ARISTOTLE UNIVERSITY OF THESSALONIKI

FRDC

Visiting scientist, Kostas Ganias - expert on fish reproductive biology related to egg production methods

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Visiting scientist, Kostas Ganias - expert on fish reproductive biology related to egg production methods
Project No 2016/103 February 2018

Visiting scientist: Dr. Konstantinos Ganias, School of Biology, Aristotle University of Thessaloniki.

Host institution: PIRSA-SARDI, Adelaide, South-Australia. Visit period: 2–16 September 2017.

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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1. Executive Summary

Foreword by A/Prof Tim Ward (SARDI Aquatic Sciences)

The Daily Egg Production Method (DEPM) is used to estimate the spawning biomass of several Australian fisheries for pelagic species, including the South Australian Sardine Fishery (SASF) and Commonwealth Small Pelagic Fishery (SPF). Dr Kostas Ganias of Aristotle University of Thessaloniki is a world leader in the reproductive biology of small pelagic fishes related to the application egg production methods. The aim of the Dr Ganias' visit to Australia was to evaluate and recommend options for improving the methods used to estimate the spawning fraction and fecundity of Australian Sardine (*Sardinops sagax*), Jack Mackerel (*Trachurus declivis*), Blue Mackerel (*Scomber australasicus*) and Redbait (*Emmelichthys nitidus*).

Dr Ganias made three key recommendations that have the potential to improve application of the DEPM to the SASF and SPF.

- 1. Consider the size of post-ovulatory follicles (POFs) and the time of sampling when allocating PoFs to daily cohorts.

This recommendation will be used to refine existing protocols for staging POFs.

A student project will also be undertaken in 2018 (by Mr Stuart Sexton, SARDI) to investigate the effects of temperature on the degeneration rates of PoFs and examine spatio-temporal relationships of spawning fraction and egg density.

- 2. Do not underake histological analysis ovaries that cannot have POFs.

The potential benefits if this recommendation will be evaluated during the application of the DEPM to the SASF during 2018.

- 3. Use a method for estimating batch fecundity that does not depend on the collection of females with hydrated females.

The application of the method suggested by Dr Ganias will be trailed in future assessments of the DEPM to SPF species (e.g. Redbait 2018).

The visit to Australia of Dr Ganias was informative. Recommendations have the potential to improve application of the DEPM to the SASF and SPF. Ongoing collborations, including joint projects and publications, are currently being planned.

2. Background

Egg production methods are amongst the most accurate fishery independent methods for assessing the stock spawning biomass, SSB, of commercially important fish stocks. Their concept was mainly shaped at the beginning of 1980s through the development of the daily egg production method (DEPM) which can be applied to any type of pelagic spawners. The DEPM has been extensively applied to fish stocks of various taxa worldwide, including Australia. Despite the long series of applications and large amount of effort and financial resources that have been invested on these assessments there are still important knowledge gaps that may lead to unreasonable increase in the cost and labor of DEPM assessments. Deficiencies might include unavailability of samples for fecundity measurements or inappropriate survey design that can lead to biased parameter estimates, etc.

The ageing of postovulatory follicles (POFs) and the subsequent assignment of spawners to daily classes remains one of the most complicated issues in the DEPM. Correct applications of the POF method presuppose the existence of a validated histological key that accurately corresponds histological POF stages to different ages. However, in many cases POF ageing is performed without prior basic knowledge on the process of POF resorption. The latter is often adopted by other populations of the same or even closely related species, e.g. the criteria developed for the Northern Anchovy (*Engraulis mordax*) by Hunter & Goldberg (1980). Apart from inaccuracy in the applied ageing keys, bias in the staging/ageing of POFs might also be introduced by other factors such as the quality of the slides, the material the ovaries have been embedded in, as well as the experience, patience and fatigue of the observer (Ganias, 2012).

The most common method used for estimating batch fecundity (BF) is to count the number of hydrated oocytes (HO) in imminent spawners (Hunter et al., 1985). 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 behavior of spawning

individuals. Hydrated females in several fishes like Sardine and Anchovy move away from the main school, thus reducing the likelihood to encounter spawning fishes (Ganias et al., 2014). Therefore, accurate BF estimates require considerable sampling and processing effort. Ganias et al. (2010) measured the spawning batch in the Atlantic Sardine (*Sardina pilchardus*) based on oocyte size frequency distributions derived from automated particle counting procedures in digital images of ovarian whole mounts. The application of this automated method allows increased numbers of samples to be analysed for BF estimates in DEPM applications by minimizing both processing effort and time.

A Technical Workshop and Stakeholder Forum on Small Pelagic Fisheries held in Adelaide in 2014 confirmed that Australia is a world leader in the application of the DEPM (Ward et al. 2015). However, experts attending the workshop identified the potential benefits of investigating opportunities for refining application of the method, including protocols and techniques used to estimate key adult reproductive parameters, namely spawning fraction and batch fecundity. Also, a recent meeting of an ICES Working Group (WGALES, Greece 2016) attended by the host PI (Ward), identified similar opportunities to refine methods used to estimate adult parameters in several European fisheries. Funding for this travel travel to Australia was needed to review current methodologies and ensure methods used in Australian fisheries are consistent with world's best practice.

3. Objectives

The planned project objectives were:

- To review methods used to estimate spawning fraction and batch fecundity for Australian species, including Australian Sardine, Jack Mackerel, and Blue Mackerel.
- To compare Australian and European experience related to application of egg production methods.

In addition, through this project the host PI and the visiting scientist managed to:

- Strengthen links and stimulate potentials for future collaboration between PIRSA-SARDI and the School of Biology of the Aristotle’s University of Thessaloniki.

- Plan potential joint publications on the comparison between the breeding strategies of European and Australian pelagic species.

4. Method

In total, I spent two weeks working with PIRSA-SARDI (2nd to 16th September 2017). Most of this time was devoted in reviewing methods used to estimate spawning fraction and batch fecundity. This included: (a) detailed microscopic observations of histological preparations of Australian Sardine (*Sadinops sagax*) ovaries and (b) testing an image analysis technique for estimating fish fecundity.

An attempt was made to improve accuracy in the ageing of the postovulatory follicles (POFs) of the Australian Sardine by including their morphological characters through the development of their size and shape. This seeks to augment the purely histological POF features that previous staging/ageing keys were based on. POFs are reabsorbing structures and thus apart from changes in their histological characteristics, such as the state of the granulosa cell layer, they also undergo significant reduction in size, until complete resorption. Therefore, their size (expressed as their cross-sectional area in histological slides) could indicate their age and consequently the daily class to which female spawners belong.

A total of 135 histological slides of Australian Sardine ovaries were used for this task. All these individuals were previously scored as having POFs. These samples were collected during the 2017 DEPM survey and originated from Scott Cove and Greenly Island. Samples from Scott Cove (n=91) were caught late at night, between 2100 and 2230, i.e. during the peak daily spawning period of Sardine. Samples from Greenly Island (n=43) were caught at dawn. The advantage of this sample scheme is that it allowed the distinction of different daily cohorts of POFs both during spawning and some hours after spawning. Therefore, it helped to accurately configure the entire range of POF degeneration, from the very fresh POFs to almost fully resorbed POFs.

Concerning fecundity calculations, two Jack Mackerel (*Trachurus declivis*) ovaries at late vitellogenic or early final oocyte maturation (FOM) stages were used to examine whether batch fecundity can be measured in non-hydrated females through an automated image analysis procedure. These ovaries were previously weighted fresh, frozen and after a while placed in flasks with 10% formalin. Fecundity measurements

were made using the gravimetric method as follows: a sub-sample of 100-150 mg tissue was taken from each ovary, weighted (1 mg), cleared from the smaller oocytes (mostly primary) using a sieve of 150 μm mesh size and then placed in a Petri dish with tap water. The Petri dish was then placed under a dissecting microscope with an attached microscopy-camera and the subsample was photographed so that all the oocytes would fit into the micrograph. In order to have micrographs of the best possible quality for subsequent oocyte counting, prior to photography each subsample was cleaned from membranes and other non-oocyte material.

In addtion to my technical/laboratory tasks, during the second week I gave a seminar in PIRSA-SARDI on issues related to the adult survey of egg production methods (see the Appendix).

Through the period of my visit I had extended conversations with Dr. Tim Ward on common problems of DEPM surveys and on ways to improve lab processing and field sampling and collaborated with members of his research group in PIRSA-SARDI, especially with Alex Ivey.

5. Results

5.1 Revision of Australian Sardine POF ageing criteria

The revision of POF ageing criteria resulted to the characterization of five different daily POF classes. Table 1 summarises the dimensional (shape, cross-sectional area) and fine histological (state of the granulosa layer) characteristics of different daily classes of Sardine POFs while Figure 1 shows the gradual stages of POF degeneration from the very young POFs to full resorption. The three different time phases per POF class (BEG, MID, END) correspond to samples caught during (latest Scott Cove samples), almost half day beyond (Greenly Island samples) and just before (earliest Scott cove sample) the daily spawning period (respectively).

As can be seen in Table 1 and Figure-1 the cross-sectional area (XSA) of POFs was shown to shrink by approximately 50% per day during the first two days after spawning. Specifically, very fresh Day-0 POFs, occurring in running females, had a mean XSA of 0.04 mm^2, which decreased to 0.018 mm^2 24 h after spawning. The latter value coincided with the XSA of the very young Day-1 POFs, which decreased to 0.011 mm^2 48 h after spawning. The per cent decrease in the XSA of Day-2 POFs was a bit lower,

reaching 0.08 mm^2 72 h after spawning. The XSA of late Day-2 POFs and beyond was smaller than 0.01mm^2 while very small POFs (<0.006mm^2) only persisted until the beginning of the Day-5 class.

Table 1. Summary of dimensional (shape, cross-sectional area) and fine histological (state of the granulosa layer) characteristics of different daily classes of Australian Sardine postovulatory follicles

POF age class	Shape	State of granulosa	Cross-sectional area (mm^2)	
Day0	Irregular	Thick and looped	BEG	0.040
			MID	0.041
			END	0.018
Day1	Rectangular	One well-formulated layer	BEG	0.018
			MID	0.015
			END	0.011
Day2	Triangular	A thin receding layer	BEG	0.012
			MID	0.011
			END	0.008
Day3	Triangular	Resorption almost completed	BEG	0.009
			MID	0.007
			END	0.006
Day4	Triangular	Resorption almost completed	BEG	0.006

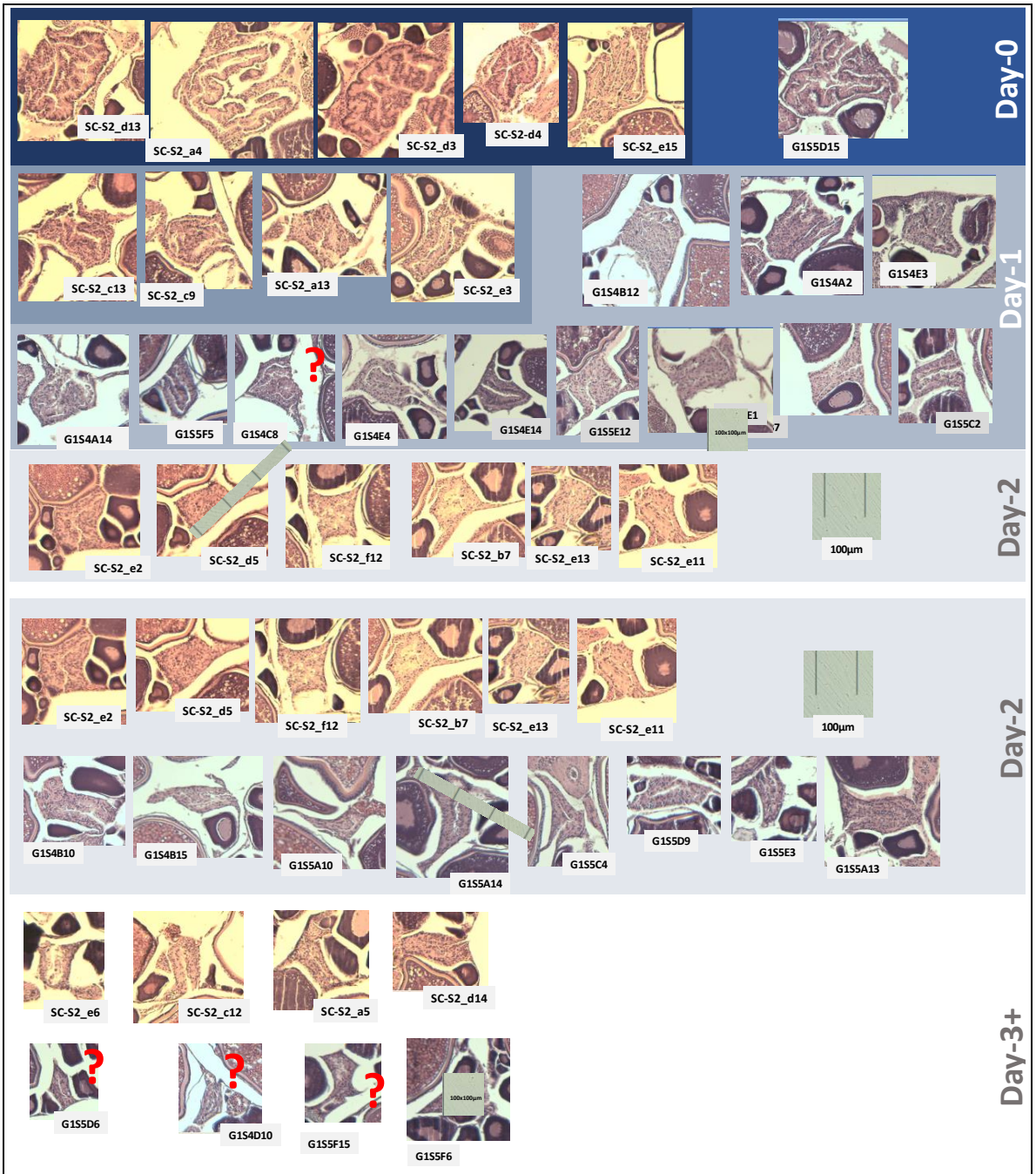

Figure 1. Light micrographs of Australian Sardine postovulatory follicles at sequential stages of resorption.

5.2. Batch fecundity measurement using particle analysis

Digital photomicrographs were processed through an automated procedure based on the ability of most image-analysis software to count and measure objects in binary or thresholded images (Fig. 2).

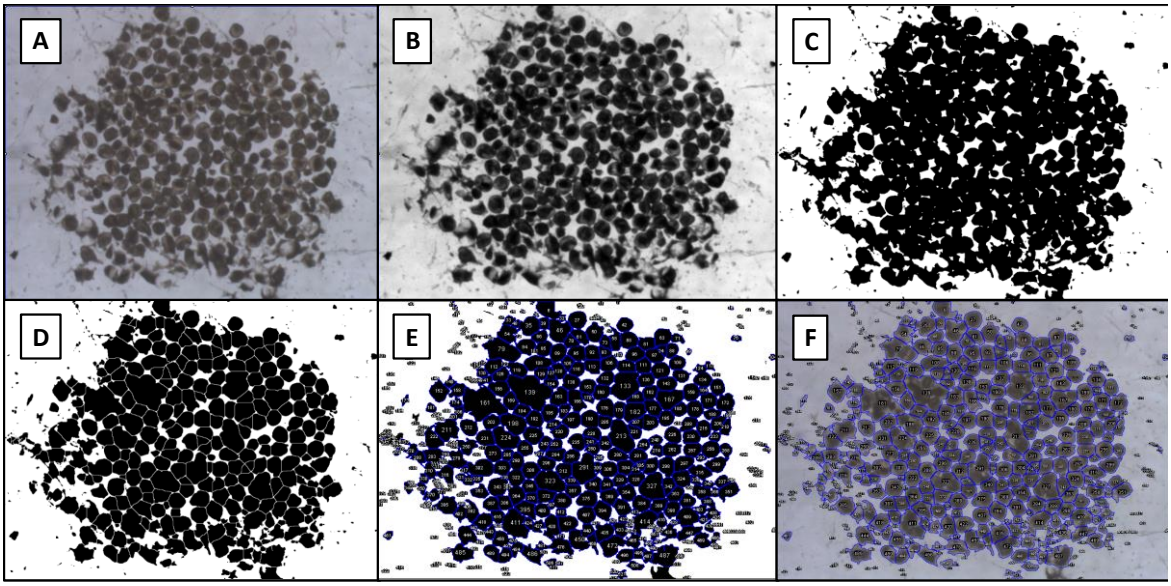

Figure 2. Consecutive phases of the processing of ovarian whole mounts of Jack Mackerel for the automated counting of oocytes.

The routine was quite similar to that described by Thorsen and Kjesbu (2001) and included consecutively the adjustment of brightness and contrast, conversion of the image type to 8-bit (Fig. 2B), restriction of colour spectrum to a region that includes all the oocytes (thresholding; Fig. 2C), separation of individual particles (segmentation; Fig. 2D) and counting of all particles above the size order of previtellogenic oocytes (Fig. 2E & F). After measuring the size of individual oocytes and producing size frequency distributions for each ovary separately, frequency histograms were produced (Fig. 3). The number of the advanced batch oocytes was measured through decomposing composite oocyte size distributions and estimating the number of particles in the distribution that corresponds to the spawning batch using a combination of the Bhattacharya's method and the NORMSEP module inside the FiSAT II software (http://www.fao.org/). Batch fecundity was estimated gravimetrically by dividing the oocyte number number with sub-sample weight and multiplying it with

whole ovary weight. Relative batch fecundity was measured by dividing fecundity with total somatic weight and ranged between 200–250 oocytes*g^{-1}.

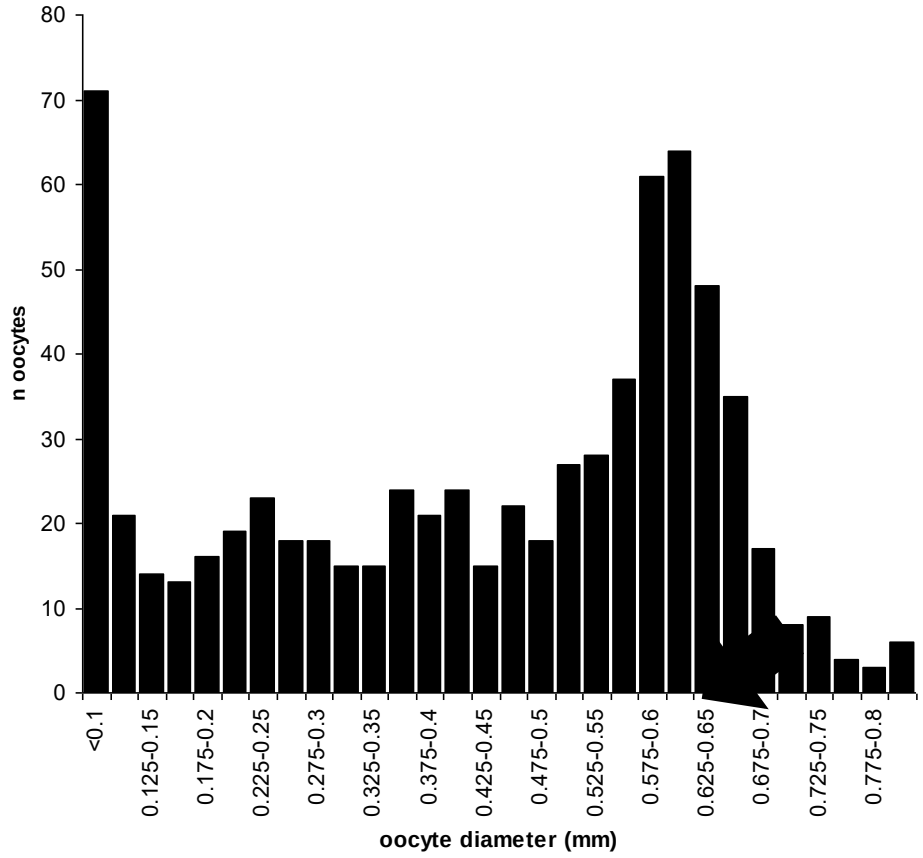

Figure 3. Oocyte size size frequency distribution from a Jack Mackerel ovary taken from automated particle analysis. The arrow corresponds to the spawning batch.

6. Discussion

The size of POFs provided an indirect, estimation of the time elapsed from spawning and may thus be used to test both the validity of POF staging criteria for identifying daily cohorts of spawners and the effect of other factors (such as temperature) in Australian Sardine DEPM applications.

Some recommendations for future assessments are:

1. To consider the possible effect of temperature in POF resorption rates by analysing years and samples caught at extreme temperatures (low/high).

2. To consider the hour of sampling during POF scorings in order to have better correspondence with the respective morpho-histological features of each resorption phase.
3. To not take into account very late triangular POFs with XSA lower than 0.01 mm^2 (marked in red in Table1), as these are generally hard to be aged and thus assigned to daily classes. These small POFs include the very late Day-2 POFs and all older classes.
4. To discard from the histological analysis all those ovaries that are impossible to have POFs such as late vitellogenic and the pre-ovulatory stages, including nucleus migration and germinal vesicle breakdown stage. This will much reduce cost and labor in spawning fraction estimations. To discard the inactive, post-spawning stages from the analysis of the spawning fraction.

Concerning the fecundity task, the resulting relative fecundity values (~200–250 oocytes*g^{-1}) where within the range of previous estimates for the same population (Ward et al., 2016), suggesting that the method worked well for estimating batch fecundity in Jack Mackerel using non-hydrated females. The main advantage of this method deals with the time that is needed for fecundity measurements. After the parameterization of the software, the process of analyzing even large volumes of image files may be carried out quite easily and quickly. In particular, the time needed (excluding the technical work required for the preparation of the whole mounts) to measure oocytes directly under the ocular microscope requires at least 5 minutes per specimen, while large numbers of photomicrographs can be analyzed using a macro in the image analysis software in only few minutes. Applications of this method may also provide large archives of digital micrographs to which someone can easily come-back for posterior analysis without depending on the status of stored biological material and existing laboratory infrastructure (precision gauges, stereoscope, etc.).

Both methods, i.e. the measurement of POF are in spawning fraction estimates and the automated procedure in BF estimation are currently used in several institutes across Europe (e.g. IPMA, Portugal; IEO, Spain) and are anticipated to improve DEPM application in Australian waters.

7. Planned publications

Two potential publications in peer-review journals were planned:

The first potential publication concerns the daily spawning dynamics and formation of spawning aggregations in Australian Sardine.

The second potential publication concerns an inter-specific and inter-population analysis of consistency in spawning frequency values among Sardine (genera *Sardinops* and *Sardina*) stocks worldwide using Australian Sardine as case study.

8. Links and future collaboration

Several forms of collaboration with Dr. Ward and PIRSA-SARDI were discussed during this visit. Apart from the aforementioned joint publications, these include the use of staff mobility programs, and the joint supervision of post-graduate and PhD projects. Moreover, close collaboration is anticipated within the framework of ongoing ICES work on the study of spawning dynamics of various commercial fish stocks.

9. Acknowledgments

First, I would like to thank Dr. Tim Ward for organizing this visit and for being an excellent host and providing all possible means to support this visit. Alex Ivey is also greatly thanked for his valuable help with histological material and data-sets.

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11. Appendix 1. Ganias seminar

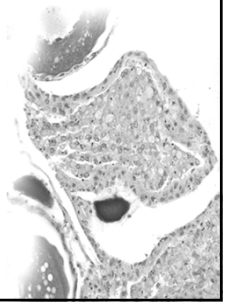
13 September 2017
PIRSA, SARDI
Adelaide, S. AUSTRALIA

Methodological advances in pelagic fish reproductive potential :

With implications for egg production methods

Kostas Ganias

ICHTHYOLOGY LAB.
School of Biology
Aristotle University of Thessaloniki,
Greece



Advances in pelagic fish reproductive potential

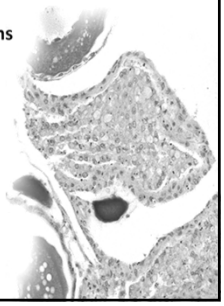
Outline

Brief introduction to egg production methods

Overview of fish spawning dynamics

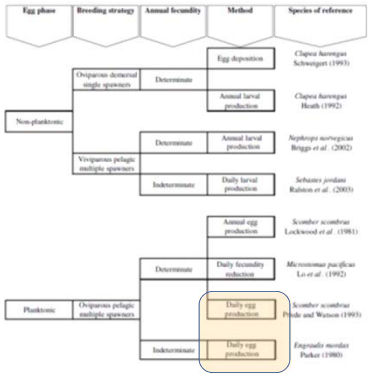
Advances in spawning frequency estimations

Advances in batch fecundity estimations



Ichtyoplankton methods

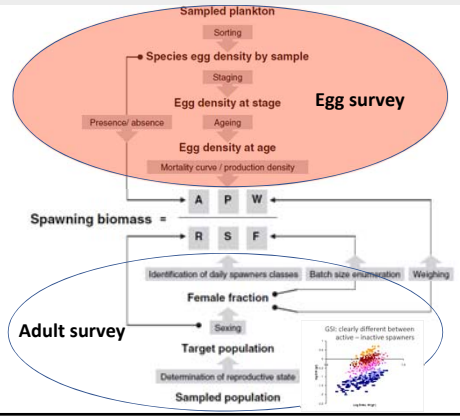
Outline



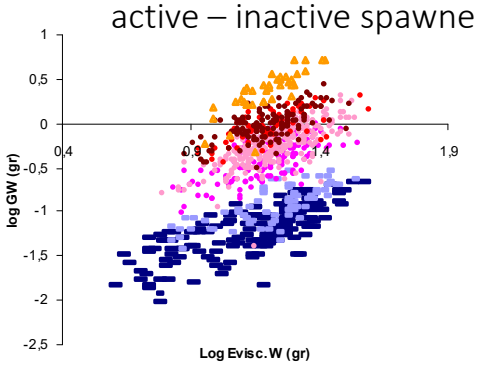
Stratoudakis et al. [2006] Fish & Fish

Ichtyoplankton methods

The DEPM Model

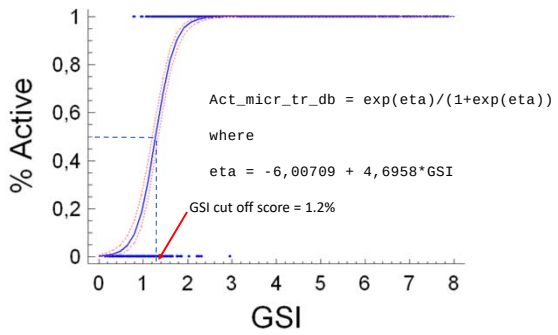


GSI: clearly different between active – inactive spawners

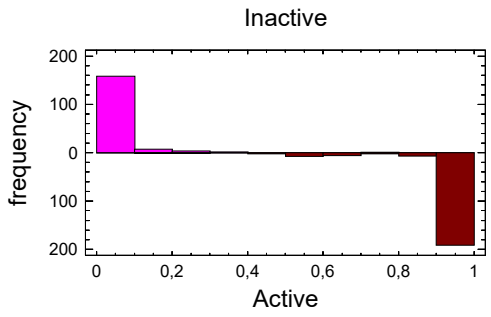


%Activity-GSI ogive

AEGEAN POPULATION

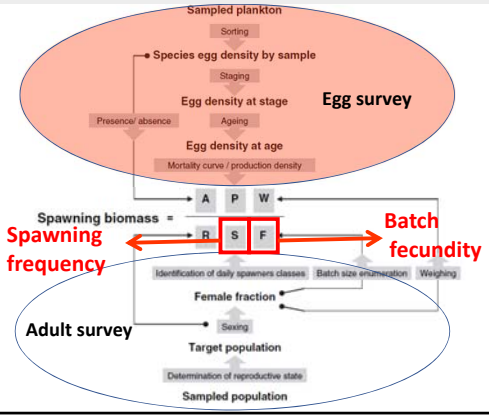


Model predictive capacity IONIAN POPULATION



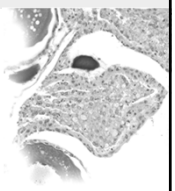
Ichtyoplankton methods

The DEPM Model



Ichtyoplankton methods

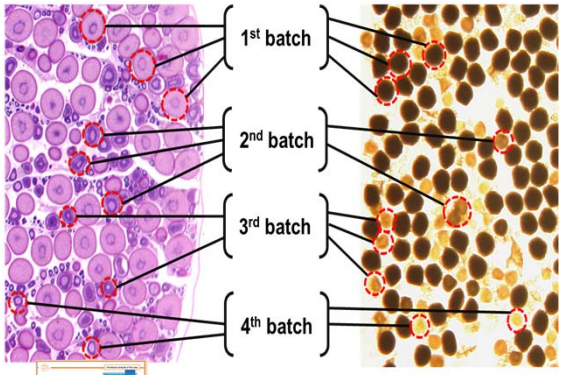
Spawning frequency and batch fecundity

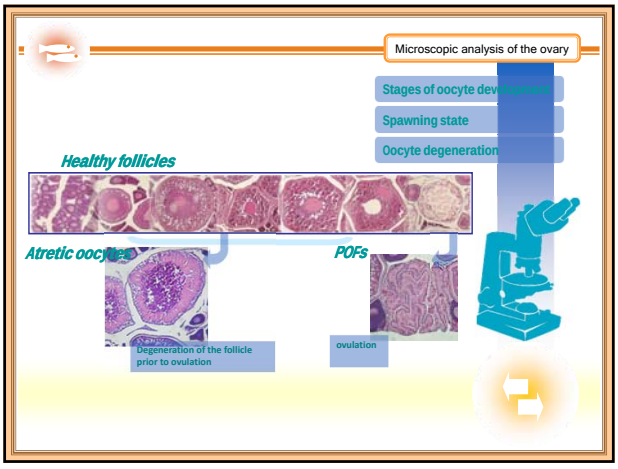


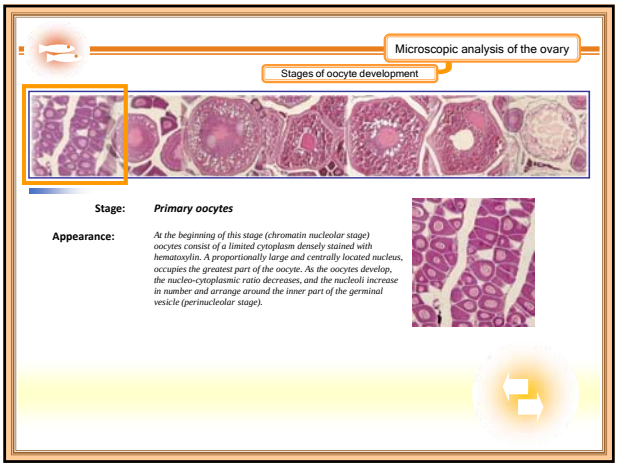
S: Expressed either as an individual (**interspawning interval**) or as a population (**spawners' fraction**) parameter

F: the number of eggs produced in a single spawning batch

Understanding and thus estimating both S and F requires some good understanding of spawning dynamics





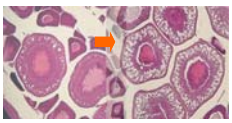


Microscopic analysis of the ovary

Stages of oocyte development

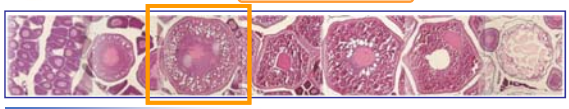
Stage: ***Cortical alveoli***

Appearance: *Unstained 'empty' vacuoles (yolk vesicles) appear in the cytoplasm and then migrate to the periphery of the cytoplasm forming at first a single layer and then several peripheral rows (cortical alveoli). By the end of this stage the zona radiata appears.*



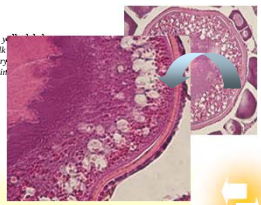
Microscopic analysis of the ovary

Stages of oocyte development



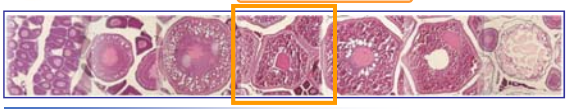
Stage: ***Primary yolk globule***

Appearance: *Yolk is incorporated to form small y within the peripheral ooplasm. Yolk ¾ of the distance from the periphery perinuclear zone. Oil droplets begin ooplasm.*



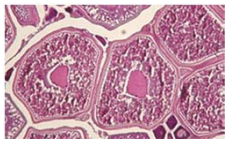
Microscopic analysis of the ovary

Stages of oocyte development



Stage : ***Secondary yolk globule***

Appearance: *Yolk globules increase in number and size, and consequently oocytes are filled with them. Oil droplets increase in number and fill the entire ooplasm.*

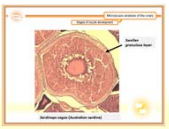


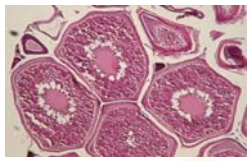
Microscopic analysis of the ovary

Stages of oocyte development

Stage : ***Tertiary yolk globule***

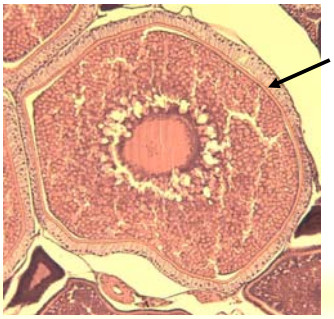
Appearance: *The oil droplets exist only in the periphery of the germinal vesicle.*





Microscopic analysis of the ovary

Stages of oocyte development



Swollen granulosa layer

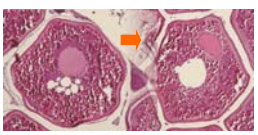
Sardinops sagax **(Australian sardine)**

Microscopic analysis of the ovary

Stages of oocyte development

Stage : ***Migratory nucleus***

Appearance: *The germinal vesicle migrates toward the animal pole and the oil droplets begin to fuse to a single oil droplet.*

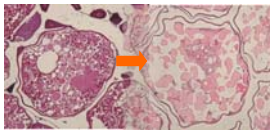


Microscopic analysis of the ovary

Stages of oocyte development

Stage : *Hydration*

Appearance: *The oocyte dramatically increases in size, the nucleus membrane disintegrates, and yolk globules are fused into large yolk plates. The hydrated oocytes will be ovulated and then spawned.*

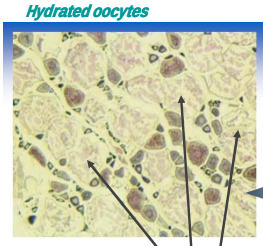


Microscopic analysis of the ovary

Spawing state

Before spawning

Hydrated oocytes



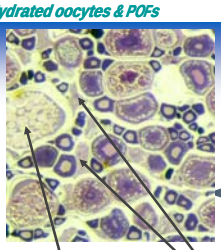
Only hydrated oocytes

Microscopic analysis of the ovary

Spawing state

At ovulation

Hydrated oocytes & POFs



Hydrated oocyte

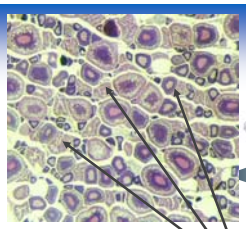
POFs

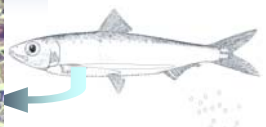
Microscopic analysis of the ovary

Spawing state

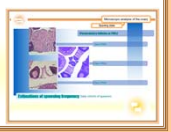
Just after spawning

POFs





POFs



Microscopic analysis of the ovary

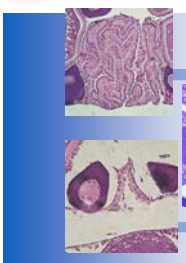
Spawing state

(Postovulatory follicles or POFs)

Day-0 POFs

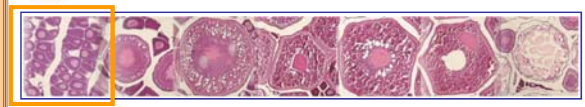
Day-1 POFs

Day-2 POFs

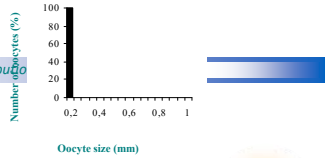


Estimations of spawning frequency Daily cohorts of spawners

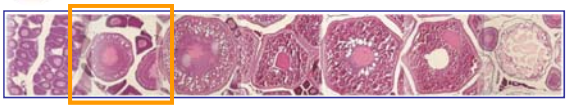
Pattern of oocyte recruitment



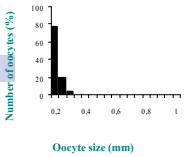
Primary oocytes *Oocyte size frequency distribution*



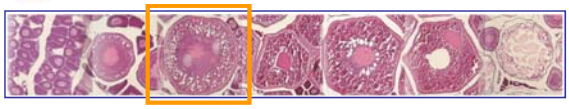
Pattern of oocyte recruitment



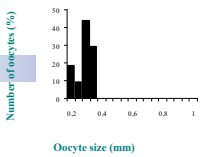
Cortical alveoli stage



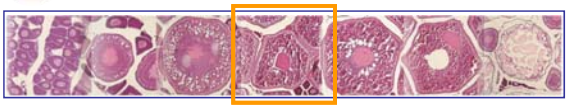
Pattern of oocyte recruitment

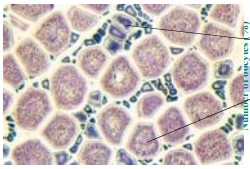


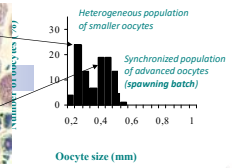
Primary vitellogenesis



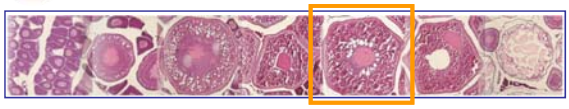
Pattern of oocyte recruitment



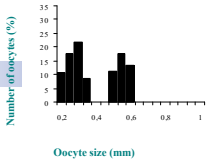




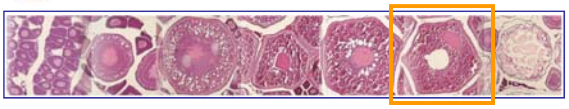
Pattern of oocyte recruitment

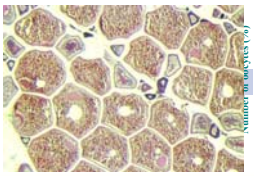


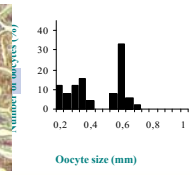
Tertiary stage



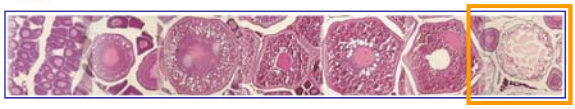
Pattern of oocyte recruitment



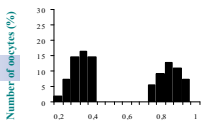




Pattern of oocyte recruitment

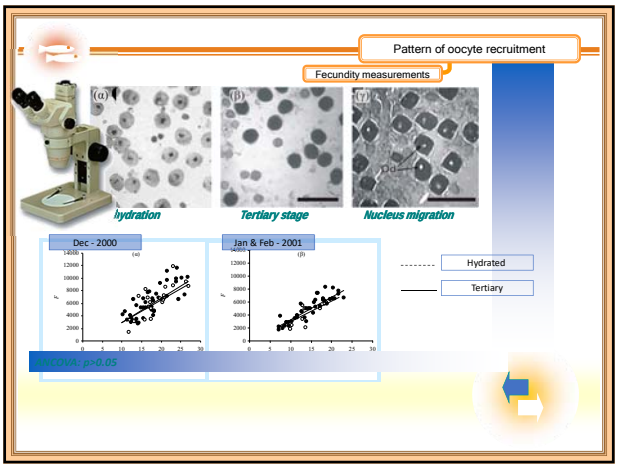


Hydration



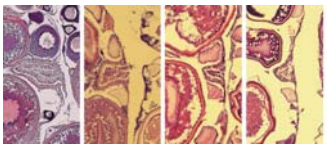
The spawning batch separates prior to hydration (tertiary stage)

Can fecundity be measured in non-hydrated females?



The POF method

POF ageing



POF staging POF-0 POF-1 POF-2 POF-3

Tank experiment (sacrificing fish at known times after spawning)

Field experiment (correspond stages to sampling times)

POF aging Day-0 Day-1 Day-2 Day-3

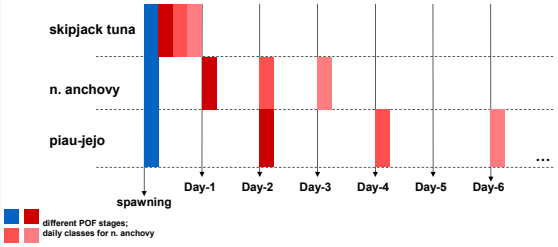
Spawning fraction Day-i / N

Bias in POF ageing

Inter-specific differences in POF resorption rates

Arbitrary use of ageing criteria from other species

The duration of POF degeneration is variable among fish species, ranging from less than 1 day in the skipjack tuna (*Katsuwonus pelamis*) (Hunter et al., 1986) to more than 7 days in the piau-jejo (*L.taeniatus*) (Santos et al., 2005).

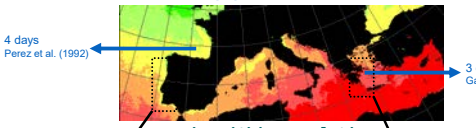


Bias in POF ageing

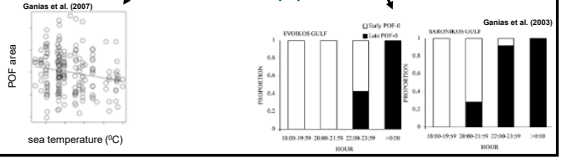
Intra-specific differences in POF resorption rates

duration of POF resorption in *Sardina pilchardus*

a. between populations



b. within populations



Background information

POFs are reabsorbing structures

Thus, apart from changes in their histological characteristics such as the state of the granulosa-cell layer, they also undergo gradual reduction in size, until complete resorption

Under similar environmental conditions POFs are postulated to shrink at a more or less constant rate

Thus, the size of the POFs could indicate their age and consequently the daily spawning class of active females

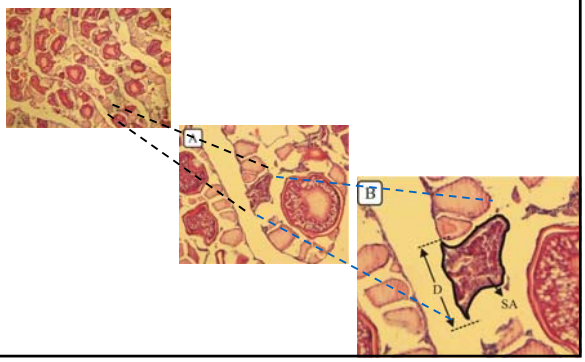
Development of a method which allowed:

To improve accuracy in the aging of the postovulatory follicles of sardine by including the evolution of their shape and their size to their morphological characterization

To explore other factors that might affect the size of POFs such as the embedding material (paraffin/resin) and ambient temperature.

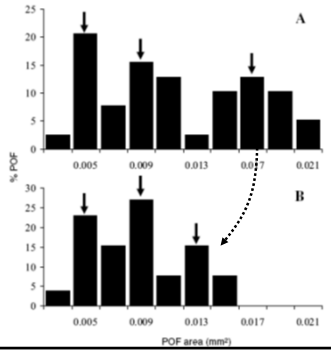
Methodology

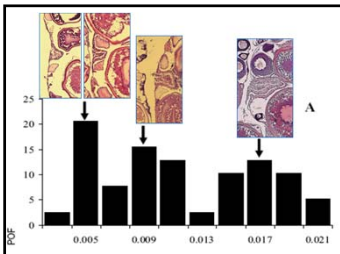
A. Identification of the appropriate POFs, and measurement of POF-XSA



Methodology

B. Validation of POF aging key



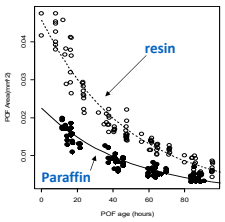


B. Validation of POF aging key

Age (d)	Shape	Material	Surface area (µm2)	State of granulosa
<1	Irregular	paraffin	16404±427	Thick and looped
		resin	35486±1472	
1-2	Rectangular	paraffin	9272±255	One well formulated layer
		resin	17650±468	
2-3	Triangular	paraffin	5910±187	A thin layer or only some
		resin	12620±303	remnants
>3	Triangular	paraffin	4160±197	Completely reabsorbed, only
		resin	7234±373	some residual vacuoles

A/ time elapsed from spawning

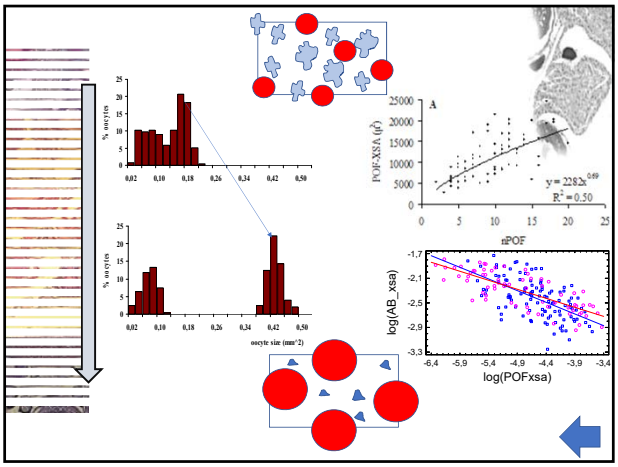
POFs shrink exponentially with time in a way that every other day the follicle surface reduces in size by almost 50%



B/ Embedding material

The rate of POF resorption, i.e. the slope of the POF surface/age relationship was not found to be significantly different between the two embedding materials.

C/ Fixative: no effect on POF-XSA

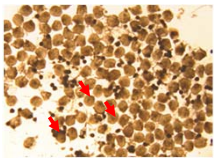


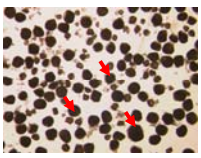
Background

Measurement of batch fecundity

Hydrated oocytes method (gravimetric/volumetric)
Hunter et al., 1985

Based on the clear size separation of the oocytes of spawning batch at hydration





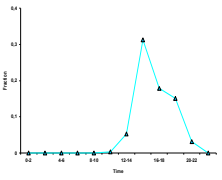
Compared to other stages of the spawning cycle

Background

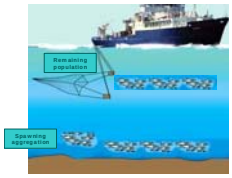
Limitations of the HOs method

Hydrated females are **scarce** because hydration stage is limited both

•**in time:**

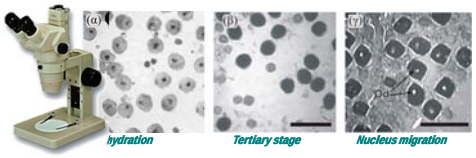


•**and space:**



Background

Use of non-hydrated females



Background

Problems with the Tertiary + MN method

•**Needs histology**
is only affordable when applied in parallel with the POF method

•Is again **limited to certain stages**

•**Can be quite labor intensive**
especially when polarized light is needed to identify tertiary & MN oocytes

•Cannot be applied to species :
a. with **continuous oocyte size frequency distributions**
b. **that lack of oil droplets** (e.g. the anchovy)

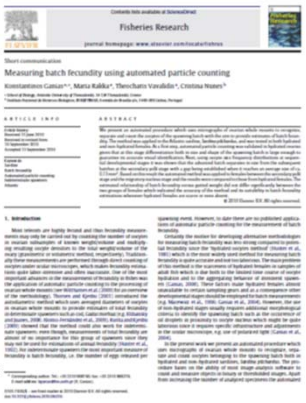
Objectives

To develop an automated procedure for measuring batch fecundity in indeterminate spawners

by analyzing digital images of ovarian whole mounts

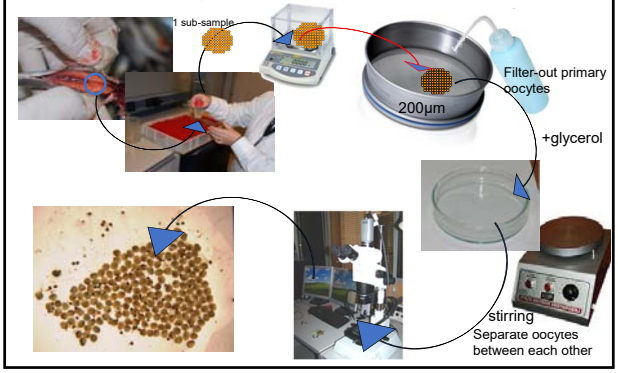
To test whether the method can be applied to non-hydrated females

To improve the samples of batch-fecundity measurements



Methodology

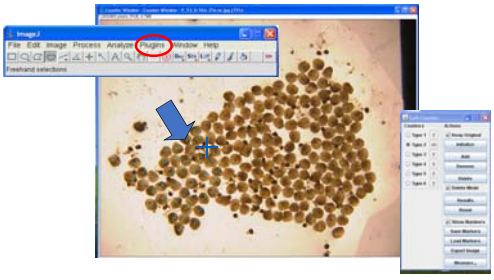
Laboratory processing of ovarian subsamples



Methodology

Validation of the automated method

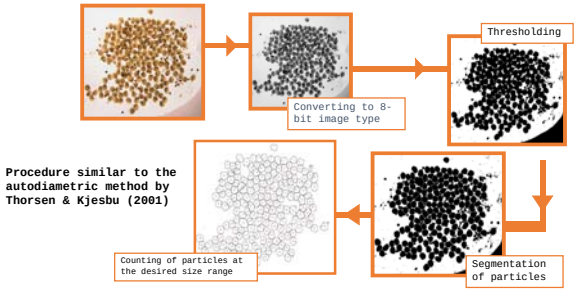
STEP -a On screen counting of hydrated oocytes using the **'Cell counter'plugin@ImageJ** (reference method)



Methodology

Validation of the automated method

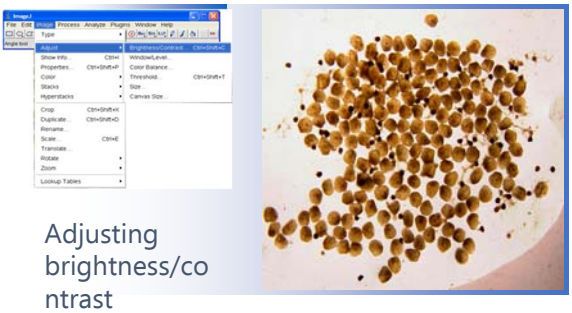
STEP 6 Development of a macro inside ImageJ for identifying and counting only the hydrated oocytes



Automatic procedure for counting hydrated oocytes through ImageJ

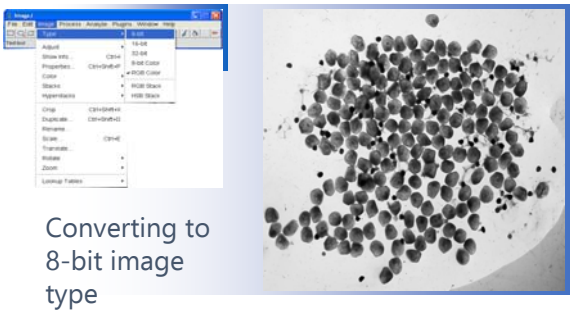
Opening of image file

Automatic procedure for counting hydrated oocytes through ImageJ (ImageJ method)



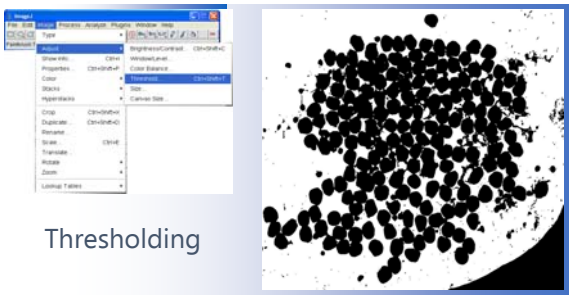
Adjusting brightness/contrast

Automatic procedure for counting hydrated oocytes through ImageJ (ImageJ method)



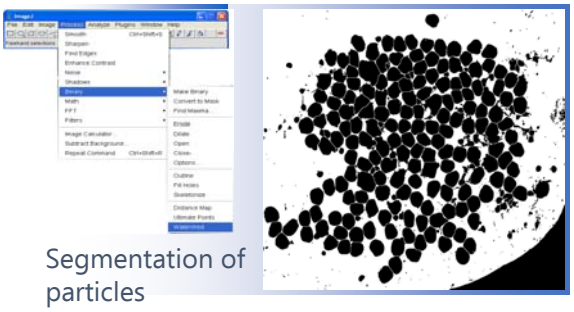
Converting to 8-bit image type

Automatic procedure for counting hydrated oocytes through ImageJ (ImageJ method)



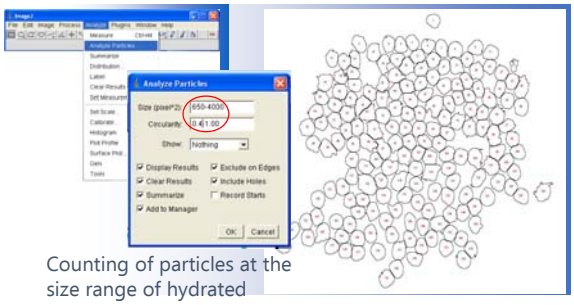
Thresholding

Automatic procedure for counting hydrated oocytes through ImageJ (ImageJ method)



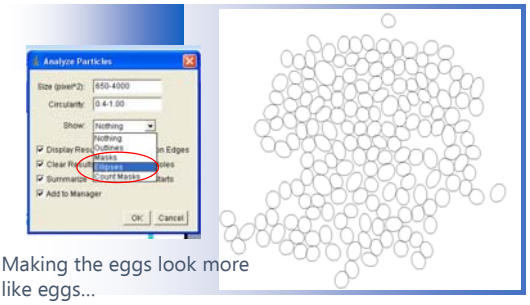
Segmentation of particles

Automatic procedure for counting hydrated oocytes through ImageJ (ImageJ method)



Counting of particles at the size range of hydrated oocytes

Automatic procedure for counting hydrated oocytes through ImageJ (ImageJ method)

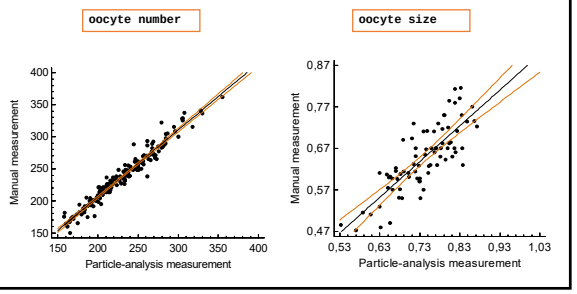


Making the eggs look more like eggs...

Methodology

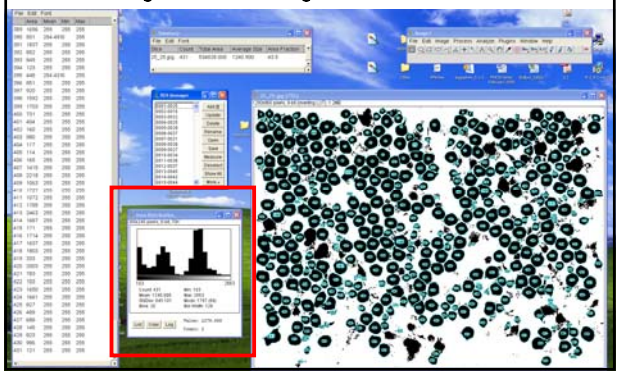
Validation of the automated method

Comparison between automated and manual measurements



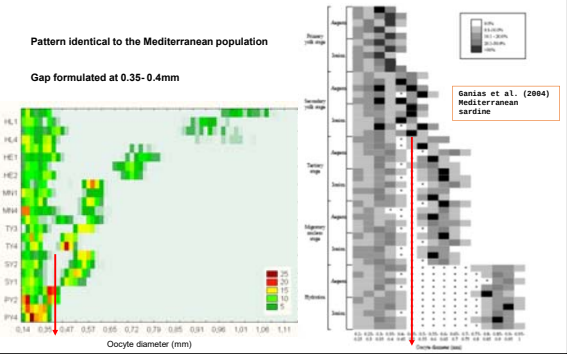
Methodology

Oocyte size frequency distributions



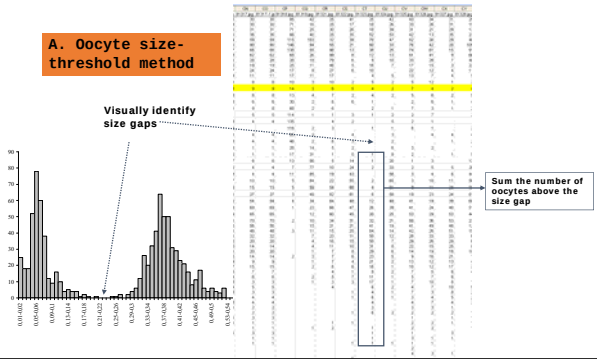
Methodology

Oocyte size frequency distributions



Methodology

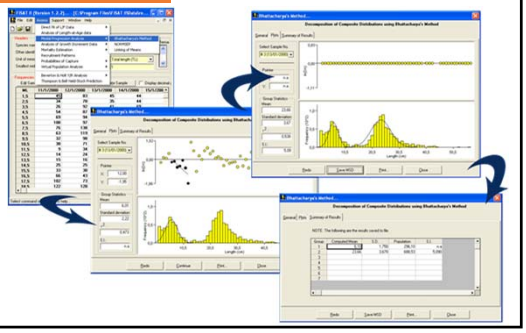
F measurement in non-hydrated ovaries



Methodology

F measurement in non-hydrated ovaries

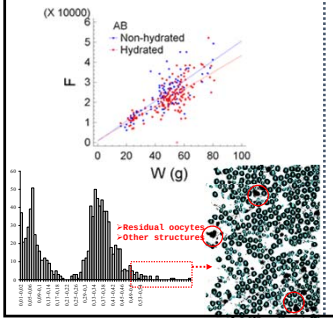
B. Bhattacharya's method



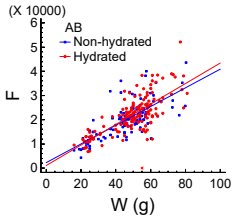
Results

Test the automated method in non-hydrated females

Using size-threshold method;
P<0.001



Using Bhattacharya's method;
P>0.5



And now...
some conclusions

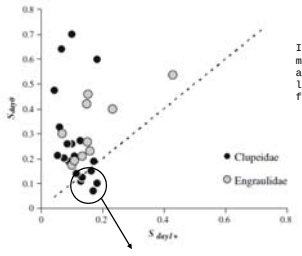
This method may...

- Increase the number of specimens for batch fecundity measurements
- increase accuracy in batch fecundity measurements
- contribute in saving time and work-load (oocyte counting 5 to 8 times faster compared to the classic method)
- provide large archives of digital microphotographs for future analysis

Ευχαριστώ πολύ!

...meaning thanks in Greek

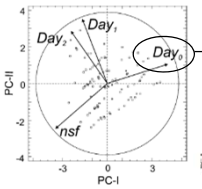
The fraction of day-0 spawners is systematically over-estimated within multiple spawning clupeoids



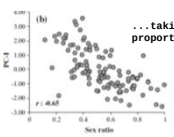
Information from 30 stocks of multiple spawning clupeoids from a wide range of geographic locations (15 species and 2 families)

Ganias (2008) MB

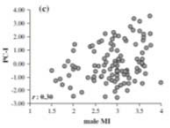
Mediterranean sardine



Actively spawning females segregate out of the remaining population...

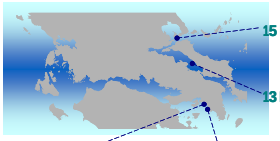


...taking with them a large proportion of males...



...that are in advanced reproductive condition

Ganias (2008) MB



15 Dec 2000
18:45 R=0.27, S_0=0.27
20:00 R=0.53, S_0=0.00
13 Dec 2000
20:00 R=0.17, S_0=0.26
22:00 R=0.35, S_0=0.21
23:30 R=0.61, S_0=0.47
3 Dec 1999
19:00 R=0.98, S_0=0.00
6 Dec 2000
20:30 R=0.34, S_0=0.22
01:00 R=0.64, S_0=0.00
00:40 R=0.36, S_0=0.23

Sequential trawl hauls over the same towing routes

R: sex ratio

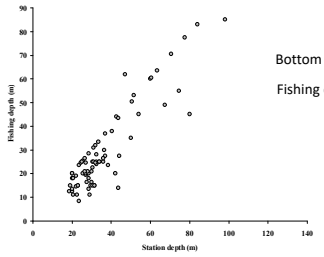
S_0: fraction of Day-0

Structures limited in space and/or ephemeral in time

A spawning aggregation is a group of conspecific fish gathered for the purpose of spawning with fish densities or numbers significantly higher than those found in the area of the aggregation during non-reproductive periods (Domeier and Colin 1997)

Ephemeral spawning aggregations

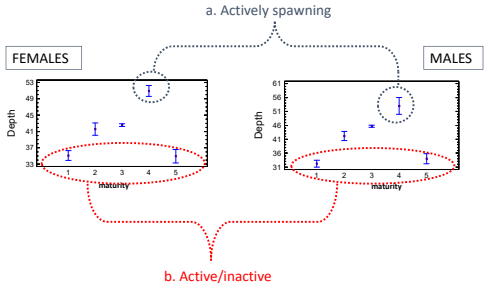
Ganias et al. (2008) MB



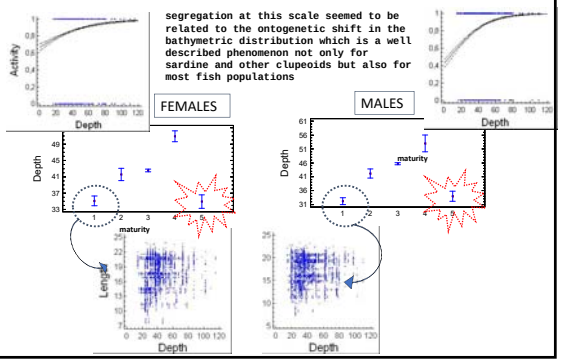
Bottom depth (289/308)

Fishing depth (78/308)

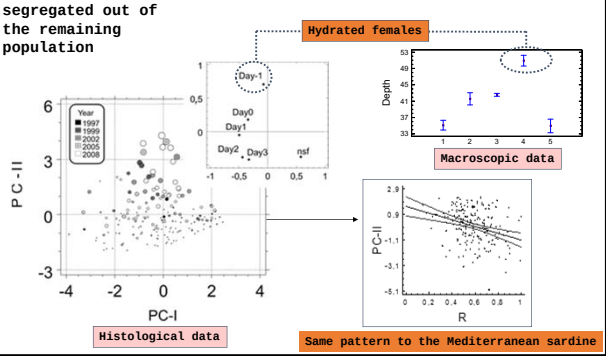
Sardines, both males and females were shown to segregate with respect to their reproductive state, with two types of segregation being generally observed:

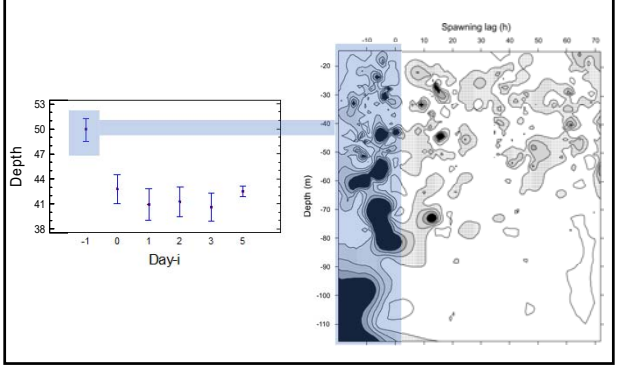


segregation at this scale seemed to be related to the ontogenetic shift in the bathymetric distribution which is a well described phenomenon not only for sardine and other clupeoids but also for most fish populations

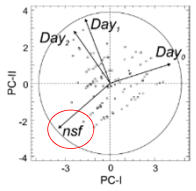


In both datasets the Day_-1 spawners were shown to be segregated out of the remaining population

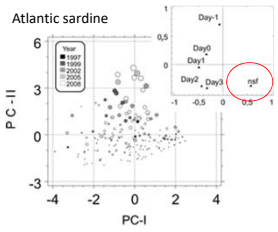




Mediterranean sardine



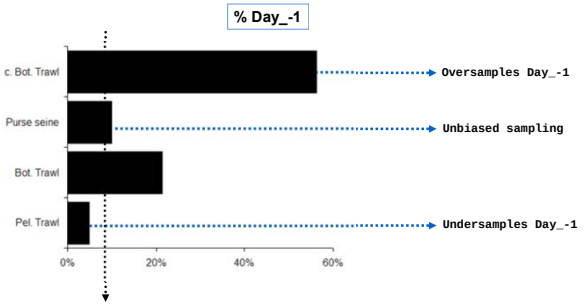
Atlantic sardine



In both populations the non-spawning (recent or imminent) individuals were clustered out of the spawning individuals

Implications

% Day_-1

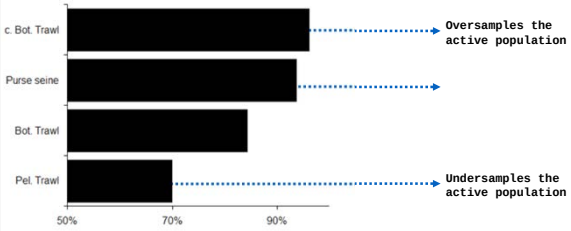


9%; average $

Biased sampling affecting the nominator of

Implications

% Active



Biased sampling affecting the denominator of

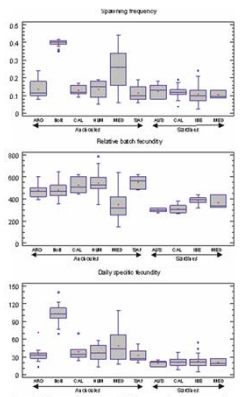


Figure 1. Box and Whisker plots for reproductive parameters of anchovy and sardine stocks from Daily egg production method (DEPM) surveys