was due to be misidentification of stage III in males (19% of total 21.4%). In autumn there was 1.3% total error in immature/mature estimation leading to a 1.3% overestimation in SSB estimate. There was no significant difference between using the macroscopic and microscopic method when distinguishing between immature and mature capelin (G-test, p=0.15 n=155).

There was a significant difference in mean gonadosomatic index (GSI) between maturity stages (ANOVA. $F_{2.93}$ = 104.65, $p < 0.05$). A post hoc comparisons shows there was a high

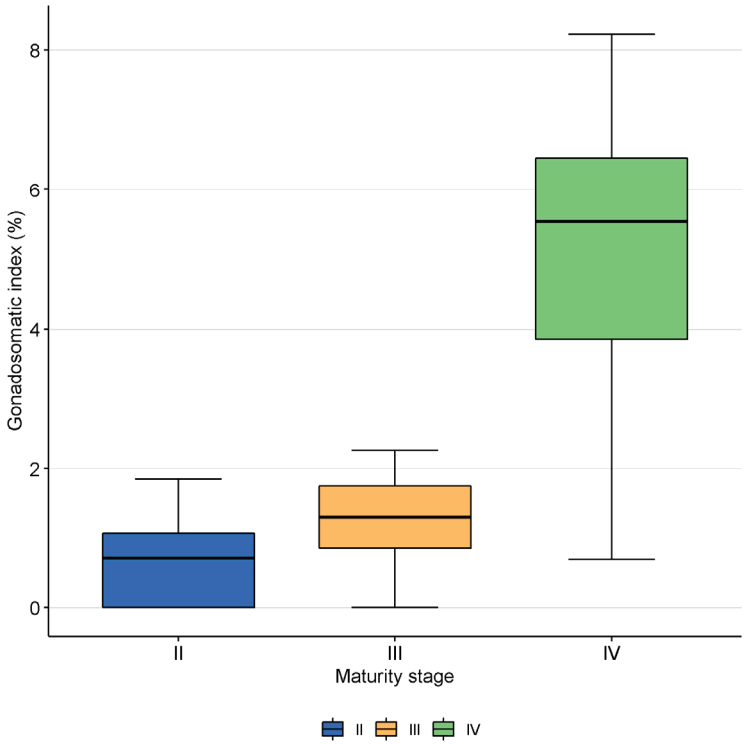



**Figure 4**. Gonadosomatic index (GSI) boxplot with error bars for female capelin of maturity stages II, III and IV.

significant difference between maturity stage III and IV (Tukey HSD test, p = 4.89e-10) with gonads in maturity stage IV on average 3.8 % higher in the GSI than those in stage III. There was however not a significant difference between maturity stage II and III (p = 0.10).

Maturity stage III does not exceed 3% in the GSI and only four individuals in stage IV are under 3% in the GSI (Figure 4). Applying this criterion to the dataset, in order to identify maturity stages using this method, results in a 5.7% staging error between maturity stages III and IV for female capelin, which is comparable to the macroscopic staging error.

To examine how the accuracy in maturity staging varied with capelin length, the capelin was split into six length groups (table in Figure 5). Figure 5 shows that the maturity stages for smaller fish (<14 cm) were more correctly assigned macroscopically than those of larger fish. The length group with the highest maturity estimation error was 14 – 15 cm capelin.

Maturity ogives for microscopic and macroscopic analyses were similar (Figure 6). The estimated length where 50% of the stock was mature (L50) was 14.6 cm for the macroscopic method and 14.5 cm for the microscopic method.

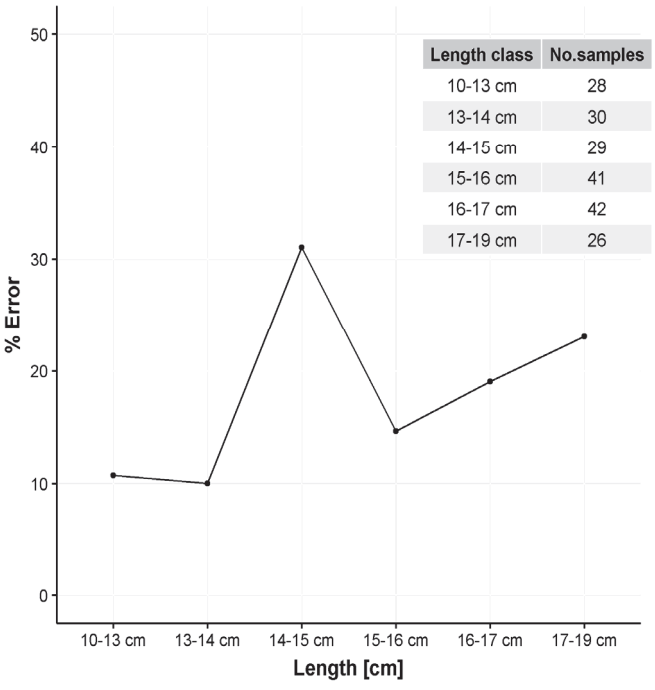



**Figure 5**. Percentage error in macroscopic identification of capelin gonads by length class. The table shows number of samples in each length class.

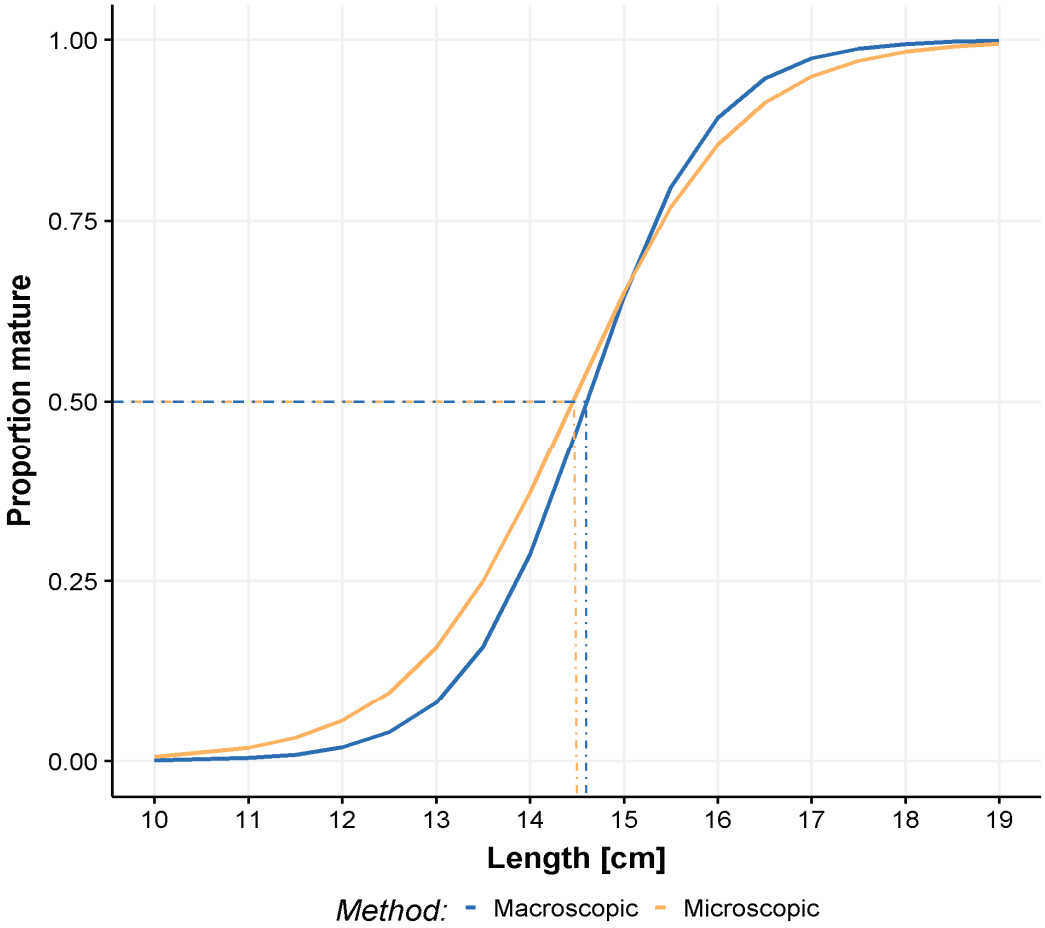



**Figure 6**. Macroscopic (blue) and microscopic (yellow) maturity ogives of male and female capelin as measured in autumn and winter surveys.

## Discussion

The most important finding of this research is that estimates of capelin maturity proportion which is essential for assessment and management of the stock, did not differ significantly between macroscopic and microscopic maturity staging. This gives support to the routinely used macroscopic determination of maturity stage in the stock assessment surveys. The main difference observed in the maturity staging between the two methods was related to developmental stage of mature gonads that does not impact the SSB estimate and therefore has little effect on stock assessment.

Our comparison of macroscopic and microscopic analyses of samples collected in September over three years, revealed that 11.4% of immature capelin was signed as mature and 7.9% vice versa. Consequently, in this case, these two types of errors balance each other out so the net impact on estimation of SSB is insignificant. In comparison to other studies, the estimated error in our study is low. For instance, in walleye pollock (*Theragra chalcogramma*), a total of 25% of macroscopic maturity stages were incorrectly assigned in comparison with histological assessment (Williams, 2007). In a study on Kattegat cod (*Gadus morhua*), macroscopic staging error was estimated to have caused a 21–35% overestimation of the female spawning stock biomass (Vitale et al., 2006). Also, a total of 53% of kingfish (*Scomberomorus commerson*) were found to be incorrectly staged macroscopically (Claereboudt et al., 2005) and a staggering 80% of female Baltic cod (*Gadus morhua*) were assigned incorrect maturity stage through macroscopic determination (Tomkiewicz et al., 2003). Common for all these species was that most of the error in staging was due to the difficulty of discriminating between immature stage and the recovering/skip spawning stage. Since capelin is considered a semelparous species (it dies after spawning), this problem does not apply.

The length at first maturity of the stock, or the maturity ogives, was estimated both from microscopic and macroscopic maturity staging (Figure 6). The results were similar, implying that the macroscopic classification is accurate and sufficient for this purpose, especially when a high number of individuals are processed. This is despite a certain identification error in routine macroscopic evaluation on capelin gonads. Consequently, moving the autumn survey forward to September had probably insignificant effects on the maturity ogive estimates.

By length class the best agreement between macroscopic and microscopic assessment of maturity was in immature fish, i.e. <14 cm, whereas the exact maturity stage of larger fish seems to be more difficult to assess. This is affirmed by the distribution of error between sexes (Figure 3). The highest error was found for larger males in stage IV (30% error). Gonadosomatic index seems to be a good indicator of maturity stage in mature female capelin and could help reduce staging error between mature fish. Unfortunately, male gonads have not been

weighted in capelin assessment surveys and were not weighted in this study. We recommend that male gonads should be routinely weighted just as female gonads and the relationship between male gonadosomatic index and maturity stage should be examined.

When determining fish maturity, the most common sources of error in macroscopic analysis are related to the lack of standardisation in the criteria used for maturity staging as well as poor interchange of criteria among observers (Vitale et al., 2006; McPherson et al., 2011). The error concerning the larger males also explains the seasonal difference error, because all males in maturity stage IV are found in winter surveys. This may be because the macroscopic difference between stage III and IV in capelin is small and indefinite. According to the current maturation key it is a simple matter of gonads filling half or 2/3 of the body cavity (Table 1). Furthermore, in males the difference is based on the colour of the testes, scaled from grey to white. These demanding and subjective determinations are largely built on experience of the researcher. To reduce errors related to these subjective staging, either more detailed classification criteria or compilation of maturity stages are required.

In an effort to unify maturity stages in pelagic fishery science, the Atlantic mackerel (*Scomber scombrus* ) and horse mackerel (*Trachurus trachurus*) have six internationally agreed maturity stages (ICES, 2007, 2015). This scale includes one maturity stage for immature fish (corresponds to stages I and II in the 8-point scale) and one stage for maturing fish (stages III and IV in the 8-point scale). McPherson et al. (2011) argued that the use of an 8-point scale for Atlantic herring (*Clupea harengus*) is simply too complicated for macroscopic determination alone. They further proposed that either the maturity readers receive more intensive training or simply that they should switch to a 4-point scale, i.e. immature, mature-active, spawning and spent/recovering. If the capelin maturity data reported here and collected with an 8-point scale were transformed in the same way to the 6-point scale the total misclassification rate would be reduced from 18% to 9.2%. A 6-point scale for Icelandic capelin would deliver less detailed data, but the data collection procedure and training of new technicians may be reduced and furthermore, result in more consistent and reliable maturity staging data.

# References


Brown-Peterson, N. J., Wyanski, D. M., Saborido-Rey, F., Macewicz, B. J., and Lowerre-Barbieri, S. K. (2011). A Standardized Terminology for Describing Reproductive Development in Fishes. *Marine and Coastal Fisheries*, 3: 52–70.

Campbell, R. C., Sokal, R. R., and Rohlf, F. J. (1970). Biometry: The Principles and Practice of Statistics in Biological Research. *Journal of the Royal Statistical Society*. Series A (General), 133: 102.

Carscadden, J. E., Gjøsæter, H., and Vilhjálmsson, H. (2013). A comparison of recent changes in distribution of capelin (*Mallotus villosus*) in the Barents Sea, around Iceland and in the Northwest Atlantic. *Progress in Oceanography,* 114: 64–83.

Claereboudt, M. R., McIlwain, J. L., Al-Oufi, H. S., and Ambu-Ali, A. A. (2005). Patterns of reproduction and spawning of the kingfish (*Scomberomorus commerson*, Lacépède) in the coastal waters of the Sultanate of Oman. *Fisheries Research*, 73: 273–282.

Costa, A. M. (2009). Macroscopic vs. microscopic identification of the maturity stages of female horse mackerel. *ICES Journal of Marine Science*, 66: 509–516.

Ferreri, R., Basilone, G., D'Elia, M., Traina, A., Saborido-Rey, F., and Mazzola, S. (2009). Validation of macroscopic maturity stages according to microscopic histological examination for European anchovy. *Marine Ecology*, 30: 181–187.

Flynn, S. R., and Burton, M. P. M. (2003). Gametogenesis in capelin, *Mallotus villosus* (Müller), in the northwest Atlantic Ocean. *Canadian Journal of Zoology*, 81: 1511–1523.

Forberg, K. G. (1982). A histological study of development of oocytes in capelin, *Mallotus villosus villosus* (Muller). *Journal of Fish Biology*, 20: 143–154.

Forberg, K. G. (1983). Maturity classification and growth of capelin, *Mallotus villosus villosus* (M), oocytes. *Journal of Fish Biology*, 22: 485–496.

Franke, T. M., Ho, T., and Christie, C. A. (2012). The Chi-Square Test: Often Used and More Often Misinterpreted. *American Journal of Evaluation*, 33: 448–458.

Hafrannsóknastofnun. (2018). *Loðna -Capelin. Ástand nytjastofna sjávar og ráðgjöf 2018*. Hafrannsóknir.

Hjálmar Vilhjálmsson. (1994). *The Icelandic capelin stock. Capelin (*Mallotus villosus Müller*) in the Iceland–Greenland–Jan Mayen area.* Rit Fiskideildar, 13: 1–281.

ICES. 2007, November 26. ICES. (2008). Report of the Workshop on Sexual Maturity Staging of Mackerel and Horse Mackerel (WKMSMAC), 26–29 November 2007, Lisbon, Portugal. *ICES CM 2007/ACFM*:26. 52 pp.

ICES. 2015, October 28. (ICES). 2015. Report of the Workshop on Maturity Staging of Mackerel and Horse Mackerel (WKMSMAC2), 28 September–2 October 2015, Lisbon, Portugal. *ICES CM 2015/SSGIEOM*:17. 93 pp.

Magallanes, E. J. T. (2016). "sizeMat": An R package to Estimate Size at Sexual Maturity. Unpublished. http://rgdoi.net/10.13140/RG.2.1.1685.7848 (Accessed 31 January 2019).

McPherson, L. R., Ganias, K., and Marshall, C. T. (2011). Inaccuracies in routinely collected Atlantic herring (*Clupea harengus*) maturity data and correction using a gonadosomatic index model. *Journal of the Marine Biological Association of the United Kingdom*, 91: 1477–1487.

Midway, S. R., and Scharf, F. S. (2012). Histological Analysis Reveals Larger Size at Maturity for Southern Flounder with Implications for Biological Reference Points. *Marine and Coastal Fisheries*, 4: 628–638.

Olafsdottir, A. H., and Rose, G. A. (2012). Influences of temperature, bathymetry and fronts on spawning migration routes of Icelandic capelin (*Mallotus villosus*): Icelandic capelin spawning migration. *Fisheries Oceanography*, 21: 182–198.

Tomkiewicz, J., Tybjerg, L., and Jespersen, A. (2003). Micro- and macroscopic characteristics to stage gonadal maturation of female Baltic cod. *Journal of Fish Biology*, 62: 253–275.

Vitale, F., Svedang, H., and Cardinale, M. (2006). Histological analysis invalidates macroscopically determined maturity ogives of the Kattegat cod (Gadus morhua) and suggests new proxies for estimating maturity status of individual fish. *ICES Journal of Marine Science,* 63: 485–492.

West, G. (1990). Methods of Assessing Ovarian development in Fishes: a Review. *Marine and Freshwater Research*, 41: 199.

Williams, K. (2007). Evaluation of the Macroscopic Staging Method for Determining Maturity of Female Walleye Pollock Theragra chalcogramma in Shelikof Strait, Alaska. *Alaska Fishery Research Bulletin*. 252–263 pp.

HAFRANNSÓKNASTOFNUN

Rannsókna- og ráðgjafarstofnun hafs og vatna