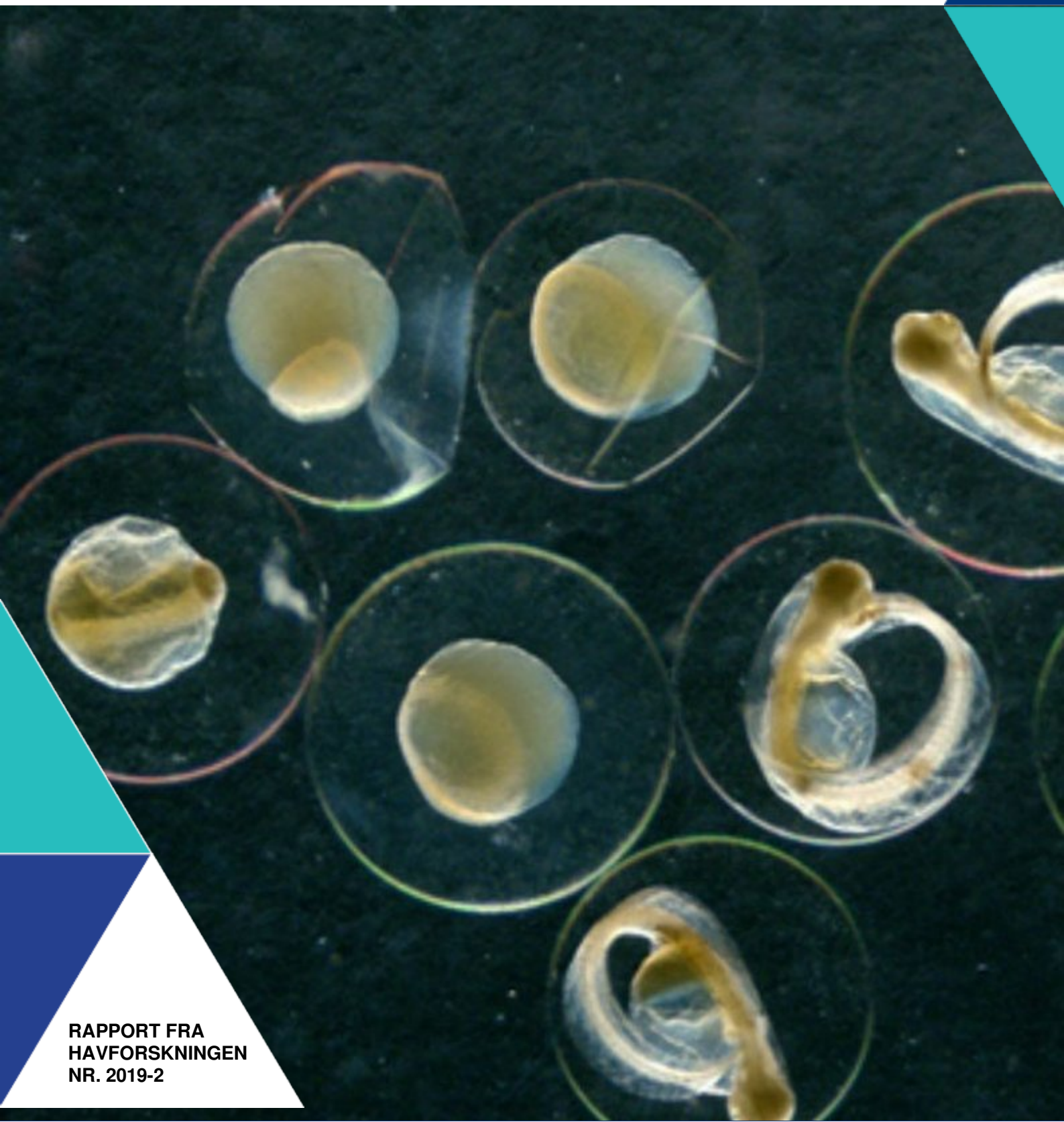
MAPPING OF FISH SPAWNING IN THE NORTH SEA

Report of the KINO-2 project for 2018

Alejandro Mateos Rivera, Bahar Mozfar, Rasmus Skern, Geir Dahle, Henning Wehde, Lisbeth Kleppe, Svein Sundby, Anders Thorsen, Bjørghild Breistein Seliussen, Lars Asplin, Gaston Ezequiel Aguirre, Stamatina Isari and Bjørn Krafft (Institute of Marine Research)



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Authors:

Alejandro Mateos Rivera, Bahar Mozfar, Rasmus Skern, Geir Dahle, Henning Wehde, Lisbeth Kleppe, Svein Sundby, Anders Thorsen, Bjørghild Breistein Seliussen, Lars Asplin, Gaston Ezequiel Aguirre, Stamatina Isari and Bjørn Krafft (Institute of Marine Research)

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Summary (English):

Increased and updated knowledge of reproductive strategies of fish is crucial to optimize the temporal and spatial planning for conducting seismic surveys; in order to reduce its potential negative ecosystems impacts. The overarching goal of this study is to investigate the current spawning times and locations for the North Sea fish stocks. Molecular barcode and traditional visual taxonomic analyses were performed on eggs and larvae, systematically collected with plankton nets along a south to north axis in the North Sea. This report describes the establishments of methodology employed and preliminary results based on samples collected during August 2017 to September 2018. In total, 129 samples have been analysed from which 22 different fish taxa are identified. Based on historic data and published literature, our results agree with the expected number of the most prevalent fish species breeding in the North Sea. Both the taxonomic and molecular methods used, demonstrate similar results in the identification of taxonomic groups. The results further demonstrate that the molecular taxonomic approach is more appropriate for identification to species levels for some taxonomic groups,

whilst the identification of egg and larvae developmental stages can only be performed using the visual taxonomic method. The two techniques complement each other to provide a detailed description of the annual fish spawning cycle for the main North Sea species. There are already emerging patterns evident in the results on the timing for breeding in some species at certain areas. However, improvements of the sampling methodology are still required, and an increased number of samples collected systematically over an extended time-period must be accomplished in order to provide appropriate data as basis for advice in timing of seismic surveys.

Summary (Norwegian):

Undersøkelse av nåværende gyte tid og lokalitet for fisk bestander i Nordsjøen.

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INTRODUCTION

The project KINO-1 (“Dynamic Mapping of North Sea Spawning”) collected historic information from various databases about fish species spawning time in the North Sea (Sundby et al., 2017). The project further describes a clear requirement for detailed information including recent data from this dynamic and complex environment. Preparations including providing sampling equipment and the training of crew onboard supply vessels for sample collection procedures of fish eggs and larvae were given by IMR personnel during spring 2017. The collection of samples from the supply vessels for the KINO-2 project started in August 2017, and the financing and official initiation of KINO-2, started 1. May 2018. KINO-2 aim at improving knowledge on spawning areas and timing for the main fish species reproducing in the North Sea.

Spawning behaviour in marine fish have demonstrated to be disturbed by high-energy acoustic sources, such as those produced from geological surveys where seismic methods are applied (Sundby et al., 2017). In the North Sea, more than 140 different fish species have been described. The most economically important fish species account for less than 10 % of the total number of species comprising around 80 % of the total North Sea fish catch in terms of biomass (Sundby et al., 2017). Despite the increased amount of studies and scientific information available concerning the biology and ecology of fish species in the North Sea, literature on spawning fish is still not very comprehensive with a distinct lack of information.

Increased temperature in the Northern part of the Atlantic Ocean over the last decades, likely affect distribution and abundance patterns of some fish species such as cod, haddock, whiting saithe and Norway pout in the North Sea (Sundby et al., 2017). These species display a decrease in biomass and a shift in the distribution towards the east, while other species such as sardine and anchovy have recently started to migrate towards the North Sea from the English Channel increasing in abundance. Consequently, these changes have altered the traditional preferred areas for feeding and spawning. Thus, new up-to-date sampling efforts of North Sea spawning locations needs to be executed. This project aims to provide improved and more detailed management advice for timing and location of seismic surveys to minimize potential negative effects on reproduction and development of early life stages of the North Sea fishes.

MATERIALS AND METHODS

Study site

Sampling sites were located within 10 nautical miles (the preferred distance is 5 nautical miles) of three different locations along a south-north latitudinal axis in the North Sea (Figure 1); Ekofisk (the southernmost location), Sleipner and Tampen (the northernmost location). The locations were selected due to: i) previous data availability for comparative purposes with the results from the former “KINO-1” project, and ii) their spatial importance for the oil industry activities.

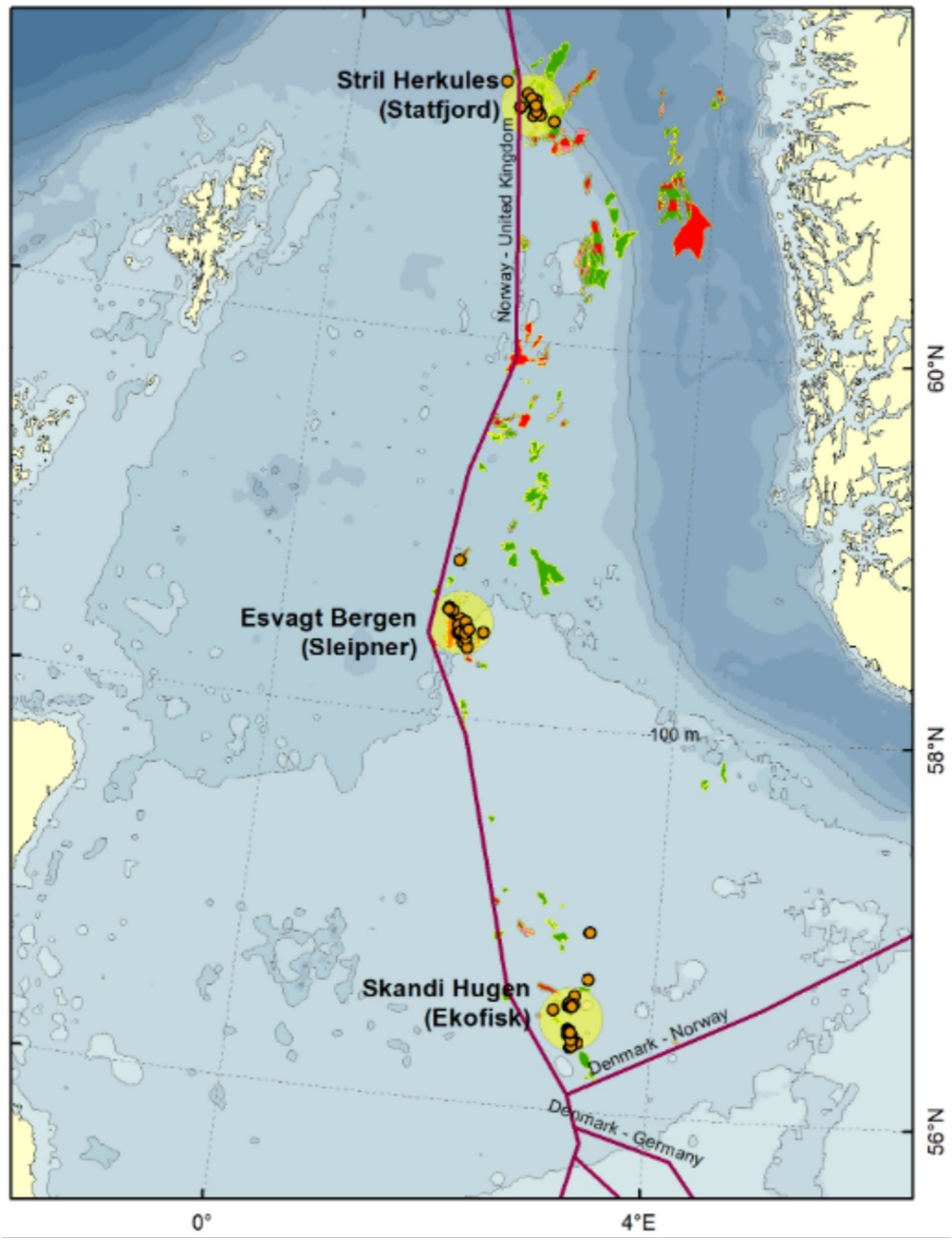


Figure 1. Map of the three different sampling sites: Ekofisk, Sleipner and Tampen (Statfjord) with the name of the boats in charge of sample collection: Skandi Hugen, Esvagt Bergen and Stril Herkules, respectively. Yellow circles represent 10 nautical miles within each sampling site, while the orange points represent the exact location where samples have been collected during August 2017 to September 2018.

Sample collection

Samples have been collected by three different supply vessels managed by Equinor. Skandi Hugen collected samples at Ekofisk, Esvagt Bergen at Sleipner and Stril Herkules at Tampen. The samples were scheduled to be collected once per week per sampling site using a WP2 net with a mesh size of 500 µm and a mouth opening of 0.57m (0.25 m^2) (Figure 2). Plankton sampling was performed by a vertical net-haul from 10 m above bottom at a wire speed of 0.5 m sec^{-1} to surface. The net configuration was chosen due to its ease of use and its resistance when used in relatively harsh weather conditions (Hassel et al., 2017). A CastAway CTD (Sontek, CA, USA) was attached to the net during

each haul providing depth profiles of temperature and salinity.

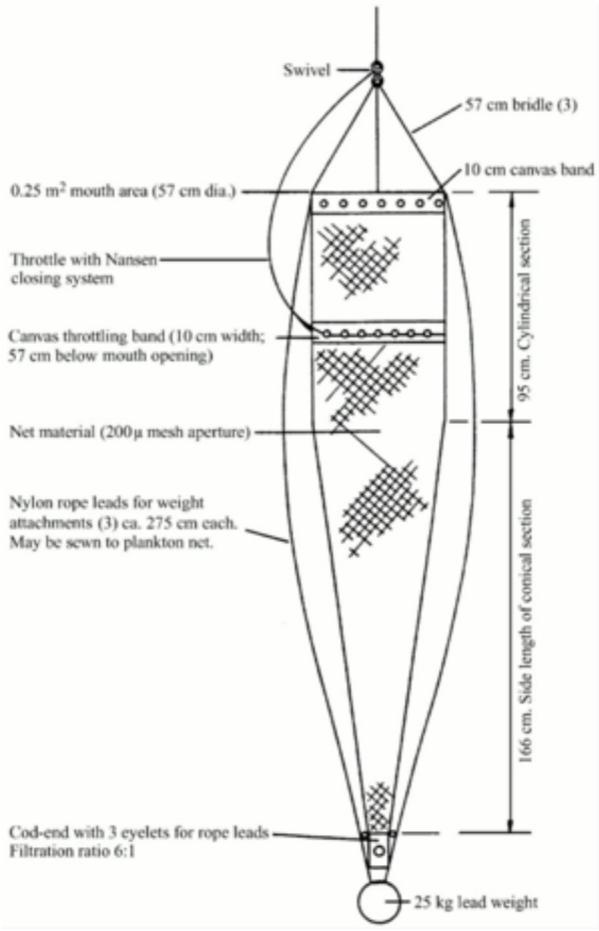


Figure 2. Diagram of a WP2 net (from Gjøsæter et al., 2000).

Each sample was sequentially poured from the cod-end through a 180 µm meshed sieve and transferred into a 100 mL square plastic bottle (Figure 3) for fixation. The first haul was preserved in 96% ethanol for the molecular taxonomic analyses, whereas a second haul was preserved in seawater borax buffered 4% formalin concentration for on shore visual taxonomic identification (Figure 3).

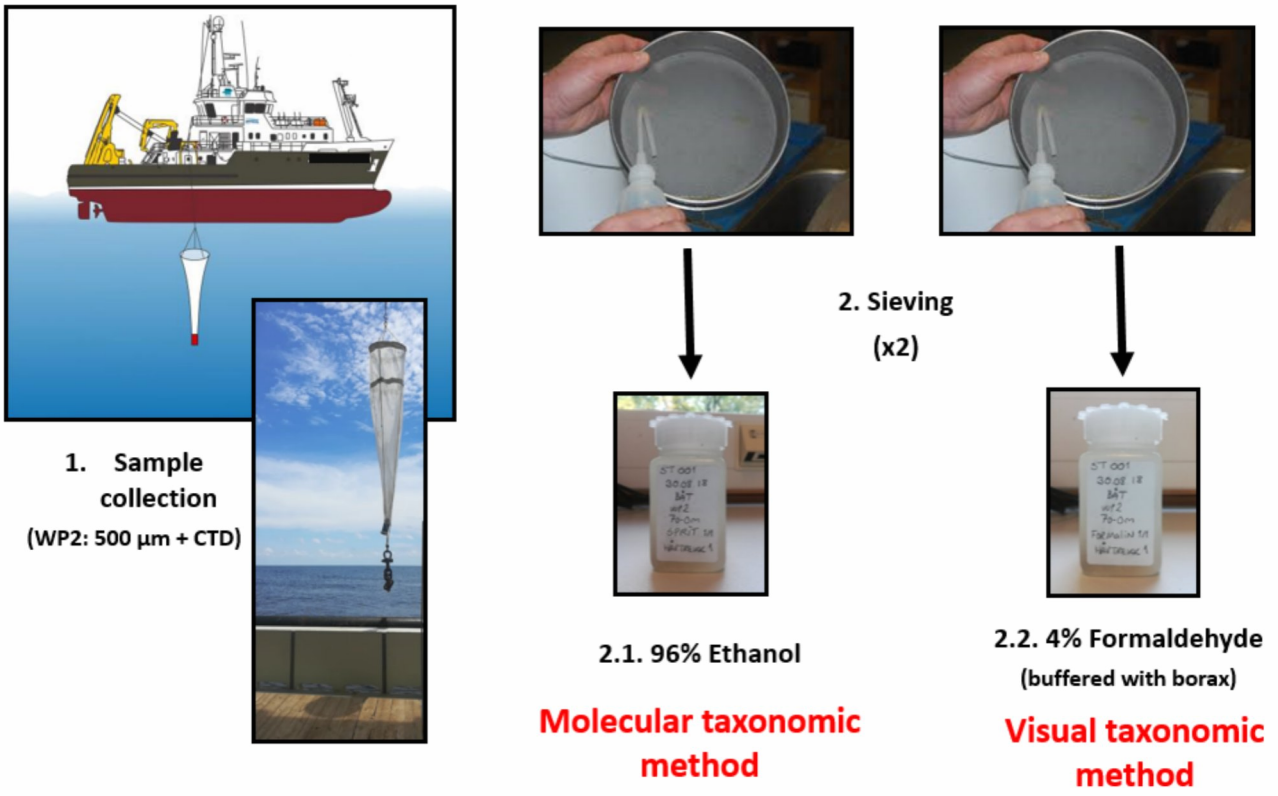


Figure 3. Diagram of the steps followed for sample collection.

Once in the laboratory, samples were poured into plankton counting chambers and the fish eggs and larvae were separated from visual inspections using a Leica M80 stereomicroscope with focusing arm (Leica Microsystems, Germany).

Visual taxonomic method

Fish eggs and larvae sorted from the samples preserved in seawater, borax buffered 4% formalin were identified to the lowest taxonomic level possible under binocular microscope following procedures described in previous studies (Russell 1976 and Munk 2005). Larval morphometry (e.g. standard length) was measured using a Leica M80 stereomicroscope with focusing arm (Leica Microsystems) using a millimeter paper.

The eggs were photographed under an Olympus SZX16 stereomicroscope (Olympus, Japan) with a 0.4575 pixels μm^{-1} resolution and subsequently measured using the open source image analysis program Image J (https://imagej.nih.gov/ij/) with the plugin ObjectJ (https://sils.fnwi.uva.nl/bcb/objectj/) and the Cindy's Fish Eggs project (https://sils.fnwi.uva.nl/bcb/objectj/examples/CindysFisheggs/Manual-Cindy-6.htm).

Egg identification was performed following the parameters described in Russel (1976) and the two main categories described in Ahlstrom and Geoffrey (1980). Briefly, eggs were classified according to: i) independent characters of the embryo (i.e. presence or absence of oil globules, egg size, egg shape, character of yolk, and width of the perivitelline space) and; ii) dependent characters of the embryo (mainly pigment patterns). Furthermore, egg staging was determined based on the criteria followed in

previous studies (Thompson and Riley 1981 and Riley 1973)

Molecular taxonomic method

The eggs and larvae separated from the individual ethanol preserved samples were transferred collectively into a 24 mL scintillation bottle containing abundant pure ethanol (99%) and stored in the fridge at 4°C until further processing. For DNA isolation, single eggs or larval eyes were placed in individual wells of the 200 µL 96 well-plates (Axygen Scientific, CA, USA) containing 75 µL of a solution 5% Chelex 100 Resin (BioRad, CA, USA) and 15 µL of Proteinase K (Qiagen, Germany). The 96 well-plates were then incubated at 56°C for 1 h followed by 10 min at 96°C. After a brief centrifugation the supernatant containing the nucleic acids were transferred into new 96 well-plates.

Following DNA isolation, PCR amplification targeting the MT-CO1 gene was performed in 12 µL reactions containing 2.4 µL 5x buffer, 1 µL of $MgCl_2$ [25 mM], 1.92 µL dNTPs [1.25 mM], 1.44 µL 10 µM primer pair combination (Table 1), 0.07 µL GoTaq G2 DNA polymerase (Promega, WI, USA), 3.17 µL dH_2O and 2 µL template DNA. The PCR conditions were i) an initial denaturation of 2 min at 95°C, followed by ii) 38 cycles of amplification (denaturation 30 s at 94°C, annealing at 52°C for 30 s and an extension of 1 min at 72°C), and iii) a final extension of 10 min at 72°C. Clean-up of the PCR products were performed mixing 5 µL of the PCR product and 2 µL ExoSap-IT PCR product Cleanup (ThermoFisher, MA, USA) followed by an incubation at 37°C for 15 min and 80°C for 15 min. Finally, sequencing was performed using 1 µL of M13F primer [0.35 µM] (Table 1) at the sequencing facility at the University of Bergen (http://www.seqlab.uib.no). Sequence analysis were performed in Geneious v8.0.5 (Kearse et al., 2012). To confirm sequence identity, they were used as queries for BLASTn (Altschul et al., 1990) against the NCBI database (http://www.ncbi.nlm.nih.gov/blast).

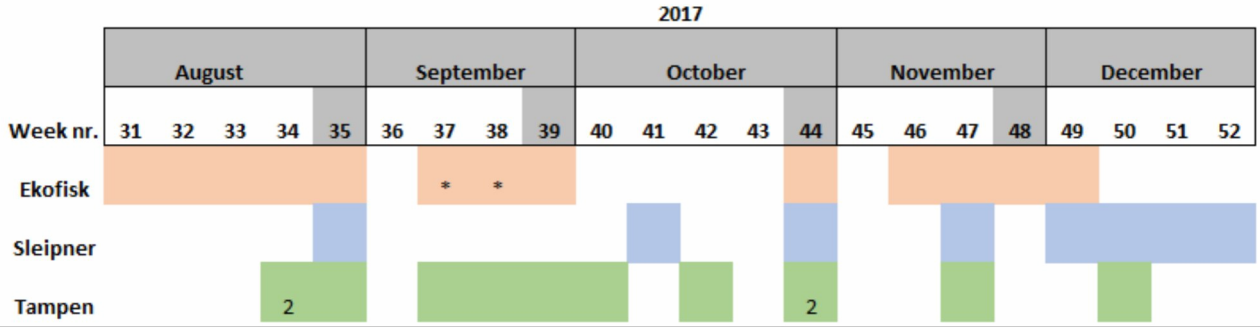
Table 1. List of primers.

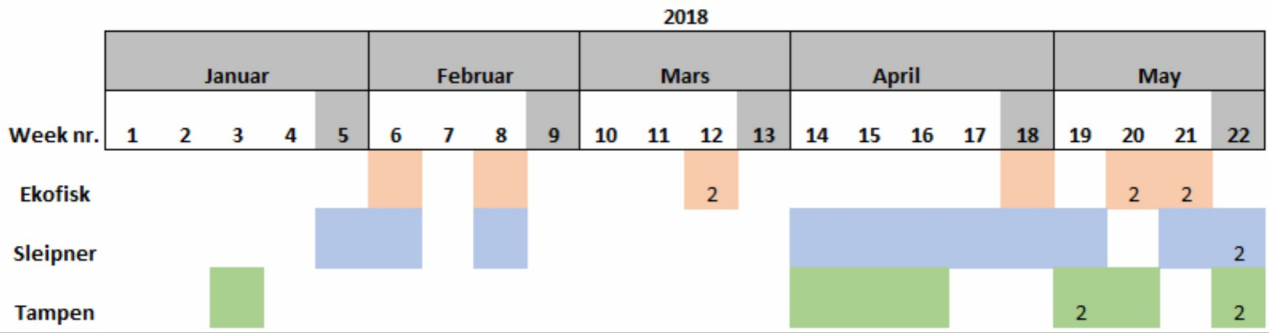
Primer	Sequence	Amount added (µL)	Reference
LepF1_t1	TGTAAAACGACGGCCAGTATTCAACCAATCATAAAGATATTGG	0.12	Ivanova et al. (2007)
VF1_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCACAAAGACATTGG	0.12	Ivanova et al. (2007)
VF1d_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCACAARGAYATYGG	0.12	Ivanova et al. (2007)
VF1i_t1	TGTAAAACGACGGCCAGTTCTCAACCAACCAIAAIGAIATIGG	0.36	Ivanova et al. (2007)
LepRI_t1	CAGGAAACAGCTATGACTAAACTTCTGGATGTCCAAAAAATCA	0.12	Ivanova et al. (2007)
VR1d_t1	CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCRAARAAYCA	0.12	Ivanova et al. (2007)
VR1_t1	CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCAAAGAATCA	0.12	Ivanova et al. (2007)
VR1i_t1	CAGGAAACAGCTATGACTAGACTTCTGGGTGICCIAAIAAICA	0.36	Ivanova et al. (2007)
M13F (-21)	CAGGAAACAGCTATGAC	1	Messing (1983)

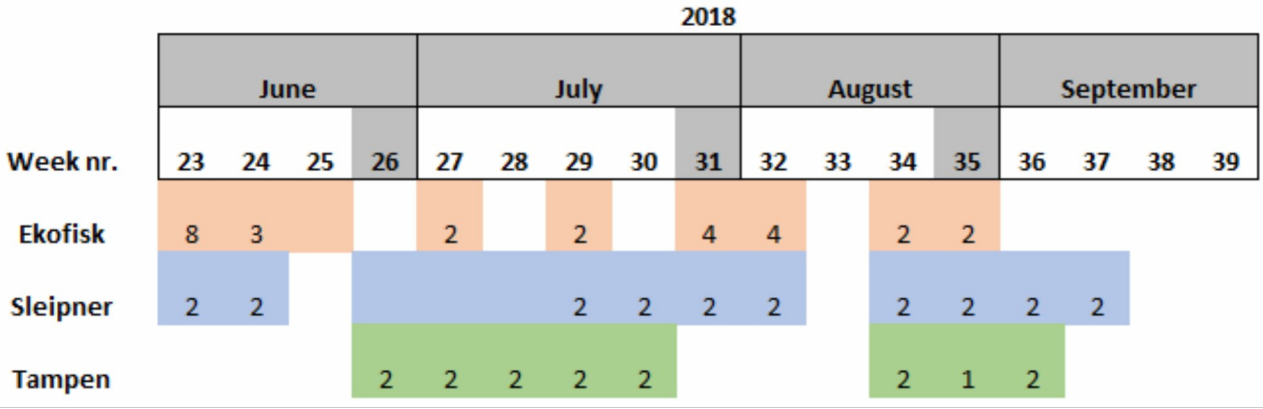
RESULTS

From samples collected from August 2017 to September 2018, a total of 129 samples have been collected and analysed (Table 2). At Ekofisk, the southernmost location, a total of 51 different samples were collected, 43 samples were collected at Sleipner, while 35 samples were collected at Tampen.

Table 2. Distribution of the samples collected per week. Asterisk indicates samples where the date has not been included in the sampling protocol. A number inside a cell indicates the number of hauls collected per week if more than one. Absence of colour indicates weeks where sampling was not conducted.







3.1 – Visual taxonomic analyses

For the visual taxonomic method, total of 1480 individuals have been analysed, consisting of 1285 fish eggs and 195 larvae. At Ekofisk, the southernmost location, there was a total of 423 individuals (386 eggs and 37 larvae), 918 individuals (802 eggs and 116 larvae) at Sleipner and 139 individuals (97 eggs and 42 larvae) at Tampen (Figure 4). No eggs or larvae have been found in samples from November and December.

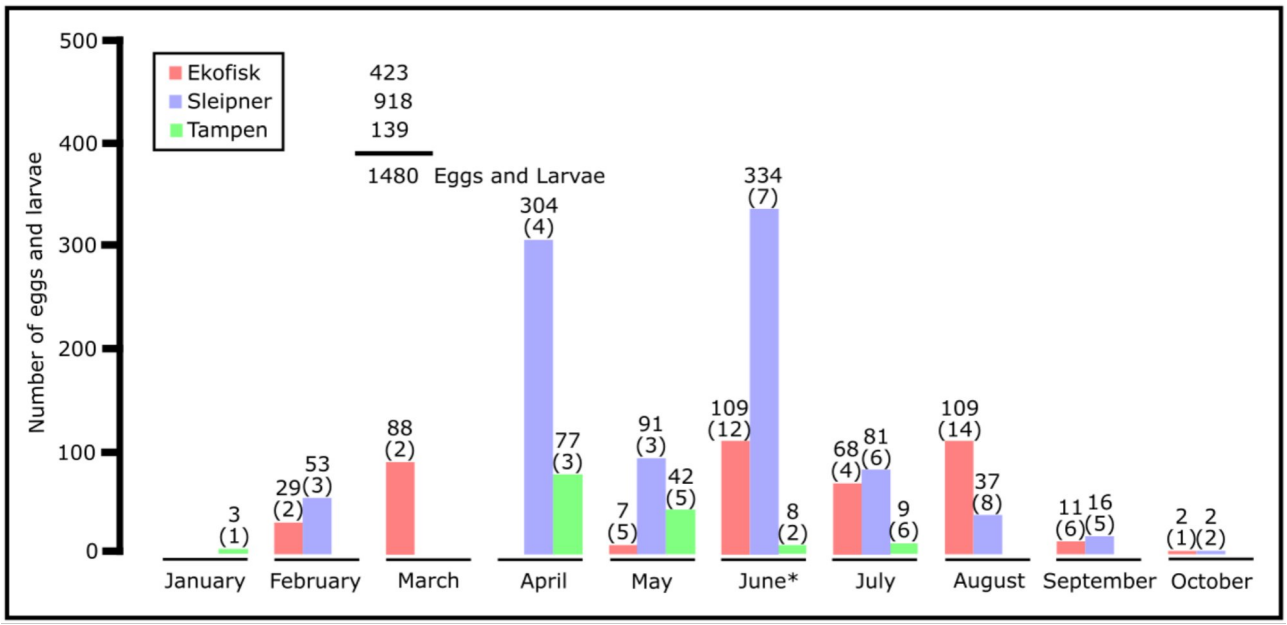


Figure 4. Total number of individuals (eggs and larvae) obtained for the visual taxonomic analyses each month per sampling site. The number of samples collected is indicated within parenthesis. November and December months are not included due to the absence of eggs and larvae in the samples.

Although most of the 1285 fish eggs have been already analysed, its identification was very challenging due to overlap in the various classifying parameters between not only species from the same family but also different families. Since identification for the fish eggs using the visual taxonomic approach was not precise and therefore could contain errors, they have been excluded from the results.

The 195 larvae were successfully assigned to species and family level, except 3 individuals which were assigned as Unknown. For the taxonomic analyses 176 larvae were classified into 16 species: Haddock (*Melanogrammus aeglefinus*), Whiting (*Merlangius merlangus*), Saithe (*Pollachius virens*), Norway pout (*Trisopterus esmarkii*), Pollack (*Pollachius pollachius*), Witch (*Glyptocephalus cynoglossus*), Long-rough dab (*Hippoglossoides platessoides*), Dab (*Limanda limanda*), Lemon sole (*Microstomus kitt*), Four-bearded rockling (*Enchelyopus cimbrius*), Ling (*Molva molva*), European hake (*Merluccius merluccius*), Mackerel (*Scomber scombrus*), Grey gurnard (*Eutrigla gurnardus*), Silver fish (*Argentina sphyraena*) and Crystal goby (*Crystallogobius linearis*). While 19 larvae were assigned to 6 families (as species identification was not possible due to damage in the larvae or difficulties in larval identification): Callionymidae, Gadidae, Gobiidae, Phycidae, Pleuronectidae and Triglidae. Overall the most abundant species were the Mackerel and the flatfish species Dab with 53 and 50 individuals, respectively (Table 3). Furthermore, only three species were found at all three sampling locations (Dab, Whiting and Lemon

sole).

Table 3. All fish larvae identified using the visual taxonomic approach in this study.

	Ekofisk	Sleipner	Tampen	Total
S. scombrus		52	1	53
L. limanda	21	25	4	50
T. esmarkii		5	14	19
M. merlangus	1	6	4	11
H. plattesoides		6	3	9
M. merluccius		4	2	6
Callionymidae		1	4	5
E. gurnardus	4			4
G. cynoglossus	1	3		4
M. kitt	2	1	1	4
Gadidae		6	1	7
Gobiidae	1	1	1	3
A. sphyraena		1	1	2
M. molva		1	1	2
Phycidae	2	2		4
Pleuronectidae			2	2
P. pollachius			2	2
Unknown	3			3
C. linearis	1			1

E. cimbrius	1			1
M. aeglefinus		1		1
Triglidae		1		1
P. virens			1	1

3.2 - Ekofisk

3.2.1 - Larvae

The lowest number of larval species were found at Ekofisk with 9 different species. Grey gurnard, Crystal goby, Four-bearded rockling and Phycidae (family level) were only found at this location (Table 4).

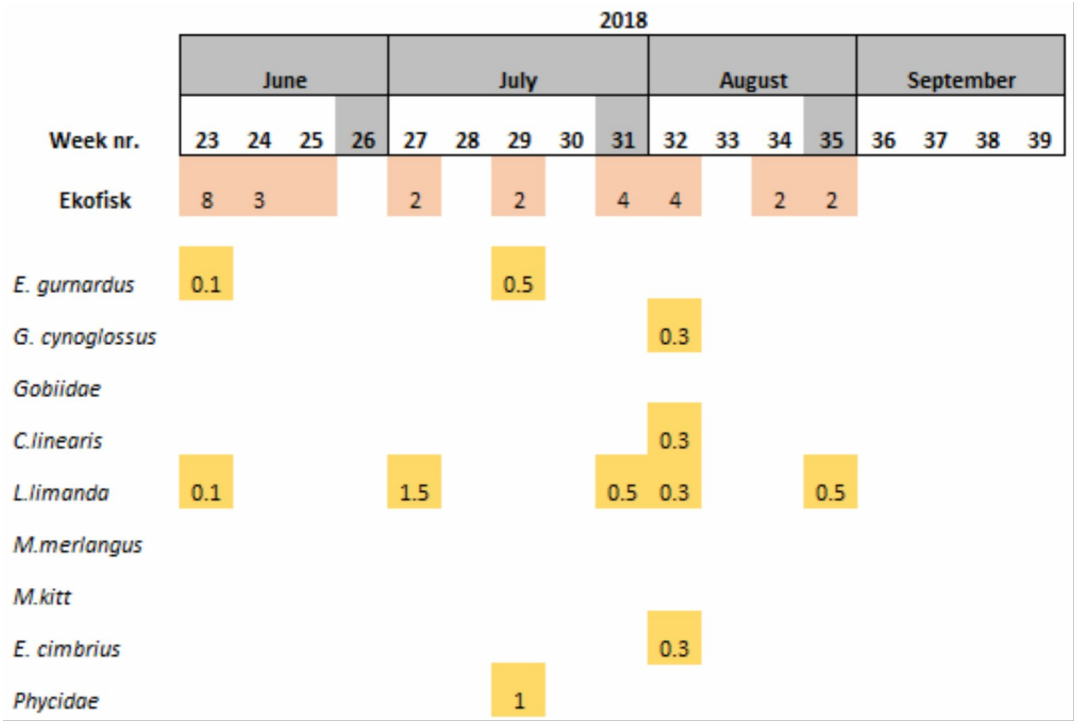
Table 4. Fish larvae identified using the visual taxonomic approach at Ekofisk. Larval numbers are adjusted to the number of samples collected that specific week.

2017

	August					September				October					November				December			
Week nr.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Ekofisk							•	•														
E. gurnardus			1	1																		
G. cynoglossus																						
Gobiidae									1													
C.linearis																						
L.limanda	4	2	3	1																		
M.merlangus	1																					
M.kitt				1				1														
E. cimbrius																						
Phycidae																						

2018

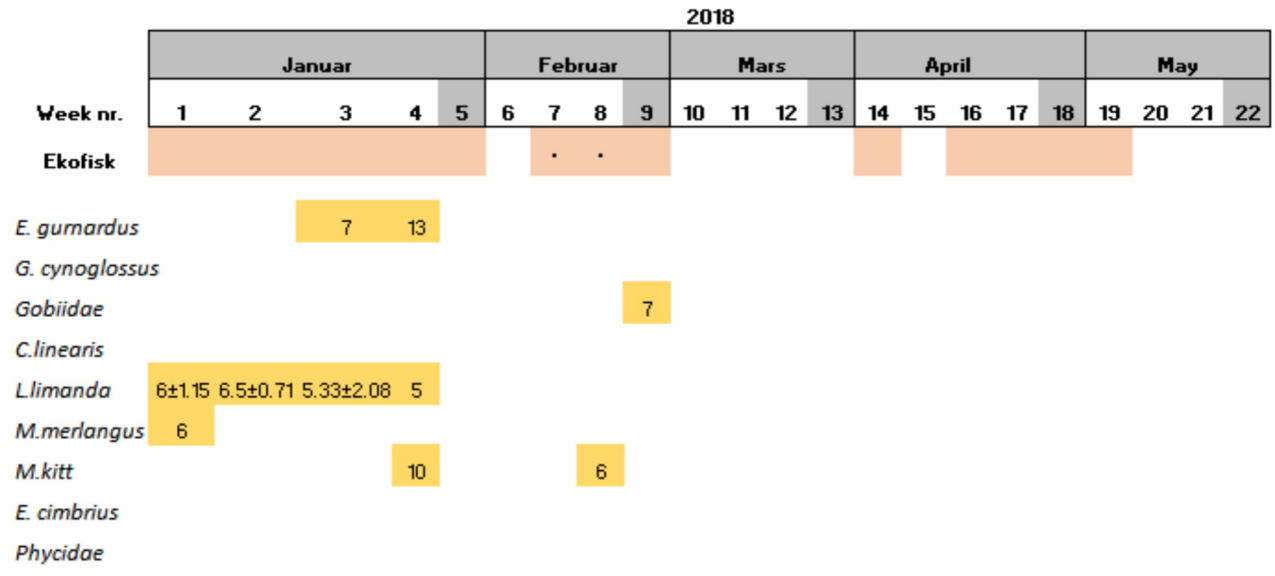
	Januar					Februar				Mars				April					May			
Week nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Ekofisk												2								2	2	
E. gurnardus																						
G. cynoglossus																						
Gobiidae																						
C.linearis																						
L.limanda						1		2														
M.merlangus																						
M.kitt																						
E. cimbrius																						
Phycidae																						



3.2.2 - Larval length

The largest larvae found at Ekofisk was one Cristal goby collected in August 2018, followed one Grey gurnard from August 2017. Larvae collected during the week 29 of 2018 were not measured (Table 5).

Table 5. Average and standard deviation of the fish larvae length identified using the visual taxonomic approach at Ekofisk.



2018

	Januar					Februar				Mars				April					May			
Week nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Ekofisk												2								2	2	
E. gurnardus																						
G. cynoglossus																						
Gobiidae																						
C.linearis																						
L.limanda						7		7														
M.merlangus																						
M.kitt																						
E. cimbrius																						
Phycidae																						

2018

	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Ekofisk	8	3			2		2		4	4		2	2				
E. gurnardus	4						x										
G. cynoglossus										6							
Gobiidae																	
C.linearis										12							
L.limanda	4				5±1.73				8±2.83	5			6				
M.merlangus																	
M.kitt																	
E. cimbrius										4							
Phycidae							x										

3.3 - Sleipner

Within the 16 species found at Sleipner, Haddock and the family Triglidae (family level) have been only found at this location (Table 6).

3.3.1 - Larvae

Table 6. Fish larvae identified using the visual taxonomic approach at Sleipner. Larval numbers are adjusted to the number of samples collected that specific week.

	2017																			
	August					September				October					November				December			
Week nr.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Sleipner																						
A. sphyraena																						
Callionymidae																						
Gadidae																						
T. esmarkii																						
G. cynoglossus																						
Gobiidae											1											
H. plattesoides																						
L. limanda																						
M. aeglefinus																						
M. merlangus																						
M. merluccius					3									1								
M. kitt					1																	
M. molva																						
Phycidae																						
S. scombrus																						
Triglidae																						


2018
Januar
Februar
Mars
April
May
Week nr.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Sleipner
2
A. sphyraena
1
Callionymidae
1
Gadidae
T. esmarkii
1 1 1
G. cynoglossus
1 1
Gobiidae
H. plattesoides
1 4 1
L. limanda
1 13 4 4 3
M. aeglefinus
1
M. merlangus
2 1 2
M. merluccius
M. kitt
M. molva
Phycidae
1
S. scombrus
11
Triglidae
1

2018

	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Sleipner	2	2					2	2	2	2		2	2	2	2		
A. sphyraena																	
Callionymidae																	
Gadidae	6																
T. esmarkii							1	1									
G. cynoglossus										1							
Gobiidae																	
H. plattesoides																	
L. limanda																	
M. aeglefinus																	
M. merlangus				1													
M. merluccius																	
M. kitt																	
M. molva					1												
Phycidae		1															
S. scombrus	27	6			5	2			1								
Triglidae																	

3.3.2 - Larval length

The largest larvae collected at this location was a single Ling individual collected in July 2018 with 14 cm, followed by one Whiting individual obtained during the week 22 of 2018 with 13 cm (Table 7).

Table 7. Average and standard deviation of the fish larvae length identified using the visual taxonomic approach at Sleipner.

	2017																			
	August					September				October				November				December			
Week nr.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Sleipner																						
A. sphyraena																						
Callionymidae																						
Gadidae																						
T. esmarkii																						
G. cynoglossus																						
Gobiidae											4											
H. plattesoides																						
L. limanda																						
M. aeglefinus																						
M. merlangus																						
M. merluccius					3.33±0.57									5								
M. kitt					4																	
M. molva																						
Phycidae																						
S. scombrus																						
Triglidae																						

2018

	Januar					Februar				Mars				April					May			
Week nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Sleipner																						2
A. sphyraena																8						
Callionymidae																						4
Gadidae																						
T. esmarkii																7		5			5	
G. cynoglossus																					4	5
Gobiidae																						
H. plattesoides															8		6±1.63	5				
L. limanda															4		4.33±0.49	4.66±0.57	6.25±0.5			6±1.00
M. aeglefinus																		9				
M. merlangus																	4±1.41	6				9.5±4.95
M. merluccius																						
M. kitt																						
M. molva																						
Phycidae																						4
S. scombrus																						4.1±0.70
Triglidae																		5				

2018

	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Sleipner	2	2					2	2	2	2		2	2	2	2		
A. sphyraena																	
Callionymidae																	
Gadidae	3.5±0.55																
T. esmarkii							3	3									
G. cynoglossus										4							
Gobiidae																	
H. plattesoides																	
L. limanda																	
M. aeglefinus																	
M. merlangus				12													
M. merluccius																	
M. kitt																	
M. molva					14												
Phycidae		6															
S. scombrus	4.04±0.52	4±0.89			7±1.22	3.5			8								
Triglidae																	

3.4 – Tampen

At the northernmost location, Tampen, 15 species were found. Saithe and Pollack were only detected at this sampling site (Table 8).

3.4.1 - Larvae

Table 8. Fish larvae identified using the visual taxonomic approach at Tampen. Larval numbers are adjusted to the number of samples collected that specific week.

	2017																			
	August					September				October					November				December			
Week nr.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Tampen				2										2								
A. sphyraena																						
Callionymidae																						
Gadidae																						
Gobiidae																						
H. plattesoides																						
L. limanda																						
M. merlangus																						
M. merluccius																						
M. kitt																						
M. molva																						
Pleuronectidae																						
P. pollachius																						
P. virens																						
S. scombrus																						
T. esmarkii																						

	2018																				
	Januar					Februar				Mars				April					May			
Week nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Tampen																			2			2
A. sphyraena																				1		
Callionymidae																1			0.5	2		
Gadidae																1						
Gobiidae																						
H. plattesoides																2			0.5			
L. limanda																				4		
M. merlangus																2			0.5	1		
M. merluccius																				2		
M. kitt																				1		
M. molva																				1		
Pleuronectidae																2						
P. pollachius																				2		
P. virens																			0.5			
S. scombrus																						
T. esmarkii																13			0.5			

	2018															
	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Tampen				2	2	2	2	2				2	1	2			
A. sphyraena																	
Callionymidae																	
Gadidae																	
Gobiidae						0.5											
H. plattesoides																	
L. limanda																	
M. merlangus																	
M. merluccius																	
M. kitt																	
M. molva																	
Pleuronectidae																	
P. pollachius																	
P. virens																	
S. scombrus						0.5											
T. esmarkii																	

3.4.2 - Larval length

The largest larvae found at Tampen was a Dab individual collected in May 2018 with 13 cm, followed by the single Silver fish collected at Tampen with 10 cm, also in May 2018 (Table 9).

Table 9. Average and standard deviation of the fish larvae length identified using the visual taxonomic approach at Tampen.

	2017																			
	August					September				October					November				December			
Week nr.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Tampen				2										2								
A. sphyraena																						
Callionymidae																						
Gadidae																						
Gobiidae																						
H. plattesoides																						
L. limanda																						
M. merlangus																						
M. merluccius																						
M. kitt																						
M. molva																						
Pleuronectidae																						
P. pollachius																						
P. virens																						
S. scombrus																						
T. esmarkii																						

2018

	Januar					Februar				Mars				April					May			
Week nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Tampen																			2			2
A. sphyraena																				10		
Callionymidae																3			4	3		
Gadidae																x						
Gobiidae																						
H. plattesoides																8±1.41			7			
L. limanda																				6.5±4.36		
M. merlangus																5±1.41			6	8		
M. merluccius																				4		
M. kitt																				6		
M. molva																				6		
Pleuronectidae																4						
P. pollachius																				7.5±0.70		
P. virens																			5			
S. scombrus																						
T. esmarkii																5.88±0.78			4			

2018

	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Tampen				2	2	2	2	2				2	1	2			
A. sphyraena																	
Callionymidae																	
Gadidae																	
Gobiidae						4											
H. plattesoides																	
L. limanda																	
M. merlangus																	
M. merluccius																	
M. kitt																	
M. molva																	
Pleuronectidae																	
P. pollachius																	
P. virens																	
S. scombrus						8											
T. esmarkii																	

3.5 - Molecular taxonomic analyses

For the molecular taxonomic method, 1493 individuals have been analysed so far, consisting of 1352 eggs and 141 larvae. At Ekofisk, the southernmost location, there was a total of 410 individuals (389 eggs and 21 larvae), 975 individuals (889 eggs and 86 larvae) at Sleipner and only 108 individuals (74 eggs and 34 larvae) at Tampen (Figure 5). No eggs or larvae have been found between October and December.

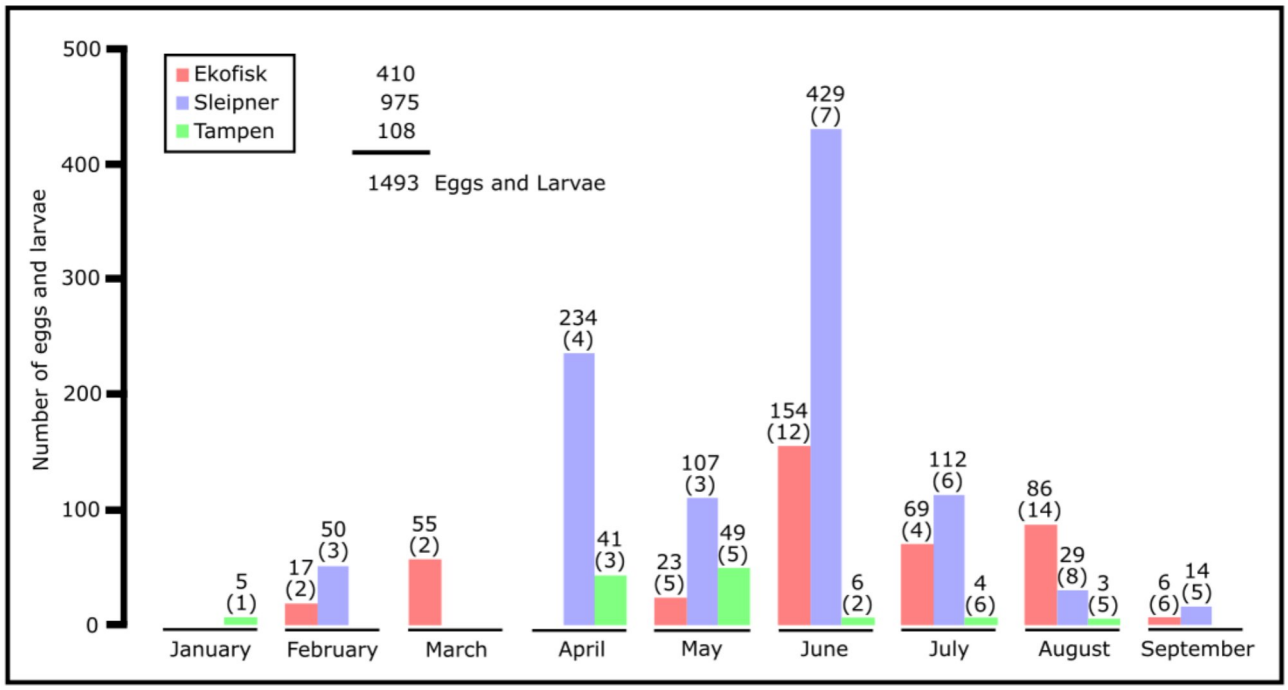


Figure 5. Distribution of the total number of individuals (eggs and larvae) obtained each month per sampling site for the molecular analyses. The number of samples collected is indicated within parenthesis. Months between October and December are not included due to the absence of eggs and larvae in the samples.

Overall, around 80% of all the eggs and larvae were successfully assigned to species level. However, we have experienced some difficulties to assign to a species level the eggs from 4 samples collected in June at Sleipner. The effectiveness of the molecular method applied to the eggs and larvae when these samples were removed from the data increased up to 92%. Furthermore, this number was increased up to more than 98.5% when the method was applied just to the larvae. Both the eggs and larvae were classified into 22 species of 10 different families: Cod (*Gadus morhua*), Haddock (*Melanogrammus aeglefinus*), Whiting (*Merlangius merlangus*), Saithe (*Pollachius virens*), Norway pout (*Trisopterus esmarkii*), Pollack (*Pollachius pollachius*), Witch (*Glyptocephalus cynoglossus*), Long-rough dab (*Hippoglossoides platessoides*), Dab (*Limanda limanda*), Lemon sole (*Microstomus kitt*), Plaice (*Pleuronectes platessa*), Four-bearded rockling (*Enchelyopus cimbrius*), Tusk (*Brosme brosme*), Ling (*Molva molva*), Three-bearded rockling (*Gaidropsaurus vulgaris*), European hake (*Merluccius merluccius*), Mackerel (*Scomber scombrus*), Norwegian topknot (*Phrynorhombus norvegicus*), Grey gurnard (*Eutrigla gurnardus*), Spotted dragonet (*Callionymus maculatus*), Silver fish (*Argentina sphyraena*) and Crystal goby (*Crystallogobius linearis*). However, 7 of these species: Cod, Saithe,

Pollack, Plaice, the Lemon Sole, the Three-bearded rockling, and the Silver fish were not found as larvae.

The largest number of different species were found at Sleipner with 17 species, probably due to the largest amount of eggs and larvae received at this location. On the other hand, 13 species were found at Ekofisk and 14 species at Tampen (Figure 6). Mackerel, Whiting, the Long-rough dab, the Norway pout, the Lemon sole and the Grey Gunard were observed in each of the three sampling locations. In addition, the Spotted dragonet and the Norwegian topknot were observed at both the southernmost and the northernmost locations, Ekofisk and Tampen, respectively, which indicates that these species could probably be present at Sleipner but they have not been found yet. Furthermore, Plaice were only found at Ekofisk, Haddock, Cod and Pollack were only found at Sleipner, while the Silver fish and the Crystal goby were only detected at Tampen (Figure 6).

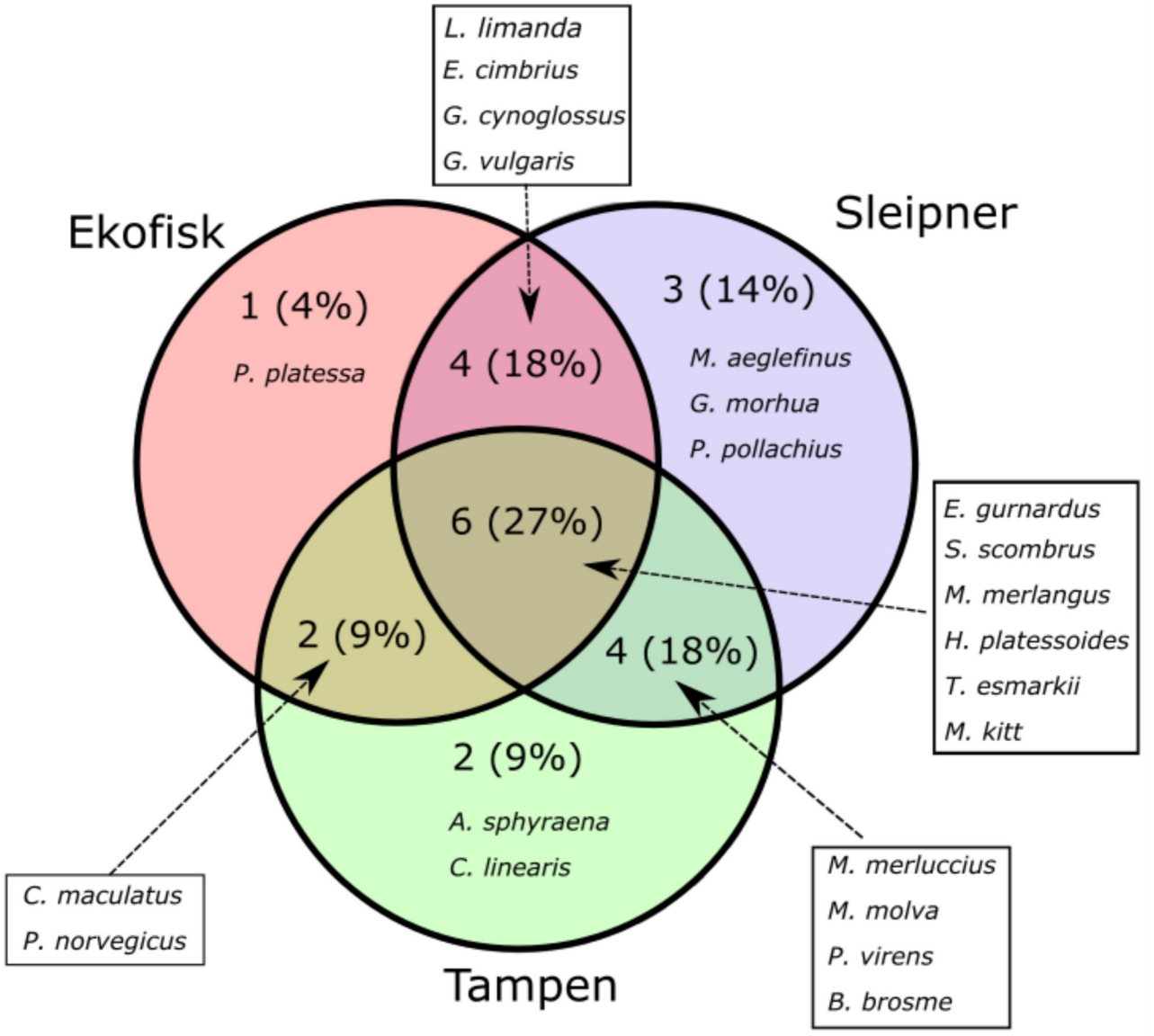


Figure 6. Diagram of the 22 species identified in this project at the three sampling sites.

The most abundant species for the molecular analyses (for both eggs and larvae) were the Dab, followed by Mackerel and Whiting (Table 10).

Table 10. Number of individuals (for both eggs and larvae) per species at the three sampling sites.

	Ekofisk	**Sleipner**	**Tampen**	**Total**
L. limanda	291	222		513
S. scombrus	19	228	17	264
M. merlangus	12	66	14	92
H. platessoides	13	36	6	55
M. merluccius		52	2	54
E. gurnardus	19	33	1	53
T. esmarkii	1	6	15	22
M. kitt	13	7	2	22
G. cynoglossus	4	18		22
M. molva		6	13	19
E. cimbrius	2	17		19
M. aeglefinus		14		14
G. morhua		11		11
C. maculatus	1		7	8
P. norvegicus	6		1	7
P. virens		1	5	6
B. brosme		2	2	4
G. vulgaris	2	1		3

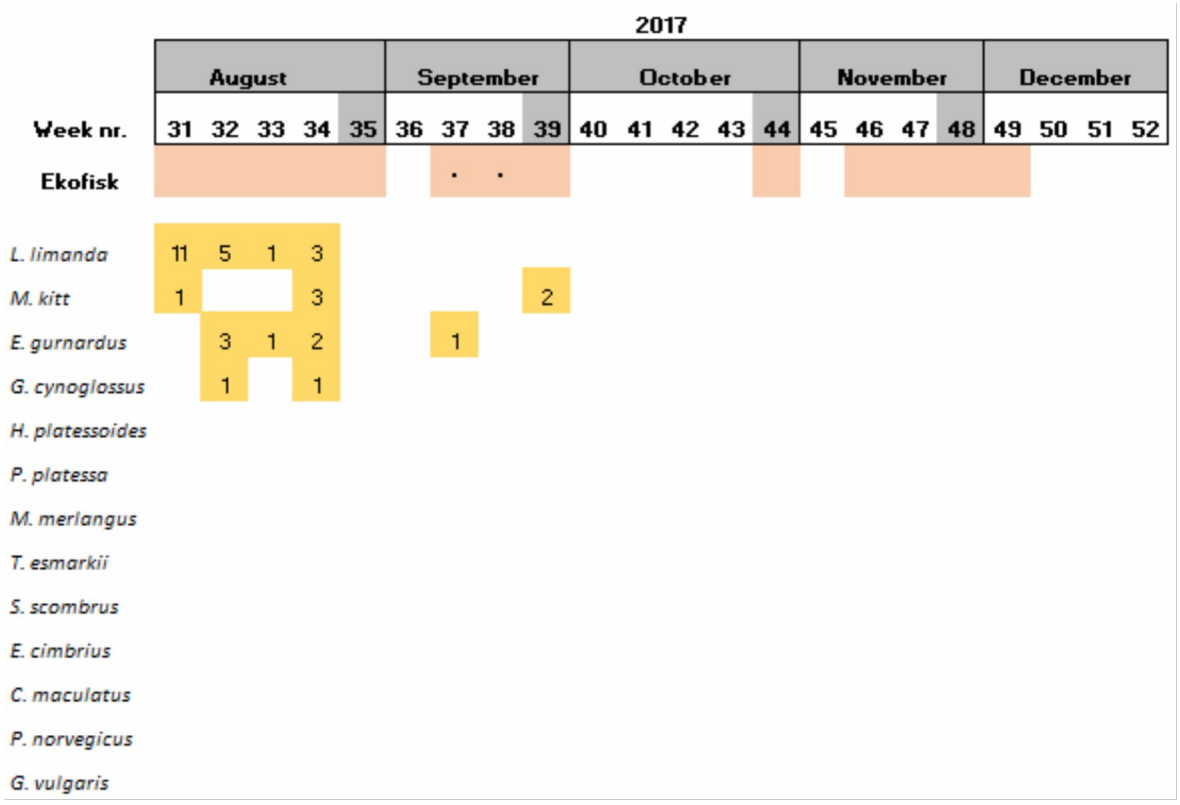
P. platessa	3			3
A. sphyraena			2	2
P. pollachius		2		2
C. linearis			1	1

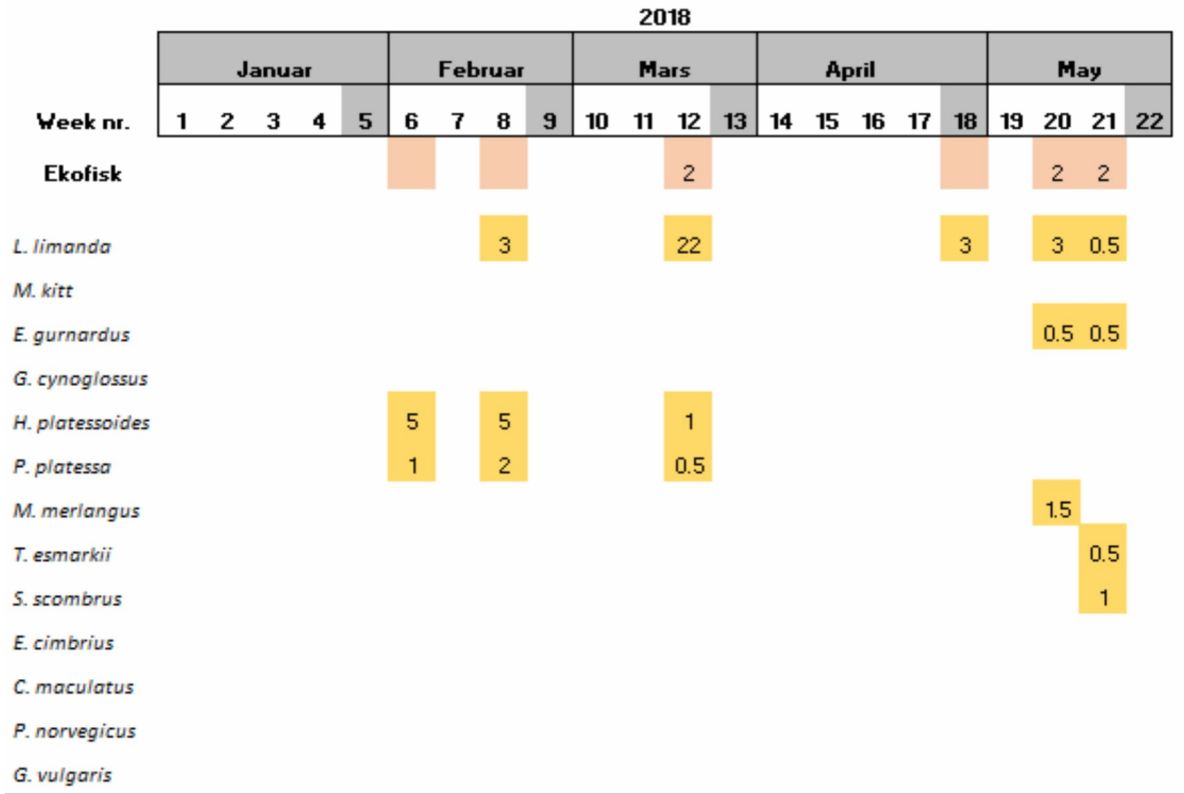
3.6 – Ekofisk

Samples at Ekofisk were collected along the whole year except in January and April. In addition, samples collected during the fourth quarter of the year (October – December) did not contain any eggs or larvae. At Ekofisk, the most abundant species was the Dab with 291, Mackerel with 19 and the Grey gurnard with also 19 individuals (Tables 11 and 12). According to our data the spawning time of Dab covers months between February and August, for Mackerel between May and June and the Grey gurnard between May and September. Some species e.g. the Norway pout, the Four-bearded rockling or the Norwegian topknot that were found only in a single month. More data about these species is required to have a better estimation of its spawning time and peak in this location.

3.6.1 - Eggs

Table 11. Fish eggs identified at Ekofisk using the molecular taxonomic approach. Egg numbers are adjusted to the number of samples collected that specific week.





2018	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Ekofisk	8	3			2		2		4	4		2	2				
L. limanda	5.4	19	10		9		19		4	5.5		2					
M. kitt		0.3			0.5		0.5					1	1				
E. gurnardus		0.7			0.5		2			0.3							
G. cynoglossus													0.5				
H. platessoides																	
P. platessa																	
M. merlangus	0.1	0.3															
T. esmarkii																	
S. scombrus	0.5	3	2														
E. cimbrius	0.3																
C. maculatus	0.1																
P. norvegicus	0.5	0.3	1														
G. vulgaris	0.1						0.5										

3.6.2 - Larvae

Table 12. Fish larvae identified at Ekofisk using the molecular approach. Larval numbers are adjusted to the number of samples collected that specific week.

2017	August					September				October					November				December			
Week nr.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Ekofisk							.	.														
L. limanda		1		2																		
M. kitt																						
E. gurnardus		1																				
G. cynoglossus		1																				
H. platessoides																						
P. platessa																						
M. merlangus		1																				
T. esmarkii																						
S. scombrus																						
E. cimbrius																						
C. maculatus																						
P. norvegicus																						
G. vulgaris																						

2018

	Januar					Februar				Mars				April				May			
Week nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Ekofisk												2							2	2		
L. limanda																		1				
M. kitt																						
E. gurnardus																						
G. cynoglossus																						
H. platessoides								1														
P. platessa																						
M. merlangus																						
T. esmarkii																						
S. scombrus																						
E. cimbrius																						
C. maculatus																						
P. norvegicus																						
G. vulgaris																						

2018

	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Ekofisk	8	3			2		2		4	4		2	2				
L. limanda	0.3	0.3			0.5					0.3							
M. kitt																	
E. gurnardus	0.1																
G. cynoglossus																	
H. platessoides																	
P. platessa																	
M. merlangus	0.5						0.5										
T. esmarkii																	
S. scombrus		0.7															
E. cimbrius																	
C. maculatus																	
P. norvegicus																	
G. vulgaris																	

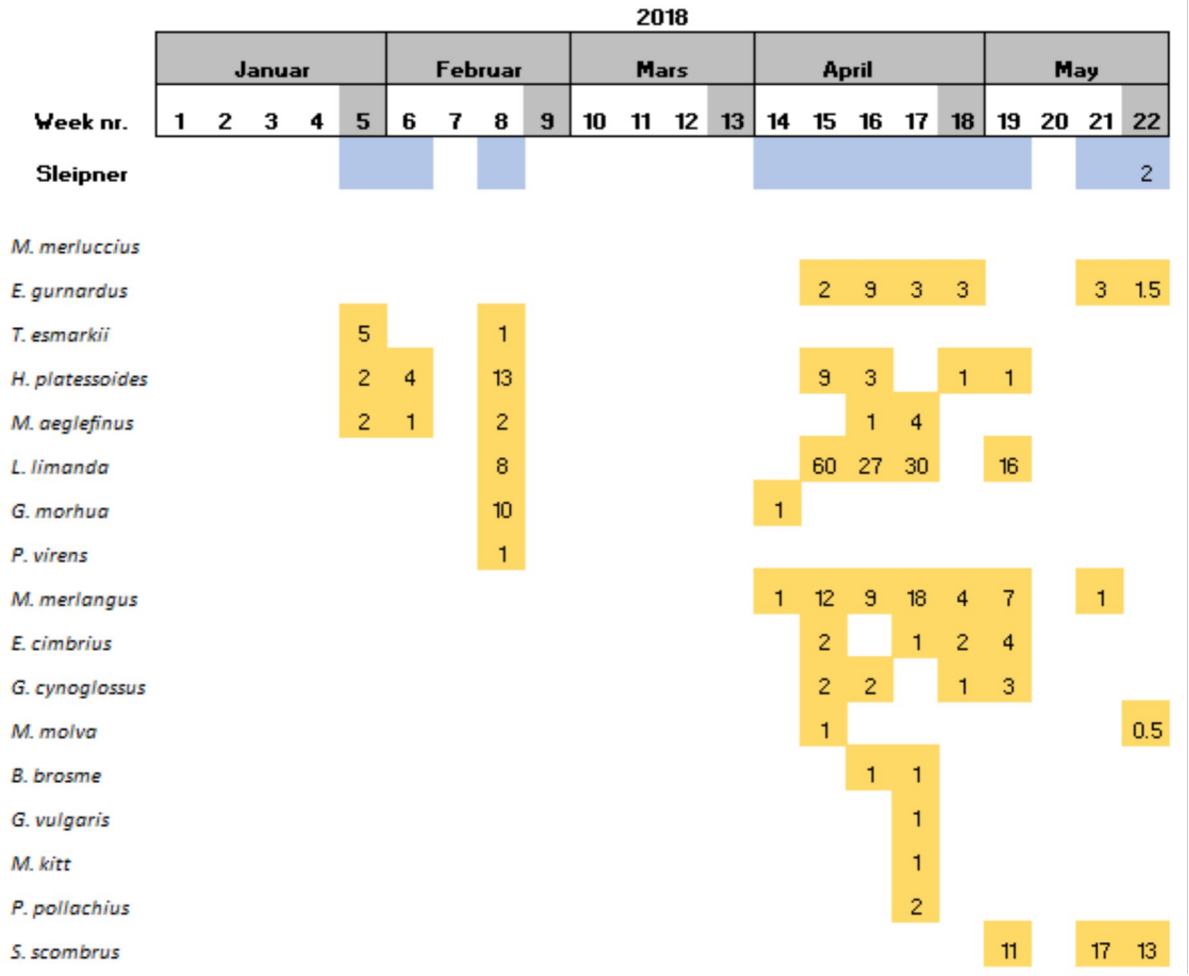
3.7 – Sleipner

At Sleipner, in addition to the absence of eggs and larvae in the samples collected between October and December, samples were not collected during January and March. The most abundant species at this location was the Mackerel with 228 individuals, followed by the Dab with 222 individuals and Whiting with 66 individuals. According to our data the spawning peak for Mackerel varies between May and August, while for Dab and Whiting the spawning time varies between April and August (Tables 13 and 14).

3.7.1 - Eggs

Table 13. Fish eggs identified at Sleipner using the molecular approach. Egg numbers are adjusted to the number of samples collected that specific week.

	2017																				
	August					September				October					November				December			
Week nr.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Sleipner																						
M. merluccius					1																	
E. gurnardus					1																	
T. esmarkii																						
H. platessoides																						
M. aeglefinus																						
L. limanda																						
G. morhua																						
P. virens																						
M. merlangus																						
E. cimbrius																						
G. cynoglossus																						
M. molva																						
B. brosme																						
G. vulgaris																						
M. kitt																						
P. pollachius																						
S. scombrus																						

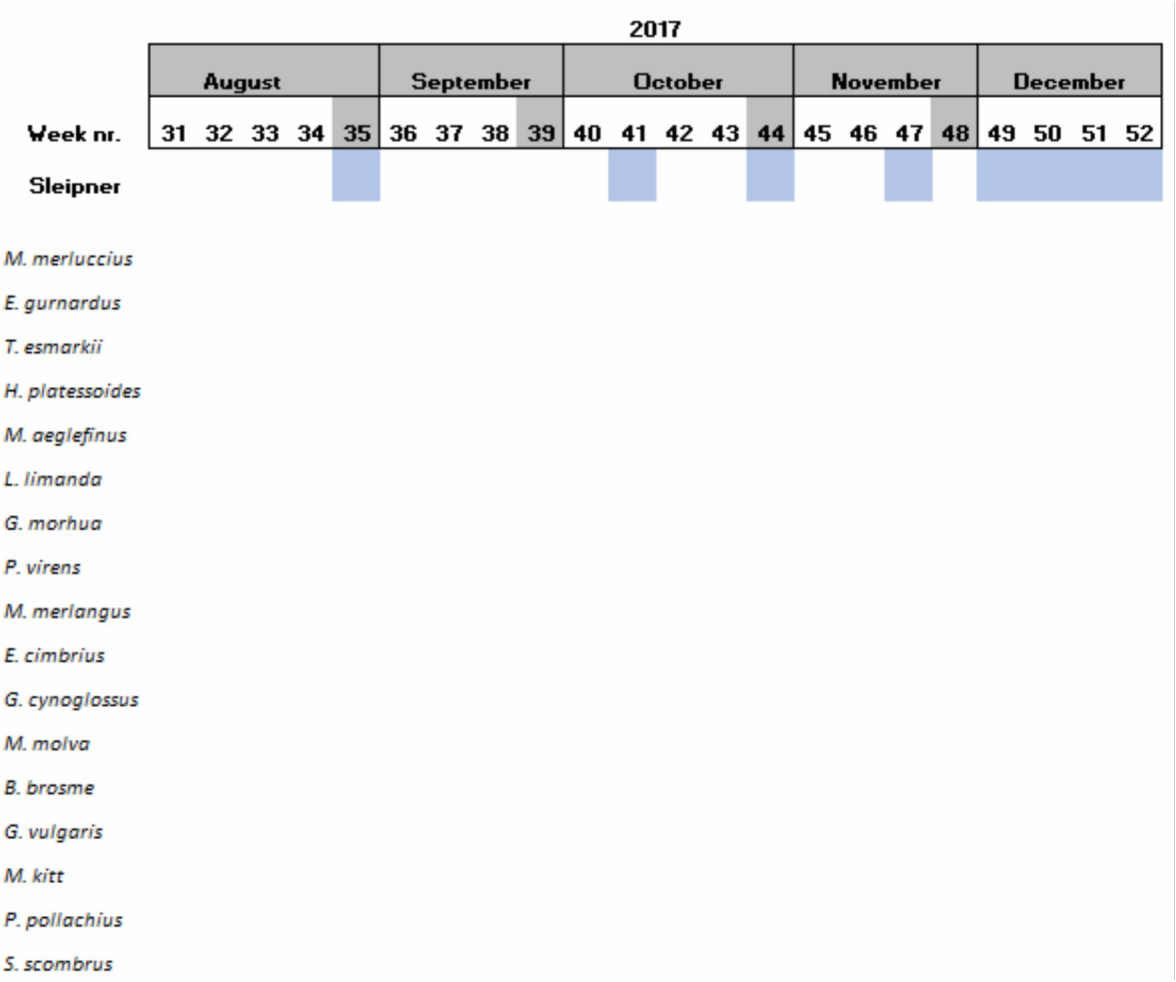

2018
Januar
Februar
Mars
April
May
Week nr.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Sleipner
2
M. merluccius
E. gurnardus
2 9 3 3 3 1.5
T. esmarkii
5 1
H. platessoides
2 4 13 9 3 1 1
M. aeglefinus
2 1 2 1 4
L. limanda
8 60 27 30 16
G. morhua
10 1
P. virens
1
M. merlangus
1 12 9 18 4 7 1
E. cimbrius
2 1 2 4
G. cynoglossus
2 2 1 3
M. molva
1 0.5
B. brosme
1 1
G. vulgaris
1
M. kitt
1
P. pollachius
2
S. scombrus
11 17 13

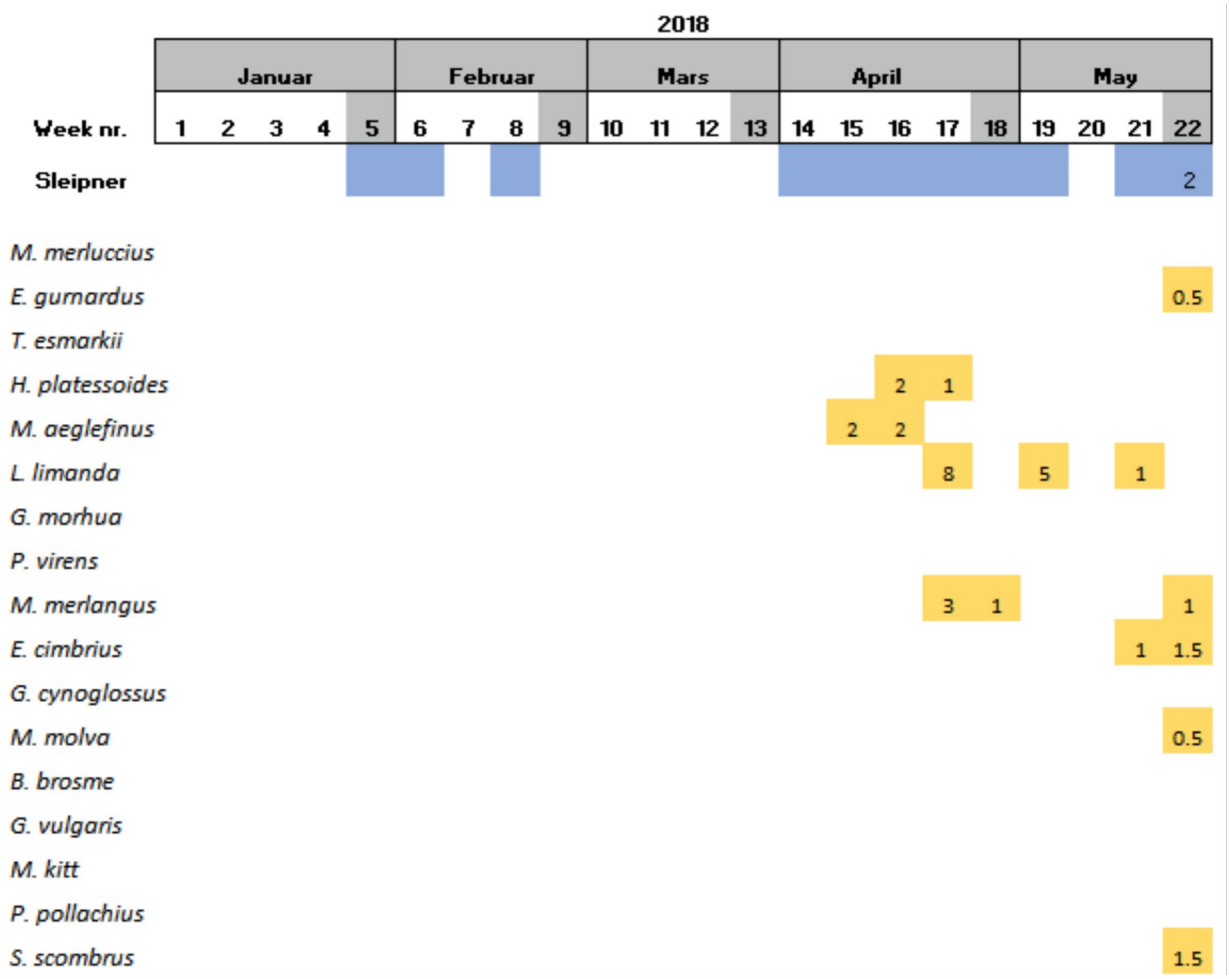
2018

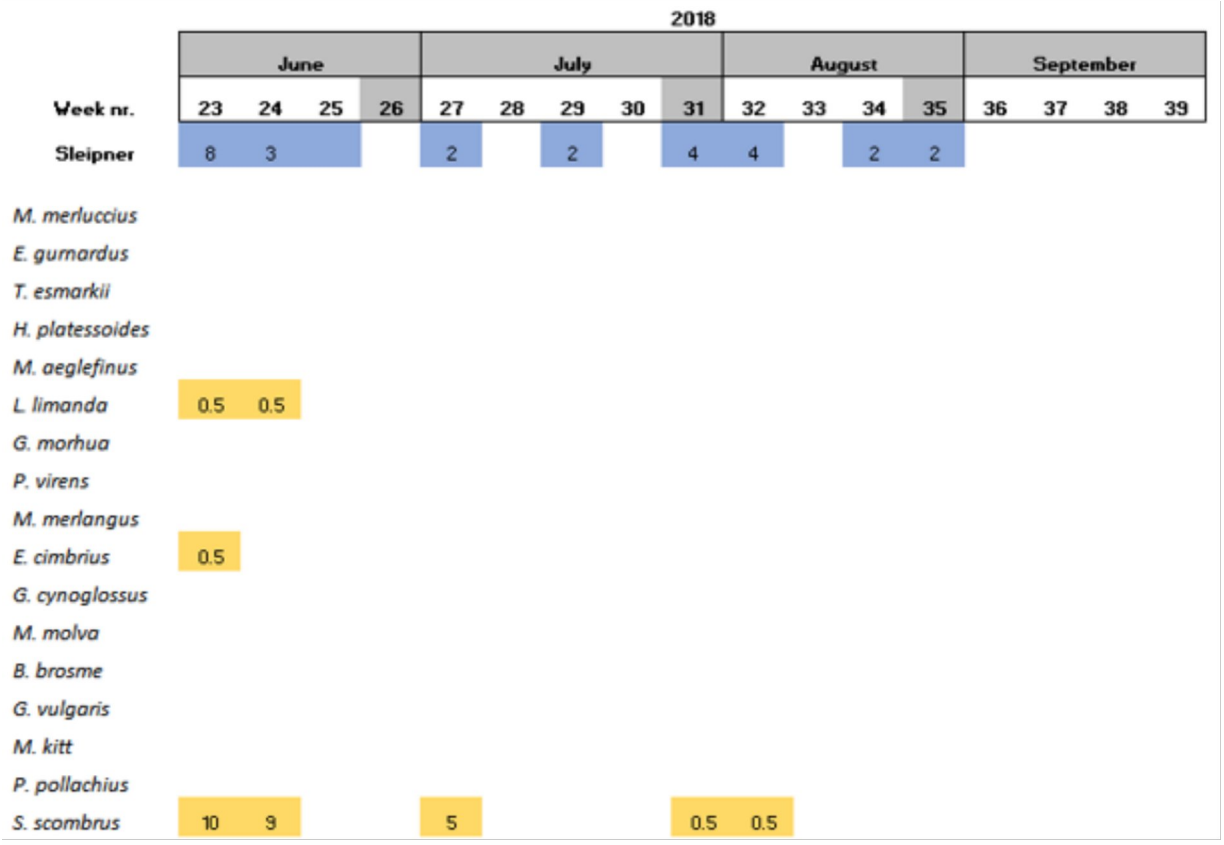
	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Sleipner	2	2					2	2	2	2		2	2	2	2		
M. merluccius		1.5		2	4		4.5	6.5	1.5	2		0.5	2.5	1	3.5		
E. gurnardus	1	1			2				0.5				0.5				
T. esmarkii																	
H. platessoides																	
M. aeglefinus																	
L. limanda	20	0.5		4	8	1	2		2	0.5		0.5					
G. morhua																	
P. virens																	
M. merlangus	1.5			1			0.5	1.5		0.5							
E. cimbrius		0.5				2											
G. cynoglossus		0.5		1	3		1	1.5				0.5					
M. molva		0.5					1										
B. brosme																	
G. vulgaris																	
M. kitt				1				1	1						0.5		
P. pollachius																	
S. scombrus	24	9.5		29	23	11	1										

3.7.2 - Larvae

Table 14. Fish larvae identified at Sleipner using the molecular approach. Larval numbers are adjusted to the number of samples collected that specific week.




2018
Januar
Februar
Mars
April
May
Week nr.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Sleipner
2
M. merluccius
E. gurnardus
0.5
T. esmarkii
H. platessoides
2 1
M. aeglefinus
2 2
L. limanda
8 5 1
G. morhua
P. virens
M. merlangus
3 1
1
E. cimbrius
1 1.5
G. cynoglossus
M. molva
0.5
B. brosme
G. vulgaris
M. kitt
P. pollachius
S. scombrus
1.5


2018
June
July
August
September
Week nr.
23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
Sleipner
8 3 2 2 4 4 2 2
M. merluccius
E. gurnardus
T. esmarkii
H. platessoides
M. aeglefinus
L. limanda
0.5 0.5
G. morhua
P. virens
M. merlangus
E. cimbrius
0.5
G. cynoglossus
M. molva
B. brosme
G. vulgaris
M. kitt
P. pollachius
S. scombrus
10 9 5 0.5 0.5

3.8 - Tampen

At the northernmost location, Tampen, samples were not collected in February and March. As the other two locations, there were no eggs or larvae in the samples collected at the end of the year, in this case between September and December. The most abundant species was also the Mackerel with only 17 individuals, followed by the Norway pout and Whiting with 15 and 14 individuals, respectively (Tables 15 and 16). All the Norway pout individuals at Tampen were all found as larvae (Table 16). However, results at this location should be treated carefully as they might be biased due to the low number of individuals. Therefore, more information is needed to provide a more accurate answer about the spawning time of the different species at this location.

3.8.1 - Eggs

Table 15. Fish eggs identified at Tampen using the molecular approach. Egg numbers are adjusted to the number of samples collected that specific week.

	2017																				
	August					September				October					November				December			
Week nr.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Tampen				2										2								
M. merluccius				0.5																		
M. kitt				0.5																		
P. virens																						
M. merlangus																						
M. molva																						
C. maculatus																						
H. platessoides																						
B. brosme																						
A. sphyraena																						
S. scombrus																						
E. gurnardus																						
C. linearis																						
T. esmarkii																						
P. norvegicus																						

2018

	Januar					Februar				Mars				April					May			
Week nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Tampen																			2			2
M. merluccius																						
M. kitt																						
P. virens			5																			
M. merlangus														2	1	6				3		
M. molva															1	4				4		
C. maculatus															1	1				1		
H. platessoides																1			0.5			
B. brosme																1						
A. sphyraena																1				1		
S. scombrus																			2	4		2
E. gurnardus																				1		
C. linearis																						
T. esmarkii																						
P. norvegicus																						

2018

	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Tampen				2	2	2	2	2				2	1	2			
M. merluccius																	
M. kitt					0.5												
P. virens																	
M. merlangus																	
M. molva																	
C. maculatus																	
H. platessoides																	
B. brosme																	
A. sphyraena																	
S. scombrus				1	0.5	1											
E. gurnardus																	
C. linearis																	
T. esmarkii																	
P. norvegicus																	

3.8.2 - Larvae

Table 16. Fish larvae identified at Tampen using the molecular approach. Larval numbers are adjusted to the number of samples collected that specific week.

2017

	August					September				October					November				December			
Week nr.	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Tampen				2										2								
M. merluccius																						
M. kitt																						
P. virens																						
M. merlangus																						
M. molva																						
C. maculatus																						
H. platessoides																						
B. brosme																						
A. sphyraena																						
S. scombrus																						
E. gurnardus																						
C. linearis				0.5																		
T. esmarkii																						
P. norvegicus																						

2018

	Januar					Februar				Mars				April					May			
Week nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Tampen																			2			2
M. merluccius																				1		
M. kitt																						
P. virens																						
M. merlangus																			0.5	1		
M. molva																2			0.5	1		
C. maculatus																1				3		
H. platessoides																1			1.5			
B. brosme																1						
A. sphyraena																						
S. scombrus																						
E. gurnardus																						
C. linearis																						
T. esmarkii																13				2		
P. norvegicus																1						

	2018															
	June				July					August				September			
Week nr.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Tampen				2	2	2	2	2				2	1	2			
M. merluccius																	
M. kitt																	
P. virens																	
M. merlangus																	
M. molva																	
C. maculatus																	
H. platessoides																	
B. brosme																	
A. sphyraena																	
S. scombrus																	
E. gurnardus																	
C. linearis																	
T. esmarkii																	
P. norvegicus																	

DISCUSSION

The use of the two different approaches for ichthyoplankton identification allowed for inter-comparisons of their applicability. Overall, it was observed that the use of the molecular taxonomic method allowed for reaching a more detailed taxonomic level vs. the visual taxonomic method. For larvae identification, more than 98% of the larvae was assigned to species level using the molecular approach, while using the taxonomic method 89% of the larvae was assigned to species level. However, the greatest difference between both methods was observed in the identification of eggs. With the molecular taxonomic approach, between 80-90% of the eggs could be assigned to species level, whilst we have encountered difficulties to assign them to species level using the visual taxonomic method. In the visual taxonomic method, several of the classifying parameters, such as egg size or presence and number of oil globules overlaps between different species or even families (Markle et al., 1984). Furthermore, some of the embryonic characters, such as pigmentations on the embryo, are only applicable in late stages of the egg development.

Although we could not establish any definitive reason regarding the eggs and larvae that were not assigned to fish species using the molecular approach, we suggested that the method did not work probably due to: i) the eggs or larvae were damaged; ii) the eggs or larvae were not properly stored (e.g. if the sample contained some sea water with the ethanol); iii) an early stage of the eggs that did not contain sufficient amount of DNA; or iv) the DNA extraction or the PCR amplification did not work.

Furthermore, the molecular taxonomic identification used less time than the visual taxonomic approach, as it allows to work with several different individuals simultaneously. However, there are also some cons with the molecular approach as, unlike the taxonomic approach, the molecular method does not allow to stage the eggs and it is highly dependent on the information available in the databases, although the latter has not been a problem so far for the fish species present in the North Sea. With all the information, we have indications of that the molecular method is the most suited method for ichthyoplankton identification except when the eggs development stage is required.

Even though the number of eggs and larvae obtained for the taxonomic and molecular approaches were similar, 1480 and 1493 respectively, there was a high variation in the samples uptake as 16 more samples were collected at Ekofisk than at Tampen. However, the increased number of samples was not indicative of a higher number of individuals. The highest number of individuals for both the taxonomic and molecular analyses were found in the 43 samples collected at Sleipner. From June 2018 two samples were collected per week at the three different locations. That is the reason for the increased number of samples between June and September with respect to the other periods. It is also noteworthy to mention the absence of eggs or larvae in the last three months in the three locations despite the 20 samples collected. Although this result suggests that there is no fish spawning time between October and December, it contradicts previous studies where winter spawning time has been observed for some of the fish species in the North Sea (Lelievre et al., 2014).

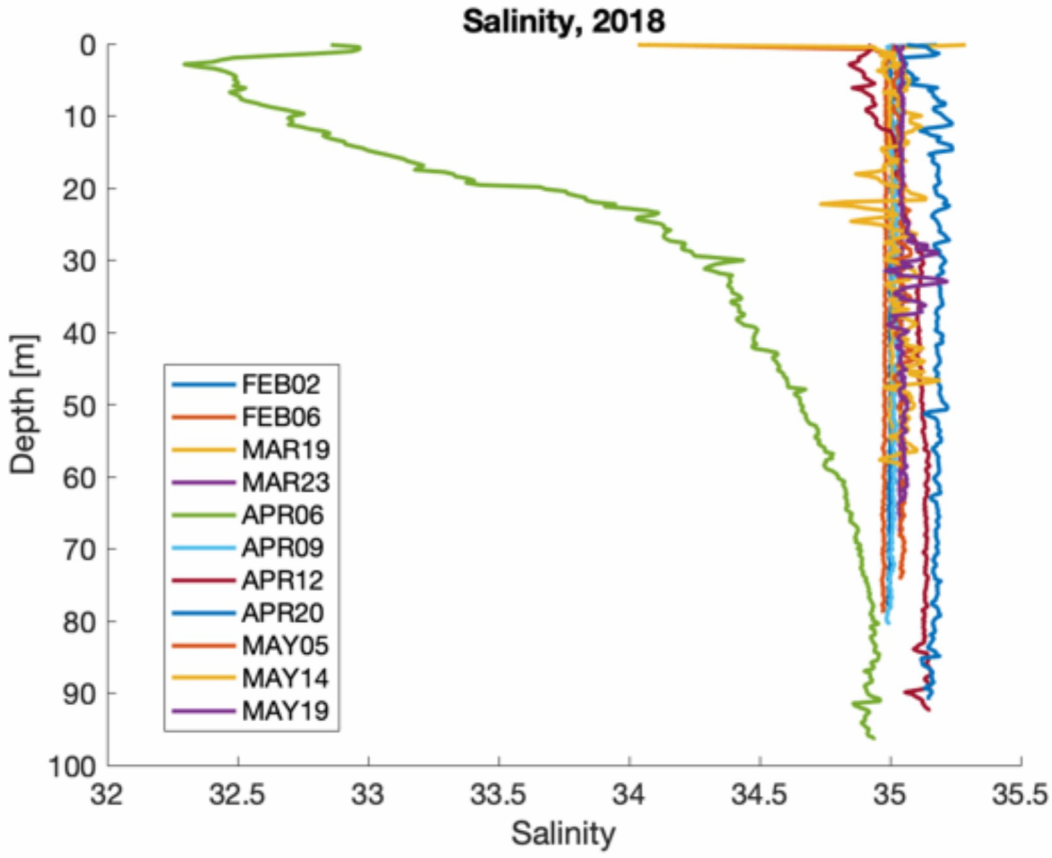
Most of the 22 species identified in this study were also identified in the previous project, “KINO-1” (Sundby et al., 2017). For some of these species, such as Dab, Mackerel or Whiting among others, we

can have a good idea about the spawning time across the year at the locations of Ekofisk and Sleipner. However, there are other species which only very few individuals were found and in most of the cases these individuals were found only during a single month. Additional data about these species is needed in order to determine its spawning time during the annual cycle. Furthermore, the reduced amount of eggs and larvae obtained in the samples collected at Tampen, did not allow us to draw any conclusions about the spawning time of the fish species at this location.

CHALLENGES

During the start period of the project, samples were collected once per sampling site per week, however it was observed that the amount of eggs and larvae in the samples was low. Consequently, in June 2018, it was decided that the samples should be collected twice per week per sampling site. The results showed that the numbers of eggs and larvae increased slightly, although still low in numbers. Furthermore, there have also been periods were the vessels have not been able to perform sampling due to i) bad weather conditions, ii) technical problems and/or iii) vessel location was outside of the 5 nautical miles from station mid-point required for this study.

In addition, we have experienced some issues with the CTD data collection. From the 129 samples collected so far, 100 are either data-empty or just contain one measurement point during the depth profile. Only 3 hauls contained complete CTD logging including GPS locations. The reason for this missing data is most likely due to deviations from the sampling procedure, as the CTD unit needs to receive proper satellite fix which takes several minutes after it has been switched on before deployment into water, it also needs to stay in the surface waters for some minutes before the descend (Figure 7).



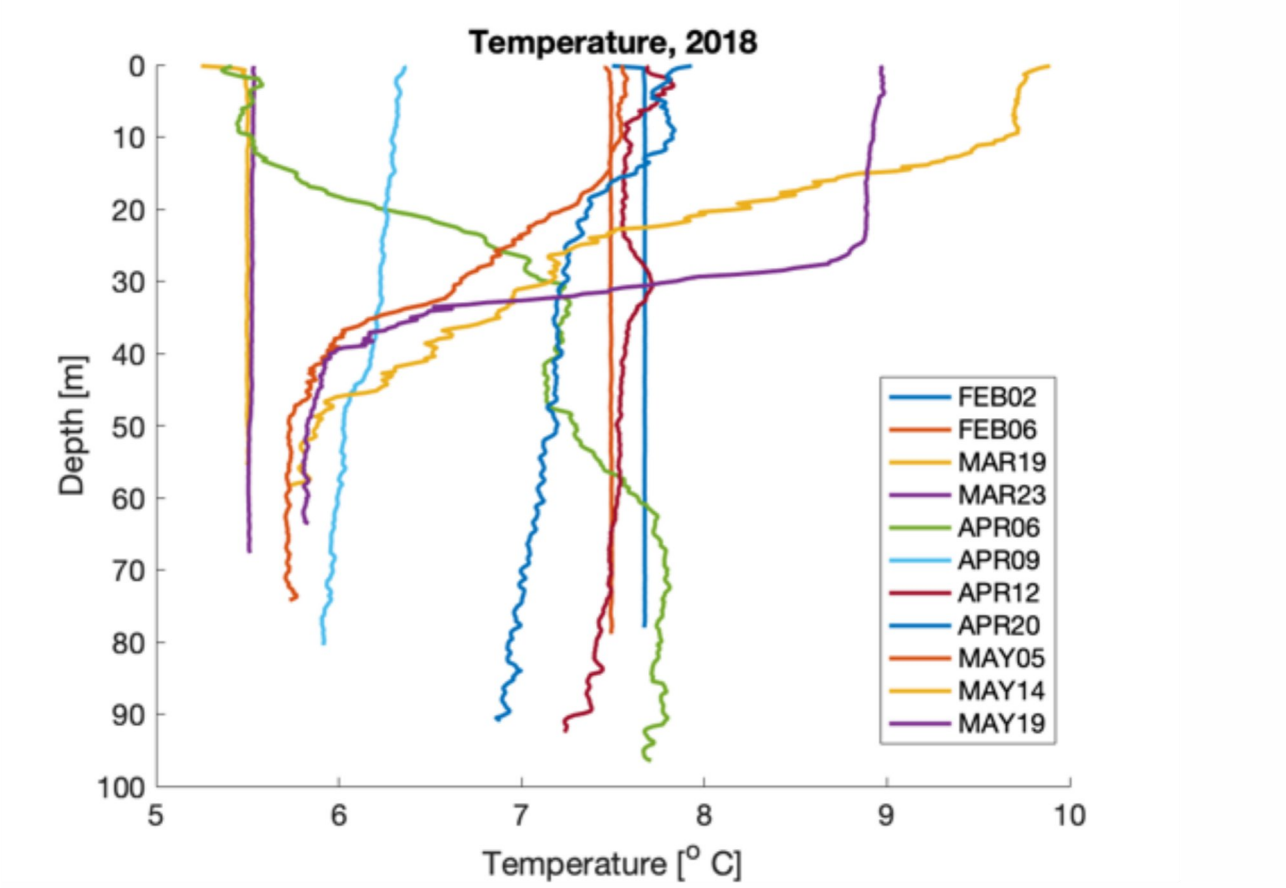


Figure 7. Results from the CTD data collected.

The challenges mentioned above indicate that communication with the crew on the vessels can be improved and further optimized. Although the vessels have signalled that there are no problems regarding sampling procedure, the low numbers of eggs and larvae and, especially, the issues regarding the CTD measurements may indicate that the sampling procedure are not followed and that repeated training courses should be organized. This can also be applied for the sample preservation and labelling of sample bottles, which also vary in quality on the received samples. To improve the procedures, we will prepare pre-filled labels for the sampling bottles and produce some simplified manuals to be hung on the walls onboard the vessels.

Plotting of the position for the stations shows that they occur within 10 nautical miles from each other at each sampling site. We believed the vessels were stationary at a position when design of method was made. Due to complex bathymetric and oceanographic conditions, especially for the Tampen area, we recommend that sampling must occur within 5 nautical miles distance around the centre position for all future sampling.

To improve the quality of the sampling, it is a strong wish that Equinor personnel are given the possibility to allocate time to meet for training and to discuss with IMR personnel how these issues can be solved and improved. Equinor have stated that they will address this internally first and return with a suggested procedure for how this best can be done.

FUTURE PERSPECTIVES

Traditional splitting of samples using a “Motoda-plankton splitter” has proven not to be so straight forward to use for non-professionals, which is the case for the crew conducting the practical sampling onboard the respective supply vessels.

To substitute the traditional splitting of samples we will initiate the use of a double WP2 net (Figure 8), instead of the single WP2 net used at present. This net-configuration will increase the amount of eggs and larvae obtained per haul and most importantly; increase the accuracy for statistical comparative analyses of the results from the two taxonomic identification techniques.

Figure 8. Set up of double-net configuration (in the picture a combination of a Juday and a WP2 net).

Regular surveys conducted by IMR visit the sampling areas of Ekofisk, Sleipner and Tampen give us the opportunity to include data collected during these surveys in 2019 and 2020 to optimize the dataset and results. We aim to coordinate such effort with the responsible IMR personnel.

As the samples from Tampen indicate, that relatively small quanta eggs and larvae are to be found in this area, we must assess whether there is a need to change the sampling location to better represent the reproductive activities in the area. A potential change of location must happen after a dialog with Equinor to discuss its practical feasibility regarding the acceptable range of movement by the supply vessels.

A second vessel for use at Tampen is currently prepared with equipment to increase the sampling activity at this area in cases when the originally designated vessel does not have the opportunity to conduct sampling (e.g. when vessel is on assignment elsewhere).

We also plan to explore other ways to improve the methodology. One test will be to use 1% formaldehyde to preserve the samples, as suggested by Lelievre et al. (2010). If successful, this method

will allow to perform both molecular and taxonomic analyses using the same eggs. In addition, we currently also test the potential of using fluorescence to identify fish species. Although this technique has never been applied on fish eggs before, will be tested during the next part of the project. In fact, we have so far tested 9 different eggs that belonged to three different species (*G. cynoglossus*, *M. kitt* and *M. merluccius*) and displayed 3 different fluorescent patterns (Figure 9). Although those results are promising, further work including more species need to be performed.

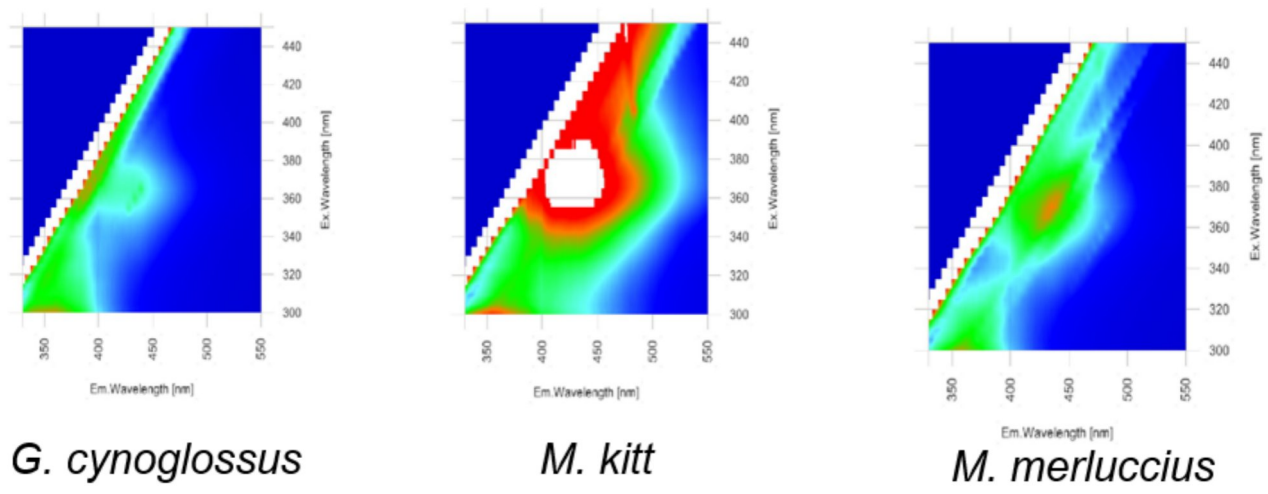


Figure 9. Fluorescent patterns of Witch, Lemon sole and European hake.

WORKSHOP/CONFERENCES PRESENTATIONS

Mateos-Rivera A, Mozfar B, Skern-Mauritzen R, Dahle G, Thorsen A, Sundby S, Glover K, Kleppe L, Wehde H, Krafft BA (2018) Assessment of the spawning time for the major North Sea fish stocks. ICES Working Group on Atlantic Larvae and Egg Survey (WGALES) - Technical University of Denmark, Copenhagen, October 2018. (Oral presentation)

Mateos-Rivera A, Mozfar B, Skern-Mauritzen R, Dahle G, Thorsen A, Sundby S, Glover K, Kleppe L, Wehde H, Krafft BA (2018) Assessment of the spawning time for the major North Sea fish stocks. Northern North Sea Planktonic Ecosystem Working Group (NNSPEWG). Institute of Marine Research, Bergen, October 2018. (Oral presentation)

ACKNOWLEDGEMENTS

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REFERENCES

Ahlstrom H, Geoffrey H (1980) Characters useful in identification of pelagic marine fish eggs. *CalCOFI Rep.* XXI.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* **215**: 403-410.

Gjøsæter H, Dalpadado P, Hassel A, Skjoldal HR (2000) A comparison of performance of WP2 and MOCNESS. *Journal of Plankton Research* **22**: 1901-1908.

Hassel A, Endresen B, Martinussen MB, Knutsen T, Johanessen ME (2017) Håndbok for plankton. Prøvetaking og analyse. Havforskningsinstituttet.

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes* **7**: 544-548.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, *et al*. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647-1649.

Lelievre S, Vaz S, Martin CS, Loots C (2014) Delineating recurrent fish spawning habitats in the North Sea. *Journal of Sea Research* **91**: 1-14.

Lelievre S, Verrez-Bagins V, Jerome M, Vaz S (2010) PCR-RFLP analyses of formalin-fixed fish eggs for the mapping of spawning areas in the Eastern Channel and Southern North Sea. *Journal of Plankton Research* **32**: 1527-1539.

Markle DF, Frost LA (1985) Comparative morphology seasonality, and a key to planktonic fish eggs from the Nova Scotian shelf. *Canadian Journal of Zoology* **63**: 246-257.

Messing J (1983) New M13 vectors for cloning. *Methods in Enzymology* **101**: 20-78.

Munk PN, Jørgen G (2005) Eggs and larvae of North Sea fishes. Frederiksberg, Denmark biofolia.

Russell FS (1976) The eggs and planktonic stages of British marine fishes. London Academic Press.

Sundby S, Kristiansen T, Nash RDM, Johannessen T (2017) Dynamic Mapping of North Sea Spawning: report of the “KINO” project. *Fisken og Havet* **2**: 183.

Thompson BM, Riley JD (1981) Egg and larval development studies in the North Sea cod (*Gadus morhua* L.). *Rapports et Procès-verbaux des Réunions Conseil International pour l'Exploration de la Mer* **178**: 553-559

APPENDIX

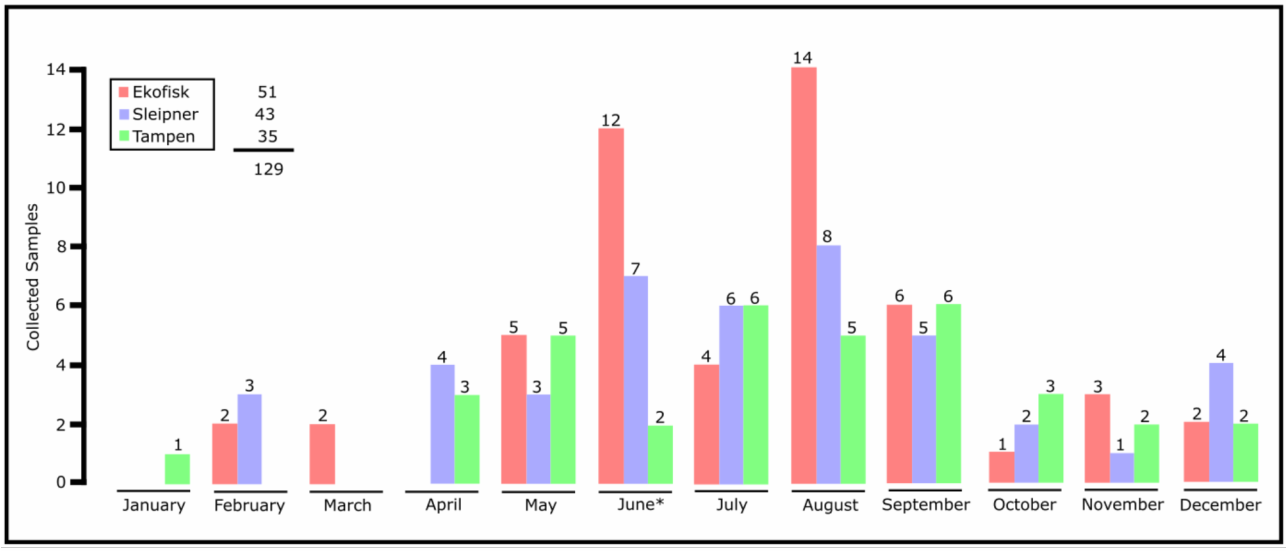


Figure A1. Distribution of the samples collected each month per sampling site throughout the year.

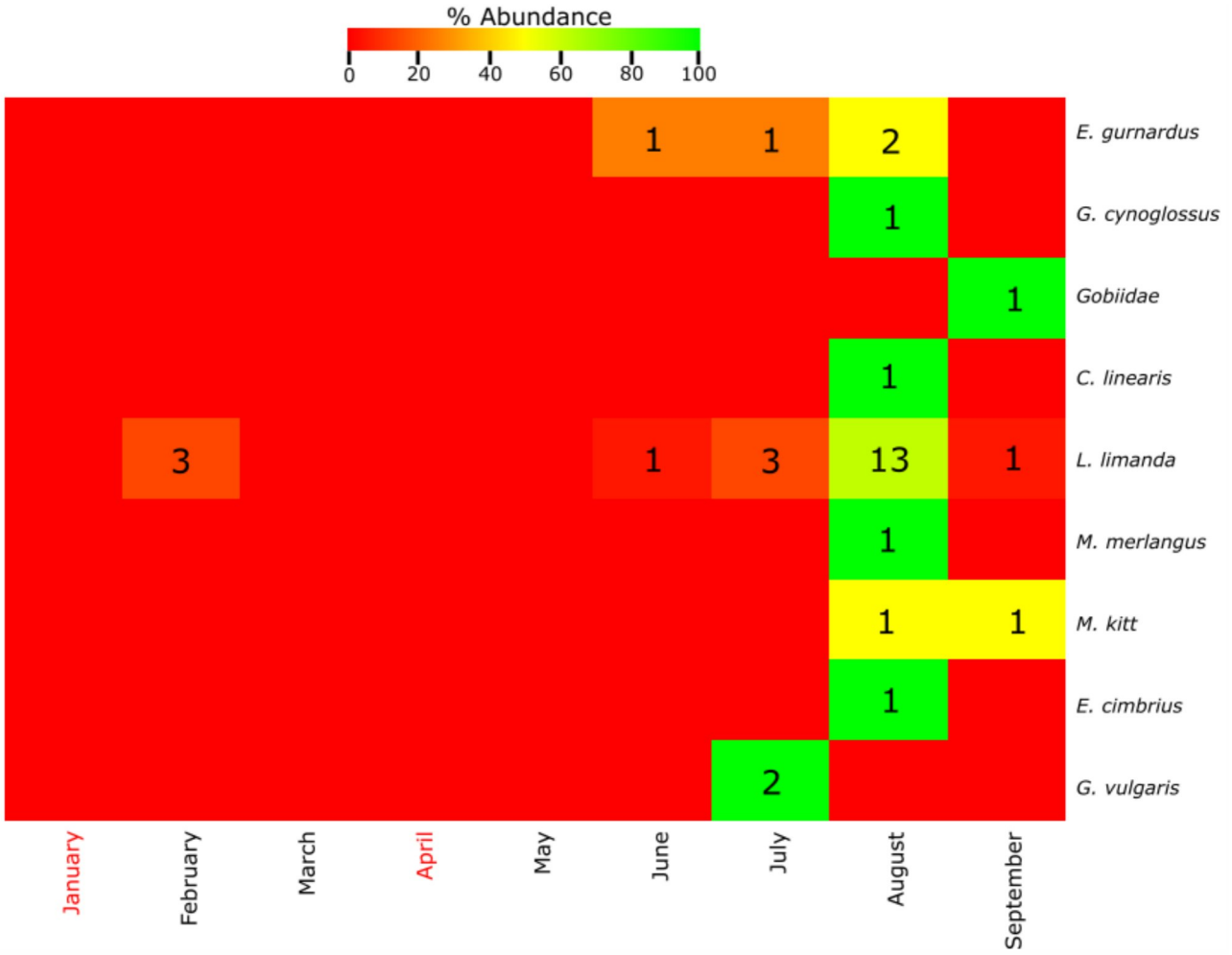


Figure A2. Heatmap of the % abundance of the fish larvae found at Ekofisk from the taxonomic approach. The total number of individuals from each species per month of the year is indicated with a number within the figure. Months with no samples collected are indicated with red color. Months between October and December are not included due to the absence of larvae in the samples.

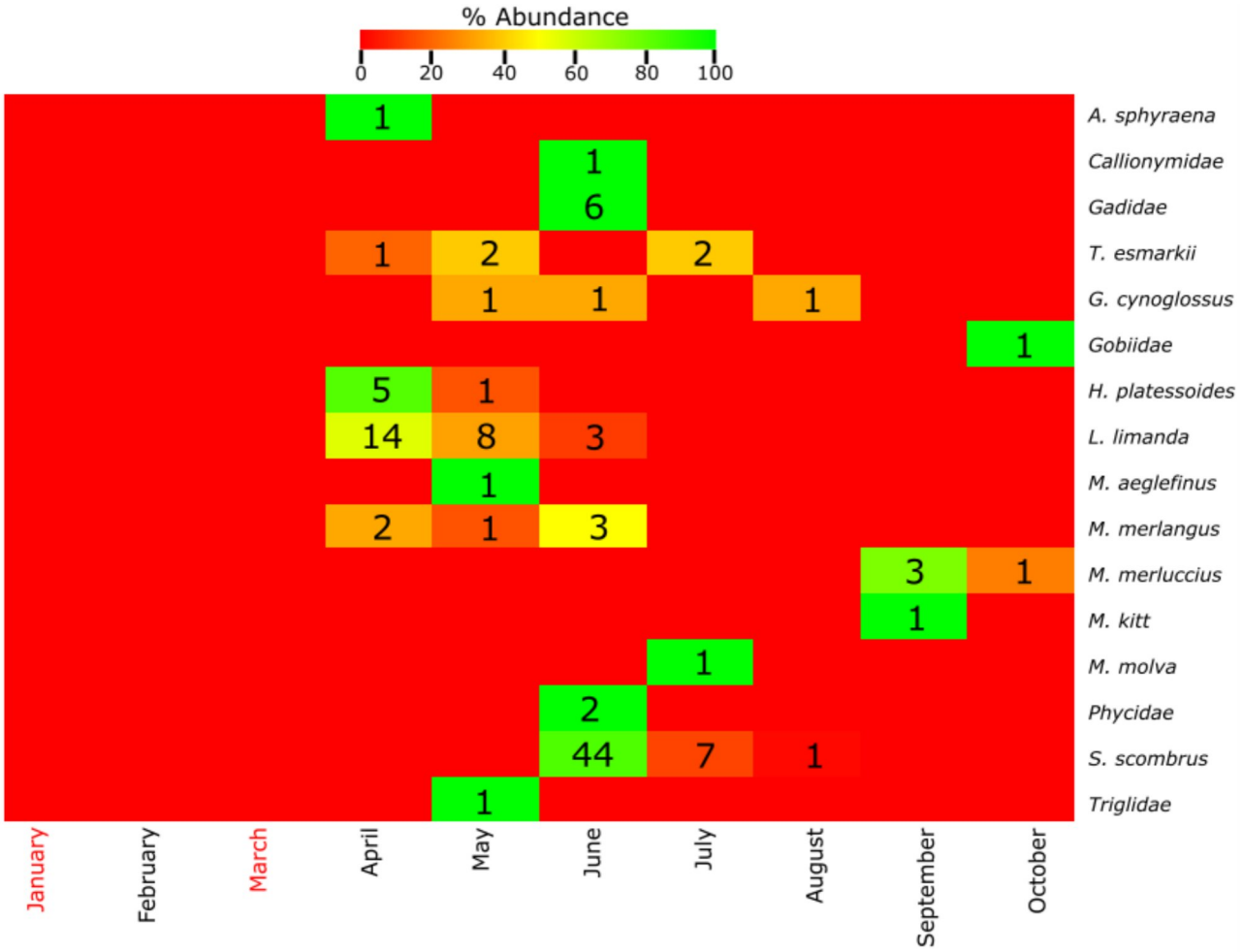


Figure A3. Heatmap of the % abundance of the fish larvae found at Sleipner from the taxonomic approach. The total number of individuals from each species per month of the year is indicated with a number within the figure. Months with no samples collected are indicated with red color. Months between October and December are not included due to the absence of larvae in the samples.

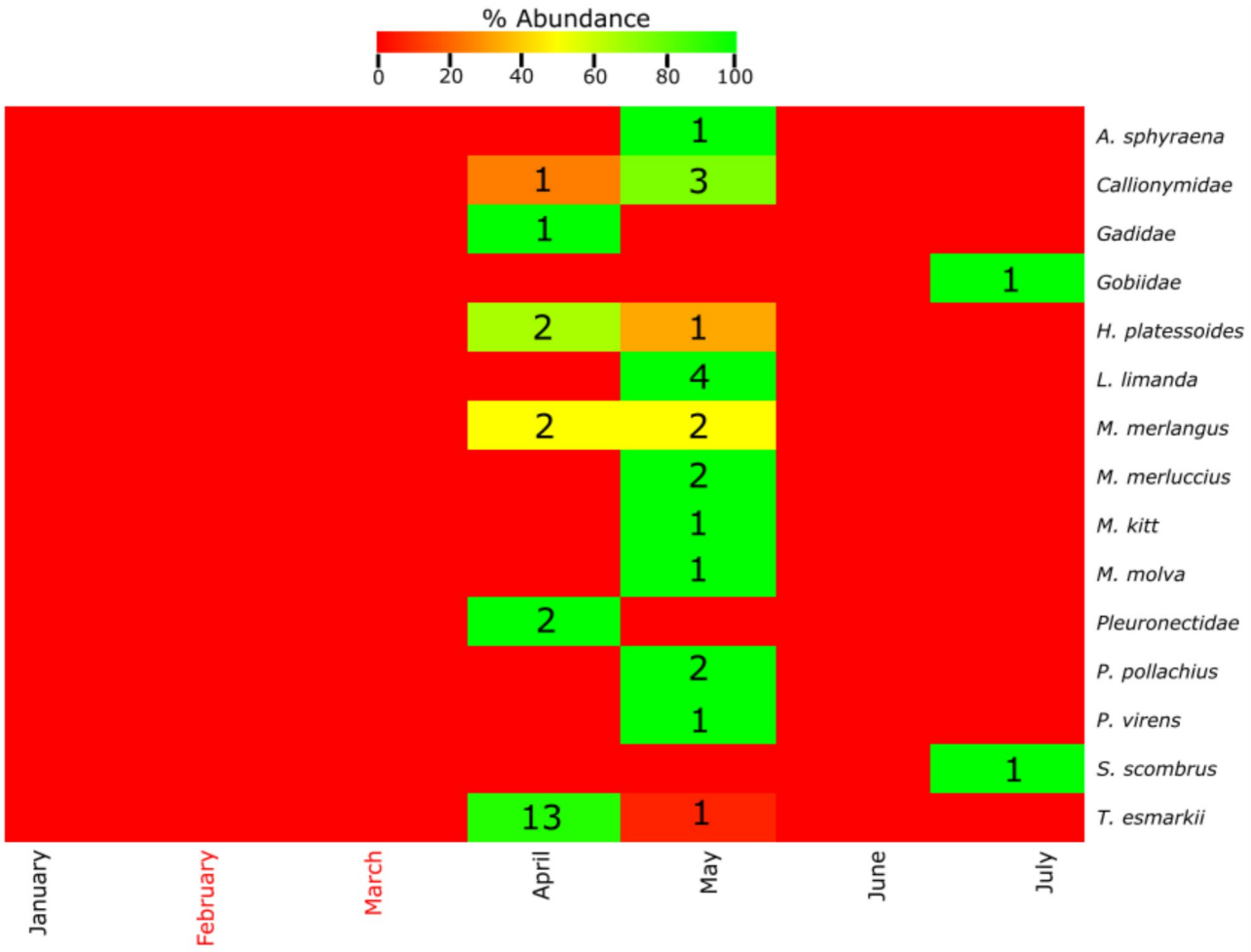


Figure A4. Heatmap of the % abundance of the fish larvae found at Tampen from the taxonomic approach. The total number of individuals from each species per month of the year is indicated with a number within the figure. Months with no samples collected are indicated with red color. Months between October and December are not included due to the absence of larvae in the samples.

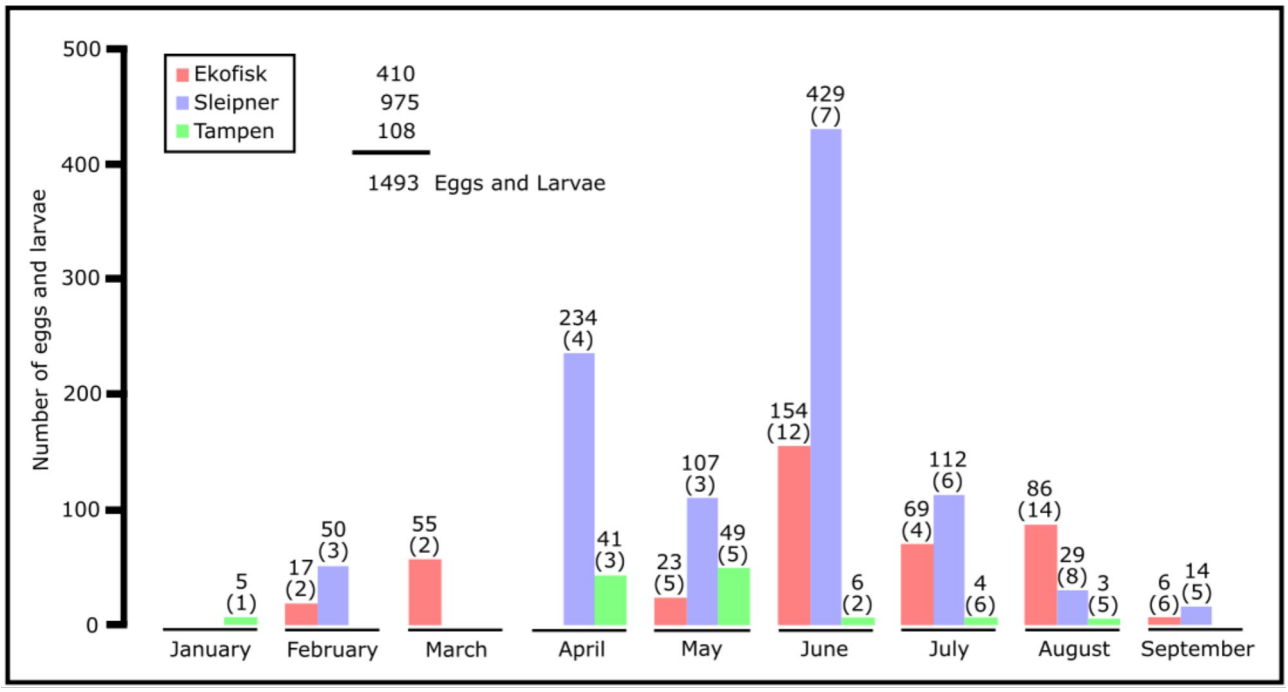
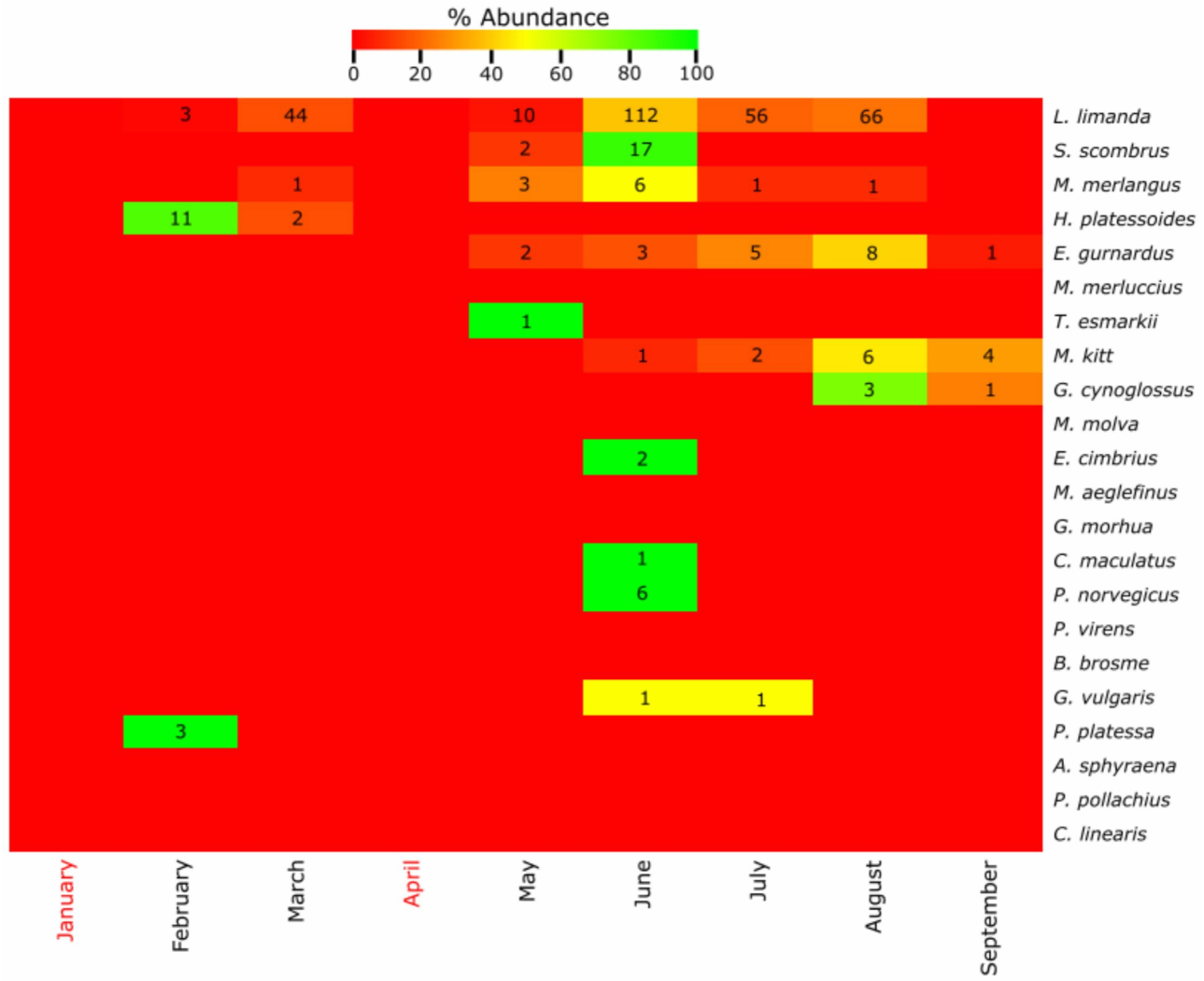


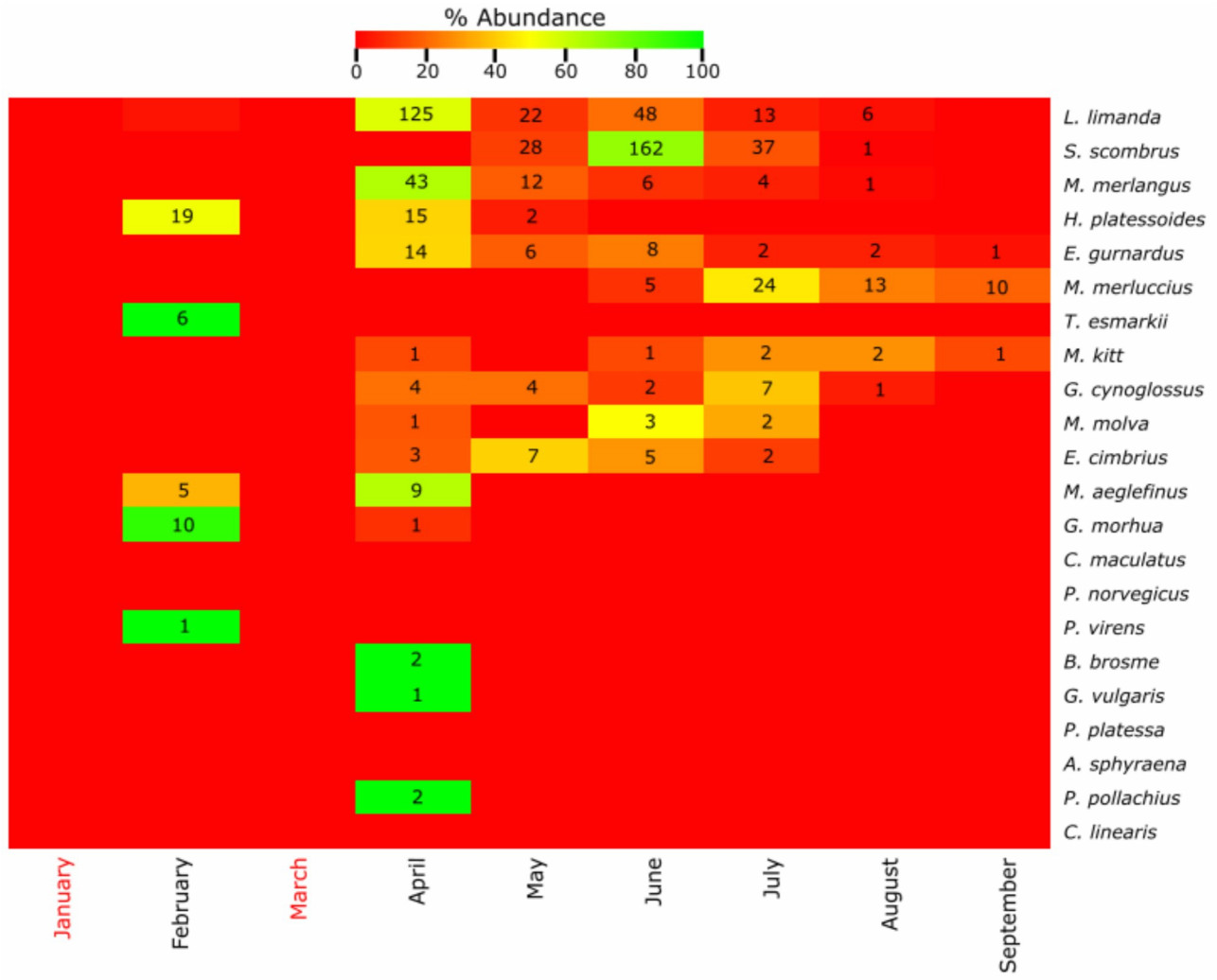
Figure A5. Distribution of the total number of eggs and larvae obtained each month per sampling site throughout the year used for the molecular analyses. The number of samples collected is indicated within parenthesis. Months between October and December are not included due to the absence of eggs and larvae in the samples.



L. limanda	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
S. scombrus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. merlangus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
H. platessoides	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
E. gurnardus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. merluccius	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
T. esmarkii	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. kitt	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP

G. cynoglossus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. molva	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
E. cimbrius	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. aeglefinus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
G. morhua	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
C. maculatus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. norvegicus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. virens	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
B. brosme	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
G. vulgaris	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. platessa	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
A. sphyraena	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. pollachius	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
C. linearis	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP

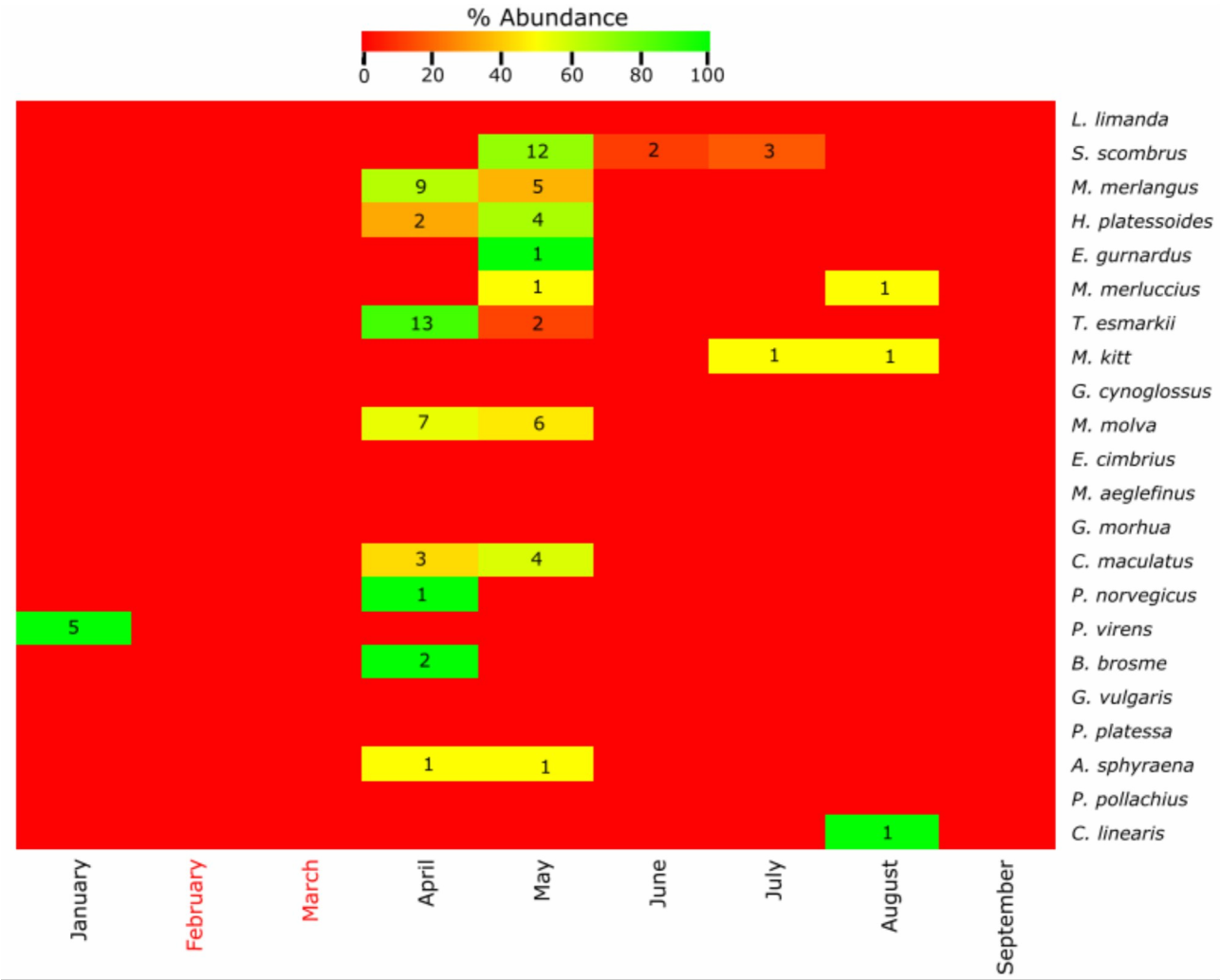
Figure A6. Top: Heatmap of the % abundance of the fish species found at Ekofisk using the molecular approach. The total number of individuals from each species per month of the year is indicated with a number within the figure. Months with no samples collected are indicated with red color. Months between October and December are not included due to the absence of eggs and larvae in the samples. Bottom: Table of the presence (yellow) or absence (white) of the fish species. Months where samples have not been collected are indicated with grey color while red color indicates fish species not found at this location.



L. limanda	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
S. scombrus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. merlangus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
H. platessoides	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
E. gurnardus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. merluccius	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
T. esmarkii	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. kitt	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
G. cynoglossus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP

M. molva	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
E. cimbrius	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. aeglefinus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
G. morhua	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
C. maculatus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. norvegicus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. virens	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
B. brosme	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
G. vulgaris	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. platessa	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
A. sphyraena	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. pollachius	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
C. linearis	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP

Figure A7. Top: Heatmap of the % abundance of the fish species found at Sleipner using the molecular approach. The total number of individuals from each species per month of the year is indicated with a number within the figure. Months with no samples collected are indicated with red color. Months between October and December are not included due to the absence of eggs and larvae in the samples. Bottom: Table of the presence (yellow) or absence (white) of the fish species. Months where samples have not been collected are indicated with grey color while red color indicates fish species not found at this location.



L. limanda	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
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M. merlangus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
H. platessoides	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
E. gurnardus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. merluccius	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
T. esmarkii	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. kitt	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
G. cynoglossus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP

M. molva	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
E. cimbrius	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
M. aeglefinus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
G. morhua	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
C. maculatus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. norvegicus	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. virens	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
B. brosme	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
G. vulgaris	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. platessa	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
A. sphyraena	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
P. pollachius	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
C. linearis	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP

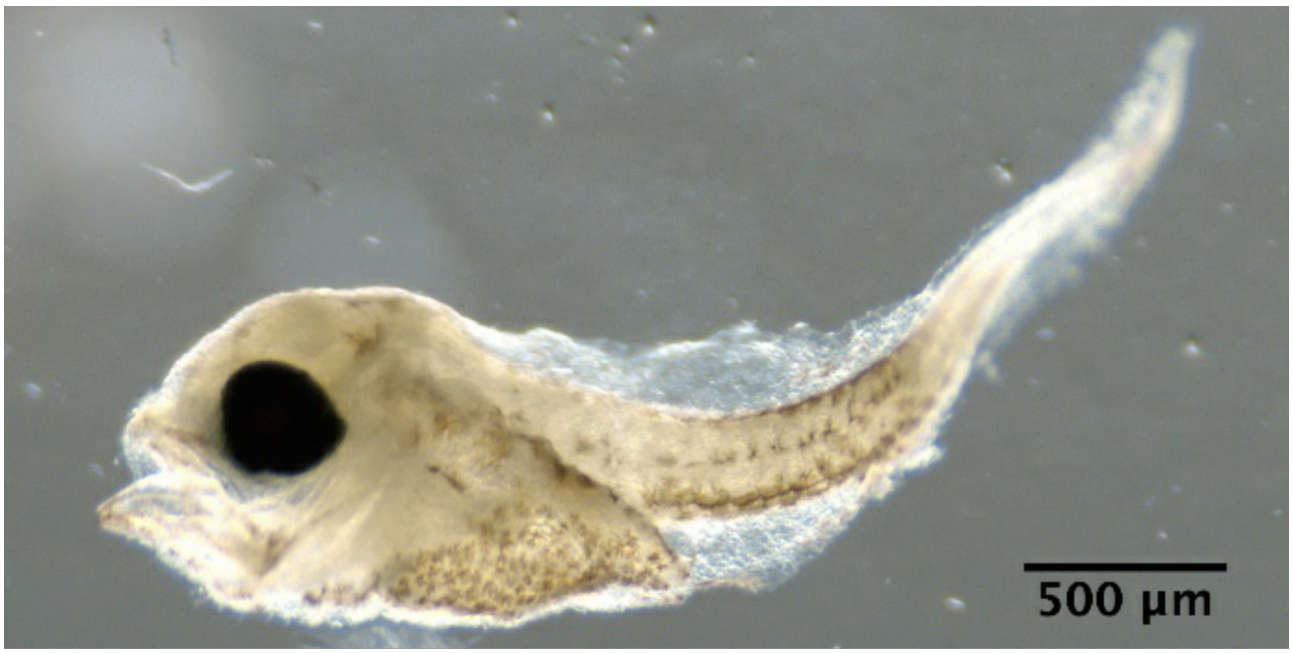
Figure A8. Top: Heatmap of the % abundance of the fish species found at Tampen using the molecular approach. The total number of individuals from each species per month of the year is indicated with a number within the figure. Months with no samples collected are indicated with red color. Months between October and December are not included due to the absence of eggs and larvae in the samples. Bottom: Table of the presence (yellow) or absence (white) of the fish species. Months where samples have not been collected are indicated with grey color while red color indicates fish species not found at this location.

Illustrations of fish larvae identified from using the visual taxonomic method

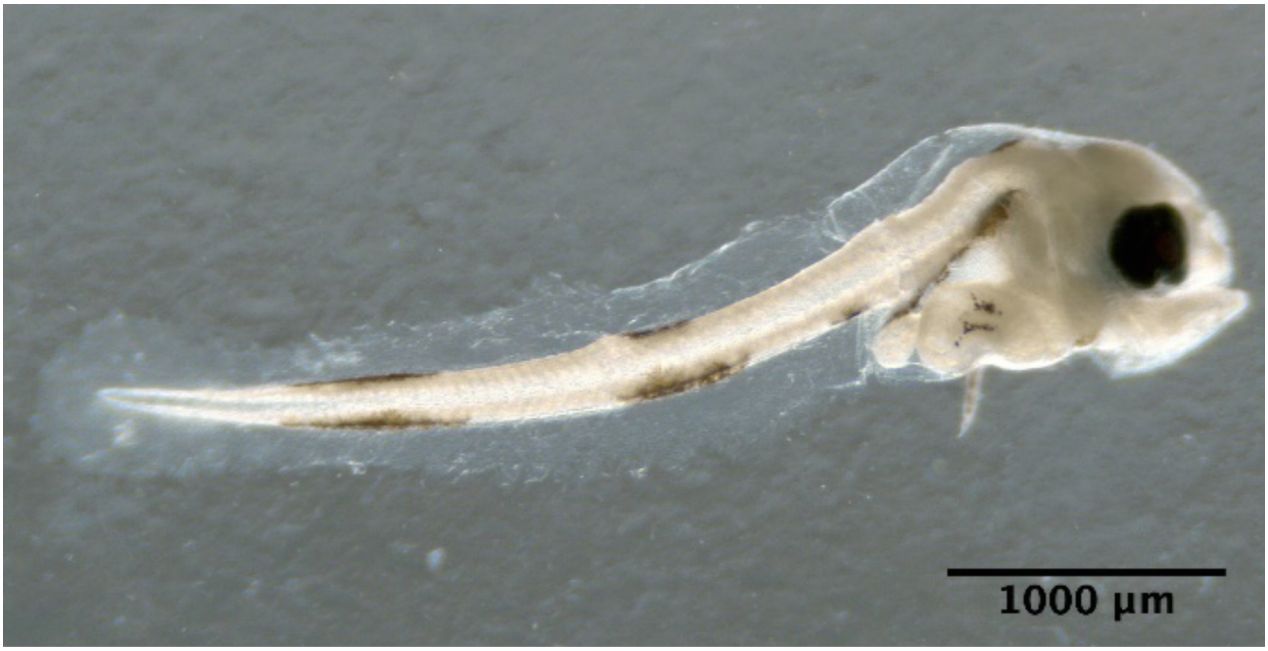
Argentinidae

Argentina sphyraena

Callionymidae

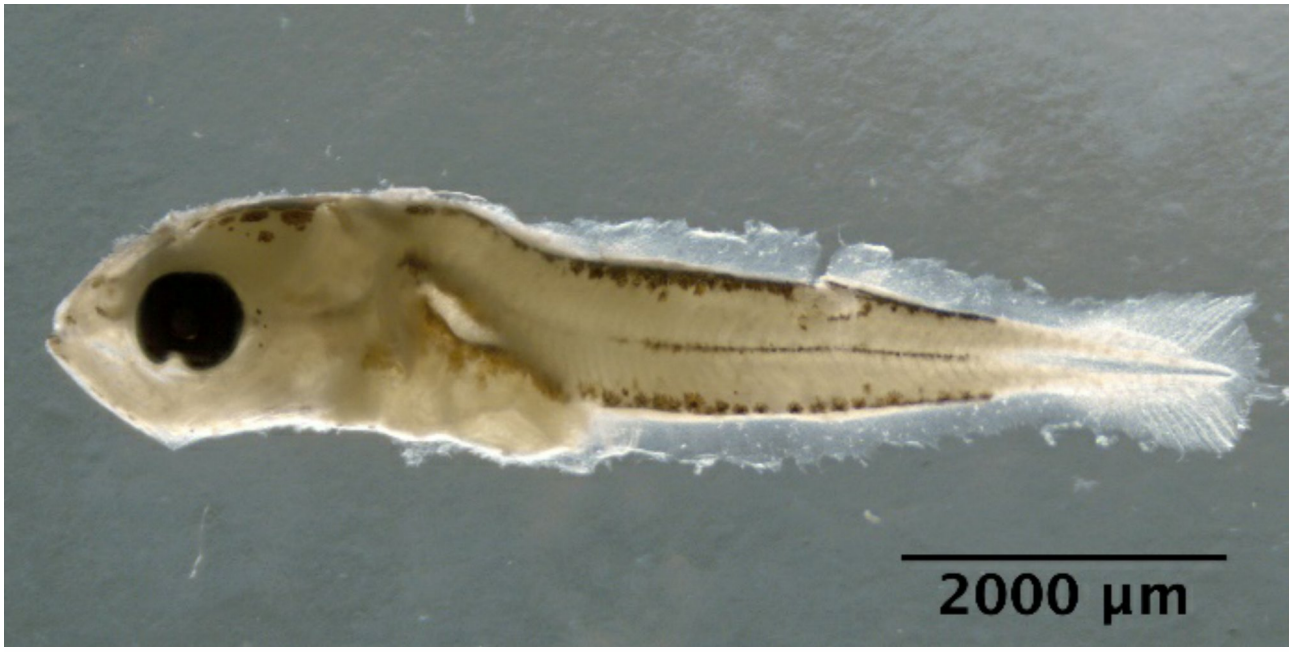


Gadidae



Pollachius virens

Trisopterus esmarkii



Pollachius pollachius

Melanogrammus aeglefinus

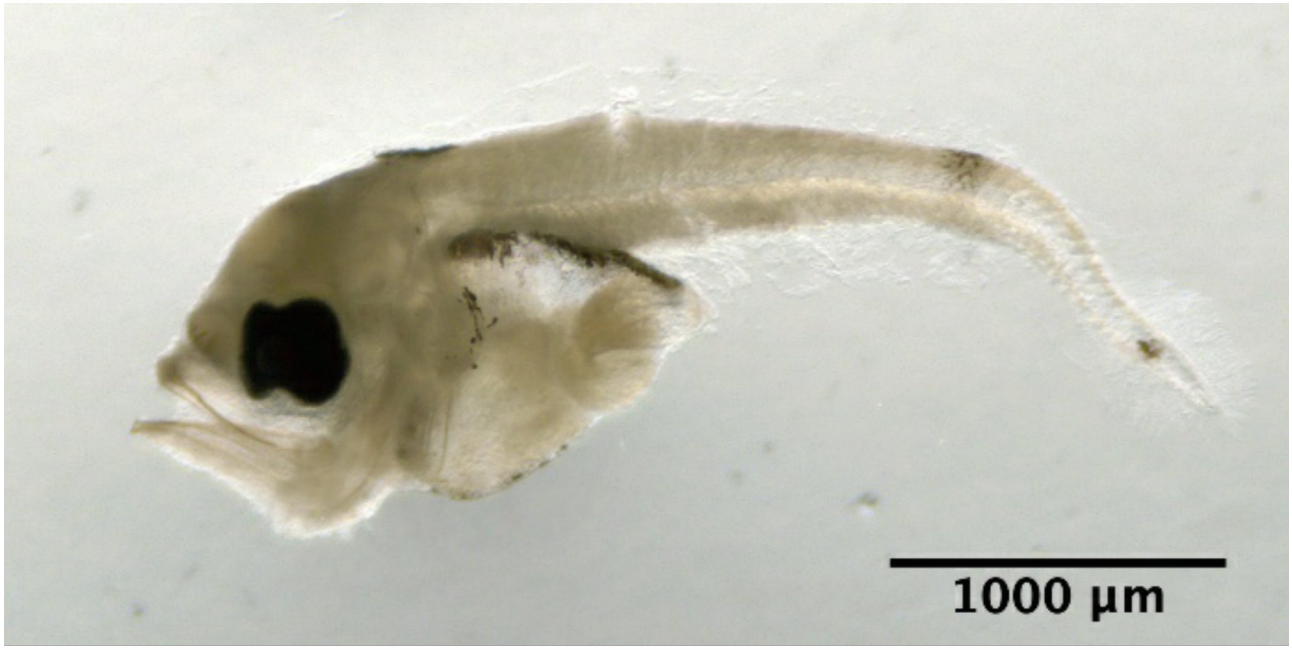
Merlangius merlangus

Molva molva

Gobiidae

Crystallogobius linearis

Merluccidae

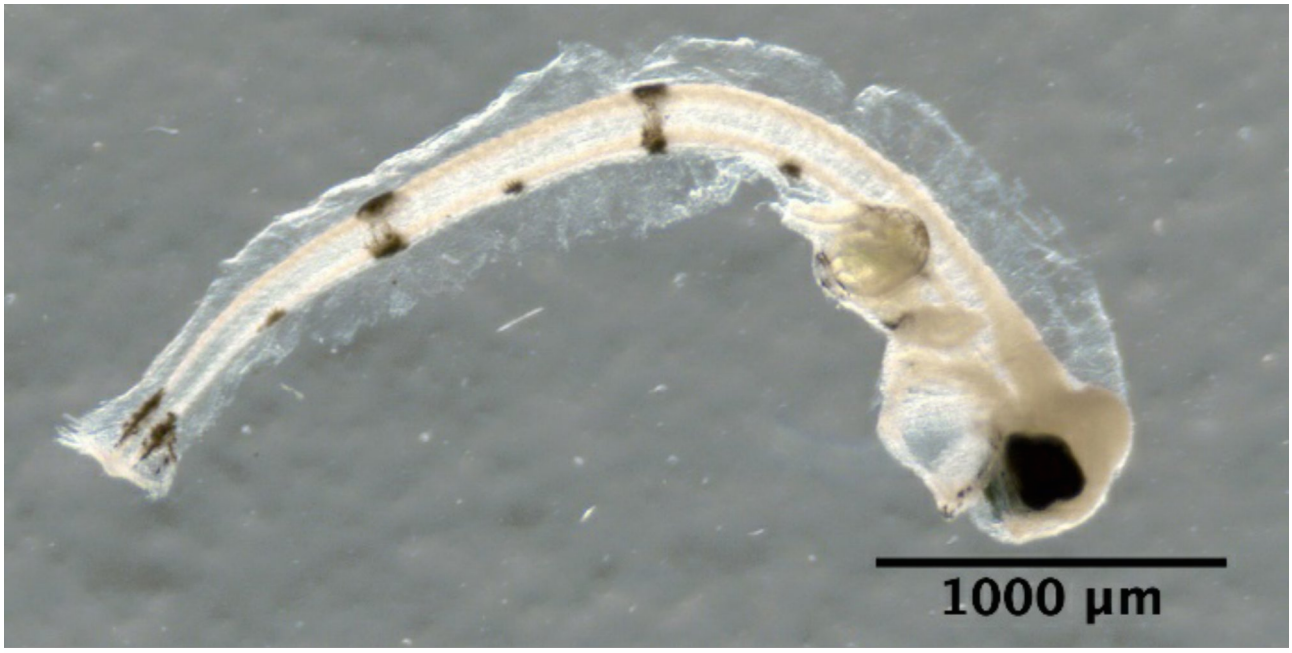


Merluccius merluccius

Phycidae

Enchelyopus cimbrius

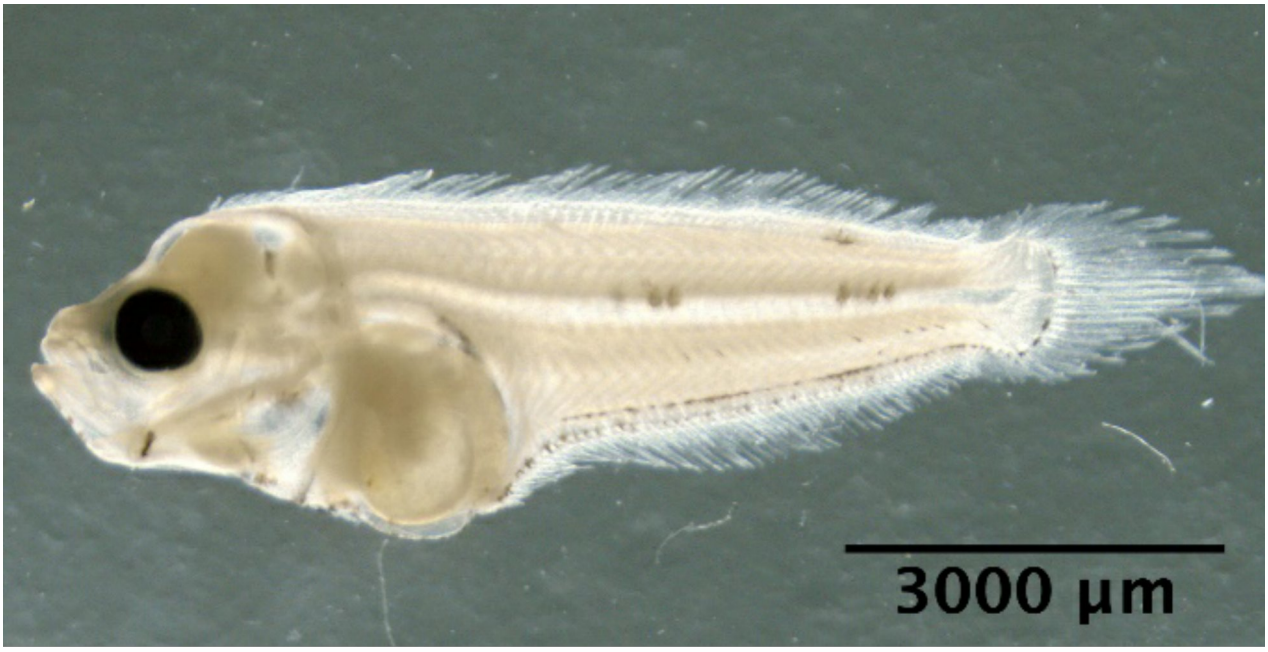
Pleuronectidae



Glyptocephalus cynoglossus

Hippoglossoides platessoides

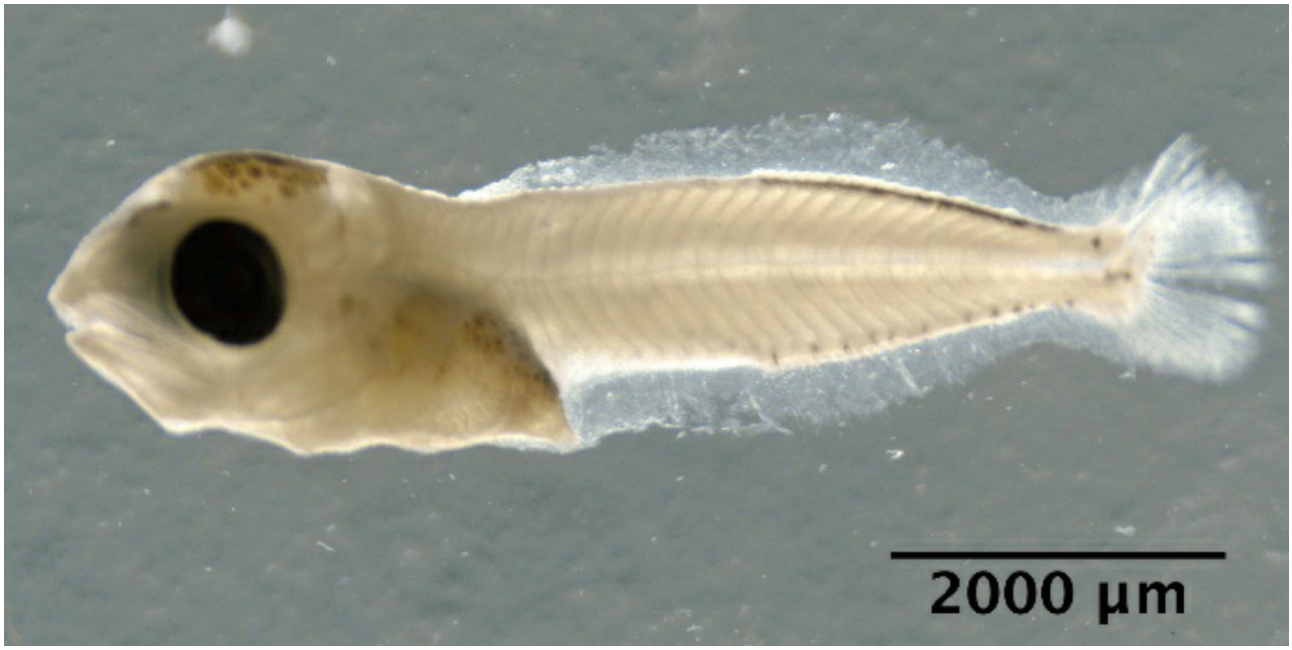
Limanda limanda



Limanda limanda

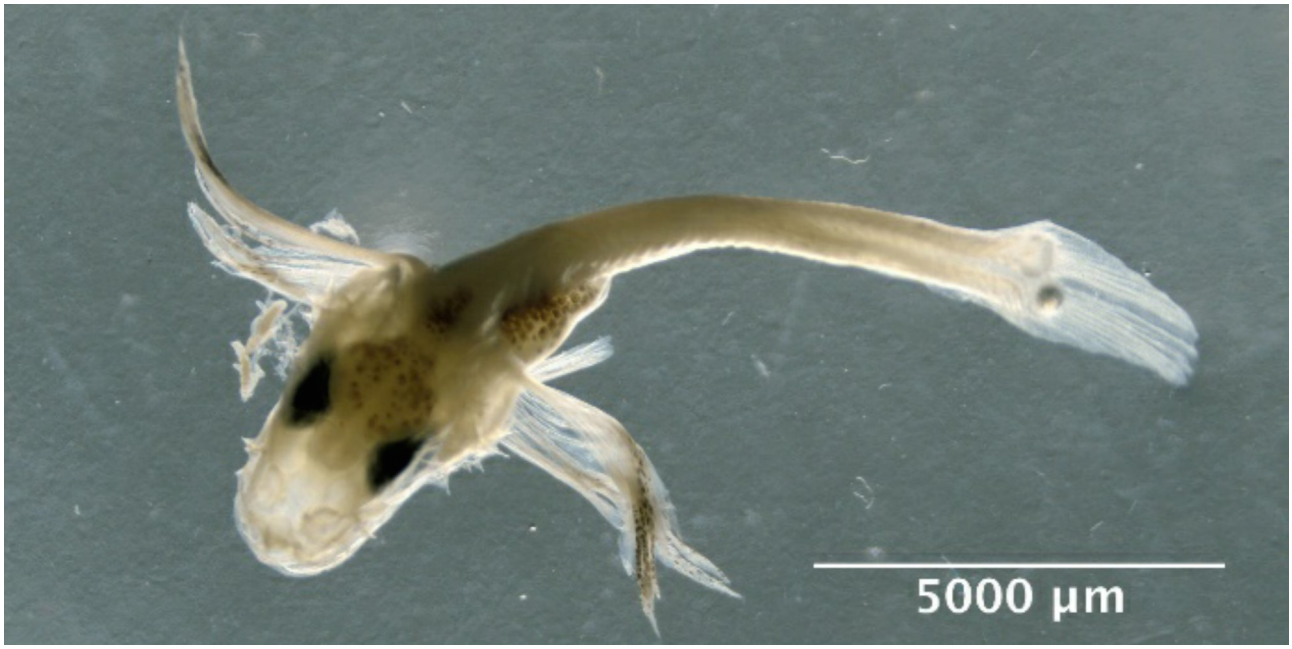
Microstomus kitt

Scombridae



Scomber scombrus

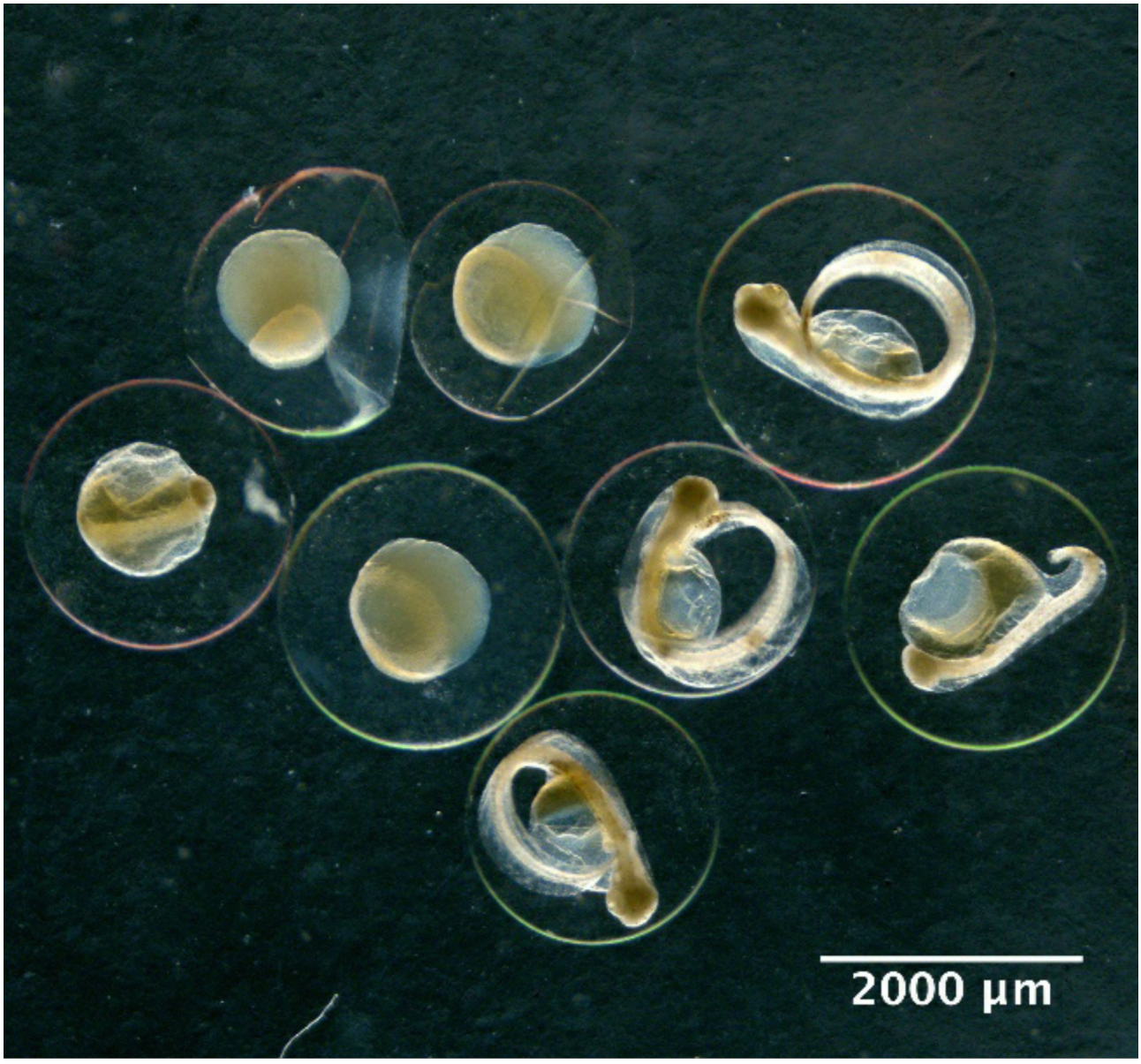
Triglidae



Eutriglia gurnardus

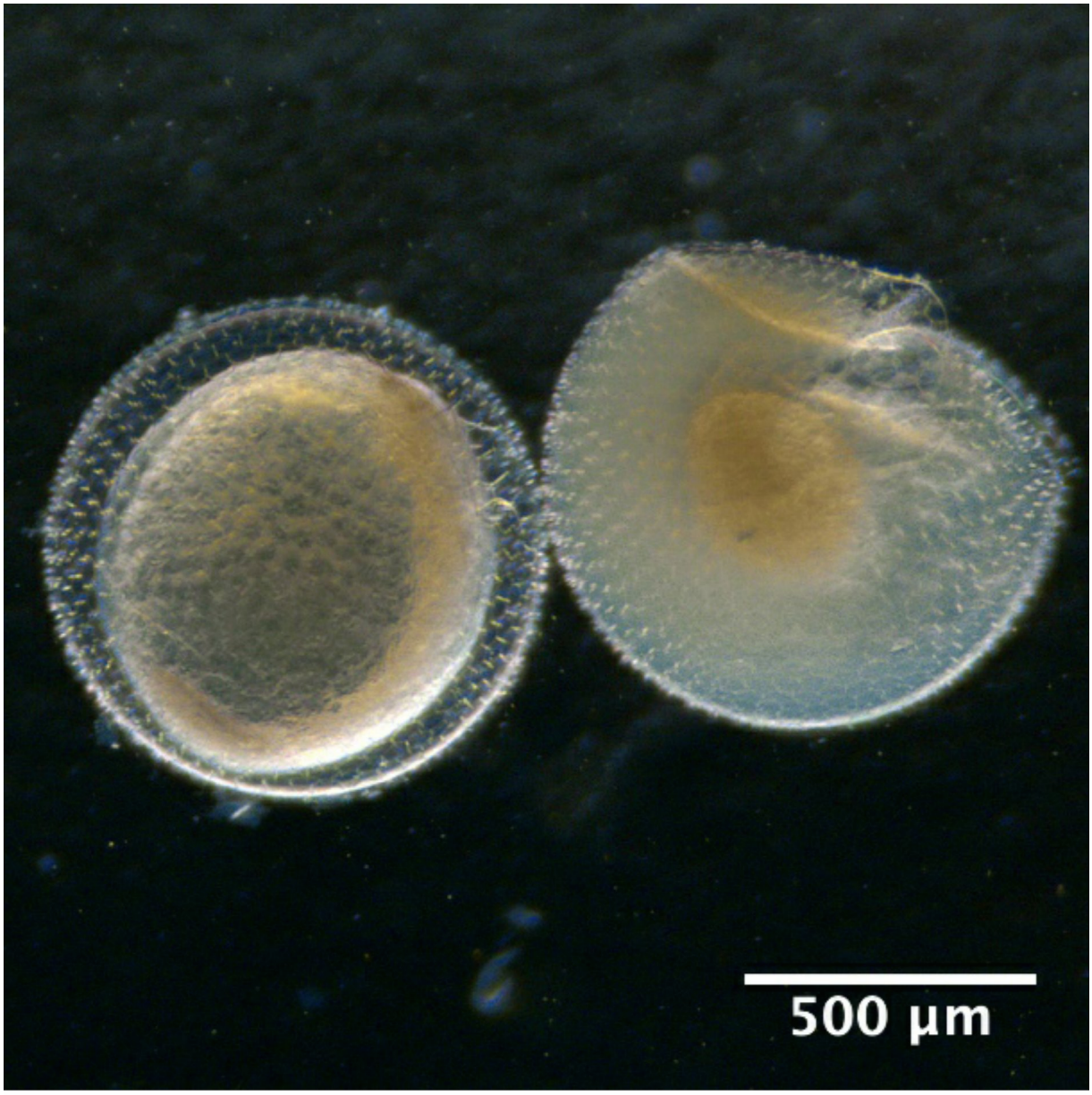
Illustrations of fish eggs identified from using the visual taxonomic method

Large egg with large perivitelline space:



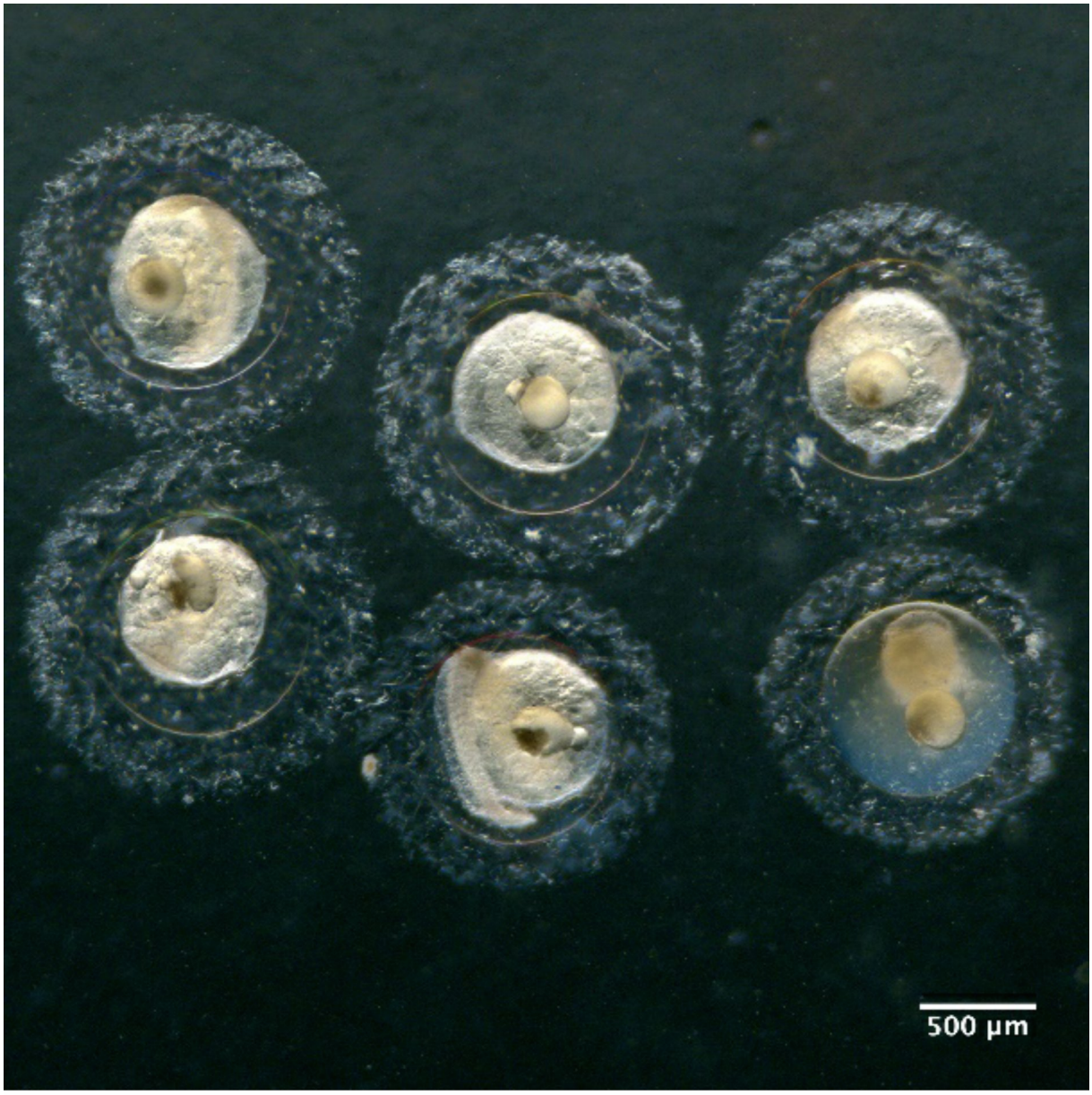
Hippoglossoides platessoides

Small eggs with sculptured membrane:



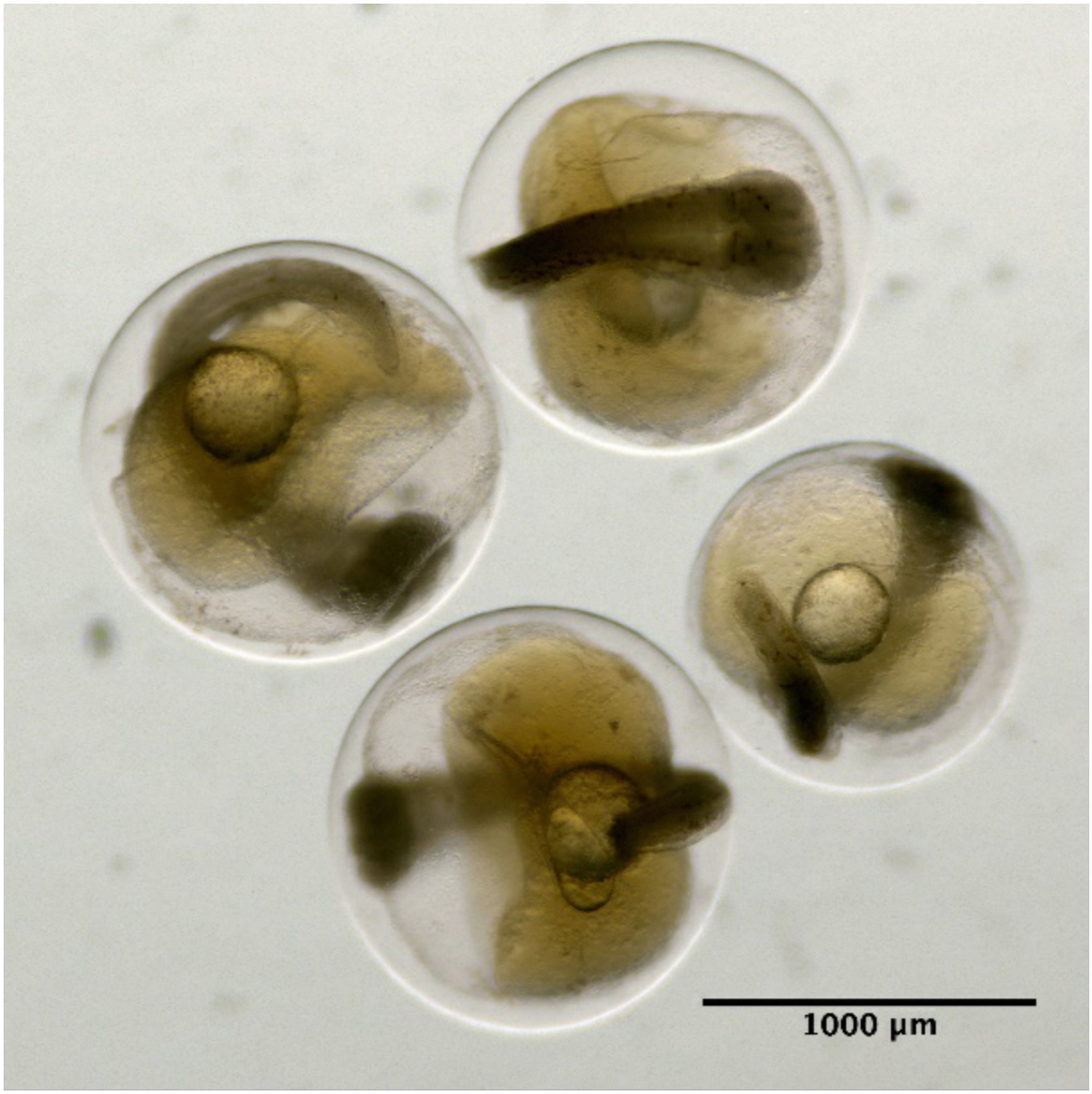
Callionymidae spp.

Eggs with one oil globule and segmented yolk:

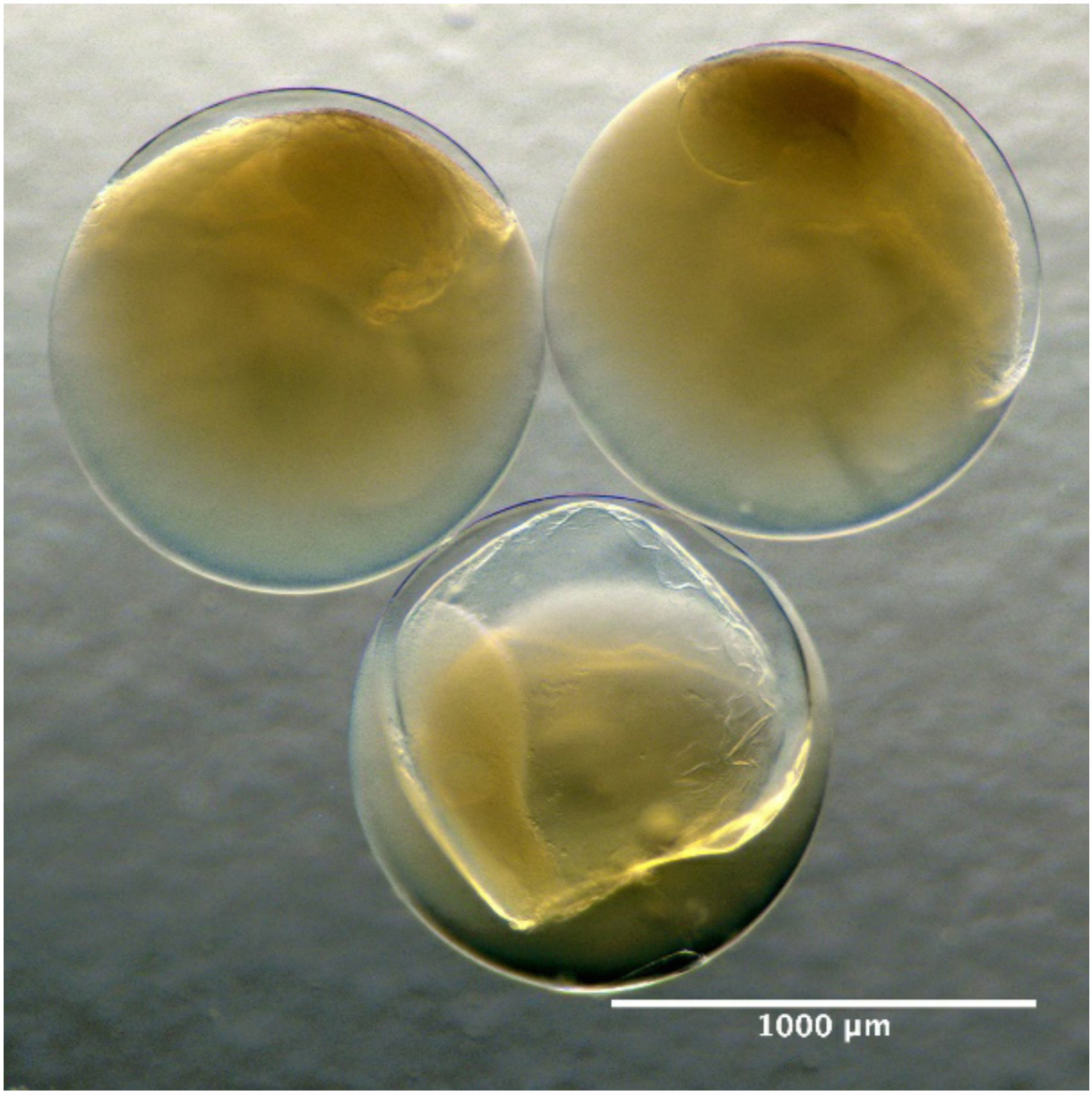


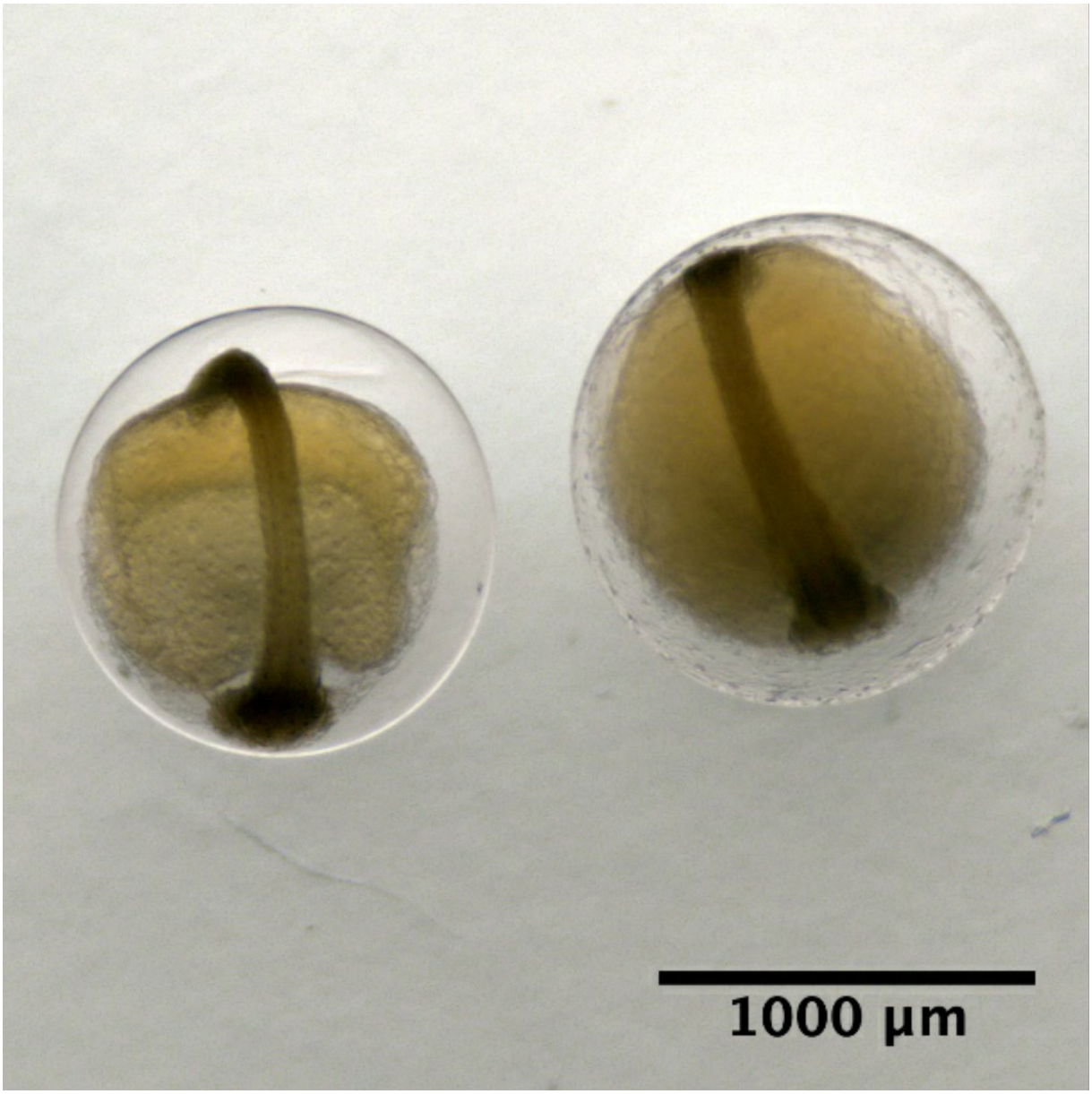
Maurolicus muelleri

Eggs with one oil globule and unsegmented yolk:



Eggs without oil globule and unsegmented yolk:




1000 µm

HAVFORSKNINGSINSTITUTTET

Postboks 1870 Nordnes
5817 Bergen
E-post: post@hi.no
www.hi.no