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Oocyte batch development and enumeration in the European anchovy (*Engraulis encrasicolus*)

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Abstract

An alternative method to the traditional hydrated oocyte (HO) method has been evaluated for the Sicilian anchovy, *Engraulis encrasicolus*. The method is based on the processing of ovarian whole mount images and the identification of the spawning batch in oocyte size frequency distributions and shows the advantage that it can be applied to various oocyte stages rather than strictly to the HO stage. Despite the peculiar elliptical shape of anchovy oocytes, this image analysis technique was fully successful since the yolked stage appeared to perform equally to the HO stage for anchovy batch fecundity measurements.

Keywords: Anchovy, ovarian whole mounts, image analysis, elliptical egg shape, batch fecundity.

Introduction

Fish fecundity constitutes a key factor in stock assessment and management of commercially exploited fish resources since it is implemented in estimations of spawning stock biomass (SSB) through 'Egg Production Methods' (EPMs) (Hunter & Lo, 1993; Armstrong & Witthames, 2012). In particular, the 'Daily Egg Production Method' (DEPM) currently represents one of the two direct methods (with echo-acoustic surveys) applied for evaluating the SSB of pelagic fish stocks, such as anchovies and sardines. DEPM applications require estimates of batch fecundity (BF), i.e. the number of eggs spawned in single spawning events.

The most common method used for estimating BF is to count the number of hydrated oocytes (HO) in imminent spawners (see Hunter *et al*., 1985, for a detailed description of the method). Despite its popularity, applications of the HO method are often limited by difficulties in obtaining field collections of hydrated females. The latter is mainly due to a) the very short daily duration of the ripe spawning phase and b) the segregative behaviour of spawning individuals. Hydrated females in anchovy and sardine populations seem to move away from the main school, thus reducing the sampling occasions to encounter spawning fishes (Ganias, 2008; Basilone *et al*., 2015). Therefore, accurate BF estimates require a high sampling effort and sometimes the number of females is too low or not representative of the whole sampled population (Murua *et al*., 2003).

Several authors have attempted to address the above issues by using other spawning phases rather than the hydration stage (e.g. Ganias *et al*., 2004). The main problem with the use of non-hydrated stages in BF evaluations is that the identification of the spawning oocyte batch is rather difficult though direct microscopic observations - especially for the stages preceding final oocyte maturation - because their size and appearance is quite similar to other oocytes. In the last decades, several studies attempted to solve this problem through estimating the spawning batch as the most advanced mode in oocyte size frequency distributions derived from automated particle counting procedures (e.g., Ganias *et al*., 2010). This image analysis procedure which is able to recognize and measure target oocytes in digital images of ovarian whole mounts, was first introduced for measuring mean oocytes diameters for evaluating total fecundity in determinate spawners (Thorsen & Kjesbu, 2001). Kurita & Kjesbu (2009) extended the applicability of this procedure to indeterminate spawners; however, estimates of total fecundity find no applicability in assessments of reproductive potential for such kind of spawners. More recently, Ganias *et al*. (2010) showed the suitability of image analysis techniques for the measurement of BF in the Atlantic sardine, *Sardina pilchardus*, which opposite to total fecundity is a crucial parameter for the reproductive potential of indeterminate spawners. The application of this method allows increasing the number of samples used for BF estimates in DEPM applications, and appears to save both work and time. Given that the new method constitutes a valid alternative to the HO method of Hunter *et al.* (1985), it would be worth testing it with other commercially important species that are assessed using the DEPM, such as the anchovy.

This study examines the applicability of the above method to the European anchovy (*Engraulis encrasicolus*), a species that may also suffer from particularly limited BF samples in DEPM applications (Somarakis *et al*., 2004; Stratoudakis *et al*., 2006). In contrast to sardines, anchovies display exceptionally oval oocyte shapes and high spawning frequency values (Ganias *et al*., 2014). The former characteristic may complicate correct automated oocyte counting while the latter characteristic should theoretically affect oocyte size frequency distributions. All these issues are considered and analysed in this paper by comparing BF estimations obtained from both hydrated and non-hydrated females during two sampling surveys (2011 and 2013) for anchovy populations in the Strait of Sicily.

Materials and Methods

Sampling and histological analysis

European anchovy individuals were sampled in the Strait of Sicily, within the framework of two combined DEPM and echo-acoustic surveys conducted in 2011 and 2013 for the estimation of the spawning biomass of the local anchovy stock. Samples were collected during the anchovy peak spawning months (Basilone *et al*., 2006) by the research vessel G. Dallaporta. The fishing gear was an experimental mid-water pelagic trawl (vertical opening of 8 m, cod-end mesh size of 18 mm), operating at 4.0 knots and equipped with a net monitoring system (Simrad ITI) used to evaluate the position of the trawl along the water column and the net mouth opening (vertical and horizontal), in order to estimate the catch efficiency of the gear. A random selection of at least 50-75 fish per trawl was performed if no hydrated females appeared in the catch; otherwise, the sample size would increase to 100 individuals per trawl. Each fish was measured onboard immediately, for total length (±1mm), total and somatic weight (±0.01g), sexed and the spawning phases were assigned macroscopically, referring to a 6-phase maturity scale (Ferreri *et al*., 2009). The fish were dissected and the ovaries were extracted and preserved in buffered formalin (4%). In 2011, 147 adult females were collected ranging between 110 and 155 mm in total length (TL) and between 7.59 and 26.81 g in total weight (TW) (Table 1). In 2013, a higher number of females were collected within the TL range 103 to 171mm and TW from 6.61 to 35.26 g (Table 1). In both years, TW of hydrated females was corrected to avoid overestimation, using the linear regression between TW and somatic weight (SW) of mature, non-hydrated females (R^2=0.987 in 2011; R^2=0.996 in 2013), according to Picquelle & Stauffer (1985). The gonadosomatic index (GSI= W_g/SW*100; where W_g=ovarian weight) was estimated for each individual.

At the IAMC-CNR laboratory, histological analyses were carried out on a total of 436 ovary slides of mature females (Table 1). Ovaries were dried of surface moisture and weighed (to the nearest 0.001 g). A small part of ovarian tissue was dehydrated, cleared in xylol, embedded in paraffin and 4 micron histological sections were obtained. Microscopic examination was used to identify the maturity stage of the most advanced group of oocytes and the presence of postovulatory follicles. Oocyte development was classified into six stages, according to Ferreri *et al*. (2009). The histological examination permitted to select a subsample of 131 ovaries from both survey years, for subsequent counting and size measurement of target oocytes (Table 1).

Measurements of oocyte size and number

Whole mount procedures were applied for estimating oocyte size and number both in recent and imminent spawners. Ovarian subsamples of 300-400 mg (W_{ss}) for each hydrated ovary (HYD) and 150-200 mg for vitellogenic ones (VIT) were considered to provide accurate measurements of oocyte size and number. The whole mount procedure was carried out as follows: subsamples were extracted from each ovary, placed in a Petri dish with distilled water and subjected to moderate stirring for about 5 minutes using a magnetic bar for gently mixing and separating oocytes. Subsamples were then examined under a stereo-microscope (Leica MZ6) and, whenever separation was not sufficient, ovarian slurries were re-subjected to stirring for a few extra minutes. When oocyte separation was complete, subsamples were manually processed under the stereo-microscope using forceps in order to remove membranes, blood vessels and aggregations of primary oocytes. To maximize oocyte visibility, samples were spread in a thin layer and were then digitally imaged at 8x magnification using a camera (Leica DCF 420) linked to the stereo-microscope (Fig. 1A).

Oocyte size and number in each gonad were measured using a semi-automated digital image analysis procedure (Ganias *et al*., 2010). In brief, after converting ovarian whole mount images to 8-bit type and adjusting the threshold so as to distinguish oocytes from the background, oocytes were separated using a segmentation algorithm and measured for their area (in mm^2) using particle analysis (Fig. 1B). Because of the peculiar elliptical shape of anchovy eggs, the circularity index was set to have a wide range of values (0.3-1.0) before counting and measuring the target cells. The same procedure was

Table 1. Information about sampling year, number of total sampled females (N), average total length (TL, in mm), average total weight (TW, in g), number of actively not hydrated spawner females analysed by image analysis method (N_{VIT}), number of hydrated females analysed by image analysis method (N_{HYD}), and average gonadosomatic index (GSI, in %) for females selected for oocytes size measurements.

Year	*N*	*TL (±sd)*	*TW (±sd)*	N_{VIT}	N_{HYD}	*GSI (cv)*
2011	147	122.72 (±8.46)	11.06 (±2.62)	24	48	12.99 (0.31)
2013	289	133.53 (±15.04)	17.09 (±5.96)	42	17	6.31 (0.51)

applied to both vitellogenic and hydrated oocytes. The resulting mask image with the outlines of the measured particles was then overlaid on the original image and any missing or false measurements were corrected manually (Fig. 1C). Oocytes were grouped into size classes of 0.05 mm^2 to characterize oocyte size frequency distributions. The latter were analyzed so as to define separate normally-distributed groups within the multimodal size distributions (Bhattacharya's method; Bhattacharya, 1967) (Fig. 1D). This allowed distinguishing the most advanced group (AG) from the less-developed groups (for more details on this analysis see Ganias *et al*., 2010). For the purposes of this study, oocyte size corresponds to the mean area of the advanced group.

For each individual, the total number of oocytes of the advanced group (N_{AG}), was estimated gravimetrically as the product of oocyte density (N_{AG}/W_{ss}) with ovarian weight, W_g. For practical purposes N_{AG} values from both HYD and VIT individuals were supposed to correspond to a single batch of oocytes and to thus express BF.

Statistical analysis

BF data were modelled as a function of developmental stage (VIT vs. HYD) and sampling year using generalized linear models (GLMs). Eviscerated weight was used as a covariate so as to account for changes in BF during the course of somatic growth. Therefore, the set of tested predictors included ovarian stage, year, somatic weight and the three interaction terms. Model diagnostics, including residual vs. predicted plots and quantile plots, suggested a Gaussian error structure and an identity link function for the GLM. Selection of the appropriate covariates was performed by stepwise (backward) entry using the Akaike information criterion (AIC). Analysis of deviance was employed to detect the relative importance of the model covariates and to calculate pseudo R^2 values in order to compare the proportion of the deviation accounted for by each of the covariates. This pseudo-coefficient of determination was calculated as the null residual deviance, i.e. the deviance in the model containing only the intercept, minus the residual deviance of each covariate divided by the null residual deviance of the model.

Results and Discussion

Examination of histological slides allowed to identify 131 out of 436 females at the vitellogenic (VIT; n=66) or hydrated (HYD; n=65) stage with no sign of recent spawning (absence of postovulatory follicles). These individuals covered a wide range of GSI values (1.54 - 24.33; Table 1) and were selected for subsequent oocyte size measurements. Several studies demonstrated the usefulness of estimating the gonadosomatic and con-

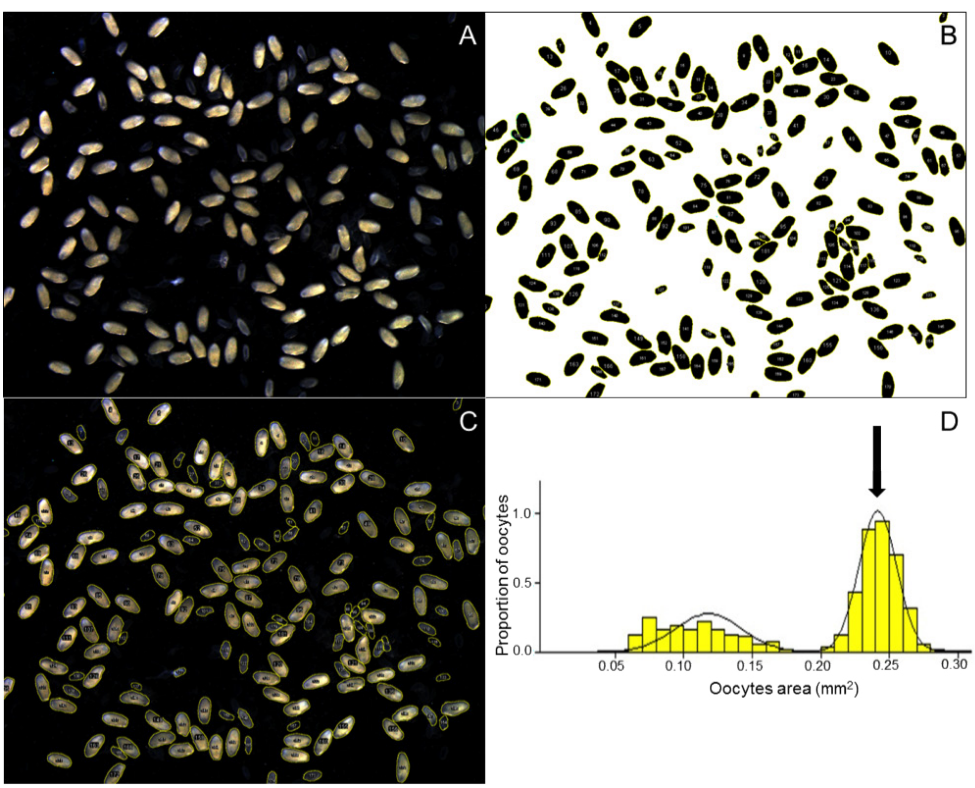

Fig. 1: Consecutive phases of the processing of ovarian whole mounts of anchovy (*Engraulis encrasicolus*) for the counting of oocytes through semi-automated image analysis procedure: A. initial picture (8x magnification); B. thresholding and particle segmentation; C. counting of oocytes through selecting particles corresponding to the size and shape of oocytes; D. separation of the advanced group -indicated by black arrow- from the smaller oocytes in the oocyte size (mm^2) frequency distribution using the Bhattacharya's method.

dition indexes, as a proxy of maturity state and reproductive potential (Trippel *et al*., 1997; Marshall *et al*., 1998), although this applicability for such purposes was questioned by some authors (DeVlaming *et al*., 1982; Erickson *et al*., 1985; West 1990), also in the case of small pelagics. For example, Yoneda *et al*. (2013) showed wide variation of GSI values between subsequent egg batches and within a short time before ovulation for the Japanese anchovy (*Engraulis japonicus*), even if relative batch fecundity did not exhibit any fluctuation. However, oocyte size is a better indicator of individual reproductive state, particularly in indeterminate spawners such as anchovy, because ovarian weight, and thus GSI, is synergistically affected by oocyte size (stage) and number (fecundity) (Ganias *et al*., 2014).

Mean oocyte sizes, obtained by the semi-automatic image analysis procedure, were pooled together in a size frequency distribution graph to highlight the evolution of the maturing oocyte batch (Fig. 2). The spawning batch starts to be easily distinguishable from the advanced nucleus migration phase (NM) (mean oocyte size: 0.24 mm^2) while hydrated oocytes (size bigger than 0.35 mm^2) are completely separated from the previous maturity stages. Such results appear comparable to other studies carried out in Atlantic waters for similar species (*Sardina pilchardus*); however, in the sardine, the spawning batch starts to separate in size at the beginning of yolk granule formation (Ganias *et al*., 2010). The difference in the pattern of oocyte dynamics may be due to differences in the spawning dynamics of the two species. For example, anchovy populations are known to exhibit higher spawning frequency values compared to sardine populations (Ganias *et al*., 2014) which is predicted to cause differences in the oocyte size frequency distribution between the two species.

GLM analysis showed that somatic weight and year were the only significant predictors of anchovy batch fecundity (Table 2, Fig. 3A and B). The calculated pseudo R-squared values showed that sampling year explained a large proportion (67.2%) of model deviance while somatic weight explained 24.3% of model deviance. On the other hand, ovarian development stage (VIT vs. HYD) was not a significant predictor of anchovy batch fecundity (Table 2), as also suggested by the high overlapping degree among BF partial residual distributions (Fig. 3A). This suggests that the yolked stage performs equally to the hydrated stage for anchovy batch fecundity measurements, as observed for other indeterminate spawners (Ganias *et al*., 2010). Additionally, none of the remaining interaction terms was significant predictor of batch fecundity (Table 2).

Finally, when pooling together both development stages (VIT and HYD), the only strong driving effect on BF was represented by the year, which showed sig-

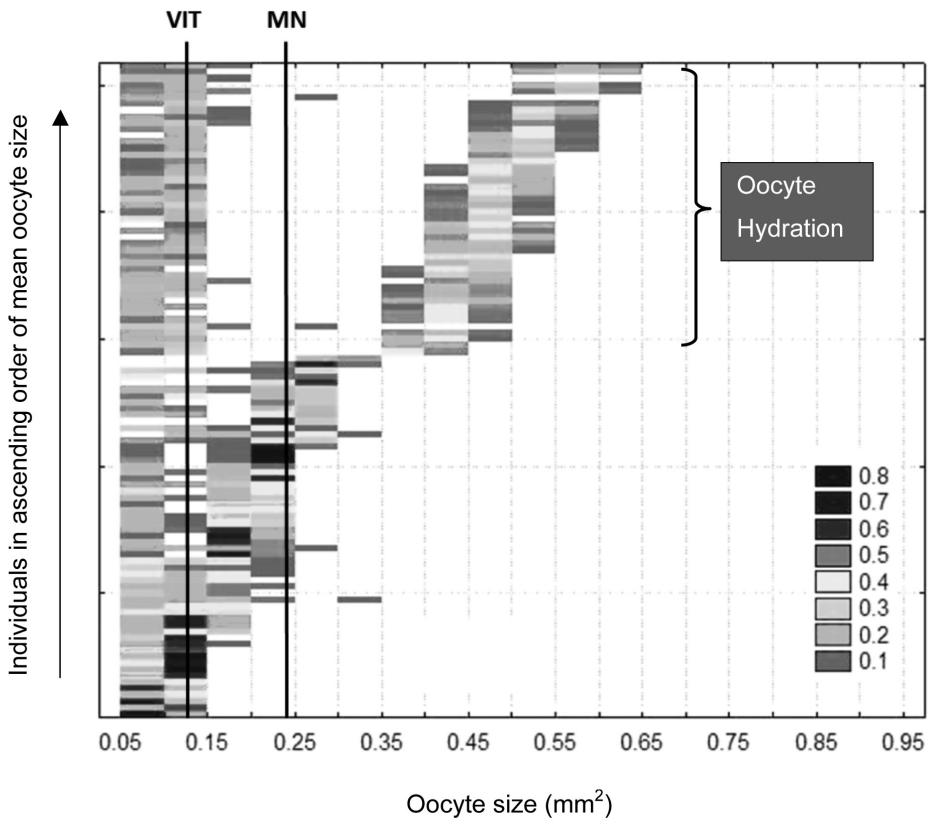

Fig. 2: Oocyte size (mm^2) frequency distributions in 131 anchovy ovaries between the early stage of vitellogenesis and hydration stage in ascending order of average oocyte size. Mean oocyte size for vitellogenic (VIT) and migratory nucleus stage (MN) oocytes is superimposed.

nificantly higher values in 2011 (Table 2, Fig. 3B). The results of this study showed not only methodological aspects but also the importance of interannual variability in BF estimation. Although interannual fluctuations are expected in such a species (Patti *et al*., 2004; Somarakis *et al*., 2004, 2012; Basilone *et al*., 2013; Bonanno *et al*., 2014; Barra *et al*., 2015), this study highlighted the relevance of such variability for BF evaluation, suggesting the requirement to obtain new estimates each year, especially when fecundity is used in stock assessment methods. Interannual variability in reproductive potential, mainly spawning frequency and fecundity, is widely related to environmental variation (Somarakis *et al*., 2004, 2012; Armstrong & Witthames, 2012). The effects of factors such as temperature and feeding conditions, particularly when a density-dependent mechanism is activated, may influence oocyte size (increasing the energy reserves comprised of yolked vesicles; Mc Bride *et al*., 2015) or the number of oocytes per batch (Somarakis *et al*., 2012).

In order to validate previous findings, whereby batch fecundity measurements in anchovy are not affected by the spawning phase of the ovary, relative fecundity was regressed against mean oocyte size (Fig. 4). The latter is a more precise determinant of ovarian phase compared to stage classification into two discrete histological descriptors (HYD vs. VIT). Fecundity estimates for Sicilian anchovy are not affected by the reproductive phase as reflected by mean oocyte diameter (slope of the regression analysis: P>0.5; Fig. 4). Moreover, the residuals of the linear regression relationship were homoscedastic, suggesting that earlier developmental phases provide equal

Table 2. Coefficients of the generalized linear model used to analyse the effect of somatic weight (W), year, developmental stage (vitellogenic versus hydrated) and the interaction between somatic weight and stage on the batch fecundity of Sicilian anchovy.

Source of variation	*Estimate*	*SE*	*T*
Null	2176.05*	869.89	2.50
W	424.27**	59.09	7.18
Year	-3726.11**	530.05	-7.03
Stage	ns		
W*stage	ns		
W*year	ns		
Year*stage	ns		

ns: non-significant; *: P<0.01; **: P<0.001

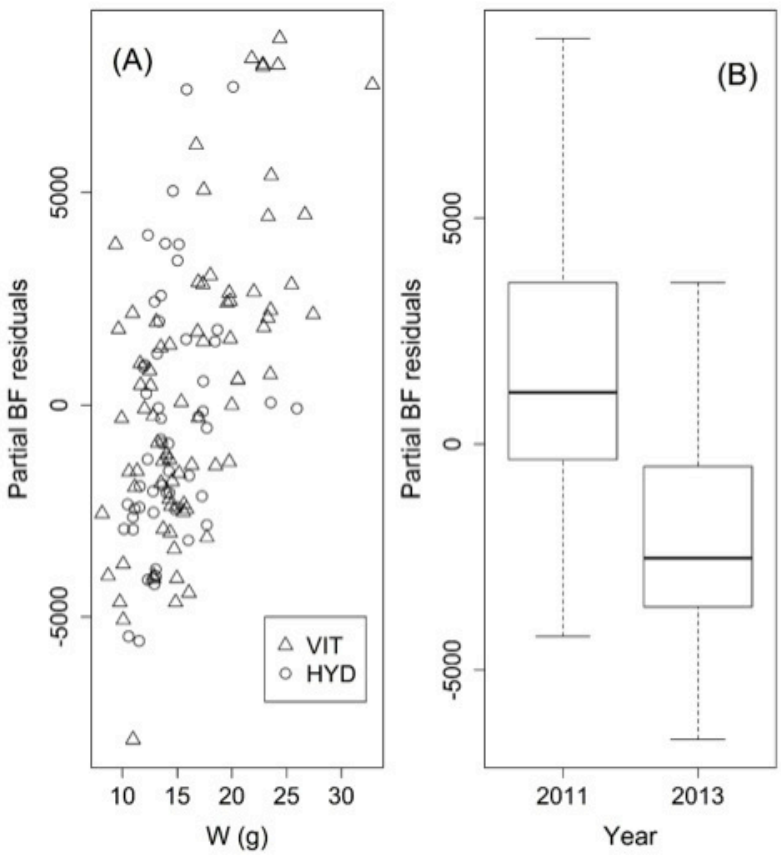

Fig. 3: (A) Relationship between the GLM derived partial batch fecundity (BF) residuals with somatic weight (W) for vitellogenic (VIT) and hydrated (HYD) ovaries. (B) Box and whisker plots of the GLM derived partial BF for the two survey years. Each box represents the 1st quartile, the median and 3rd quartile, whiskers represent the lowest and highest data within 1.5 X interquartile range of the 1st and 3rd quartiles.

measurements of batch fecundity to late hydration stages. Thus, separation of the spawning batch using the Bhattacharya procedure in vitellogenic females is equally effective as the identification of the hydrated batch.

Generally, the daily spawning period of the European anchovy was identified after sunset, both in the Atlantic and in the Mediterranean, including the study area (Basilone *et al*., 2015 and references therein). The greatest problem with using the hydrated stage is that it is particularly rare in adult anchovy samples, because of the short duration of this active spawning phase and because hydrated females showed preference for a spawning area away from the rest of the stock (Picquelle & Stauffer, 1985; Basilone *et al*., 2015). Thus, the estimate of BF by means of the vitellogenic stage, as supported by present results, would be greatly improved, allowing a huge number of individuals to be analyzed for such a purpose.

The success of the present application may be partially attributed to the very low prevalence and intensity of ovarian atresia in anchovy samples. Low atresia levels at spawning peak consist a general characteristic of anchovy populations (Somarakis *et al*., 2004, Ganias *et al*., 2014) that seems to be linked to the direct energetic support of egg production from food intake during the spawning period (McBride *et al*. 2015). However, even if atretic follicles were prevalent and abundant in our whole mount preparations they would have been recognizable by their irregular shape, relatively smaller size and uneven transparency (Hunter & Macewicz, 1985; Óskarsson *et al*., 2002). This suggests that automated particle counting could be equally successful for measurements of BF in species or populations with high proportions of atretic oocytes, allowing to avoid histological procedures, which usually require bigger investments both in time and costs.

The semi-automated image analysis procedure used for anchovy oocytes had already been applied to this species although only to evaluate vitellogenic oocyte growth (Schismenou *et al*., 2012), through the measurement of the long and short axes of oocytes. This study applied a similar procedure for a new application on European anchovy: it estimates oocyte area in order to identify the advanced batch and provide BF evaluations. The oocytes and eggs of anchovy display a peculiar elliptical shape instead of the typical spherical eggs generally found in most fish species. However, present results show that such a peculiar trait does not affect the applicability of image analysis methodology, including the evaluation of fecundity. This is probably due to the correction factor for circularity, applied during the analyses, which allowed obtaining accurate results. This study demonstrated that semi-automatic egg counting can be applied to both hydrated and non-hydrated females to measure batch fecundity accurately, including species with elliptical egg shape. Moreover, the method is much more effective compared to the traditional hydrated methods, due to the potential increase of number of individuals for fecundity estimates.

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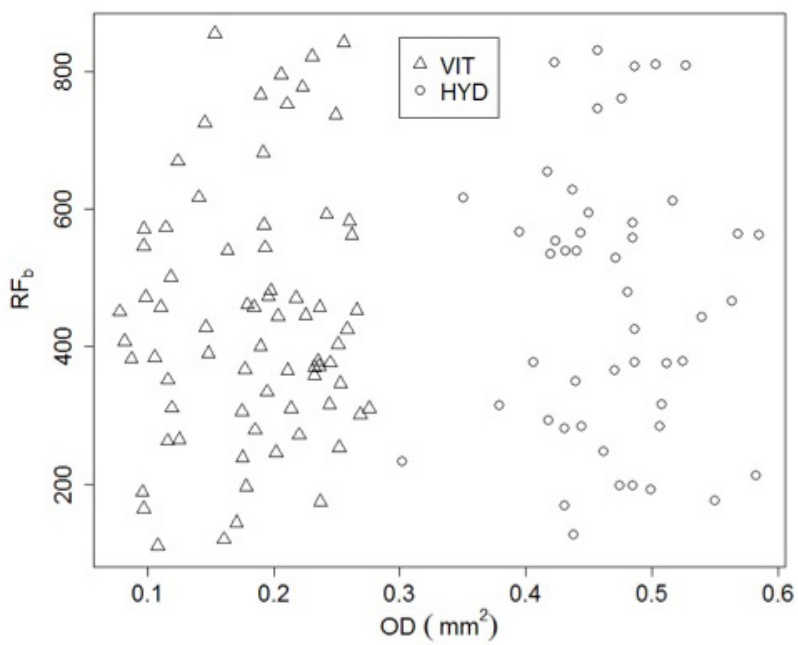

Fig. 4: Relationship between anchovy relative batch fecundity (RF_b) and mean oocyte size (OD) of the advanced batch for vitellogenic (VIT) and hydrated ovaries (HYD).

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References

Armstrong, M.J., Witthames, P.R., 2012. Developments in understanding of fecundity of fish stocks in relation to egg production methods for estimating spawning stock biomass. *Fisheries Research*, 117-118, 35- 47.

Barra, M., Petitgas, P., Bonanno, A., Somarakis, S., Woillez, M. *et al*., 2015. Interannual Changes in Biomass Affect the Spatial Aggregations of Anchovy and Sardine as Evidenced by Geostatistical and Spatial Indicators. *PLoS ONE*, 10 (8): e0135808.

Basilone, G., Guisande, C., Patti, B., Mazzola, S., Cuttitta, A. *et al*., 2006. Effect of habitat conditions on reproduction of the European anchovy (*Engraulis encrasicolus*) in the Strait of Sicily. *Fisheries Oceanography*, 15 (4), 271-280.

Basilone, G., Bonanno, A., Patti, B., Mazzola, S., Barra, M. *et al*., 2013. Spawning site selection by European anchovy (*Engraulis encrasicolus*) in relation to oceanographic conditions in the Strait of Sicily. *Fisheries Oceanography*, 22 (4), 309-323**.**

Basilone, G., Ganias, K., Ferreri, R., D'Elia, M., Quinci, E.M. *et al*., 2015. Application of GAMs and multinomial models to assess the spawning pattern of fishes with daily spawning synchronicity: A case study in the European anchovy (*Engraulis encrasicolus*) in the central Mediterranean Sea. *Fisheries Research*, 167, 92-100.

Bhattacharya, C.G., 1967. A simple method of resolution of a distribution into Gaussian components. *Biometrics*, 23, 115-135.

Bonanno, A., Giannoulaki, M., Barra, M., Basilone, G., Machias, A. *et al*., 2014. Habitat selection response of small pelagic fish in different environments. Two examples from the oligotrophic Mediterranean Sea. *PLoS ONE*, 9 (7), e101498.

DeVlaming, V., Grossman, G., Chapman, F., 1982. On the use of the gonadosomatic index. *Comparative Biochemistry and Physiology*, 73, 31-39.

Erickson, D.L., Hightower, J.E., Grossman, G.D., 1985. The relative gonadal index: an alternative index for quantification of reproductive condition. *Comparative Biochemistry and Physiology*, 81, 117-120.

Ferreri, R., Basilone, G., D'Elia, M., Traina, A., Saborido-Rey, F. *et al*., 2009. Validation of macroscopic maturity stages according to microscopic histological examination for European anchovy. *Marine Ecology*, 30 (Suppl. 1), 181-187.

Ganias, K., 2008. Ephemeral spawning aggregations in the Mediterranean sardine, *Sardina pilchardus*: a comparison with other multiple-spawning clupeoids. *Marine Biology*, 155, 293-301.

Ganias, K., Somarakis, S., Machias, A., Theodorou, A., 2004. Pattern of oocyte development and batch fecundity in the Mediterranean sardine. *Fisheries Research*, 67 (1), 13-23.

Ganias, K., Rakka, M., Vavalidis, T., Nunes, C., 2010. Measuring batch fecundity using automated particle counting. *Fisheries Research*, 106, 570-574.

Ganias, K., Somarakis, S., Nunes, C., 2014. Reproductive Potential. p. 79-121. In: *Biology and Ecology of Sardines and Anchovies*. Ganias, K. (Ed.). Boca Raton, FL: CRC Press.

Hunter, J.R., Lo, N.C.H., 1993. Ichthyoplankton methods for estimating fish biomass: introduction and terminology. *Bulletin of Marine Science*, 53 (2), 723-727.

Hunter, J.R., Lo, N.C.H., Leong, R.H.J., 1985. Batch fecundity in multiple spawning fishes. In: Lasker, R. (Eds.), An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*. NOAA Technical Report No. NMFS, 36, 67-77.

Hunter, J.R., Macewicz, B.J., 1985. Rates of atresia in the ovary of captive and wild northern anchovy, Engraulis mordax. *Fishery Bulletin*, 83, 119-136.

ICES, 2008. Report of the Workshop on Small Pelagics (*Sardina pilchardus, Engraulis encrasicolus*) maturity stages (WKSPMAT). ICES REPORT, 10-14 November 2008, Mazara del Vallo, Italy. pp.82.

Kurita, Y., Kjesbu, O.S., 2009. Fecundity estimation by oocyte packing density formulae in determinate and indeterminate spawners: theoretical considerations and applications. *Journal of Sea Research*, 61, 188-196.

Marshall, C.T., Kjesbu, O.S., Yaragina, N.A., Solemdal, P., Ulltang, O., 1998. Is spawner biomass a sensitive measure of the reproductive and recruitment potential of Northeast Arctic cod? *Canadian Journal* of Fisheries and *Aquatic* Sciences, 55 (7), 1766-1783.

McBride, R.S., Somarakis, S., Fitzhugh, G.R., Albert, A., Yaragina, N.A. *et al*., 2015. Energy acquisition and allocation to egg production in relation to fish reproductive strategies. *Fish* and Fisheries, 16, 23-57.

Murua, H., Kraus, G., Saborido-Rey, F., Witthames, P.R., Thorsen, A., Junquera, S., 2003. Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. *Journal of Northwest Atlantic Fishery Science*, 33, 33-54.

Óskarsson, G.J., Kjesbu O.S., Slotte, A., 2002. Predictions of realised fecundity and spawning time in Norwegian spring-spawning herring (*Clupea harengus*). *Journal of Sea Research*, 48, 59- 79.

Patti, B., Bonanno, A., Basilone, G., Goncharov, S., Mazzola, S. *et al*., 2004. Interannual fluctuations in acoustic biomass estimates and in landings of small pelagic fish populations in relation to hydrology in the Strait of Sicily. *Chemistry* and Ecology, 20 (5), 365-375.

Picquelle, S.J., Stauffer, G., 1985. Parameter Estimation for an Egg Production Method of Anchovy Biomass Assessment. In: *An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, Engraulis mordax*. Lasker, R. (Eds.). NOAA Technical Rep. NMFS., 36, 7-16.

Schismenou, E., Somarakis, S., Thorsen, A., Kjesbu, O.S., 2012. Dynamics of de novo vitellogenesis in fish with indeterminate fecundity: an application of oocyte packing density theory to European anchovy, *Engraulis encrasicolus*. *Marine Biology*, 159, 757-768.

Somarakis, S., Palomera, I., Garcia, A., Quintanilla, L., Koutsikopoulos, C. *et al*., 2004. Daily egg production of anchovy in European waters. *ICES Journal of Marine Science*, 61, 944-958.

Somarakis, S., Schismenou, E., Siapatis, A., Giannoulaki, M., Kallianiotis, A. *et al*., 2012. High variability in the Daily Egg Production Method parameters of an eastern Mediterranean anchovy stock: Influence of environmental factors,

fish condition and population density. *Fisheries Research*, 117- 118, 12- 21.

Stratoudakis, Y., Bernal, M., Ganias, K., Uriarte, A., 2006. The daily egg production method: recent advances, current applications and future challenges. *Fish* and Fisheries, 7, 35-57.

Thorsen, A., Kjesbu, O.S., 2001. A rapid method for estimation of oocyte size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. *Journal of Sea Research*, 46, 295-308.

Trippel, E.A., Kjesbu, O.S., Solemdal, P., 1997. Effects of adult age and size structure on reproductive output in marine fishes. p. 31-62. In: *Early life history and recruitment in fish populations*. Chambers, R.C., Trippel, E.A. (Eds.). Chapman and Hall, New York.

West, G., 1990. Methods of assessing ovarian development in fishes: a review. *Australian Journal of Marine & Freshwater Research*, 41, 199-222.

Yoneda, M., Kitano, H., Selvaraj, S., Matsuyama, M., Shimizu, A., 2013. Dynamics of gonadosomatic index of fish with indeterminate fecundity between subsequent egg batches: application to Japanese anchovy *Engraulis japonicus* under captive conditions. *Marine Biology*, 160, 2733-2741.