Master Thesis

Investigations on the reproduction of the electric lanternfish *Electrona risso* (Cocco, 1829) in the subtropical-tropical North Atlantic

Photography by K. Wieben

by Kim Lea Wieben

Supervisors

Prof. Dr. Oscar Puebla, Geomar

Dr. Heino Fock, Thünen Institute of Sea Fisheries

GEOMAR
Helmholtz-Zentrum für Ozeanforschung Kiel

THÜNEN
Johann Heinrich von Thünen-Institut
Bundesforschungsinstitut für Ländliche Räume, Wald und Fischerei

Kim Lea Wieben

Adelheidstraße 20-22

24103 Kiel

Germany

Tel.: +49 174 8811812

Email: kim.wieben@hotmail.de

Christian-Albrechts-University of Kiel

Faculty of Mathematics and Natural Sciences, Department of Marine Sciences

Study path: M.Sc. Biological Oceanography

Student number: 1014609

Prof. Dr. Oscar Puebla

GEOMAR Helmholtz Centre for Ocean Research Kiel

Wischhofstraße 1-3

24148 Kiel

Germany

Tel.: +49 431 6004559

Dr. Heino Fock

Thünen Institute of Sea Fisheries

Palmaille 9

22767 Hamburg

Germany

Tel.: +49 40 38905169

„Ich erkläre, dass ich meine Masterarbeit „Investigations on the reproduction of the electric lanternfish *Electrona risso* (Cocco, 1829) in the subtropical-tropical North Atlantic" selbstständig und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe und dass ich alle Stellen, die ich wörtlich oder sinngemäß aus Veröffentlichungen genommen habe, als solche kenntlich gemacht habe. Die Arbeit hat bisher in gleicher oder ähnlicher Form noch keiner Prüfungsbehörde vorgelegen. Ich versichere, dass die eingereichte schriftliche Fassung der auf dem beigefügten Medium gespeicherten Fassung entspricht.

Datum, Unterschrift

Contents

List of Figures

List of tables

Index of Abbreviations

A	atretic oocytes
ca	cortical alveoli
CA	cortical alveoli oocyte
df	degrees of freedom
ETRA	Eastern Tropical Atlantic Province
GSI	gonadosomatic index
GVB	germinal vesicle (nucleus) breakdown oocyte
HYD	hydrolysed oocyte
hyg	hydrated yolk granules
IQR	inter-quartile range
ITCZ	intertropical convergence zone
MN	migratory nucleus oocyte
nu	nucleus
OG	oogonia
OMZ	oxygen minimum zone
PG	primary growth oocyte
POF	postovulatory follicle
SD	standard deviation
SL	standard length
VTG1-3	primary to tertiary vitellogenic oocyte
WTRA	Western Tropical Atlantic Province
yg	yolk granules
ym	yolk mass
yv	yolk vesicles

Abstract

The main objective of this thesis was the analysis of the female reproductive cycle of *Electrona risso*, a subtropical-tropical myctophid. Myctophidae are one of the dominating groups in the mesopelagic zone of the world's oceans and represent an important link between trophic levels. Samples were collected during the 383. research cruise of the fishery research vessel "Walther Herwig III" in March and April 2015. Sampling was conducted at 18 stations in the North Atlantic from the equator to the Bay of Biscay with *Electrona risso* present between 0° and 17°N (n = 918). Histological cross-sections of female gonads revealed that *Electrona risso* is a batch spawner with a group-synchronous egg development and a determinate fecundity. The length distribution (30.51 - 81.22 mm SL), showed two major cohorts with the older one reaching maximum reported length. The length at first maturity (L_{50} = 55.5 mm SL) separated both cohorts, showing that only the older cohort was capable of spawning, indicating only one single spawning period with probably death shortly after. The spawning season could not be determined, but active spawning was observed in late March and in early April. The overall sex ratio and the sex ratio over length did not significantly differ from parity, but females appeared to dominate in small and large size classes. A profound age and growth analysis is still missing and should be investigated in a future study, together with the reproductive pattern of *E. risso* populations in other regions.

1 Introduction

1.1 Myctophidae

Myctophidae are one of the dominating groups in the mesopelagic zone of the world's oceans (Gjøsæter & Kawaguchi, 1980). The mesopelagic zone extends from 200 to 1,000 m depth and separates the epipelagic photic zone at the surface and the bathypelagic aphotic zone in the deep sea. Even though some light reaches the mesopelagic zone, the phytoplankton production is low. Thus, it is called the ocean's twilight zone. As a result of the present light and food conditions, most zooplankton and nekton undertake large vertical migrations to the productive epipelagic zone during night time (Marshall, 1979), a process called diel vertical migration (DVM). Feeding in the epipelagic zone at night and excreting in the mesopelagic zone during daytime leads to a transfer of a significant amount of carbon and nutrients to the bathypelagic (Longhurst et al., 1988; Dam et al., 1995).

The marine family Myctophidae is distributed in all oceans and constitutes 32 genera with at least 240 species (Nelson, 2006). One characteristic of lanternfishes is the presence of photophores on their body. Photophore arrangement sometimes is the only identifier to distinguish between species morphologically. Lanternfishes feed on various types of zooplankton (Pusch et al., 2004; Dalpadado & Gjøsæter, 1988) and serve as prey for larger animals. For instance, some Antarctic seal species, penguins and cephalopods feed primarily on myctophids (Rodhouse et al., 1992; Cherel et al., 1997; Guinet et al., 1996). Thus, myctophids represent an important link between lower and higher trophic levels. Nevertheless, the role of mesopelagic fish in the world oceans and their basic biology yet remain mostly unknown, even their total biomass is still in discussion. The former estimate of total biomass was 1,000 million tons globally, but acoustic surveys revealed an underestimation by at least one order of magnitude (Irigoien et al., 2014).

1.2 Myctophidae in commercial fisheries

Myctophids are of little commercial importance, though several countries have attempted to establish a fishery. Soviet-Russian fishing activities on lanternfishes off West Africa have been reported (Gjøsæter & Kawaguchi, 1980) and South African catches peaked with 42,000 tons landed in 1973 for *Lampanyctodes hectoralis* (Prosch, 1991). Most of the species are not edible for human consumption due to their high amount of wax esters (Gjøsæter & Kawaguchi, 1980), but the fish meal and fish oil industry at that time indicated interest to exploit this resource (Haque et al., 1981). However, myctophid exploitation for human

consumption will likely yield cascading effects for the ecosystem, because myctophids often are the major prey item for higher trophic levels. In the Australian Coral Sea for example, *Diaphus danae* specimens form large spawning aggregations, which are fed on by spawning aggregations of yellowfin and bigeye tuna, which are popular edible fish species (Flynn & Paxton, 2012). Cascading effects are particularly known for the removal of top predators, e.g. sharks (Baum & Worm, 2009), but also for midtrophic levels, like zooplankton and planktivorous fish (Möllmann et al., 2008).

Exploitation of the deep sea fish community is often poorly or not managed at all, with possibly severe impacts on the stocks (Koslow et al., 2000). Even when a mesopelagic fish stock is managed, the consequences of this exploitation are often unknown. Analyses of fishery on mid-trophic level species similar to myctophids, showed negative impacts on various parts of the ecosystem (Smith et al., 2011), even though the fishery was at maximum sustainable yield (MSY) level. One of the major difficulties in preserving and managing the mesopelagic fish fauna is of course the lack of information on life history traits e.g. the reproductive biology. In the light of the high commercial potential of myctophids and their role in ecosystems, it is urgent to close this knowledge gap.

To conclude, investigations on the reproduction of myctophids are not only a contribution to basic knowledge, but also important for commercial fisheries as myctophids could become an important resource for fishmeal production. Moreover, myctophids are an important trophic link in the ecosystem and due to commercial fishing activities on their predators, human consumption of fish is also affected.

1.3 The reproductive cycle in female teleosts

The reproductive cycle in females is usually as described in Fig. 1. Immature females enter the reproductive cycle and start maturing until they are mature, i.e. capable of spawning. After spawning they have spent ovaries und are then resting. In multiple spawning fish, females enter the cycle again in the next spawning season, whereas one-time spawners finish the cycle after they have spawned.

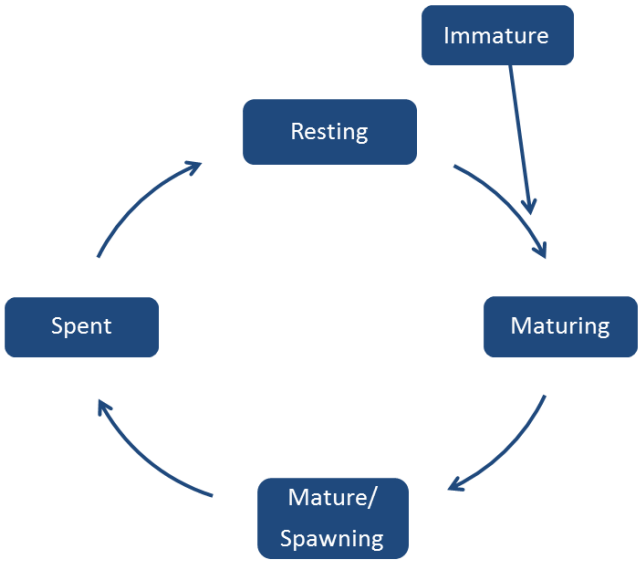


Figure 1: Reproductive cycle in female fish. Based on Brown-Peterson et al. (2011), adapted to the terminology postulated by Murua et al. (2003).

1.3.1 Spawning type

Beside the different number of spawning cycles, two types of spawning can be distinguished in teleost fishes; total spawning and batch spawning. In total spawning, all eggs are spawned at once at one point in each spawning season (Holden & Raitt, 1974). Total spawners are also characterised by a synchronous ovary, meaning that all oocytes are developed at the same time (Wallace & Selman, 1981). In contrast to a synchronous ovary in total spawners, batch spawners release their eggs in groups ("batches") over a period of time within the spawning season (Holden & Raitt, 1974). The oocytes are either developed group-synchronously or asynchronously. Group-synchronous ovaries have at least two populations of oocytes, one population of larger oocytes ("clutch") and oocytes in various oogenetic stages out of which the clutch is recruited (Wallace & Selman, 1981), like in Atlantic cod *Gadus morhua* (Murua & Sabrido-Rey, 2003). In asynchronous ovaries all oogenetic stages of oocytes are present and appear to be in a random mixture (Wallace & Selman, 1981), like in Atlantic mackerel *Scomber scombrus* (Murua & Sabrido-Rey, 2003).

To determine the spawning type it is essential to prepare histological cross-sections of the gonads, since the differentiation of cell types in the gonad is necessary for that analysis. As stated above, in total spawners there is only one type of oocyte present in the ovary at each maturity stage, whereas in batch spawning species there are groups of oocytes, representing

different oogenetic stages for each maturity stage. Histological sections further allow examination of other components, like atretic oocytes and post-ovulatory follicles (POFs). Atretic eggs "[..] are maturing eggs which for one reason or another may be completely resorbed." (Bagenal, 1978). As the oocyte structure is altered within the resorption process (Murua et al., 2003), atresia can be detected in histological sections. POFs are the remaining follicles after the hydrated oocyte has been spawned (Hunter & Macewicz, 1985). POFs in combination with different oocyte types present in the ovary indicate batch spawning as reproductive strategy rather than total spawning.

Froese & Pauly (2013) reported that maturity is closely related to growth and mortality. "A species which, for a single life-time spawning event, transforms a certain fraction of its body weight into gonads, maximises its expected output and thus its fitness if it matures, spawns and dies at the size and age of maximum growth rate." (Froese & Pauly, 2013). They further reported that one-time spawners mature close to 0.67 asymptotic length, whereas nonguarding multiple spawners mature at a significantly lower size.

1.3.2 Oogenesis

The process by which primordial germ cells (PGCs) become eggs is called oogenesis (Patino & Sullivan, 2002). Patino & Sullivan (2002) further described oogenesis in six steps; (1) formation of PGCs (germline segregation), (2) transformation of PGCs into oogonia (sex differentiation), (3) transformation of oogonia into oocytes (onset of meiosis), (4) growth of oocytes while under meiotic arrest, (5) resumption of meiosis (maturation), and (6) expulsion of the ovum from its follicle (ovulation). In this study, I focussed on the steps 3 to 6, which were also described and explained by Le Menn et al. (2007). First, oogonia (OG) (see 1.3.4) transform into primary growth oocytes (PGs). Wallace and Selman (1981) refer to this stage as 'primary growth stage', whereas other authors call it 'perinucleolar stage', referring to the nucleoli, located in the periphery of the nucleus (Dietrich & Krieger, 2009; West, 1990). Females with only PGs present in their ovary are still immature and not yet able to spawn (Murua et al., 2003). Oocytes then morph into cortical alveoli oocytes (CAs), where first cortical alveoli appear and the females are subsequently in the maturation process, called "maturing" (Murua et al., 2003). After the cortical alveoli stage, oocytes enter the vitellogenesis, characterised by the packing of vitellogenin, which is sequestered from the maternal blood, into yolk vesicles (Wallace & Selman, 1981). The yolk vesicles are usually distributed in the centre of the oocytes, the remaining space in the oocytes is filled with yolk

granules and cortical alveoli (Wallace & Selman, 1981). During the ongoing vitellogenesis the yolk granules grow and assemble around the nucleus (also called germinal vesicle). After the vitellogenesis the nucleus moves to the animal pole of the oocyte (further called migratory nucleus oocytes (MNs)) and the yolk granules begin hydration. With oocytes reaching this stage, females are now called "mature" (Dietrich & Krieger, 2009). Afterwards, the nucleus disintegrates (oocytes in this stage are further called germinal vesicle breakdown oocytes (GVBs)) and the yolk vesicles fuse into one single yolk mass in the centre (Dietrich & Krieger, 2009). The oocytes further hydrate und become ready to be spawned. During spawning the oocytes are released from their follicles, which remain as POFs in the gonad. The POFs are degraded within the next few days, e.g. within 48 hours in northern anchovy (Hunter & Macewicz, 1985). For a detailed description of the cellular processes inside the oocytes during each stage view Le Menn et al. (2007), Kagawa (2013) or Wallace & Selman (1981).

The numerous studies on reproduction in teleosts led to a large number of terms, definitions and descriptions. Dodd summarised this problem already in 1987 by saying that "[o]varian terminology is confused and confusing.". Various authors attempted to postulate a standardised terminology (Murua et al., 2003; Brown-Peterson et al., 2011). In this study, I have adopted the terminology of Murua et al. (2003).

1.3.3 The gonadosomatic index (GSI)

Another tool to study reproduction, in particular to support the assessment of maturity, is the gonadosomatic index (GSI). The GSI is the ratio of the gonad weight divided by the total body weight of the fish (Hunter & Macewicz, 1985). It increases with maturity (June, 1953) and can be used to detect hydrated ovaries since the wet weight of hydrated ovaries is much higher than that of other maturity stages (Hunter & Macewicz, 1985). It has been used as a basis for models to determine the maturity stage (McPherson et al., 2011), especially when histological sections are lacking (McQuinn, 1989) or macroscopical estimates are uncertain (Vitale et al., 2006). However, its validity is not yet fully resolved. DeVlaming et al. (1982) postulated four criteria that have to be met for the GSI to be an appropiate method of describing and comparing reproduction. The fourth criterion is that "[t]he linear, arithmetic relationship of gonadal weight to body weight does not change with stage of gonadal development." This criterion often impedes the validity of the GSI, because ovary weight usually increases faster with fish length than somatic weight (Hunter & Macewicz, 1985).

Hence, small fish usually have a lower GSI than larger fish in the same reproductive stage and this effect increases with ongoing maturation (DeVlaming et al. 1982). Thus, the GSI should be used cautiously.

1.3.4 Fecundity

Beside the spawning mode, the examination of fecundity is essential to describe reproductive patterns. Two types of fecundity are differentiated; determinate and indeterminate fecundity. Indeterminate fecundity is defined as a fecundity that is not fixed before the onset of spawning (Hunter et al., 1992) and is evidenced by the presence of oogonia in each maturity stage. Oogonia are diploid cells which derive from primordial germ cells (Kagawa, 2013) and are the precursors of oocytes. If no oogonia are present in later stages, the species has a determinate fecundity, as no further oocytes, apart from those already visible, could be recruited in this spawning period.

1.4 The electric lanternfish *Electrona risso* (Cocco, 1829)

This study focussed on reproduction of *Electrona risso*, one out of five species in the *Electrona* genus. *Electrona risso* is mainly distributed in the temperate to tropical regions of the Eastern North Atlantic, but also occurs in various other areas (Tab. 1).

Table 1: Distribution of *Electrona risso*.

Area	Authors	Comments
Eastern North and South Atlantic	Hulley, 1992	
Western North Atlantic	Podrazhanskaya, 1993	
Eastern North Pacific	Aron, 1960	with uncertainties
Western North Pacific	Kubodera et al., 2007	in the diet of sharks
	Kubota & Uyeno, 1972	
	Wang & Chen, 2001	
Eastern South Pacific	Craddock & Mead, 1970	
Mediterranean Sea	Tåning, 1918	
	Karakulak et al., 2009	in the diet of bluefin tuna
	Cuttitta et al., 2004	larval *E. risso*
Indian Ocean	Nafpaktitis & Nafpaktitis, 1969	

By now, only little information is given on the reproduction of this species. Length at maturity in *E. risso* was reported to be 59 mm (Hulley, 1981) with a reported maximum length of 82 mm (Hulley, 1990). The only reported maximum age of *E. risso* (600 days) was estimated by otolith analysis of individuals with 60 mm SL (Linkowski, 1987). The eggs were described by Sanzo (1939) with a diameter of 0.80 to 0.84 mm.

1.5 Research Questions

The main objective of this thesis is to analyse the reproductive cycle of female *E. risso*, i.e. understanding reproductive activity in relation to its life cycle, of which the latter is usually indicated by the age and size distribution. In this case, however, only the length distribution is available. First, I will investigate the maturation process in females and differentiate the maturity stages macroscopically, including the examination of egg sizes. Second, I will examine the reproductive mode, i.e. if *E. risso* is a batch spawner or a total spawner. This includes the examination of histological sections. After that I will examine the applicability of the GSI as an indicator for the maturity stage. Third, I will analyse the length frequency distribution, the length at maturity and the sex ratio. What does the length frequency distribution tell about the life cycle of *E. risso*? At which size do females mature? Does the sex ratio vary in different size classes and if so, is *E. risso* a sexually dimorphic fish?

2 Material and Methods

2.1 Sampling

Samples have been collected during the 383. research cruise of the fishery research vessel "Walther Herwig III" from 01.03.2015 until 24.04.2015 starting in Bremerhaven and returning to Bremerhaven, Germany. Mesopelagic sampling was done in the second leg of WH383 from Dakar to Bremerhaven.

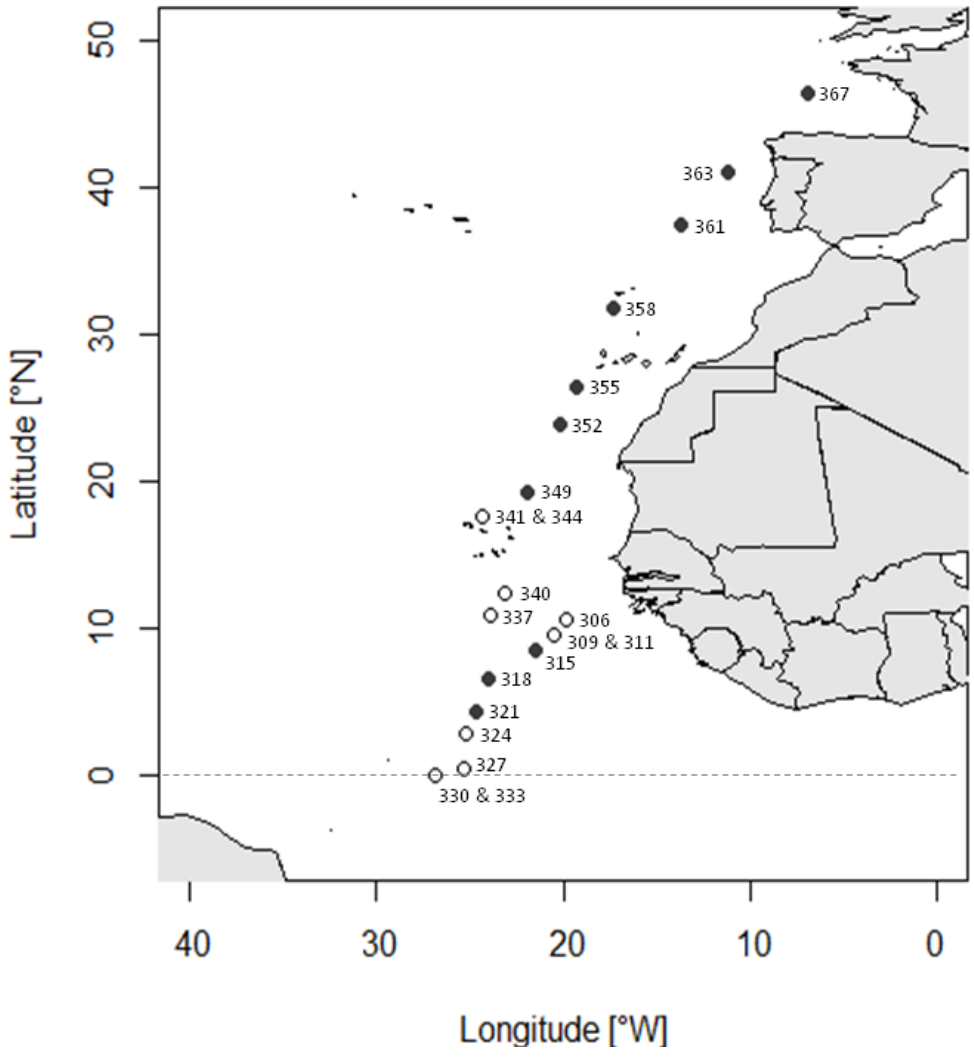


Figure 2: Mesopelagic sampling stations of WH383 in March-April 2015. Circles: Sampling stations, open circles: Stations, where *Electrona risso* specimens were caught, dashed line: equator. Stations with two station numbers, e.g. 309 & 311, were 24h-stations with day- and night-sampling.

Sampling was conducted at 18 stations covering a latitudinal gradient in the North Atlantic from the equator to the Bay of Biscay (Fig. 2), including six stations (306, 309, 311, 315, 337

and 340) in an oxygen minimum zone (OMZ). Sampling was usually performed during nighttime with three additional daytime hauls on the 24h-stations 309 & 311, 330 & 333 and 341 & 344 (Tab. 2). Fish were caught with a pelagic trawl equipped with a multiple opening-closing device with three net bags ("multi-sampler") allowing precise sampling in three predefined depths (Tab. 2), which were chosen based on echo sound signals. *Electrona risso* specimens (n= 936) were sorted out and fixed in a 4% formalin freshwater liquid buffered with phosphate for later analysis in the lab.

2.1.1 Preconditions for pooling samples

In reproduction studies, sampling should provide an accurate representation of the fish population, i.e. sampling should span the entire range in body length and should also allow the analysis of seasonal and regional variabilities (Murua et al., 2003). *Electrona risso* was chosen, because it was an abundant species and the taxonomical determination was fast and with high certainty. In order to increase sample size and thus precision and accuracy of the description of the reproductive cycle, specimens from all stations were pooled after checking for differences in maturity between the stations with highest abundances (Station 309 & 311 vs. 330 & 333). For these stations, day and night samples were pooled (see Tab. 2 and Tab. 4). Testing of differences of maturity stages between stations was done with a chi² test of homogeneity (see Appendix II.1).

2.2 Length and Weight Determination

Weight measurements were carried out as 'wet weight' after carefully dabbing each item. Wet weight is recorded to the nearest 0.001 g. Length is measured as 'standard length' (SL), and measured to the nearest 0.01 mm by means of an electronic caliper. The abdominal cavity was opened for a macroscopic sex determination using a scalpel. Female specimens were photographed in order to document the size of the gonads proportionally to the body. In females, the gonads were removed and weighed to the nearest 0.001 g.

Not in all of the 936 specimens a complete analysis was possible. In some specimens the caudal part of the body was ripped off in the net so the length and the weight could not be determined. In other specimens the abdominal cavity was already opened and the gonads

could not be weighed. Those specimens were excluded from the analysis resulting in a new sample size of n = 918.

After the sex determination of the fully intact specimens, the sex ratio was analysed. Statistical analysis was performed using the R software (Version 3.0.3, R Core Team. 2013). Fish were divided into 1 mm length classes and the sex ratio was calculated. To check for differences between length classes, a chi² test of homogeneity was performed (see Appendix II.2).

2.3 Macroscopic determination of maturity and determination of the oocyte diameter

The gonadal stage was determined under a binocular (Leica M80) based on a macroscopical description of Gartner (1993) for female gonads of various myctophid species. As female *E. risso* gonads deviated from Gartner's description, a new species-specific description was developed. Pictures of the gonads and oocytes were taken with the Leica Application Suite software (version 3.4.0) with a Leica DFC420 camera attached to a Leica M80 binocular. Photographs were analysed with the software ImageJ version 1.49 (http://imagej.nih.gov/ij/), which was also used to measure oocyte diameters. Per stage at least five oocytes from different individuals have been measured and the mean was calculated. The oocytes in stage I were too small to be measured properly with the software and therefore were referred to as <0.2 mm.

2.4 Calculation of the gonadosomatic index (GSI)

This study focussed on female *Electrona risso* specimens, so the following steps of analysis were applied solely to female individuals.

After the weight determination the gonadosomatic index (GSI) was calculated (1).

$$GSI = \frac{GW}{TW} \times 100 \quad (1)$$

Where:

GSI = gonadosomatic index

GW = gonadal weight (wet weight) in g

TW = total body weight (wet weight) in g

2.5 Microscopic analysis of oogenesis and histology

Determination of the gonadal status was supported using histological cross-sections of the gonads. Female *Electrona risso* gonads in the predefined stages were sectioned at 2 µm and stained with progressive Gill Hematoxilyn (Gill, 2010) and eosin at the Institute of Pathology (University Medical Centre Schleswig-Holstein, Kiel). The processing of histological cuts followed the standard procedure (Mulisch & Welsch, 2010):

1. Removal of tissue
2. Fixation (Formalin)
3. Drainage of sample in solutions with increasing ethanol concentration } embedding processor
4. Infiltration of tissue with paraffin and embedding of sample
5. Sectioning of samples with a microtome at 2 µm
6. Mounting of sections on glass microscope slides and drying at 54 °C in a drying and heat chamber
7. Removal of paraffin with xylene
8. Watering of sections in solutions with decreasing ethanol concentration } automatic slide stainer
9. Staining with hematoxilyn and eosin in an automatic slide stainer
10. Watering of sections in solutions with increasing ethanol concentration
11. Enclosing of sections with medium and cover glass with coverslipping machine

See Appendix I for a detailed description of the material and the processes inside the embedding processor and the automatic slide stainer.

Photographs of the sections were taken with the Leica camera attached to a Leica DM2000 light microscope, similar to the gonad and oocyte pictures. Pictures were also edited and analysed with the ImageJ software.

2.6 L_{50} and the ratio $L_{50/Lmax}$

All specimens above stage I (immature) were considered mature (see 3.2 and 3.3) and used to calculate the L_{50}. The L_{50} was calculated with a logistic regression model with a binomial error distribution and a logit link-function. Maturity was categorised as either 0 for immature females or 1 for mature females. In order to extend the range of numbers, the values were logit transformed. The logistic regression model (2) was fitted to the maturity state of females with the standard length as response variable, similar to García-Seoane et al. (2014).

$$logit(p) = \log\left(\frac{p}{1-p}\right) = a + b \times x \quad (2)$$

Where:

p = probability of maturity

$a + b \times x$ = logistic regression

The R code is presented in the Appendix II.3.

The $L_{50/max}$ ratio, after the description by Froese and Pauly (see 1.3.1), was calculated as (3).

$$L_{50/\max} = \frac{L_{50}}{L_{max}} \quad (3)$$

Where:

L_{50} = length at which 50 % of females are mature

L_{max} = maximum observed length

Froese & Pauly (2013) used length at maturity L_m instead of L_{50} for the analysis, with L_m defined as the mean length at which fish of a given population become sexually mature for the first time (Froese & Binohlan, 2000), but de facto L_{50} is the first age at maturity observed in a population. Further, Froese and Pauly (2013) used L_∞ instead of L_{max}. L_∞ is not available for *E. risso*, as there is no profound growth analysis given, therefore L_{max} was used.

2.7 Fecundity

The common method for estimating the fecundity is the gravimetric method (Hunter and Goldberg, 1980). With this method fecundity is calculated as the product of gonad weight and oocyte density, where oocyte density is the number of oocytes per gram of gonadal tissue. The gravimetric method is based on an extrapolation of the total number of oocytes via the oocyte density per gram tissue calculated from five subsamples. The gravimetric method for fecundity determination requires hydrated and fully intact gonads (Hunter et al., 1985) and it can also be applied to batch spawning species. In order to estimate the batch fecundity, only the hydrated oocytes are counted (Hunter et al., 1985).

In this study, one female in maturity stage VI was chosen. Only five females in stage VII had been sampled (Fig. 6), of which three gonads were slightly damaged and therefore some eggs

already slipped out and the two intact gonads were used for the histological analysis. As stated above, only intact gonads can be used. As a result of that, one female in stage VI (without hydrated oocytes) was used for fecundity analysis. Only one half of the gonad was used for the analysis, as the second half was damaged and some eggs already slipped out. Therefore only a tentative value is given.

For the counting, oocytes were placed into a counting chamber for zooplankton and were carefully removed from other tissue. Due to the small size of the female gonad and problems to collect proper subsamples without causing further disintegration of the tissues, counting was carried out for the whole gonad. The number of oocytes was then doubled to estimate the total batch fecundity. Relative fecundity was calculated as the number of eggs per gram total body weight (including gonadal weight).

3 Results

3.1 Sampling results

In total n = 936 *E. risso* specimens have been sampled (Tab. 2), of which n = 918 specimens were in a sufficient condition for the further analysis of the reproductive cycle.

Table 2: Station details and *Electrona risso* sample size.

Station	Date	Time [hh:mm, UTC]	N [decimal degrees]	W [decimal degrees]	Depth [m]	n *Electrona risso* ♀	n *Electrona risso* ♂
					50-59	-	-
306	23.03.2015	22:00	10.50	19.80	165-175	-	-
					410-418	36	25
309	24.03.2015	12:00	9.50	20.50	340-360	9	12
					390-415	21	35
					500-540	2	6
311	24.03.2015	22:00	9.50	20.50	50-60	-	-
					245-270	64	52
					398-423	16	7
					46-65	-	-
324	28.03.2015	22:00	2.73	25.20	140-169	-	-
					455-470	8	6
					60-65	-	-
327	29.03.2015	22:00	0.33	25.33	380-415	53	45
					480-500	20	18
330	30.03.2015	12:00	0.00	26.90	360-400	76	118
					480-490	12	16
					640-660	1	2
333	30.03.2015	23:15	0.00	26.90	50-60	-	-
					370-410	68	96
					480-510	-	-
					50-60	-	-
337	02.04.2015	22:00	10.78	23.90	375-410	17	17
					590-610	-	-
					55-68	-	-
340	03.04.2015	22:00	12.27	23.08	380-405	28	25
					555-580	-	1
					330-350	2	4
344	08.04.2015	12:00	17.60	24.30	400-420	-	-
					500-550	-	-
						Σ 433	**Σ 485**

3.2 Description of the maturity

Seven maturity stages (I-VII) could be distinguished in female *Electrona risso* specimens, based on a macroscopical analysis. The size of the gonads ranged from small, immature gonads (Fig. 3 I) to ripe and large gonads, filling up most of the cavity (Fig. 3 VII). The differences in the size of the gonads between the stages were too little to differentiate between stages without uncertainty, therefore the oocytes had been analysed under a binocular (Fig. 4). The most advanced oocytes in the first stage were smaller than 0.2 mm and translucent. In the stages II-IV the oocytes became bigger (up to 0.4 mm) and opaque. In maturity stage V a bubble-like structure was formed in the centre of the oocytes (Fig. 4 V), which became larger and morphed into one single mass in stage VI (Fig. 4 VI). The majority of oocytes in the most advanced stage (Fig. 4 VII) were translucent with diameters of 0.73 ± 0.05 (mean ± SD) mm.

3.3 Description of the oogenesis

In order to gain more information on the processes inside the oocytes, the histological sections were analysed microscopically. Oogenesis in *E. risso* followed the usual pattern in teleosts, described in 1.3.2. Females in the first stage were immature and not yet capable of spawning. Almost all oocytes were primary growth oocytes (PGs), only few cortical alveoli oocytes (CAs) were present (Fig. 5 I). Maturation started with the vitellogenesis, which began in stage II. Three oogenesis stages could be distinguished within the vitellogenesis and are defined as maturity stages II-IV (Fig. 5 II-IV). Each stage was characterised by the dominant presence of primary, secondary and tertiary vitellogenic oocytes (VTG1-3). Though maturation already started in these stages, PGs and CAs were still present. Stage V was characterised by the migration of the nucleus to the periphery of the oocyte, called the migratory nucleus stage (Fig. 5 V). Also PGs were still present. Stage V was followed by stage VI, where the most advanced oocytes were germinal vesicle breakdown oocytes (GVB) (Fig. 5 VI). Similar to the stages before, various groups of oocytes could be distinguished, e.g. VTGs. In stage VII the maturation is over and the female was ready to spawn, which was obvious by the abundant presence of hydrated oocytes (HYD) (Fig. 5 VII). A summary of the macroscopic and microscopic description of the gonads and information on the oocyte diameter can be found in Table 3.

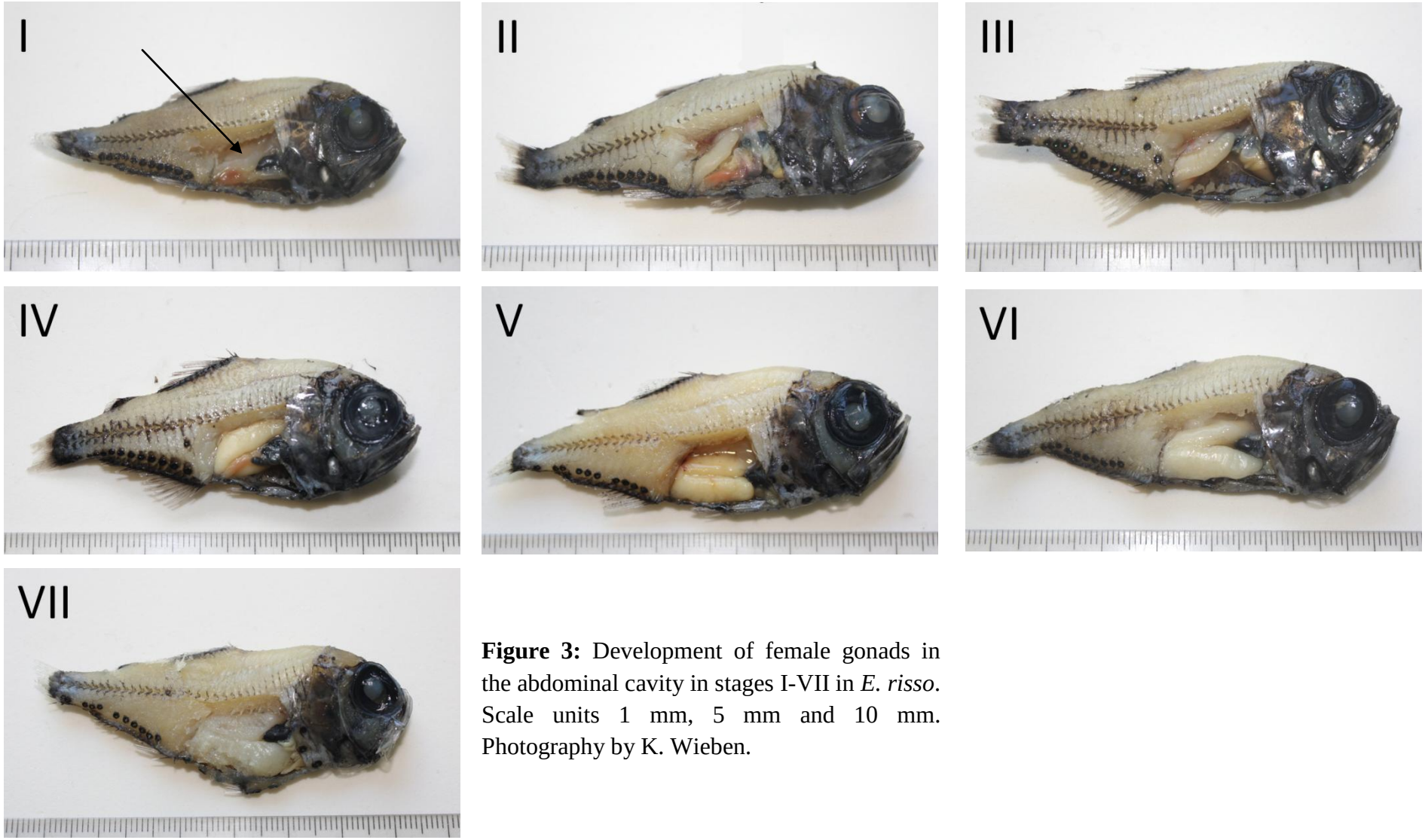


Figure 3: Development of female gonads in the abdominal cavity in stages I-VII in *E. risso*. Scale units 1 mm, 5 mm and 10 mm. Photography by K. Wieben.

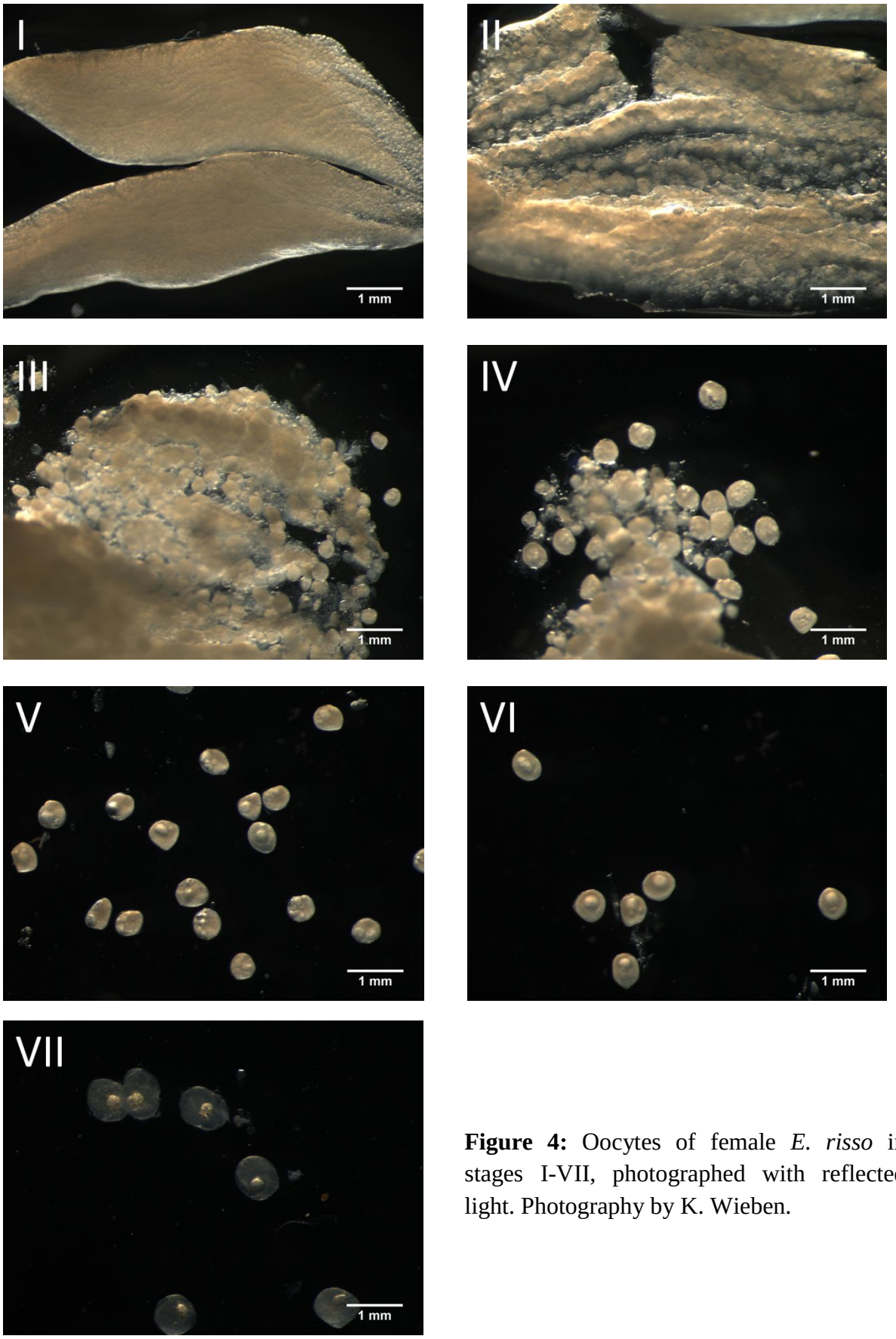
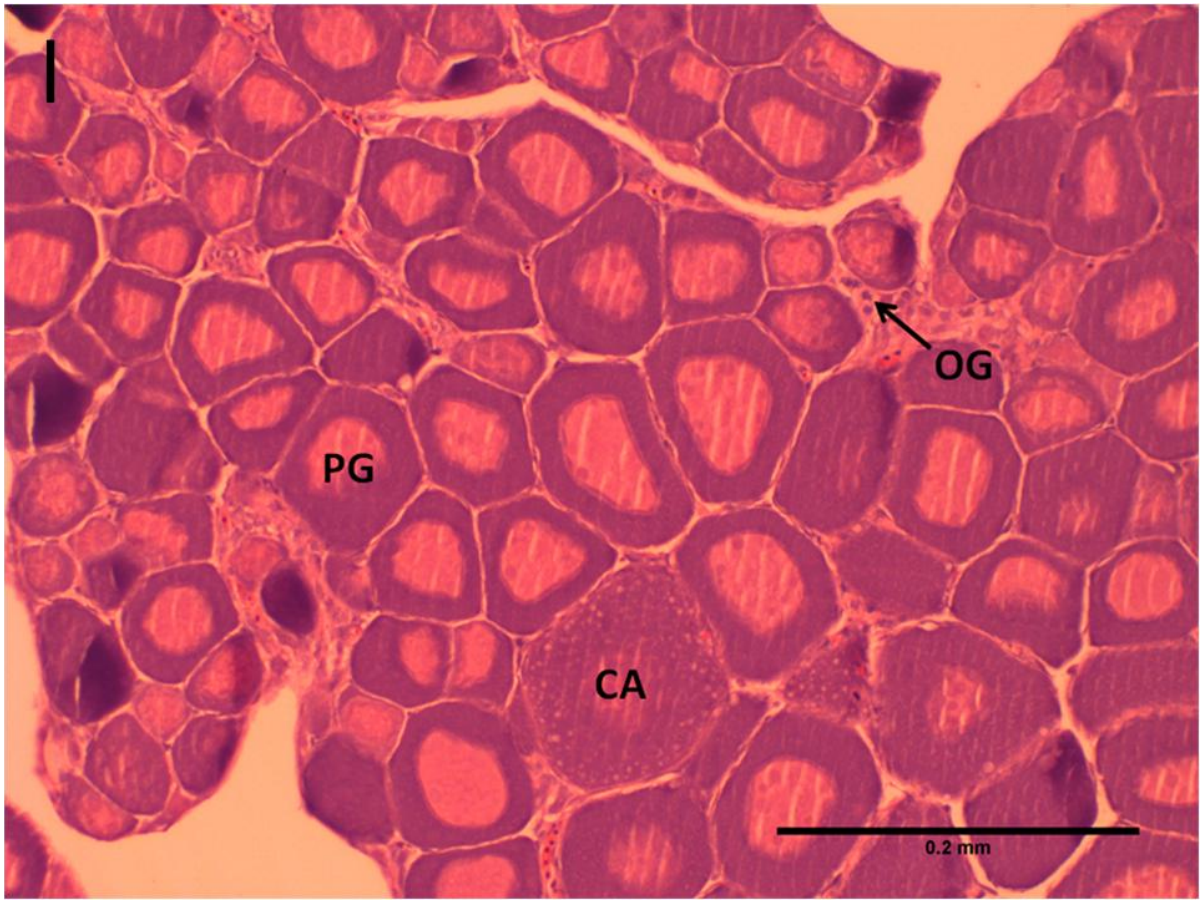
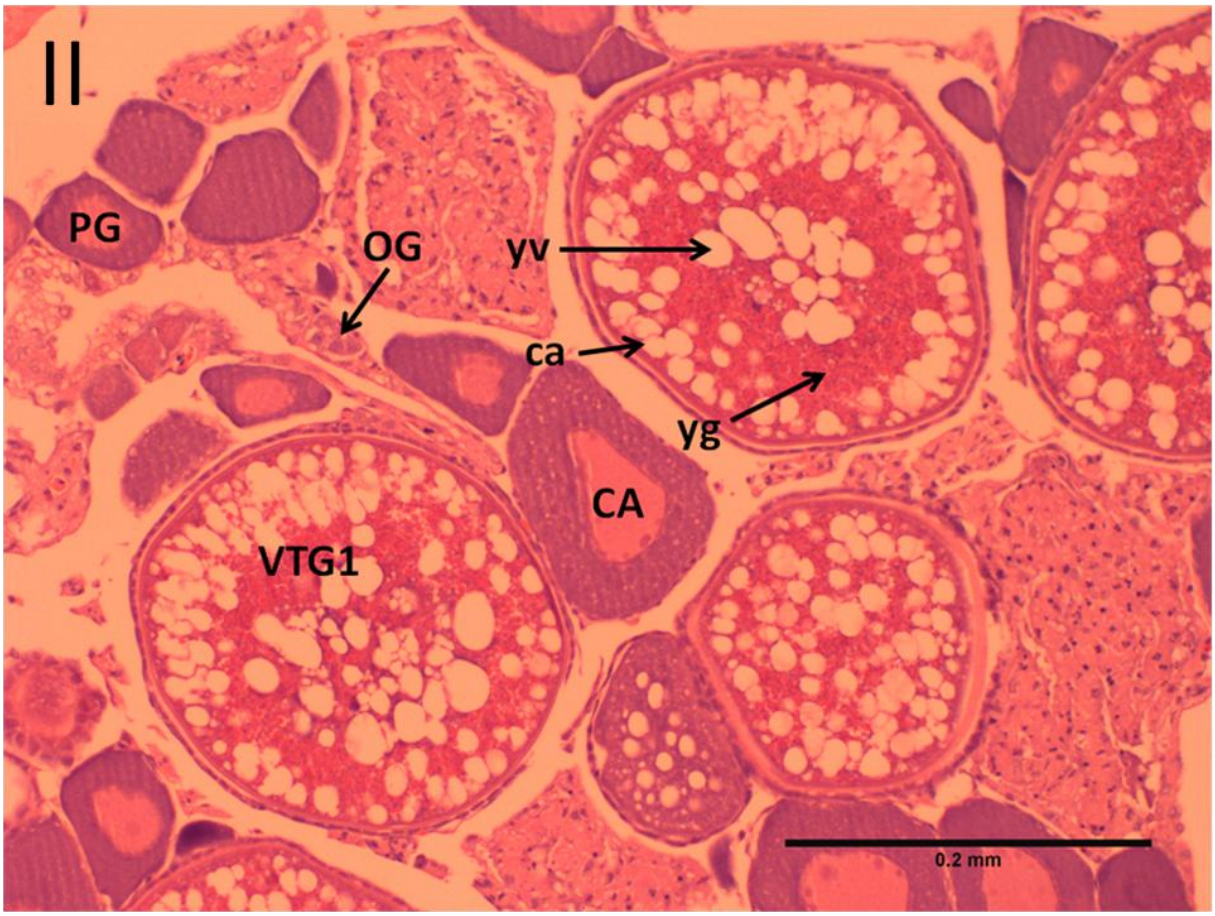


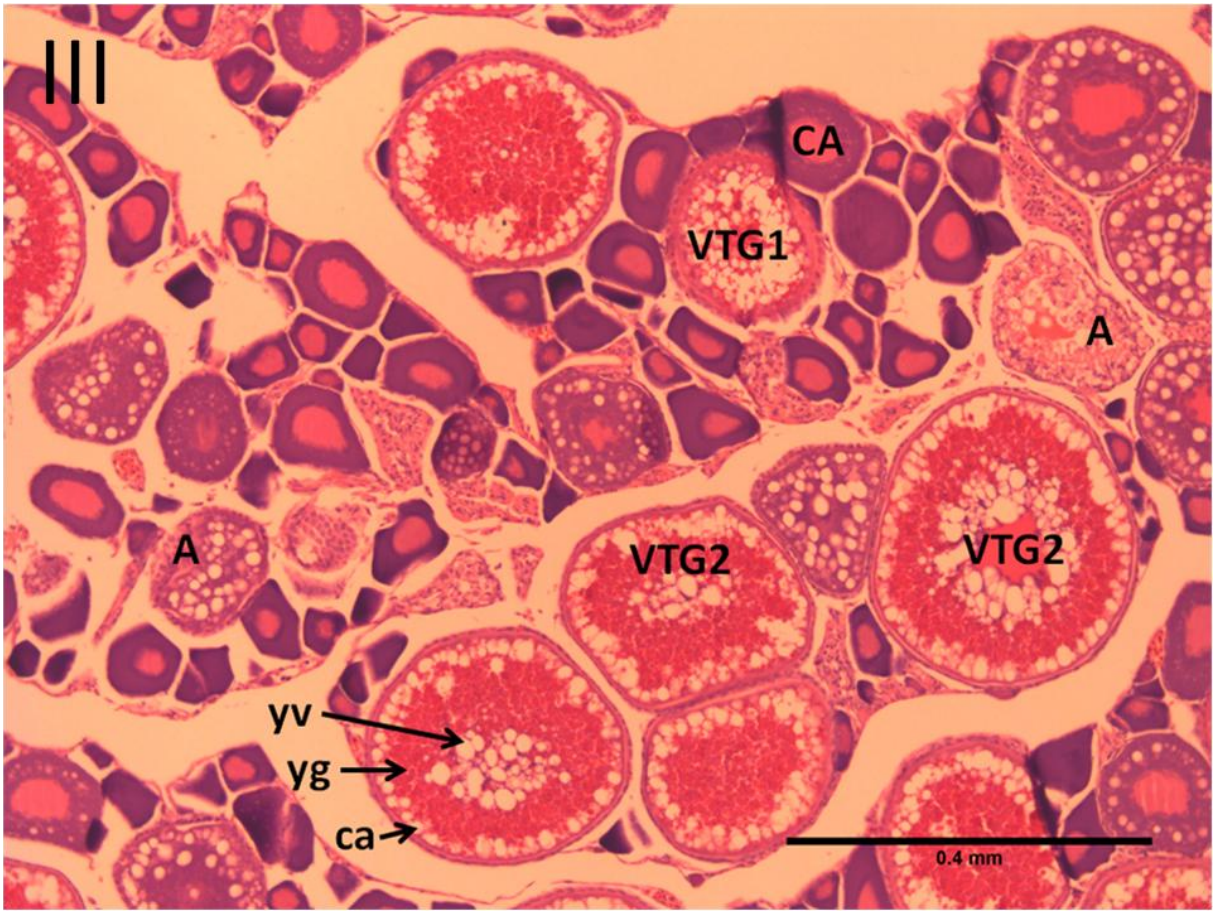
Figure 4: Oocytes of female *E. risso* in stages I-VII, photographed with reflected light. Photography by K. Wieben.

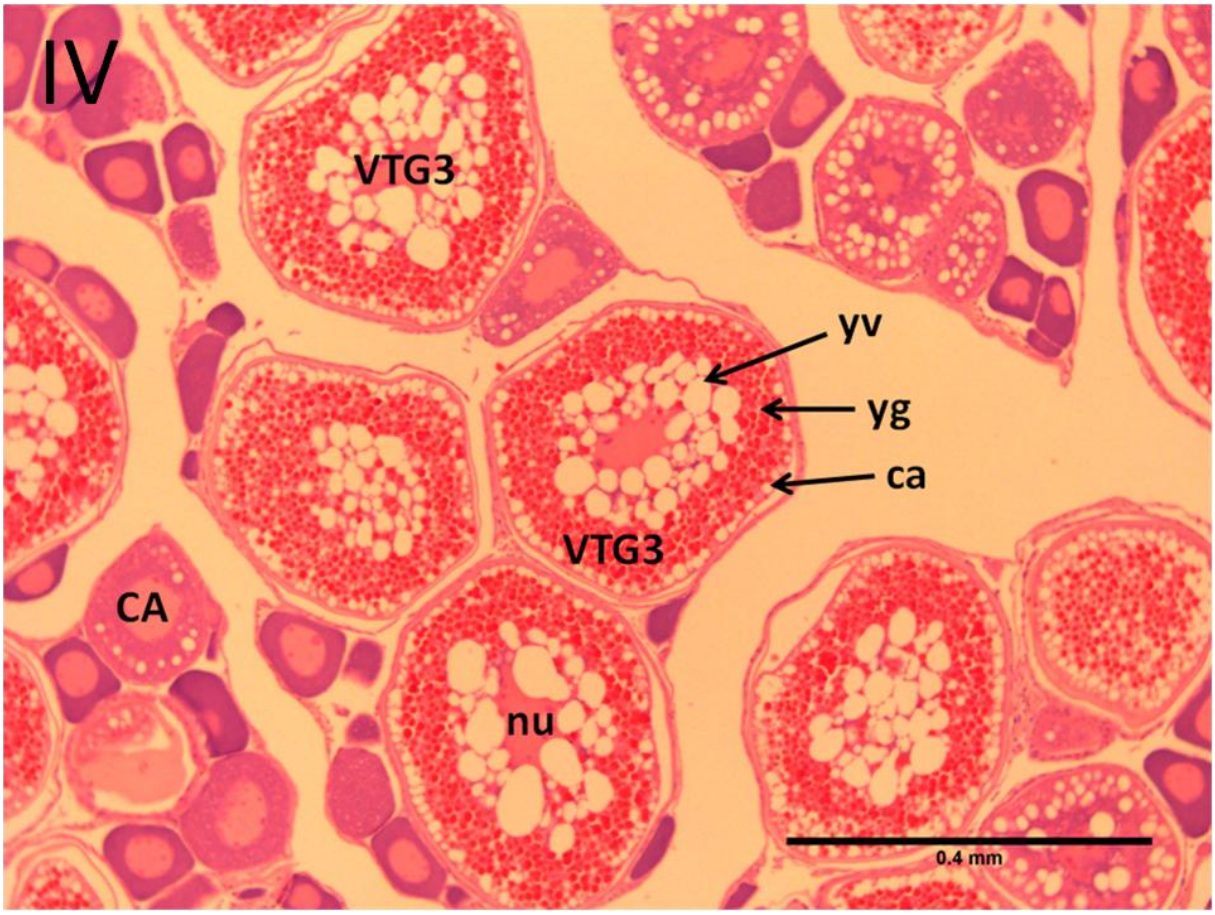
Beside the different oogenesis stages, other oocyte types could be distinguished. Oogonia (OG) were only present in females in stage I and II and lacked in stages III-VII. Post-ovulatory follicles (POFs) were present in stage VI, but their age could not be determined. Also atretic oocytes could be determined. Although the differentiation between atretic oocytes and POFs was difficult, atresia was determined certainly in stage III (Fig. 5 III).

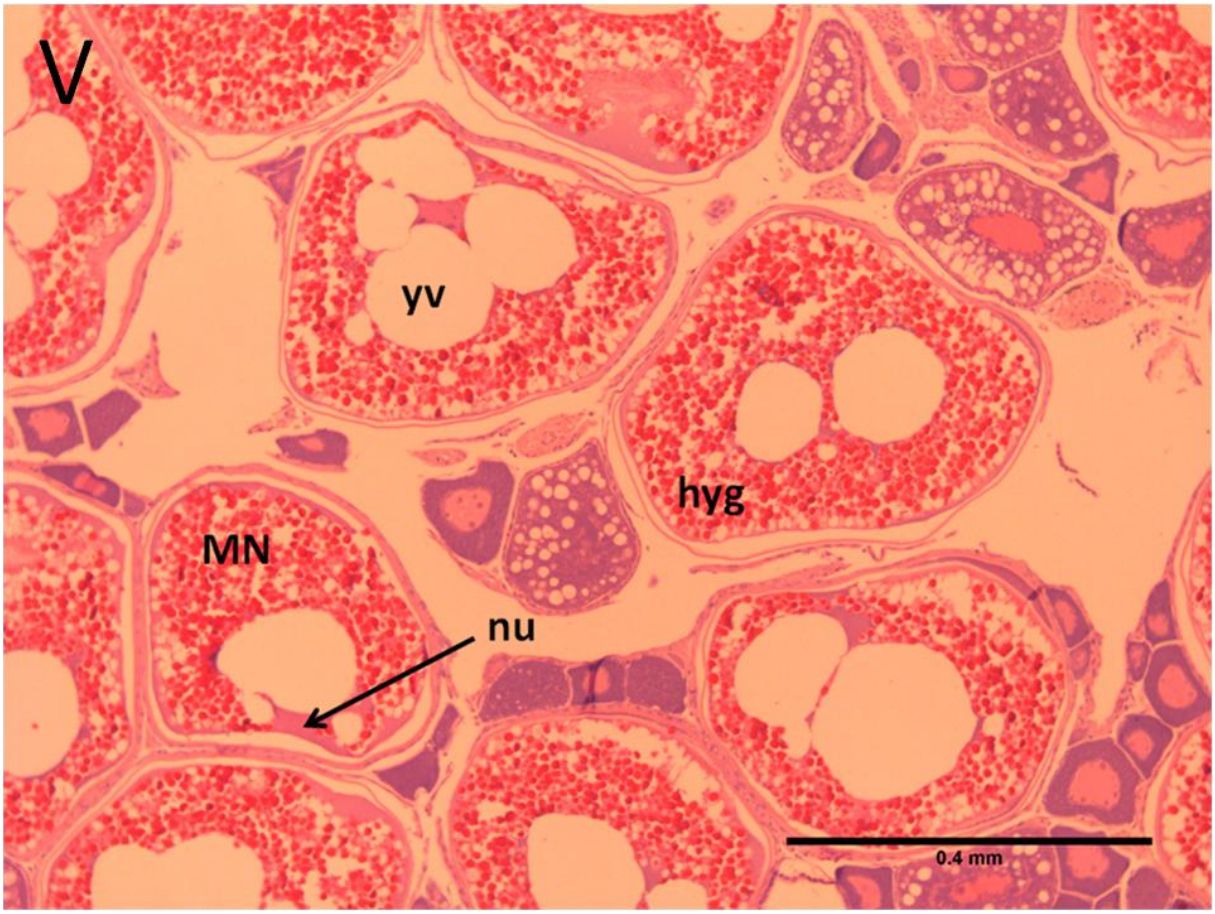
The cross-sections showed that in all stages dominant groups of oocytes were present and at least two different groups were evident. This allows assigning *E. risso* to the category of fish with a group-synchronous egg development as a precondition for batch spawning. The presence of hydrated oocytes and POFs in connection with earlier oogenetic stages gives evidence of batch spawning.

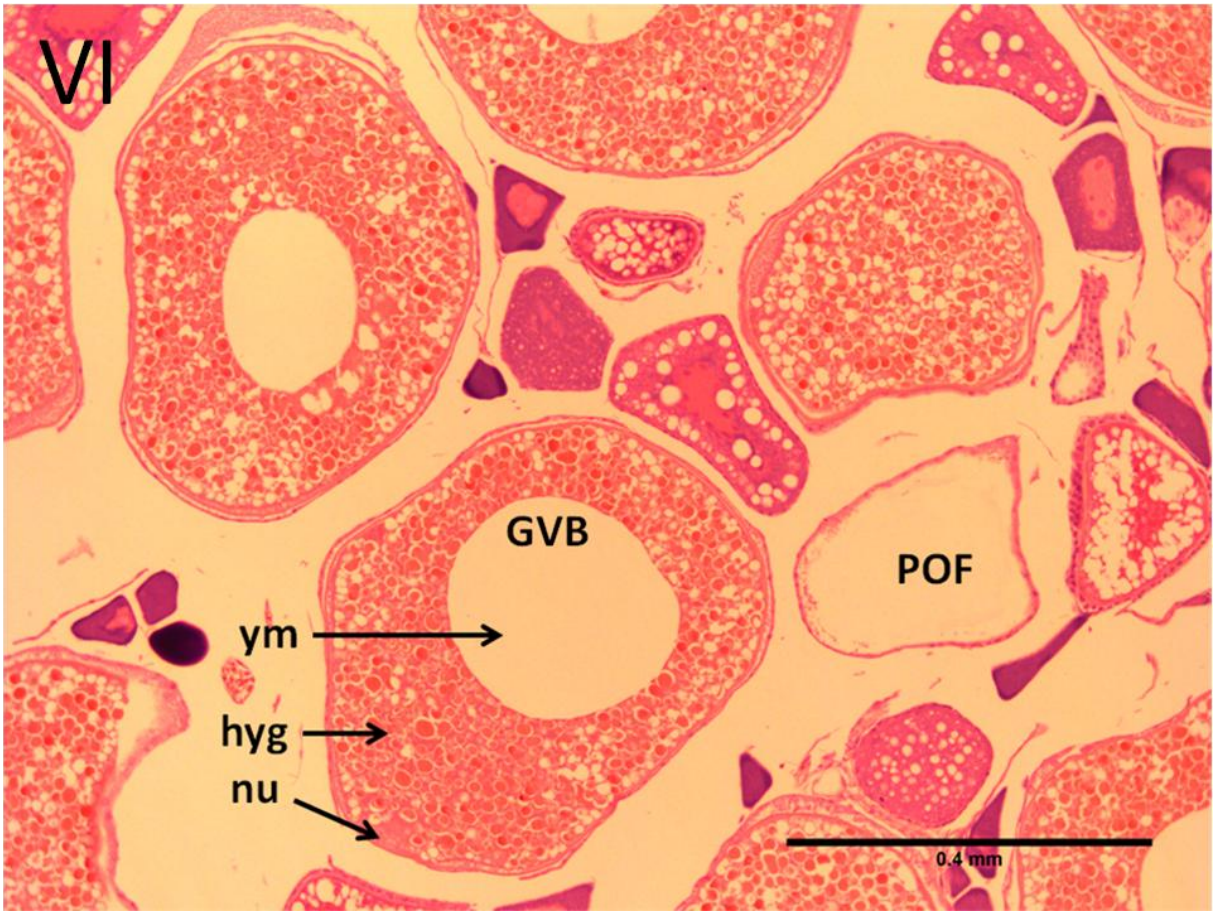

I
OG
PG
CA
0.2 mm


II
PG
OG
yv
ca
yg
CA
VTG1
0.2 mm


III
CA
VTG1
A
A
VTG2
VTG2
yv
yg
ca
0.4 mm


IV
VTG3
yv
yg
ca
VTG3
CA
nu
0.4 mm


V
yv
hyg
MN
nu
0.4 mm


VI
GVB
POF
ym
hyg
nu
0.4 mm

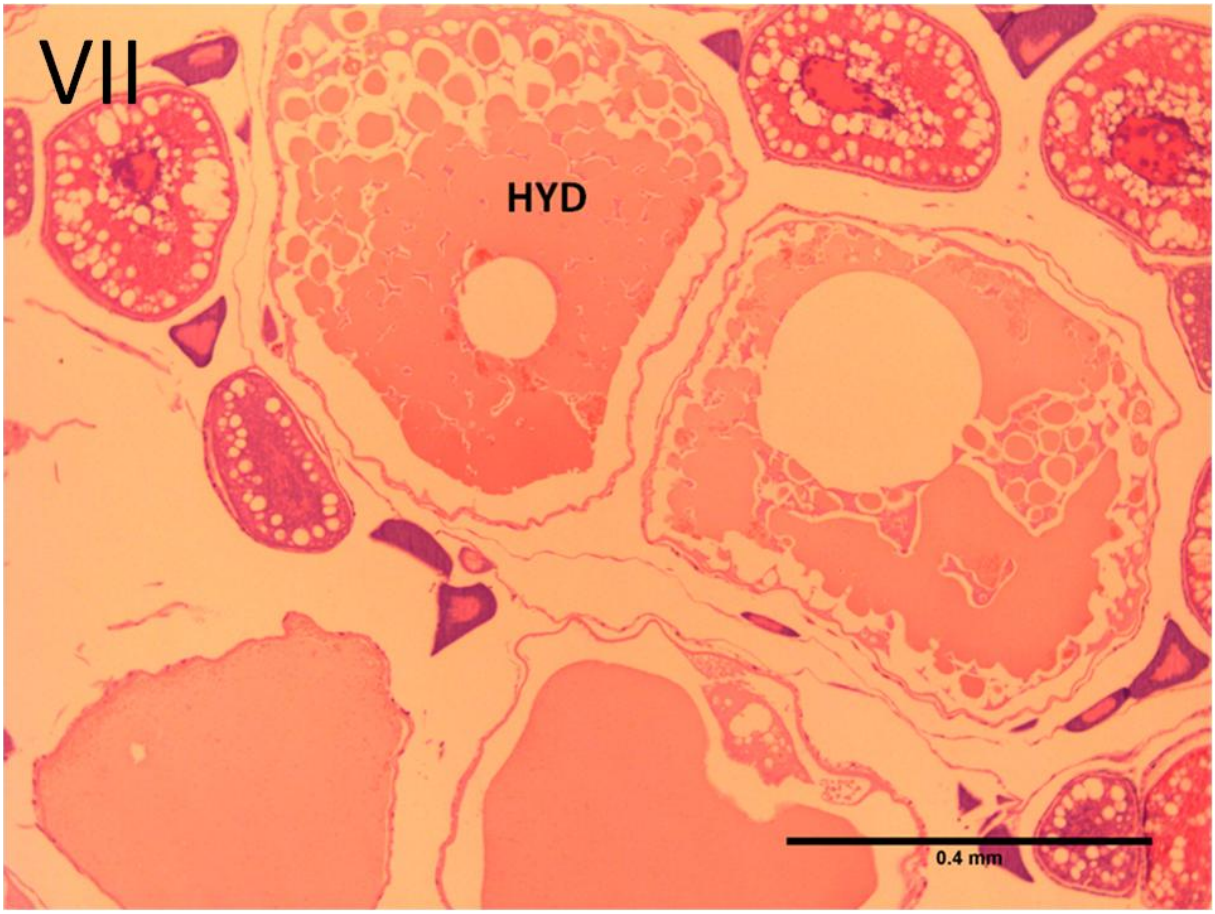


Figure 5: Histological sections of *E. risso* ovaries, stages I-VII, stained with HE and sectioned at 2 µm. Scale bar in I-II: 0.2 mm, in III-VII: 0.4 mm. A = atretic oocytes, CA = cortical alveoli oocyte, ca= cortical alveoli, GVB = germinal vesicle (nucleus) breakdown oocyte, HYD = hydrolysed oocyte, hyg = hydrated yolk granules, MN = migratory nucleus oocyte, nu = nucleus, OG = oogonia, PG = primary growth oocyte, POF = postovulatory follicle, VTG1-3 = primary to tertiary vitellogenic oocyte, yg = yolk granules, ym = yolk mass, yv = yolk vesicles. Photography by K. Wieben.

Table 3: Maturity Stages in female *E. risso.*

	Stage	Diameter ± SD[mm] of most advanced oocyte	Macroscopic Description	Microscopic Description
immature	I	<0.2	Ovaries thin, ribbonlike and translucent. Small, clear oocytes, only visible under higher magnification	Mainly primary growth oocytes (PGs) present, also few cortical alveoli oocytes (CAs). Frequent presence of oogonia (OG).
maturing	II	0.20 ± 0.04	Ovaries still ribbonlike, but extending posteriorly Oocytes are bigger, but still not visible without magnification. Larger oocytes are opaque and do not have a clear centre	Additional to PGs and CAs also early vitellogenic oocytes (VTG1s) are present. First yolk granules and vesicles become visible.
	III	0.27 ± 0.05	Ovaries are enlarged and oval in cross section, off-white colour. Oocytes are opaque and visible to naked eye, some of them with clear structures in the centre.	Largest oocytes in vitellogenic stage II (VTG2s), with cortical alveoli in the periphery and yolk vesicles in the centre. No longer presence of oogonia.
	IV	0.39 ± 0.04	Ovaries still oval in cross section, more golden in colour. Clear bubble-like structures in the centre of oocytes.	Largest oocytes in vitellogenic stage III (VTG3s), with diminished cortical alveoli in the periphery and enlarged yolk vesicles around the nucleus in the centre.
mature	V	0.48 ± 0.06	Ovaries almost circular in cross section, golden colour. Some oocytes with single clear circular centre.	Most oocytes in the migratory nucleus stage (MNs). Cortical alveoli start dissolving. Yolk vesicles merge into few larger vesicles. Yolk granules start hydrating.

mature	VI	0.54 ± 0.05	Ovaries fill up more than 2/3 of the abdomial cavity, off-white in colour. All oocytes with single clear circular centre.	Most oocytes in the germinal vesicle breakdown stage (GVBs). Nucleus disintegrates. Cortical alveoli fully dissoluted. One large yolk mass in the centre. Yolk granules continue hydrating. First appearence of POFs.
spawning	VII	0.73 ± 0.05	Ovaries fill up most of the abdomial cavity, off-white in colour. Already hydrated oocytes are translucent circular opaque centre.	Majority of oocytes are in hydration process, some already fully hydrated (HYDs).

3.4 Gonadosomatic index (GSI)

The GSI was positively correlated with maturity in females (Fig. 6) and ranged between minimum <1 in stage I and maximum 9.1 in stage VII. The GSI in the three vitellogenic stages II-IV ranged between 0.6 and 4.6. In stage V and stage VI the GSI lay between 1.9 to 4.9 and 2.8 to 6.7. All GSI ranges overlapped, except for the GSI range in stage VII, with values around a median of 8.6.

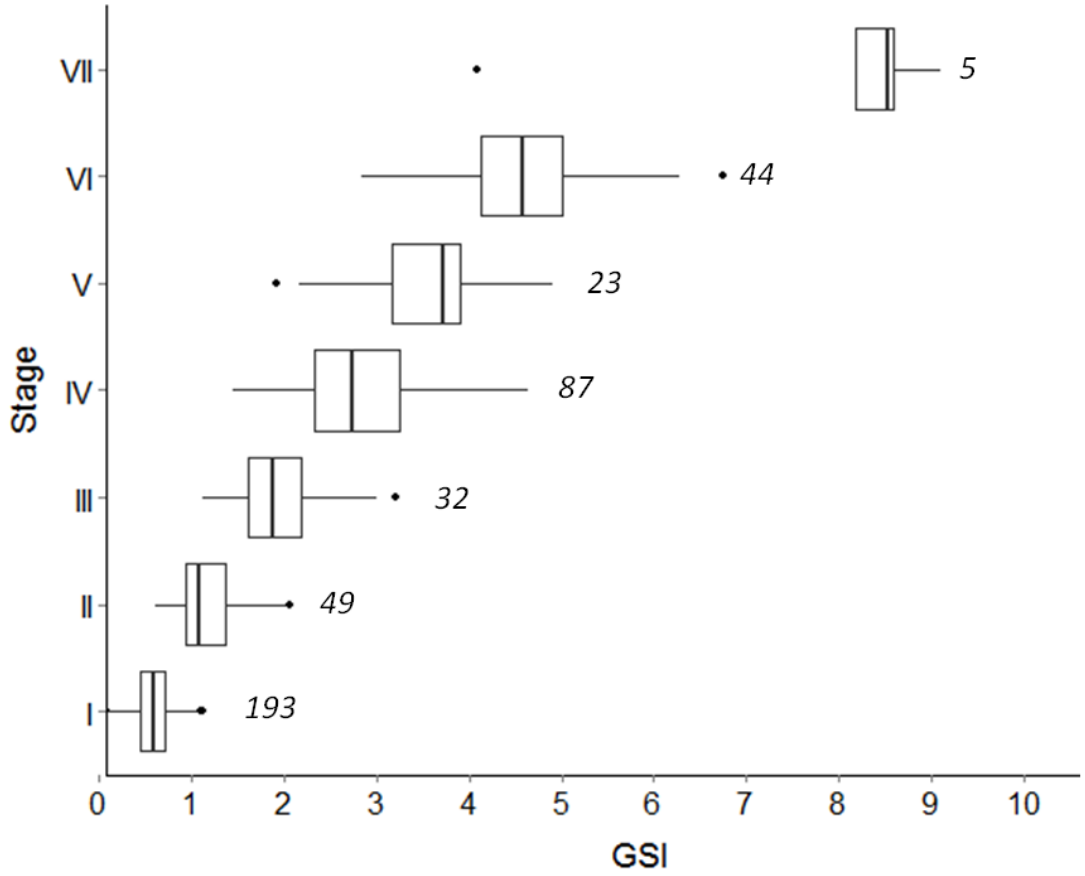


Figure 6: Range of GSI values of female *E. risso* in maturity stages I-VII, displayed as box-whisker-plots. Edges of boxes: first and third quartiles, upper whisker reaches to the highest value within 1.5*IQR, lower whisker reaches to the lowest value within 1.5*IQR, points: outliers (data beyond the end of the whiskers), numbers: sample sizes.

3.5 Analysis of the reproductive cycle

3.5.1 Testing for differences in maturity stages between stations

Before all samples were pooled, differences between stations were tested. Stations 309 & 311 were pooled and compared to 330 & 333 (see Tab. 2). These stations contained the most specimens and were geographically separated, so if there was a difference in maturity between all stations, it should be pronounced and detectable here. In total, 112 female *E. risso* from the first stations were compared to 157 females from the latter stations (Tab. 4). The chi²-Test was not significant ($\chi^2 = 35$, df = 30, p-value = 0.2426), showing that there was no significant difference in maturity between these stations. This allowed us to pool all samples in order to increase the overall sample size for the analysis of the reproductive cycle.

Table 4: Number of *E. risso* females in each maturity stage in the combined stations.

Stations	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII	∑
St. 309+311	80	5	5	12	2	8	0	112
St. 330+333	43	24	14	35	13	23	5	157

3.5.2 Length Frequency Distribution

A total of n = 918 *Electrona risso* specimens were measured and weighed. The length frequency distribution (Fig. 7) ranged from 30.51 mm standard length (SL) to 81.22 mm SL. Two peaks at 51 mm and 64 mm appeared, indicating two major cohorts. The distribution was skewed to the left, probably indicating a further cohort at about 40 mm.

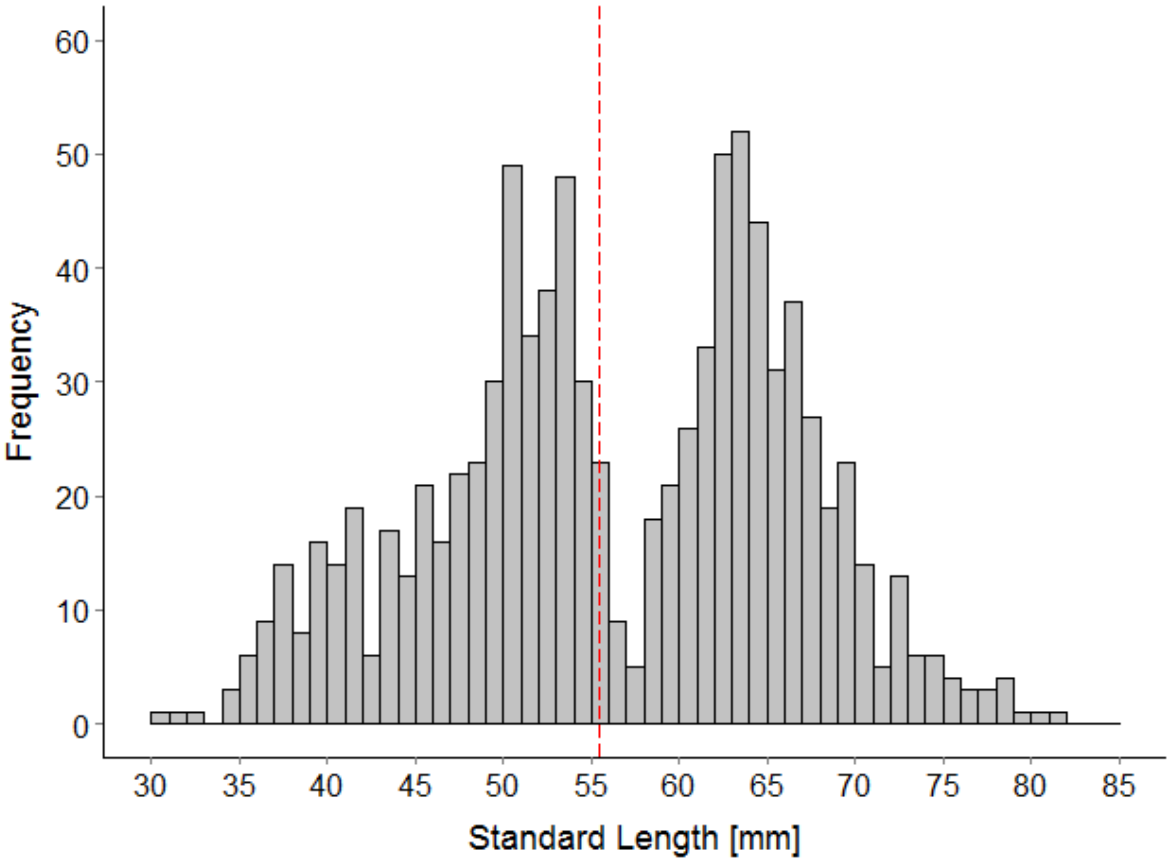


Figure 7: Length Frequency Distribution of *E. risso*, including males and females. Red dashed line: L_{50}, estimated by a logistic regression model (see 3.5.4).

3.5.3 Sex ratio

Out of 918 specimens, 433 female specimens were available, resulting in a sex ratio of 0.89:1 females to males. The overall sex ratio did not significantly differ from parity ($\chi^2 = 2.95$, df = 1, p-value = 0.09). Considering the sex ratio by length class, it appears that females dominated in smaller and larger length classes and males in specimens from 78 to 82 mm (Fig. 8), but the sex ratio did not significantly differ between the length classes ($\chi^2 = 48.69$, df = 50, p-value = 0.53). This is partly due to the fact that in length classes where either sex dominated, only few specimens were available (see Fig. 7).

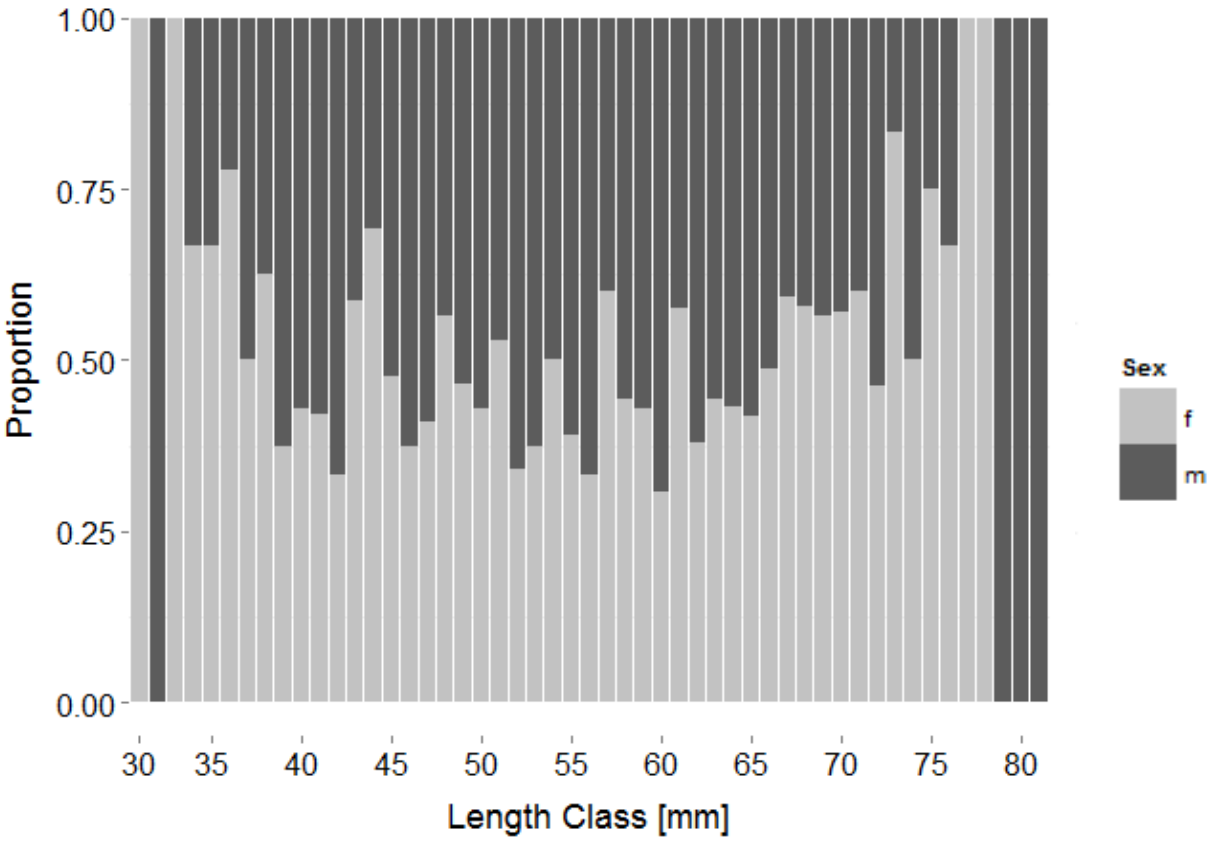


Figure 8: Sex ratio. Light grey bars: Female *E. risso*, dark grey bars: Male *E. risso*.

3.5.4 L_{50} and $L_{50/max}$

The length at which 50 % of the females start maturation could be determined to be at 55.5 mm (Fig. 9). In females smaller than 49 mm all individuals were immature, whereas all females larger than 64 mm were mature (with an exception of the length classes 70 and 71 mm).

The L_{50} separates the two major cohorts in the length distribution (see Fig. 7, red dashed line), so that the cohort with the peak at 64 mm can be understood as the mature and reproducing cohort.

The evaluation of the logistic regression model with the summary(model) function in R showed that the length has a significant influence on the probability of maturity (p-value < $2 * 10^{-16}$). Furthermore, including the length in describing the probability of maturity, reduced the deviance strongly, while losing one degree of freedom (Null deviance: 417.8 on 46 df, residual deviance: 33.5 on 45 df) (detailed information in Appendix II.3).

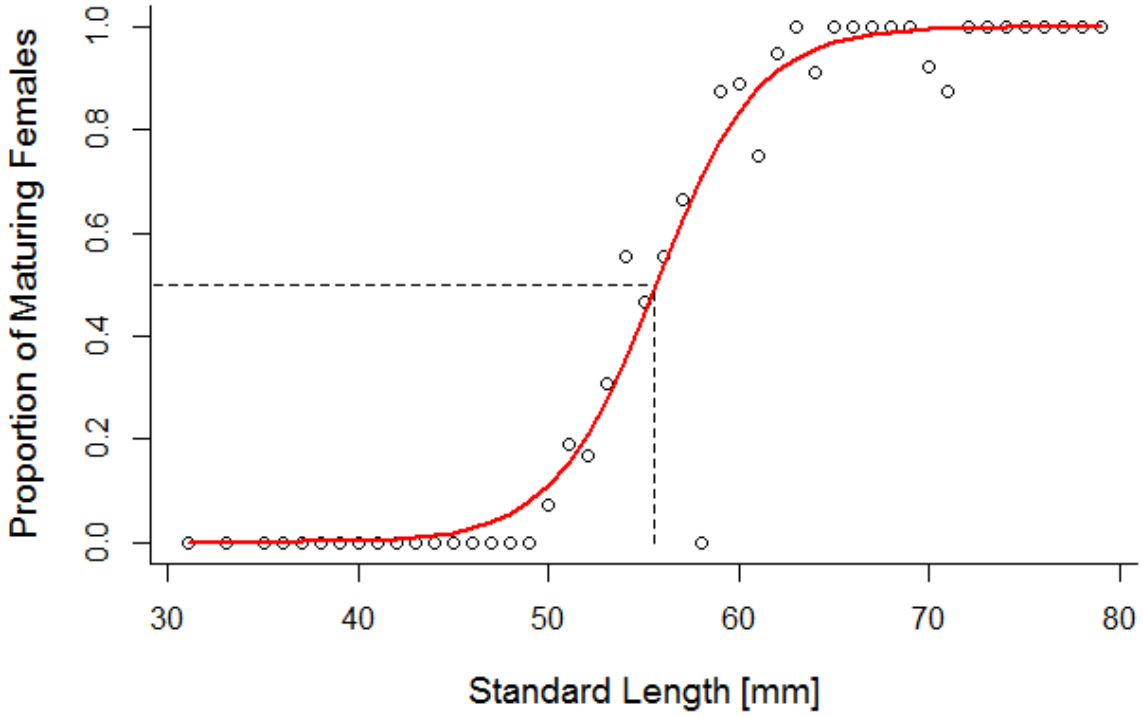


Figure 9: Determination of L_{50} in female *E. risso*. Red line: fitted values from the logistic regression model.

With the L_{50} (55.5 mm) and the maximum length L_{max} measured in this study (81.22 mm), the $L_{50/max}$ was calculated. This calculation showed that *E. risso* females mature at 0.68 maximum length. This value coincides well with the 0.68 $L_{50/max}$ that was postulated for one-time spawners by Froese & Pauly (2013).

3.6 Batch Fecundity

The female (n=1) chosen for the fecundity analysis was in stage VI with 63.7 mm SL and 7.544 g total weight. Tentative batch fecundity was calculated to be 2668, with a relative batch fecundity of 354 eggs g^{-1} body weight.

4 Discussion

This study provides the first ever reported description of the maturity stages in *Electrona risso* females. *E. risso* could be identified as a batch spawner with a group-synchronous egg development. The fact that *E. risso* has a determinate fecundity indicates that there is just one spawning season during which eggs are released in batches. This is strengthened by the length frequency distribution showing two major cohorts, with only the older one capable of reproducing. How these main conclusions were drawn in detail and how they match the results of previous research will be shown in the following.

4.1 Sampling limitations

The sample size varied strongly between the stations. Pooling of stations was the only option to achieve a sufficient sample. Before pooling, differences in maturity between the stations had to be checked. A comparative analysis of maturity between stations was only possible in the two stations with highest abundances. There was no significant difference in maturity, although the two stations were located approximately 1500 km apart. The fact that there was no difference in maturity despite the distance of the stations, led to the conclusion that pooling all stations was justified.

Pooling all stations was also necessary to cover a wide body length range, which is essential in reproduction studies. The samples in this study ranged from small and immature females to actively spawning females, with some of them also reaching the reported maximum length. Therefore, in terms of length and maturity, our samples are well-representing the population of *E. risso* in the subtropical-tropical eastern North Atlantic.

Although there was no significant difference in maturity between the two stations with highest abundances, possible local or regional differences should be kept in mind. One possible influence could be that the stations were spread over a gradient of 18 latitudinal degrees and a sampling time frame of more than two weeks. The stations lay in a triangle where different water masses meet, so biotic and abiotic factors could vary between the stations. Especially traits like the time of onset of spawning could be influenced by differences in temperature and prey availability (further details in 4.3.3). Moreover, some stations were located in an oxygen minimum zone (OMZ). Stramma et al. (2012) described the negative influence of the OMZ in the tropical northeast Atlantic on tropical pelagic fishes like tuna and billfishes. But how

oxygen depletion influences reproduction in pelagic fishes or how mesopelagic fish in particular are influenced, is still unknown. The influence of these regional and local factors on abundance, length, maturity etc. should be the subject of future studies with *E. risso*.

The low and varying sample size also inhibited the analysis of the 24h-stations, so differences between the day and night catches could not be analysed. Moreover, the sampling depths varied, because the overall aim of the hauls was not to investigate the vertical distribution of fishes, but to catch a high biomass. The vertical distribution and possible sex dependent differences should be investigated in the future.

4.2 Life History Traits

4.2.1 Length, age and L_{50}

The *E. risso* specimens in this study ranged between 30.51 and 81.22 mm SL. Specimens in this study reached the reported maximum length of 82 mm (Hulley, 1990) and were larger than those sampled in the Eastern North Atlantic by Linkowski (1987), which ranged from 14 to 72 mm. Linkowski (1987) also did an age determination based on analysis of daily increments of otoliths. Together with the results of Linkowski (1987), I am able to presume the life cycle of *E. risso*. Linkowski (1987) reported an age of 600 days in specimens with 60 mm SL. As the individuals in this study grew up to 20 mm larger, I estimate that *E. risso* gets approximately two years old.

The length frequency distribution showed two major peaks at 51 and 64 mm, indicating two cohorts. A possible third peak could be seen around 40 mm, but it was not pronounced enough to analyse it. The analysis of the L_{50} showed that females start maturation at 55.5 mm, which is slightly lower than the L_{50} of 59 mm, reported by Hulley (1981). The evaluation of the logistic regression model showed that length significantly influenced the probability of maturity. The overall approach with the logit transformation of the data and the logistic regression model fitted to the maturity with standard length as response variable was good and it is very likely that the L_{50} was estimated correctly. The L_{50} from this study lay between the two major peaks in the length frequency distribution, separating them in one immature cohort and one reproducing cohort. Overall, I conclude that *E. risso* has an approximate life span of two years, matures after one year and spawns in its second year in one single spawning period (further information on the reproductive strategy in 4.3).

The calculation of the $L_{50/max}$ revealed, that *E. risso* matures at 0.68 maximum length, which is close to 0.67 for one-time spawners postulated by Froese & Pauly (2013). This result also strengthens the theory that *E. risso* only spawns in one single spawning period. However, a profound age and growth analysis is still missing.

4.2.2 Sex ratio

The overall sex ratio (0.89 f : 1 m) did not significantly differ from parity. This is the common case in many myctophid species. Gartner (1993) reported that five out of seven tropical myctophid species did not differ from parity. Also in tropical *Benthosema fibulatum* from the Arabian Sea (Hussain, 1992) and *Diaphus suborbitalis* from the equatorial Indian Ocean (Lisovenko & Prut'ko, 1987) there was no significant difference. Contrary to that, Flynn and Paxton (2012) reported a strong dominance of females over males (23:1) in spawning aggregations of *Diaphus danae*. But they suggested that a sex dependent vertical stratification led to this sampling bias, in accordance with Go (1981) and Hulley & Prosch (1987). This also strengthens that the vertical distribution of *E. risso* should be investigated in a future study.

Beside the overall sex ratio, size-dependent variations were analysed, i.e. sexual dimorphism. The dominance of males in size classes of 79-82 mm in this study was most likely due to the low abundance of large specimens. There was no significant difference in the sex ratio between the length classes, though the p-value was close to the significance level. Still, an overall pattern of females dominating small and large size classes was visible. This was also observed in *Lampanyctodes hectoris* (Prosch, 1991) and in *Benthosema pterotum* (Sassa et al., 2014). Flynn and Paxton (2012) observed even non-overlapping size classes with larger females than males in *Diaphus danae*, although this might be due to the low sample size of males. Clarke (1983) investigated sex ratios and sexual differences in size in 22 mesopelagic fish species. He concluded that females might grow faster and have a longer lifespan which leads to their dominance in larger size classes. Gartner (1993) rejected this theory by stating that his data show that sexual differences in size have little ecological significance. Whether or not sex-related size differences in myctophids have a biological reason or if it is just due to a sampling bias, remains unclear and cannot be answered with data from this study.

4.3 Reproductive cycle

4.3.1 Maturity Stages

The analysis of the histological cross-sections showed that maturation in *E. risso* females followed the usual process in teleosts. The first and immature stage with dominantly primary growth oocytes was followed by three stages in which oocytes fulfilled vitellogenesis. Subsequently in stage V, the nucleus moved to the periphery of the oocytes and broke down in stage VI. The last and most mature stage was the hydration stage and the oocytes were ready to be released. In this stage the mean egg diameter was 0.73 ± 0.05 mm (mean ± SD), which is close to 0.80 - 0.84 mm, which was the egg diameter reported by Sanzo (1939). The difference is due to the fact, that in this study the oocytes were still in the gonad and some of them not fully hydrated yet, whereas Sanzo (1939) measured planktonic eggs.

In the present study no ‘spent’ or ‘resting’ stages could be determined. This might be due to the high probability of misinterpretation and the confusion with early maturity stages (Murua et al., 2003). Another explanation is that there are simply no stages like ‘spent’ or ‘resting’ in *E. risso*. Dalpadado (1988) encountered the stage 'spent' in her study on *Benthosema pterotum*, but only few females in this stage were present. She hypothesised that the reason for a low number of spent gonads is either that females only spawn once and shortly die afterwards or that they spawn multiple times, recover rapidly and leave no trace of previous spawning. Although *E. risso* was identified as a batch spawner with multiple egg releases, it spawns in just one single season. Therefore, I assume that in *E. risso* no stage such as ‘spent’ or ‘resting’ exists, because females might die shortly after they ended their spawning period. This is also confirmed by the length frequency distribution, showing that no larger specimens than those in the second peak were found. Moreover, the second peak almost reached the reported maximum length, so it is unlikely that there are larger and resting females than those that were sampled. Therefore, the reproductive cycle of *E. risso* deviates from the usual one shown in figure 1. Reproduction in *E. risso* appears to be rather a path than a cycle (Fig. 10).

Figure 10: Scheme of the reproductive path of *E. risso*.

4.3.2 Spawning Type

The histological cross-sections also revealed the spawning type of *E. risso*. The combination of the presence of POFs and the presence of various types of oocytes in each gonadal stage strongly indicates batch spawning. Oocytes mature group-wise and are spawned in batches. The presence of different groups of oocytes was only observed visually. Oocyte-frequency measurements would have given more profound and quantitative results, however the visual observation was sufficient enough for a qualitative evaluation of the spawning type.

Beside the developing oocytes, also degenerating oocytes were investigated. But the analysis of atretic oocytes and POFs in *E. risso* gonads appeared to be difficult. Especially the differentiation between them was challenging and uncertain, hence their analysis was kept short in this study. Nevertheless, it would have been helpful to analyse the POFs in regard to their age, because it indicates the time between the spawning events. POFs are visible for a few days after spawning and have age-depending morphological characteristics. A detailed analysis of the POFs and also of atretic oocytes would be useful and could be subject of a subsequent study.

Reproductive strategies of myctophids have been studied in various regions and for various species around the world. Both types of spawning, total and batch spawning, occur in this family, but batch spawning seems to be the more common mode, especially in tropical and subtropical waters. Gartner (1993) distinguished between two reproductive patterns; a protracted spawning season of 4-6 month, with spawning every 1-4 days and a restricted spawning season with spawning once or twice a year. His definition of a protracted spawning season matches well the description of the reproductive strategy of batch spawning species. In his study he reported that *Benthosema subortbitale*, *Lampanyctus alatus*, *Lepidophanes guentheri* and *Notolychnus valdiviae* have a protracted spawning season, whereas only *Ceratoscopelus sp*. and maybe also *Diaphus dumerli* have a restricted spawning period. Also *Benthosema pterotum* from the East China Sea was shown to be batch spawners (Sassa et al., 2014). Dalpadado (1988) and Hussain (1992) reported on the reproduction of two *Benthosema* species from the Arabian Sea and observed that both species spawn several times a year, but both authors could not certainly state a spawning type. Interestingly, the reproductive strategy seems to be affected by regional conditions (e.g. temperature, salinity, etc.). The abundant myctophid *Benthosema glaciale* from cold water populations and from temperate water populations spawns in batches, but the two populations differ in length of their spawning

season (Garcia et al., 2014). Therefore, other populations of E. risso outside the tropical and subtropical region should be investigated.

4.3.3 Estimation of the Spawning Season

In temperate and polar regions the reproductive cycle is usually driven by day length, temperature and food availability (Bye, 1984). Season-driven synchronised spawning enhances the genetic mixing in the population (Bye, 1984) and leads to higher fertilization probabilities, which is of high importance in the deep sea environment. In the tropics (and also in the deep sea) temperature and light conditions remain more or less constant throughout the year, but still many fish species have a well-defined annual reproduction cycle (Mead et al., 1964). The sampling stations from this study were located in the transition zone of the Western Tropical Atlantic Province (WTRA) and the Eastern Tropical Atlantic Province (ETRA). Although light and temperature are rather constant, both provinces show seasonal fluctuations in primary production and chlorophyll-a concentrations, as shown by Longhurst (1998). He further described, that this area is primarily influenced by seasonally varying strength of the hemispheric trade winds and position of the intertropical convergence zone (ITCZ). Because of that, the thermocline changes in depth, causing variability in primary production. Food availability is one of the most important factors for recruitment in fish, described in the match/mismatch hypothesis by Cushing (1990). In this hypothesis, Cushing postulates that a mismatch of fish larvae hatching and food availability results in a lower recruitment, whereas a match enhances the recruitment. Therefore, spawning season and high food availability should overlap. With the data available, it was not possible to determine the spawning season in *E. risso*, but it can be certainly said that females were actively spawning during late March and early April. Only few females were caught, which were ready to spawn, but as it takes only hours for germinal vesicle breakdown oocytes to transform into hydrated oocytes, it is clear that samples were taken during active spawning activities. This is also strengthened by the presence of POFs in not yet hydrated gonads, so at least one batch was already released. During the time of the year when the samples were taken, the chlorophyll-a concentration in the WTRA is usually high, whereas in ETRA it is usually low. It is necessary to further investigate the spawning season of *E. risso* in the North Atlantic and put it into context with the food availability.

4.3.4 Fecundity

Two aims of this study were the determination of the fecundity type and an estimation of the fecundity. The first aim could be reached by an analysis of the histological sections. Oogonia were only present in the first two stages and lacked in advanced stages and as stated in 1.3.4, this is a strong evidence for a determinate fecundity. The number of eggs was determined, so no further oocytes could be produced apart from those already present. This fecundity type fits well in the picture of the reproductive strategy of *E. risso*. As they have only one spawning season in their life, there is no need for further oocytes in later seasons and hence no need for oogonia in later stages of their life. This also strengthens the hypothesis that females die shortly after spawning.

In the literature there is little information on the fecundity type of myctophids, but in some species it was guessed by their reproductive strategy. García-Seoane et al. (2014) argued that *Benthosema glaciale* must have an indeterminate fecundity, because it would not be typical for a temperate species to spawn only those few times, that can be extrapolated from the number of groups of oocytes present in the gonad. I would not recommend to rely on interpretations like that, but to check for oogonia. A small number of groups of oocytes present in the gonad does not necessarily mean that there are only few spawning events, because sometimes only small batches are released.

The second question, the estimation of the fecundity, could be answered by using a modified version of the gravimetric method. The most advanced oocytes of the chosen stage VI gonad were counted. For this step the oocyte-frequency measurements also would have been useful. Another prerequisite of the gravimetric method is testing for a position or side effect (Hunter et al., 1985). It is further recommended to test for size differences of oocytes within one gonad half, as the hydration process is not constant throughout the gonad, but the gonads rather hydrate from the periphery to the center. Moreover, differences between the gonad halves should be checked, although variations do not seem to be common (Sassa et al., 2014; Hunter et al., 1985). Murua et al. (2003) also recommend checking for POFs in the hydrated gonad before the fecundity analysis. Only gonads without POFs, and therefore females without previous spawning activities, should be used for fecundity estimations. The estimation of the *E. risso* female fecundity in this study is based on the analysis of only one female and of course this is not valid in terms of statistics. Clarke (1984) reported high variations in the fecundity between similar-sized individuals of one myctophid species, which makes large sample sizes even more important. To conclude the methodological discussion, a

profound analysis of the fecundity in *E. risso* with a sufficient sample size and with all prerequisites being checked, is still lacking. Nevertheless, our estimation of the fecundity is the first one ever reported for female *E. risso*; hence, it is worth consideration.

When comparing fecundities, it is important to take the body length into consideration. Fecundity usually increases with body length (Bagenal, 1978), which was also shown in several myctophid species (Gartner, 1993). In myctophids, the fecundity usually ranges between a few hundred eggs and a few thousand eggs (Clarke, 1984; fecundity determination in 22 myctophid species). In this study a batch fecundity of 2,668 eggs and a relative fecundity of 354 eggs g^{-1} was estimated, fitting well into the general scale. When Gartner (1993) investigated the reproductive strategies of seven myctophid species in the Guld of Mexico, he reported fecundities ranging from 62-106 in *Notolynchus valvidae* and up to 3,287-12,626 in *Ceratoscopelus warmingii*. *Lepidophanes guentheri* (653-2,294) and *Myctophum affine* (536-3,037) reached similar values of fecundity as *E. risso* in this study and both species are of similar size (12-62 and 12-67 mm SL as compared to for *E. risso*). *Benthosema glaciale* females in the Mediterranean Sea and the North Atlantic have a rather low fecundity with a mean of 491 ± 228 (mean ± SD, García-Seoane et al., 2014), whereas *Benthosema pterotum* females from the Indian Ocean had a fecundity up to 3,000 (Dalpadado, 1988). The fecundity of female *B. pterotum* was also estimated in the East China Sea and was reported lower with values between 253 and 1,942 (Sassa et al., 2014). The highest fecundity in myctophids was found in *Diaphus danae* in the Australian Coral Sea (Flynn & Paxton, 2012). One female with 106 mm SL had a batch fecundity of 25,803 eggs. Summarizing this, the results from this study fit well into the overall scale of fecundity in myctophids.

4.4 Gonadosomatic index (GSI)

All ranges of the GSI per stage overlapped. Only the GSI range for females with hydrated oocytes (Stage VII) clearly separates from the others, which was expected (see 1.3.3). This shows that the GSI should not be used solely to detect stages. Beside the use of the GSI for stage detection, the GSI can be used to compare maturity levels, but its validity is still questioned (see 1.3.3). Contrary to that, the GSI in the Mediterranean sardine, *Sardina pilchardus*, was found to be a proper index for maturity (Somarakis et al., 2004). The authors focussed on the fourth criteria of deVlaming et al. (1982) and figured out that ovarian growth was isometric in all maturity stages of *S. pilchardus*, except for the hydration stage in which

growth was allometric. They concluded that the GSI is an appropriate index to describe ovarian activity except for the hydration stage. In this study none of the criteria by deVlaming et al. (1982) were checked, so the GSI values should be used cautiously.

Nevertheless, I want to compare our results with GSI values from other studies with myctophids. Overall, the GSI values from this study fit well into the general frame for myctophids. In *Benthosema glaciale,* García-Seoane et al. (2014) observed the highest mean GSI throughout the year during the spawning season. With a value of 3.25, it was much lower than the GSI for females with hydrated gonads in this study, which was around 8.6. This could be explained by that they calculated an overall mean for the females in this season, whereas in this study I calculated a stage specific mean. Hussain (1992) also reported much lower GSI values for *Benthosema fibulatum* with a maximum of 3.9. In *Benthosema pterotum* Sassa et al. (2014) observed a GSI ranging from 10 to 16 in females with hydrated oocytes, which is higher than in *E. risso* from this study. The highest GSI value reported for a myctophid is 34.01 in *Diaphus danae* by Flynn & Paxton (2012), but the majority of GSI values in their study ranged between 8 and 18. To summarise this, the GSI values for *E. risso* in the tropical eastern North Atlantic are located well within the GSI range for myctophids.

4.5 Conclusion and Outlook

In this study many puzzle pieces were put together to reveal the reproductive strategy of *E. risso* females in the subtropical-tropical North Atlantic. *E. risso* is a short-lived fish with just one spawning season, in which females release their eggs in batches. Some parts of the puzzle could not yet be revealed, e.g. the length of the spawning season. As actively spawning females were found in late March and early April, I would recommend sampling repetitively from early March to end of May. With these samples also age and growth could be determined, which supports the analysis of the spawning season. Another missing puzzle piece is the fecundity. Fecundity analysis should be repeated with a higher number of females with fully hydrated oocytes. *E. risso* is widely distributed in the world's oceans and as already said in 4.1, it would be interesting to investigate their abundance and reproduction in regions beyond subtropical and tropical seas.

Although some knowledge gaps remain, the results of this study will be helpful for future investigations on myctophid reproduction. High resolution photographs of the gonads, of the oocytes and of the histological sections were provided in this study, which will be a useful

tool for other scientists interested in reproduction. Moreover, the results will contribute to the knowledge of the functioning of the mesopelagic ecosystem. Beside the gain for the basic research, the results of this study can also be used for the evaluation of a commercial fishery of myctophids. I would advise against a commercial fishery on *E. risso*, because their recruitment relies on the reproductive output of only one single spawning season. As they appear to die shortly after the end of this spawning season, it is likely that they would be caught before they were able to release all their eggs. The withdrawal of an economically profitable amount of *E. risso* specimens would have unknown consequences for the mesopelagic ecosystem.

5 References

Aron, W. (1962). The distribution of animals in the eastern North Pacific and its relationship to physical and chemical conditions. *Journal of the Fisheries Board of Canada*, *19*(2), 271-314.

Bagenal, T. B. (1978). Aspects of fish fecundity. In: Gerking, S.D. (Ed.). Ecology of freshwater fish production. *Blackwell Scientific Publications.*

Baum, J. K. & Worm, B. (2009). Cascading top-down effects of changing oceanic predator abundances. *Journal of Animal Ecology*, *78*(4), 699-714.

Brown-Peterson, N. J., Wyanski, D. M., Saborido-Rey, F., Macewicz, B. J. & Lowerre-Barbieri, S. K. (2011). A standardized terminology for describing reproductive development in fishes. *Marine and Coastal Fisheries*, *3*(1), 52-70.

Bye, V. J. (1984). The role of environmental factors in the timing of reproductive cycles. In: Potts, G.W. & Wootton, R.J. (Eds.) Fish reproduction: strategies and tactics. *Academic Press.*

Cherel, Y., Guinet, C. & Tremblay, Y. (1997). Fish prey of Antarctic fur seals *Arctocephalus gazella* at Ile de Croy, Kerguelen. *Polar Biology*, *17*(1), 87-90.

Clarke, T. A. (1984). Fecundity and other aspects of reproductive effort in mesopelagic fishes from the North Central and Equatorial Pacific. *Biological oceanography*, *3*, 147-165.

Clarke, T.A. (1983). Sex ratios and sexual differences in size among mesopelagic fishes from the central pacific ocean. *Marine Biology. 73*, 147-165.

Craddock, J. E. & Mead, G. W. (1970). Midwater fishes from the eastern South Pacific Ocean. *Anton Bruun Rep*, *3*, 3-3.

Cushing, D. H. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in marine biology*, *26*, 249-293.

Cuttitta, A., Arigo, A., Basilone, G., Bonanno, A., Buscaino, G., Rollandi, L., Garcia Lafuente, J., Garcia, A., Mazzola, S. & Patti, B. (2004). Mesopelagic fish larvae species in the Strait of Sicily and their relationships to main oceanographic events. *Hydrobiologia*, *527*(1), 177-182.

Dalpadado, P. (1988). Reproductive biology of the lanternfish *Benthosema pterotum* from the Indian Ocean. *Marine Biology*, *98*(3), 307-316.

Dalpadado, P. & Gjøsæter, J. (1988). Feeding ecology of the lanternfish *Benthosema pterotum* from the Indian Ocean. *Marine Biology*, *99*(4), 555-567.

Dam, H. G., Roman, M. R. & Youngbluth, M. J. (1995). Downward export of respiratory carbon and dissolved inorganic nitrogen by diel-migrant mesozooplankton at the JGOFS Bermuda time-series station. *Deep Sea Research Part I: Oceanographic Research Papers*, *42*(7), 1187-1197.

Devlaming, V., Grossman, G. & Chapman, F. (1982). On the use of the gonosomatic index. *Comparative Biochemistry and Physiology Part A: Physiology*, *73*(1), 31-39.

Dietrich, D. & Krieger, H. O. (2009). Histological analysis of endocrine disruptive effects in small laboratory fish. *John Wiley & Sons*.

Dodd, J. M. (1963). The ovary. In: Pang, P. K. T., & Schreibman, M. P. (Eds.). Vertebrate endocrinology: fundamentals and biomedical implications. *Academic Press.*

Flynn, A. J. & Paxton, J. R. (2013). Spawning aggregation of the lanternfish *Diaphus danae* (family Myctophidae) in the north-western Coral Sea and associations with tuna aggregations. *Marine and Freshwater Research, 63*(12), 1255-1271.

Froese, R. & Pauly, D. (2013). Fish Stocks. Volume 3, p.477-487. In: S. Levin (Ed.). Encyclopedia of biodiversity. *Academic Press.*

Froese, R. & Binohlan, C. (2000). Empirical relationships to estimate asymptotic length, length at first maturity and length at maximum yield per recruit in fishes, with a simple method to evaluate length frequency data. *Journal of Fish Biology*, *56*(4), 758-773.

García-Seoane, E., Bernal, A. & Saborido-Rey, F. (2014). Reproductive ecology of the glacier lanternfish *Benthosema glaciale*. *Hydrobiologia*, *727*(1), 137-149.

Gartner, J. V. (1993). Patterns of reproduction in the dominant lanternfish species (Pisces: Myctophidae) of the eastern Gulf of Mexico, with a review of reproduction among tropical-subtropical Myctophidae. *Bulletin of Marine Science*, *52*(2), 721-750.

Gill, G. W. (2010). H&E staining: oversight and insights. *Connection*, *14*, 104-114.

Gjøsæter, J. & Kawaguchi, K. (1980). A review of the world resources of mesopelagic fish (No. 193-199). *Food & Agriculture Org*.

Go, Y. B. (1981). Spawning Pattern of a Mesopelagic Micronektonic Fish, *Diaphus suborbitalis*, in Suruga Bay, Japan. *Bulletin of the Marine Resources Research Institute, Jeju National University, 5*, 1-8.

Guinet, C., Cherel, Y., Ridoux, V. & Jouventin, P. (1996). Consumption of marine resources by seabirds and seals in Crozet and Kerguelen waters: changes in relation to consumer biomass 1962–85. *Antarctic Science*, *8*(1), 23-30.

Haque, A., Pettersen, J., Larsen, T. & Opstvedt, J. (1981). Fishmeal and oil from lantern fish (Myctophidae) with special emphasis on protein quality. *Journal of the Science of Food and Agriculture*, *32*(1), 61-70.

Holden, M. J. & Raitt, D. F. S. (1974). Manual of fisheries science. Part 2 - Methods of resource investigation and their application. *FAO Fisheries Technical Papers (FAO)*.

Hulley, P.A. (1981). Results of the research cruises of FRV "Walther Herwig" to South America LVIII. Family Myctophidae (Osteichthyes, Myctophiformes). *Archiv für Fischereiwissenschaft 31*, 1-300.

Hulley, P.A. (1990). Myctophidae. In: J.C. Quero, J.C. Hureau, C. Karrer, A. Post and L. Saldanha (Eds.) Check-list of the fishes of the eastern tropical Atlantic (CLOFETA). JNICT, Lisbon; SEI; Paris; and UNESCO, Paris. Vol. 1.

Hulley, P. A. (1992). Upper-slope distributions of oceanic lanternfishes (family: Myctophidae). *Marine Biology*, *114*(3), 365-383.

Hulley, P. A. & Prosch, R. M. (1987). Mesopelagic fish derivatives in the southern Benguela upwelling region. *South African Journal of Marine Science*, *5*(1), 597-611.

Hunter, J. R. & Goldberg, S. R. (1980). Spawning incidence and batch fecundity in northern anchovy, *Engraulis mordax*. *Fishery Bulletin*, *77*(3), 641.

Hunter, J. R., Lo, N. C. & Leong, R. J. (1985). Batch fecundity in multiple spawning fishes. *NOAA Technical Report NMFS*, *36*, 67-77.

Hunter, J. R. & Macewicz, B. J. (1985). Measurement of spawning frequency in multiple spawning fishes. *NOAA Technical Report NMFS*, *36*, 79-94.

Hunter, J. R., Macewicz, B. J., Lo, N. H. & Kimbrell, C. A. (1992). Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fishery Bulletin*, *90*(1), 101-128.

Hussain, S. M. (1992). The reproductive biology of the lantern fish Benthosema fibulatum from the northern Arabian Sea. *Fisheries research*, *13*(4), 381-393.

Irigoien, X., Klevjer, T. A., Røstad, A., Martinez, U., Boyra, G., Acuña, J. L.,Echevarria, F., Gonzales-Gordillo, J.I., Hernandez-Leon, S., Agusti, S., Aksnes, D.L., Duarte, C.M. & Kaartvedt, S. (2014). Large mesopelagic fishes biomass and trophic efficiency in the open ocean. *Nature communications*, *5*.

June, F. C. (1953). Spawning of yellowfin tuna in Hawaiian waters. *Fishery bulletin. United States Fish and Wildlife Service*, *54*, 47-64.

Kagawa, H. (2013). Oogenesis in Teleost Fish. *Aqua-bioscience monographs ABSM*, *6*(1), 99-127.

Karakulak, F. S., Salman, A. & Oray, I. K. (2009). Diet composition of bluefin tuna (*Thunnus thynnus* L. 1758) in the Eastern Mediterranean Sea, Turkey. *Journal of Applied Ichthyology*, *25*(6), 757-761.

Koslow, J., Boehlert, G. W., Gordon, J. D. M., Haedrich, R. L., Lorance, P. & Parin, N. (2000). Continental slope and deep-sea fisheries: implications for a fragile ecosystem. *ICES Journal of Marine Science: Journal du Conseil*, *57*(3), 548-557.

Kubodera, T., Watanabe, H. & Ichii, T. (2007). Feeding habits of the blue shark, *Prionace glauca*, and salmon shark, *Lamna ditropis*, in the transition region of the Western North Pacific. *Reviews in Fish Biology and Fisheries*, *17*(2-3), 111-124.

Kubota, T. & Uyeno, T. (1972). On the occurrence of the lanternfish *Electrona rissoi* in Japan. *Japanese Journal of Ichthyology*, *19*, 125-128.

Le Menn, F., Cerdà, J. & Babin, P. J. (2007). Ultrastructural aspects of the ontogeny and differentiation of ray-finned fish ovarian follicles. In: Le Menn, F., Cerdà, J., & Babin, P. J. (Eds.) The Fish Oocyte. *Springer Netherlands.*

Linkowski, T. B. (1987). Age and growth of four species of Electrona (Teleostei, Myctophidae). In: Kullander, S. O. & Fernholm, B. (Eds.) V Congress of European Ichthyologists proceedings: Congressus Europaeus Ichthyologorum, Stockholm, 1985. *Dept. of Vertebrate Zoology, Swedish Museum of Natural History.*

Lisovenko, L. A. & Prut'ko, V. G. (1987). Reproductive biology of *Diaphus suborbitalis* (Myctophidae) in the equatorial part of the Indian Ocean. 2. Fecundity and reproductive potential. *Journal of Ichthyology*, *27*, 1-12.

Longhurst A. (1998). Ecological Geography of the Sea. *San Diego, Academic Press.*

Longhurst, A. R. & Harrison, W. G. (1988). Vertical nitrogen flux from the oceanic photic zone by diel migrant zooplankton and nekton. *Deep Sea Research Part A. Oceanographic Research Papers*, *35*(6), 881-889.

Marshall, N. B. (1979). Development in deep-sea biology. *Blandford, London*

McQuinn, I. H. (1989). Identification of spring-and autumn-spawning herring (*Clupea harengus harengus*) using maturity stages assigned from a gonadosomatic index model. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*(6), 969-980.

Mead, G. W., Bertelsen, E. & Cohen, D. M. (1964). Reproduction among deep-sea fishes. In *Deep Sea Research and Oceanographic Abstracts*, 11(4), 569-596.

Möllmann, C., Müller-Karulis, B., Kornilovs, G. & St John, M. A. (2008). Effects of climate and overfishing on zooplankton dynamics and ecosystem structure: regime shifts, trophic cascade, and feedback loops in a simple ecosystem. *ICES Journal of Marine Science: Journal du Conseil*, *65*(3), 302-310.

Mulisch, M. & Welsch, U. (2010). Romeis-mikroskopische technik. *Springer-Verlag.* 18. Auflage.

Murua, H. & Saborido-Rey, F. (2003). Female reproductive strategies of marine fish species of the North Atlantic. *Journal of Northwest Atlantic fishery science*, *33*, 23-31.

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.

Nafpaktitis, B. G. & Nafpaktitis, M. (1969). Lanternfishes (Family Myctophidae) collected during cruises 3 and 6 of the R/V Anton Bruun in the Indian Ocean (No. 5). *Los Angeles County Museum of Natural History.*

Nafpaktitis, B.G., Backus, R.H., Craddock, J.E., Haedrich, R.L., Robison, B.H. & Karnella, C. (1963). Fishes of the western North Atlantic. Order Iniomi (Myctophiformes): Part 7, Neoscopelidae and Myctophidae and Atlantic Mesopelagic Zoogeography, 35-36.

Nelson, J. S. (2006). Fishes of the World. *John Wiley & Sons.*

Patiño, R. & Sullivan, C. V. (2002). Ovarian follicle growth, maturation, and ovulation in teleost fish. *Fish Physiology and Biochemistry*, *26*(1), 57-70.

Podrazhanskaya, S. G. (1993). Feeding habits of Mesopelagic species of fish and estimation of plankton graze in the northwest Atlantic. *NAFO Scientific Council Studies*, *19*, 79-85.

Prosch, R. M. (1991). Reproductive biology and spawning of the myctophid *Lampanyctodes hectoris* and the sternoptychid *Maurolicus muelleri* in the southern Benguela ecosystem. *South African Journal of Marine Science*, *10*(1), 241-252.

Pusch, C., Hulley, P. A. & Kock, K. H. (2004). Community structure and feeding ecology of mesopelagic fishes in the slope waters of King George Island (South Shetland Islands, Antarctica). *Deep Sea Research Part I: Oceanographic Research Papers*, *51*(11), 1685-1708.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/. R version 3.0.3 (2014-03-06)

Rodhouse, P. G., White, M. G. & Jones, M. R. R. (1992). Trophic relations of the cephalopod *Martialia hyadesi* (Teuthoidea: Ommastrephidae) at the Antarctic Polar Front, Scotia Sea. *Marine Biology*, *114*(3), 415-421.

Sanzo, L. (1939). Nuovo contributo alla conoscenza dello sviluppo *Myctophum rissoi* (Cocco). *Accad. Gioenia Catania, Atti*, *14*, 515-519.

Sassa, C., Ohshimo, S., Tanaka, H. & Tsukamoto, Y. (2014). Reproductive biology of *Benthosema pterotum* (Teleostei: Myctophidae) in the shelf region of the East China Sea. *Journal of the Marine Biological Association of the United Kingdom*, *94*(02), 423-433.

Smith, A. D., Brown, C. J., Bulman, C. M., Fulton, E. A., Johnson, P., Kaplan, I. C., Lozano-Montes, H., Mackinson, S., Marzloff, M., Shannon, L.J., Shin, Y.-J. & Tam, J. (2011). Impacts of fishing low–trophic level species on marine ecosystems. *Science*, *333*(6046), 1147-1150.

Somarakis, S., Ganias, K., Tserpes, G. & Koutsikopoulos, C. (2004). Ovarian allometry and the use of the gonosomatic index: a case study in the Mediterranean sardine, *Sardina pilchardus*. *Marine Biology*, *146*(1), 181-189.

Stramma, L., Prince, E. D., Schmidtko, S., Luo, J., Hoolihan, J. P., Visbeck, M., Wallace, D.W.R., Brandt, P. & Körtzinger, A. (2012). Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. *Nature Climate Change*, *2*(1), 33-37.

Tåning, Å. V. (1918). Mediterranean Scopelidae:(Saurus, Aulopus, Chlorophthalmus and Myctophum). *AF Høst & søn.*

Vitale, F., Svedäng, H. & 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: Journal du Conseil*, *63*(3), 485-492.

Wallace, R. A. & Selman, K. (1981). Cellular and dynamic aspects of oocyte growth in teleosts. *American Zoologist*, *21*(2), 325-343.

Wang, J. T. M. & Chen, C. T. (2001). A review of lanternfishes (families: Myctophidae and Neoscopelidae) and their distributions around Taiwan and the Tungsha Islands with notes on seventeen new records. *Zoological studies Taipei*, *40*(2), 103-126.

West, G. (1990). Methods of assessing ovarian development in fishes: a review. *Marine and Freshwater Research*, *41*(2), 199-222.

Acknowledgements

This master thesis is part of the climate change project PREFACE ("Enhancing **Pre**diction o**f** Tropical **A**tlantic **C**limat**e** and its impacts"). PREFACE aims to close knowledge gaps according to the functioning of ecosystem climate and aims to improve climate models. The working group at the Thünen Institute of Sea Fisheries focuses on climate induced shifts in distributional ranges of mesopelagic fishes. This thesis on the reproduction of a myctophid in the subtropical-tropical North Atlantic will contribute to the knowledge of the functioning of the mesopelagic ecosystem.

I would like to thank Prof. Dr. Oscar Puebla for accepting this Master Thesis and supervising me. Moreover, I express my gratitude to my supervisor Dr. Heino Fock for guiding me through this thesis and always encouraging me to bring out the best of me. A special thank goes to the captain and the crew of the fishery research vessel “Walther Herwig III” and the scientific participants of WH383. Without their expertise and manpower I would not have been able to collect the samples for this thesis. Additionally, I thank the Institute of Pathology at the University Medical Centre Schleswig-Holstein, especially Prof. Dr. Christoph Röcken and Sandra Krüger, for their support of this thesis by producing the histological cross-sections. Furthermore, I thank Stephanie Czudaj, who introduced me to the wonderful world of mesopelagic fish. I give many thanks to Maik Tiedemann, who answered all my questions not matter of which kind they were. I would also like to thank him for his numerous tips on writing and how to stay motivated. A special thank goes to my friends and my family for their support.

Appendix I: Histology material and methods

Table 5: Material and devices

Company	Adress	Product
BINDER GmbH	Im Mittleren Ösch 5 78532 Tuttlingen Germany	Drying and Heating Chamber ED23
Carl Roth GmbH + Co. KG	Schoemperlenstr. 1-5 76185 Karlsruhe Germany	Microscope Slides H870.1
Leica Microsystems GmbH	Ernst-Leitz-Strasse 17-37 35578 Wetzlar Germany	Binocluar M80
		Camera DFC420
		Microscope DM2000
		Microtome RM2245
MEDITE Inc.	4203 SW 34th Street Orlando, FL 32811 USA	Cooling Plate COP 30
		Coverslipping Machine Promounter RCM 2000
		Tissue Floating Bath TFB 45
Merck KGaA	64271 Darmstadt Germany	Microscopy Certistain Eosin Y (yellowish) (C.I. 45380) for microscopy
Sakura Finetek USA, Inc.	1750 West 214th Street Torrance, CA 90501 USA	Embedding Processor Tissue-Tek VIP
SIGMA-ALDRICH International GmbH	Wassergasse 7 9000 St. Gallen, Switzerland	Hematoxilyn solution, Gill No. 3
Thermo Fisher Scientific Inc.	81 Wyman Street Waltham, MA 02454 USA	Automatic Slide Stainer Varistain 24-4

Table 6: Procedure inside the embedding processor

Step	Solution	Concentration [%]	Duration [hh:mm]	Temperature [°C]
1	ethanol	5	8:00	40
2	ethanol	70	2:00	40
3	ethanol	96	2:00	40
4	ethanol	96	1:30	40
5	ethanol	100	1:30	40
6	ethanol	100	1:30	40
7	ethanol	100	1:30	40
8	xylene		1:00	40
9	xylene		1:30	40
10	xylene		1:30	40
11	paraffin		2:00	60
12	paraffin		2:00	60
13	paraffin		2:00	60
14	paraffin		2:00	60

Table 7: Processes inside the automatic slide stainer

Step	Solution	Concentration [%]	Duration [hh:mm]
1	xylene		03:00
2	xylene		03:00
3	ethanol	100	01:00
4	ethanol	96	01:00
5	ethanol	70	01:00
6	water		01:00
7	hematoxylin		08:00
8	water		00:15
9	HCl-ethanol		00:20
10	water		05:00
11	eosin solution*		00:15
12	water		00:50
13	ethanol	70	00:20
14	ethanol	96	00:20
15	ethanol	100	01:00

*250 ml eosin (0.5%) + 0.2 ml acetic acid (100%)

Appendix II: Statistic protocols

II.1 Precondition for pooling samples: Chi²-Test of homogeneity

Is there a difference in maturity between the two stations with highest abundances (Station 309 & 311 vs. 330 & 333)?

R script:

```
######insert dataset##################
#import dataset "Data"


######define the stations###############
tab1 <- table(Data$Station, Data$stage)
tab1

st309 <- tab1[2,2:8]  #defining St. 309
st333 <- tab1[7,2:8]  #defining St. 333
st311 <- tab1[3,2:8]  #defining St. 311
st330 <- tab1[6,2:8]  #defining St. 330

st309st311 <- st309+st311   #pooling St. 309 and St. 311
st330st333 <- st330+st333   #pooling St. 330 and St. 333


######stats############################

chisq.test(st309st311,st330st333)
```

```
>       Pearson's Chi-squared test

data:  st309st311 and st330st333
X-squared = 35, df = 30, p-value = 0.2426
```

II.2 Sex ratio: Chi²-Test of homogeneity

Does the overall sex ratio differ from parity? Does the sex ratio differ between the length classes?

R scipt:

```
######insert dataset######################
#import dataset "Data"

######editing the dataset##################

#categorizing the standard length into length classes
Data <- within(Data,{
  length1 <- NA
  length1[SL > 30 & SL <=31] <- 31
  length1[SL > 31 & SL <=32] <- 32
  length1[SL > 32 & SL <=33] <- 33
  length1[SL > 33 & SL <=34] <- 34
  length1[SL > 34 & SL <=35] <- 35
  length1[SL > 35 & SL <=36] <- 36
  length1[SL > 36 & SL <=37] <- 37
  length1[SL > 37 & SL <=38] <- 38
  length1[SL > 38 & SL <=39] <- 39
  length1[SL > 39 & SL <=40] <- 40
  length1[SL > 40 & SL <=41] <- 41
  length1[SL > 41 & SL <=42] <- 42
  length1[SL > 42 & SL <=43] <- 43
  length1[SL > 43 & SL <=44] <- 44
  length1[SL > 44 & SL <=45] <- 45
  length1[SL > 45 & SL <=46] <- 46
  length1[SL > 46 & SL <=47] <- 47
  length1[SL > 47 & SL <=48] <- 48
  length1[SL > 48 & SL <=49] <- 49
  length1[SL > 49 & SL <=50] <- 50
  length1[SL > 50 & SL <=51] <- 51
  length1[SL > 51 & SL <=52] <- 52
  length1[SL > 52 & SL <=53] <- 53
  length1[SL > 53 & SL <=54] <- 54
  length1[SL > 54 & SL <=55] <- 55
  length1[SL > 55 & SL <=56] <- 56
  length1[SL > 56 & SL <=57] <- 57
  length1[SL > 57 & SL <=58] <- 58
  length1[SL > 58 & SL <=59] <- 59
  length1[SL > 59 & SL <=60] <- 60
  length1[SL > 60 & SL <=61] <- 61
  length1[SL > 61 & SL <=62] <- 62
  length1[SL > 62 & SL <=63] <- 63
  length1[SL > 63 & SL <=64] <- 64
  length1[SL > 64 & SL <=65] <- 65
  length1[SL > 65 & SL <=66] <- 66
```

```
 length1[SL > 66 & SL <=67] <- 67
 length1[SL > 67 & SL <=68] <- 68
 length1[SL > 68 & SL <=69] <- 69
 length1[SL > 69 & SL <=70] <- 70
 length1[SL > 70 & SL <=71] <- 71
 length1[SL > 71 & SL <=72] <- 72
 length1[SL > 72 & SL <=73] <- 73
 length1[SL > 73 & SL <=74] <- 74
 length1[SL > 74 & SL <=75] <- 75
 length1[SL > 75 & SL <=76] <- 76
 length1[SL > 76 & SL <=77] <- 77
 length1[SL > 77 & SL <=78] <- 78
 length1[SL > 78 & SL <=79] <- 79
 length1[SL > 79 & SL <=80] <- 80
 length1[SL > 80 & SL <=81] <- 81
 length1[SL > 81 & SL <=82] <- 82
 length1[SL > 82 & SL <=83] <- 83
 length1[SL > 83 & SL <=84] <- 84
 length1[SL > 84 & SL <=85] <- 85
 length1[SL > 85 & SL <=86] <- 86
})

#categorise the sex
Data<- within(Data,{
 sex <- NA
 sex[stage == "m" ] <- "m"
 sex[stage != "m"] <- "f"
})

######Stats#################################

#chi square test: overall sex ratio
tbl1<-table(Data$length1,Data$sex)
chisq.test(tbl1)

>       Pearson's Chi-squared test

data:  ratio
X-squared = 48.6927, df = 50, p-value = 0.5259

#chi square test: sex ratio over length classes
tbl2 <- table(Data$sex)
chisq.test(tbl2)

>       Chi-squared test for given probabilities

data:  number
X-squared = 2.9455, df = 1, p-value = 0.08612
```

II.3 L_{50}: Logistic regression model

At which size do *E. risso* females mature?

R scipt:

```
######insert dataset######################
#import dataset "DataF" with only females

######editing the dataset#################

#categorizing the standard length into length classes
DataF <- within(DataF,{
  length1 <- NA
  length1[SL > 30 & SL <=31] <- 31
  length1[SL > 31 & SL <=32] <- 32
  length1[SL > 32 & SL <=33] <- 33
  length1[SL > 33 & SL <=34] <- 34
  length1[SL > 34 & SL <=35] <- 35
  length1[SL > 35 & SL <=36] <- 36
  length1[SL > 36 & SL <=37] <- 37
  length1[SL > 37 & SL <=38] <- 38
  length1[SL > 38 & SL <=39] <- 39
  length1[SL > 39 & SL <=40] <- 40
  length1[SL > 40 & SL <=41] <- 41
  length1[SL > 41 & SL <=42] <- 42
  length1[SL > 42 & SL <=43] <- 43
  length1[SL > 43 & SL <=44] <- 44
  length1[SL > 44 & SL <=45] <- 45
  length1[SL > 45 & SL <=46] <- 46
  length1[SL > 46 & SL <=47] <- 47
  length1[SL > 47 & SL <=48] <- 48
  length1[SL > 48 & SL <=49] <- 49
  length1[SL > 49 & SL <=50] <- 50
  length1[SL > 50 & SL <=51] <- 51
  length1[SL > 51 & SL <=52] <- 52
  length1[SL > 52 & SL <=53] <- 53
  length1[SL > 53 & SL <=54] <- 54
  length1[SL > 54 & SL <=55] <- 55
  length1[SL > 55 & SL <=56] <- 56
  length1[SL > 56 & SL <=57] <- 57
  length1[SL > 57 & SL <=58] <- 58
  length1[SL > 58 & SL <=59] <- 59
  length1[SL > 59 & SL <=60] <- 60
  length1[SL > 60 & SL <=61] <- 61
  length1[SL > 61 & SL <=62] <- 62
  length1[SL > 62 & SL <=63] <- 63
  length1[SL > 63 & SL <=64] <- 64
  length1[SL > 64 & SL <=65] <- 65
  length1[SL > 65 & SL <=66] <- 66
  length1[SL > 66 & SL <=67] <- 67
  length1[SL > 67 & SL <=68] <- 68
  length1[SL > 68 & SL <=69] <- 69
```

```
 length1[SL > 69 & SL <=70] <- 70
 length1[SL > 70 & SL <=71] <- 71
 length1[SL > 71 & SL <=72] <- 72
 length1[SL > 72 & SL <=73] <- 73
 length1[SL > 73 & SL <=74] <- 74
 length1[SL > 74 & SL <=75] <- 75
 length1[SL > 75 & SL <=76] <- 76
 length1[SL > 76 & SL <=77] <- 77
 length1[SL > 77 & SL <=78] <- 78
 length1[SL > 78 & SL <=79] <- 79
 length1[SL > 79 & SL <=80] <- 80
 length1[SL > 80 & SL <=81] <- 81
 length1[SL > 81 & SL <=82] <- 82
 length1[SL > 82 & SL <=83] <- 83
 length1[SL > 83 & SL <=84] <- 84
 length1[SL > 84 & SL <=85] <- 85
 length1[SL > 85 & SL <=86] <- 86
})


#categorise maturity: 0=immature, 1=mature
DataF <- within(DataF,{
 mat <- NA
 mat[stage == "w7"] <- "1"
 mat[stage == "w6"] <- "1"
 mat[stage == "w5"] <- "1"
 mat[stage == "w4"] <- "1"
 mat[stage == "w3"] <- "1"
 mat[stage == "w2"] <- "1"
 mat[stage == "w1"] <- "0"
})

#shows countdata of immature and mature females per lengthclass
tbl1 <- xtabs(~ length1+mat, data=DataF)

#tbl1 was manually typed out and then insertet as a dataset "tbl1"

######Stats#############################

model1 <- glm(cbind(mat.1, mat.0) ~ length,
          family=binomial(logit), data=tbl1)

summary(model1)

> Call:
glm(formula = cbind(mat.1, mat.0) ~ length, family = binomial(logit),
    data = countdata2)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-2.71750  -0.46677  -0.01468   0.22236   1.73574
```

```
Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) -20.64065    1.99318  -10.36   <2e-16 ***
length        0.37114    0.03585   10.35   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 417.778  on 46  degrees of freedom
Residual deviance:  33.517  on 45  degrees of freedom
AIC: 73.423

Number of Fisher Scoring iterations: 6
```