WORKSHOP 2 ON THE IDENTIFICATION OF CLUPEID LARVAE (WKIDCLUP2)

VOLUME 4 | ISSUE 31

ICES SCIENTIFIC REPORTS

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International Council for the Exploration of the Sea
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H.C. Andersens Boulevard 44-46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

ISSN number: 2618-1371

ICES Scientific Reports

Volume 4 | Issue 31

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Recommended format for purpose of citation:

ICES. 2022. Workshop 2 on the identification of clupeid larvae (WKIDCLUP2).
ICES Scientific Reports. 4:31. 69 pp. http://doi.org/10.17895/ices.pub.20054435

Editors

Matthias Kloppmann

Authors

Nevena Almeida • Paula Alvarez • Maria Manuel Angelico • Samantha Barnett • Ewout Blom
Josselin Caboche • Annegret Finke • Vivian Fischbach • Anne Georgi • Elisabete Henriques
Hannah Holah • Luisa Iglesias • Erika Kolemij • Amy Larter • Isabel Lee-Elliott • Valerie Lefebvre
Andrejs Makarčuks • Ineke Pennock • James Pettigrew • Isabel Riveiro • Birgit Suer • Maik Tiedmann
Cindy van Damme • Malin Werner

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Contents

i Executive summary

The Workshop 2 on the Identification of Clupeoid larvae (WKIDCLUP2) is part of a series of workshops that aim to calibrate fish larvae identification. Different clupeoid (herring, sprat, sardine and anchovy) larvae surveys are carried out on the Northeast Atlantic Shelf and provide essential data for the assessment of fish, herring in particular, stocks in the North Sea, Irish Sea and the Baltic. In recent years, other clupeoids besides herring are occurring in the survey samples in increasing numbers. Since clupeoid larvae can easily be mixed up, effective quality control and proper larvae identification is essential to reliable survey results. These identification workshops are repeated regularly in order to maintain consistency of identification across the community as well as to train and improve the skills of new survey participants. As part of this workshop, the WebApp SmartDots was adapted to be utilized for ichthyoplankton identification based on images.

Identification agreement generally varies from 56-94% depending on the species and participation cohort. Based on 60 different fish larvae evaluated in 2020, overall agreement in identifying clupeid and discriminating them from other, non-clupeid larvae among all participants was 81.7%. Agreement for herring larvae was 86%, for sprat 80%, for sardine 86% and for anchovy 71%. For 2 reading rounds in 2021, 120 fish larvae were used for each. Overall agreement during the first round was 72.5%, 81% for herring larvae, for sprat 56 %, for sardine 67 % and for anchovy 72 %. During the second round, overall agreement increased to 81.7%, 85% in herring, 83% in sprat, 69% in sardine, 82% in anchovy and 94% in non-clupeoids.

Subsequent analysis myotome counts, which was facilitated through the SmartDots WebApp during all larvae reading events, showed that particularly in those specimens that showed low agreement in correct identification, variation of counts was high. Consequently, techniques for their correct determination were discussed and existing information on clupeoid larvae identification updated accordingly. New, previously unpublished information on specific pigmentation discriminating between sprat and sardine was added to existing larval descriptions.

The only species for which larvae abundance indices are currently used in the assessment is herring. Two of those surveys are conducted in the North Sea, the third in the Baltic. Based on the reassuring results of the workshop identification trials, the potential error caused by misidentification of the larvae can be considered as low or negligible for all of these surveys.

Though the agreement in correctly identifying larvae of clupeoid fish increased considerably in comparison to the first WKIDCLUP in 2014, the results of this workshop underscored the importance of carrying out such events regularly. Increasing spatial and temporal overlaps in distribution of larval clupeoids due to warming oceans demonstrates the need to have ongoing discussion and training for consistency in identification. Through adaptation of the SmartDots WebApp to ichthyoplankton identification trials, such workshop should also become more feasible as online events and, thereby, receive higher attendance.

ii Expert group information

Expert group name	WORKSHOP 2 ON THE IDENTIFICATION OF CLUPEOID LARVAE (WKIDCLUP2)
Expert group cycle	Annual
Year cycle started	2020
Reporting year in cycle	2/2
Chairs	Matthias Kloppmann, Germany
Meeting venue(s) and dates	1-2 September 2020, Bremerhaven, Germany, as video conference (27 participants)
	30 August-3 September 2021, Bremerhaven, Germany, as video conference (25 participants)

1 Clupeoid Larvae identification and description (ToR b)

WKIDCLUP2 updated the overview (ICES, 2014) of the reference literature used and the characteris-tics for identification of the different clupeoid larvae. The most used references for identification of fish larvae in the Northeastern Atlantic and Mediterranean are: Ehrenbaum (1905-1909), Russell (1976), Fahay (1983), Moser *et al.* (1984), Munk and Nielsen (2005).

Before identification of fish larvae some background information is needed. To get acquainted to major fish larvae description literature, the introduction chapter of Russell (1976) gives all the back-ground information on larvae characteristics and different development stages and should be read by anyone who wants to identify fish larvae. Also, information on the timing of sampling and the area where samples were collected should be available. However, and not only in the light of the warming climate, shifts in spawning area and season should always be anticipated in clupeid fish (see e.g. Peck *et al*., 2021 for review). A profound knowledge of the dynamics of clupeiform populations is, therefore, of advantage for successful identification of their larvae.

A fish larva is the active immature form of a fish and differs greatly from the adult. It is the stage between egg, starting with hatching, and metamorphosis when the species-specific adult morphology is attained. The larval stage is characterised by progressive changes throughout its duration:

- Organs develop and become functional
- Pigmentation changes and becomes stronger
- Fins develop and often change their relative position. Most conspicuous is the development of the caudal fin with flexion of the urostyle.

The above characteristics, which change with the different developmental stages, can and should all be used for the species identification of fish larvae.

The yolk-sac stage is the transitional stage between the egg and larval stage. Very often, particularly in larvae which hatch from pelagic eggs, these larvae lack functional eyes and mouth and the fins are not developed. The characteristics known already from the eggs are retained during this stage (e.g. yolk segmentation, oil globule). During this stage the characteristic pigmentation develops. Yolk-sac larvae from demersal eggs generally hatch at a further advanced development stage com-pared to larvae from pelagic eggs. Their eyes are often already fully pigmented and functional.

Most fish larvae live in and have to adapt to a completely different environment compared to their adult conspecifics. They develop typical larval characters that can be used for identification. Several larval characters which need to be utilised for identification are:

- Body shape
- Fins and fin fold
- Eyes
- Spines
- Fin rays and fin ray counts
- Body proportions
- Myotome counts
- Pigmentation patterns

Four major groups can be identified from the shape of the body:

- Long, slender and elongated: Clupeidae, Engraulidae, Ammodytidae,
- Laterally compressed and dorsal-ventrally high: Pleuronectidae
- More typically fish like forms: e.g. Gadidae
- Some conspicuously aberrant forms: e.g. *Lophius sp.*, *Zeus faber*

1.1 Clupeoid larvae general characteristics

The common characteristic for all larvae of clupeiform fish is their tubular shape of the body, the slender head and the long gut covering > 75 % of total body length. They can be differentiated from other long and slender fish larvae by the length of their gut, which is only between 25 and 50 % of body length in Stichaeids, Pholids, Lumpenids and Ammodytids, by their pigmentation, which is much stronger in Argentinids, or they morphology of their eyes, which is elliptical and often stalked in larvae of more oceanic species like e.g. Sternoptychids, Stomiids or Gonostomatids. It has to be born in mind that body proportions of clupeiform larvae change during development thus the anus moves forward and the myotome count decreases with age.

Primary characteristics

The ultimate primary characteristic in discriminating the clupeid larvae (herring, sprat and sardine) from each other is the number of myotomes between the nape and the anus. It is important that my-otome counting is done correctly (i.e. start and end are well identified). For myotome count in the larvae trunk (from the back of the head to anus), see figures 1.1 to 1.3 (read also description in Russell, 1976). The small undeveloped myotomes at the head in front of the first vertebra should be excluded from the counts. The first myotome is the myotome behind the head at the first vertebra. The last myotome to be counted in the trunk is the one which on the ventral side is aligned with the anus. (Thus, the middle of this myotome is still in front of the anus.) The only engraulid of the area (anchovy, *Engraulis encrasicolus*) is characterized by the size of its head, which is larger compared to the other species. In later stages of anchovy, the anus is always situated underneath the dorsal fin. The primary characteristics are summarized in table 1.1.

Figure 1.1 Myotome counting in clupeids: Start and end point of number of myotomes in the trunk.

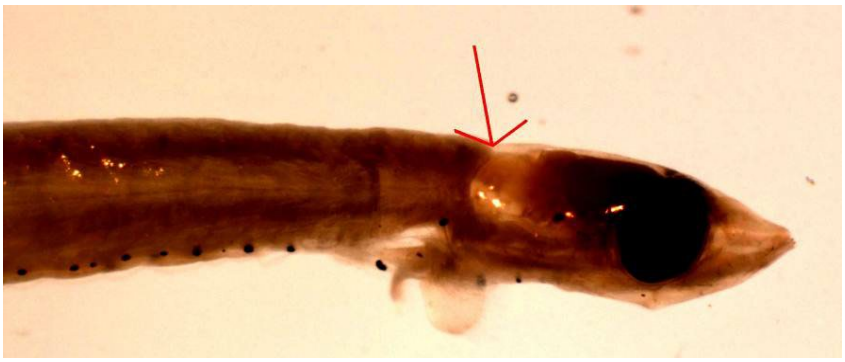


Figure 1.2 Myotome counting in clupeids: The first myotome after the head.

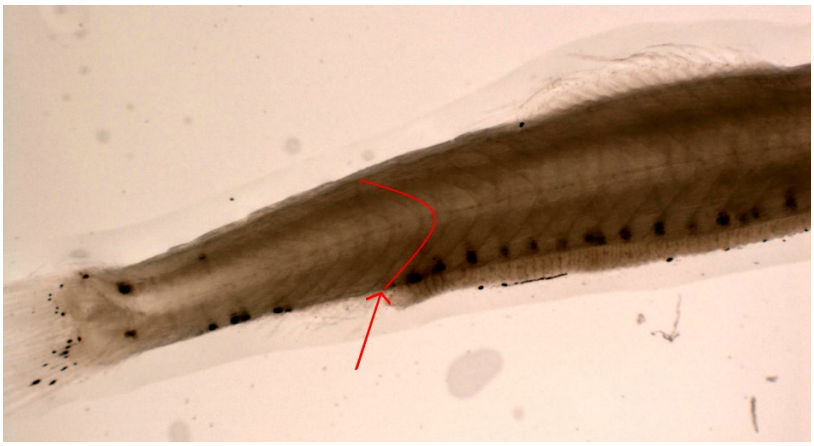


Figure 1.3. Myotome counting in clupeids: The last myotome at the anus.

Table 1.1: Primary characteristics of clupeoids (slightly modified from Russell, 1976).

Development stage (total length)	Herring	Sprat	Pilchard/Sardine	Anchovy
Yolk sac	Yolk not segmented	Yolk segmented	Yolk segmented	Yolk segmented, oblong shape
< 10 mm				
No. myotomes in trunk	47	37	41-42	
10-20 mm				
No. myotomes in trunk	46-47	35-37	41-42	
Position pelvic fin	Not appeared yet	Appears at 17.5-20 mm, 4-5 myotomes behind the pylorus	Appear at 18-20 mm, level with the pylorus	
Dorsal fin				Rear edge of dorsal fin overlaps with the anal fin
20-40 mm				
No. myotomes in trunk	41-46	31-35	36-41	
Position pelvic fin	4-8 myotomes behind the pylorus	4-5 myotomes behind the pylorus	Level with the pylorus	
Length of tail from anus to base of caudal fin	Greater than 6 times in total length	Less than 6 times in total length		

Secondary characteristics

Herring is always bigger at any developmental stage compared to the other species. Herring have pigmented eyes at hatching while other species' eyes do not gain pigmentation until later (5 mm). Herring attain flexion stage later (17 mm) than other species so larvae at 11-13 mm with flexion will not be herring (Munk and Nielsen, 2005). Pigmentation in anchovy larvae occurs in much less regular assemblages as in herring, sprat and sardine.

(Note: In southern Iberia also the clupeoids *Sardinella aurita* and *S. maderensis* can be found.

Apart from references given above, other useful descriptions for clupeoid larvae can be found in Fage (1920), D'Ancona (1931), and Saville (1964).

1.2 Identification to key to small Northeast-Atlantic clupeoid larvae

Compiled by H.-Ch. John, January 2004, after Ehrenbaum (1905-1909, 1936), Fage (1920), D´Ancona (1931), Lebour (1921). Supplemented by details on herring and sardinella.

Vertebrae counts including urostyle:

Alosa fallax, **Twaite Shad,** 55 – 59 (larvae almost pigmentless)

Alosa alosa, **Allis Shad,** 57 – 58 (larvae almost pigmentless)

} Anadromous species, larvae unlikely to occur in the marine environment

Clupea harengus, **Herring,** 55 – 58, hatches at large size (5 – 7 mm NL, i.e. notochord length, yolk only present in small larvae, bubbly and structured, no oil globule) eyes and intestines heavily pigmented.

Sardina pilchardus, **Sardine,** 50 – 53 (*Sardinella aurita* 48, occurring only from the Mediterranean southwards)

Sprattus sprattus, **Sprat,** 46 – 50

Engraulis encrasicolus, **Anchovy,** 46 - 48

<u>Yolk sack larvae:</u>

Yolk conspicuously segmented, no oil globule, eyes unpigmented, **preanal length < 80 % NL**:
Engraulis encrasicolus

Yolk less structured, **no oil globule**, eyes unpigmented, **preanal length ≥ 85 % NL**:
Sprattus sprattus

Yolk segmented, **Oil globule present**, eyes unpigmented, **preanal length ≥ 83 % NL**, intestinal pigment develops early:
Sardina pilchardus ***(***but also *Sardinella aurita)***

Yolk segmented, no oil globule, **eyes pigmented**, preanal length >80% NL, much larger than other clupeids at this stage (5 - 9mm TL):
Clupea harengus

<u>Early preflexion larvae and later:</u>

Gut with **< 80 % NL conspicuously shorter than** in all other species. Dorsal fin developing already at 6 mm NL. Dorsal and anal fins complete at 11 mm, **Anus below or** immediately in front of end of dorsal fin. Pelvic fins (developing at 15 mm) at pylorus.
Engraulis encrasicolus

Gut long > 85 % NL, **Pylorus at Myosept # 21,** foregut therefore conspicuously long compared to sprat, sardinella or sardine. Dorsal fin developing at 12 mm, anal fin at 16 mm. **Dorsal fin starts at 33rd myomer (**30 at transformation). **Anus** distinctly behind end of dorsal fin at **47th myomer** (43rd at transformation). After transformation pre-anal myotome count decreases and distance between dorsal and anal fin is reduced. **Pelvics** (developed at 21-22 mm) **4-8 myotomes behind pylorus.** Intestinal pigment present from ~20mm TL.
Clupea harengus

Gut long > 85 % NL, **Pylorus at Myosept # 15**, dorsal fin (and almost at the same time the anal fin) develops at 9 mm NL, start of dorsal fin at **myomer 28**. Anus distinctly behind end of dorsal fin at **37th myomer**. **Pelvic fins develop at 18 mm, behind pylorus at 18th myomer**. Supraintestinal hindgut-melanophores develop late only at differentiation of dorsal actinotrychs into pterygiophore and ray.
Sprattus sprattus

Gut long > 83 % NL, **pylorus (**and pelvic fin at 20 mm**) at myosept # 18**, dorsal fin develops at 8.5 mm, anal fin later at 12 mm, **start of dorsal fin at 31st myomer**. Anus distinctly behind end of dorsal fin at **42nd**. Intestinal pigment stronger and much earlier than in *Sprattus sprattus*, but otherwise very similar appearance. The presence of a continuous series of **supraintestinal hindgut-melanophores** before and during dorsal fin development can be utilized as a differentiating character to the sprat:
Sardina pilchardus
(Sardinella similar but tail pigment develops later, pelvic fins slightly behind pylorus, less vertebrae, ventral hindgut-melanophores spot-like, in sardine dashes).

1.3 Descriptions of the four workshop target species

Herring *Clupea harengus*

Distribution of adults

Herring is a comparatively large pelagic and planktivorous clupeoid species with a sub-arctic/boreal to temperate distribution pattern. In the North Eastern side of the Atlantic, herring occurs between the Barents Sea in the Northeast and the Northern border of the Bay of Biscay in the South. It consists of a number of stocks with specific spawning sites and spawning time. It ranges from Iceland and southern Greenland southward to the northern Bay of Biscay and eastward to Spitsbergen and Novaya Zemlya in Russia, including the North Sea and Baltic Sea (Whitehead, 1984a, 1985). In the western North Atlantic, herring occurs from southwestern Greenland and Labrador southward to South Carolina, USA (Figure 1.4).

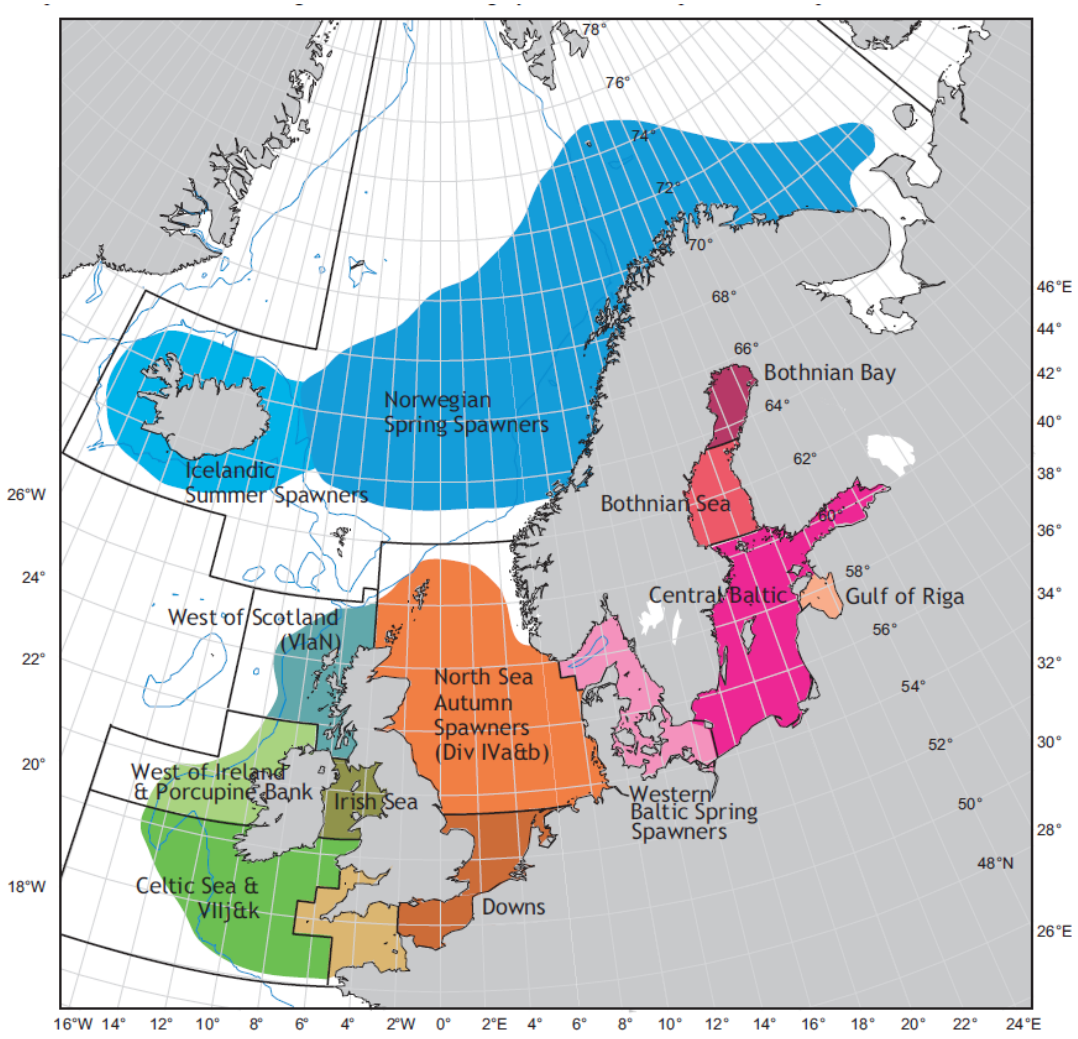


Figure 1.4: Spatial distribution of herring stocks in the Northeastern Atlantic (von Dorrien *et al*., 2013).

Temporal distribution of spawning

Herring spawn at almost any time of the year, and there is possibly no month of the year at which none of the different herring stocks is spawning (see Russell, 1976; Sinclair, 1988). The spawning seasons of the major herring stocks is summarized in Table 1.2.

Table 1.2 The spawning seasons of different herring stocks (References: Jakobsson *et al*. 1969, Sinclair 1988, Holst *et al*. 2004, modified & extended pers. comm. by Enda O'Callaghan, Birgit Suer, Dorothee Moll)

Area	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
English Channel	■	■										■
North Sea	■							■	■	■	■	■
Norwegian Coast		■	■									
Iceland		■	■				■					
Buchan / Shetland								■	■			
Central North Sea								■	■	■		
Clyde Sea					■	■						
Irish Sea									■	■		
Celtic Sea	■											■
West of Ireland					■	■	■	■	■			
Western Baltic Sea Spring Spawning		■	■	■	■							
Central Baltic					■	■						
Bothnian Sea									■	■		

Recent changes observed in herring spawning in the Baltic Sea:

During recent years (2011-2013), herring larvae have been observed during ichthyoplankton surveys conducted in the Bornholm Basin in November, which may indicate increased spawning activity and an increased stock size of the autumn spawning herring (Bastian Huwer, pers. comm.). Preliminary results show that larval abundance increased in the vicinity of Bornholm/Christiansø, while larval sizes are decreasing, indicating that larvae are hatching in the vicinity of Bornholm Island.

In consensus to historical reports (e.g. Rechlin, 1991) spring spawning herring arrive early on their inshore spawning grounds if the preceding winter is mild. However, the past decade was dominated by a succession of mild winters relative to the temperature-based threshold for initial spawning in Greifswald Bay (Germany) with significant consequences on the year-class strength of recruits (Polte *et al.*, 2021). The mechanism for increased early life stage mortality can as of yet only be hypothesized but it might be related to an asynchrony among first-feeding larvae and their planktonic prey induced by the shift of spawning phenology.

Eggs

- demersal eggs attached to substrata like gravel, broken shells or submerged aquatic vegetation, autumn spawning herring favor steeper and deeper waters and generally on gravel (Baltic Sea)
- 0.9-1.5 mm in diameter, size is variable, depending on time of spawning and spawning cohorts

Larvae

Primary characteristics – as observed in formaldehyde preserved larvae and summarized in the following table 1.3:

Table 1.3: Primary characteristics of herring larvae of the different size classes

	Yolk-sac (5-9mm)	<10mm	10-15mm	15-20mm	>20mm
Myotome count in trunk		47	46-47	46-47	41-46
Fin development	The caudal fin is present and begins to develop from hatching	The caudal fin continues to develop	The dorsal fin begins to develop at 10-12 mm	The anal fin begins developing around 16 mm	The pelvic fin develops 4-8 myotomes behind the pylorus Dorsal fin is complete at 28-29mm Notochord flexion is complete by 21mm Caudal fin is definitely incised by this stage
Pigmentation	Yolk-sack herring have pigmented eyes but otherwise are less pigmented than other clupeids at this stage.	Larvae are less pigmented than other clupeids at this stage	More pigmentation begins to develop around the caudal and ventral areas	Pigmentation is becoming more comparable to other clupeids by 20mm	
Other identifying features	Herring larvae hatch at 5-9mm (typically 5-6)			Gills form and become visible in this stage	The pylorus is found at myosept 21

The pre-anal length decreases with growth after larvae reached 15 mm length (Schnakenbeck, 1929), and the distance in myotomes between the posterior margin of the dorsal and anterior margin of the anal fin also decreases (Table 1.4). These distances are given below, though may not be precise in every case as rate of development may differ between individuals and populations.

Table 1.4: Myotome counts in trunk and between dorsal and anal fins for different sizes of herring larvae.

Length (SL)	Pre-anal myotomes	Myotomes between dorsal and anal fin
15	47	
20	47	8
22		7
25		5
26		4
27	45	
35	43	
40	42	

Primary characteristics as observed in Western Baltic herring (Schnakenbeck, 1929):

- Herring larvae possess a yolk-sac at 5-9 mm, also the caudal fin starts to differentiate
- The dorsal fin develops at 10-15 mm, with the notochord flexion starting when all elements of the dorsal fin are present, at 16 mm and is completed by 18 mm.
- The anal fin develops from 16-19 mm and finally the pelvic fin (visibly) develops at 22 mm. At 30 mm the larvae transitioned into the juvenile fish.

The length measurements are mean values and are dependent on environmental factors such as temperature, the order of development however should stay the same during different environmental conditions.

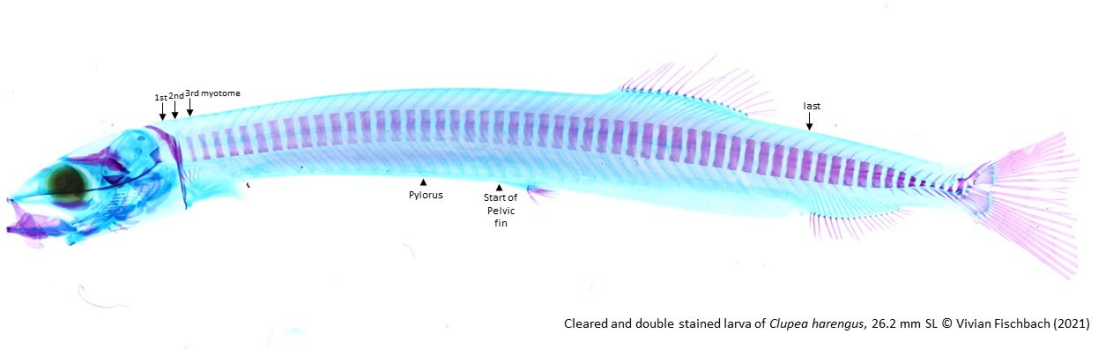
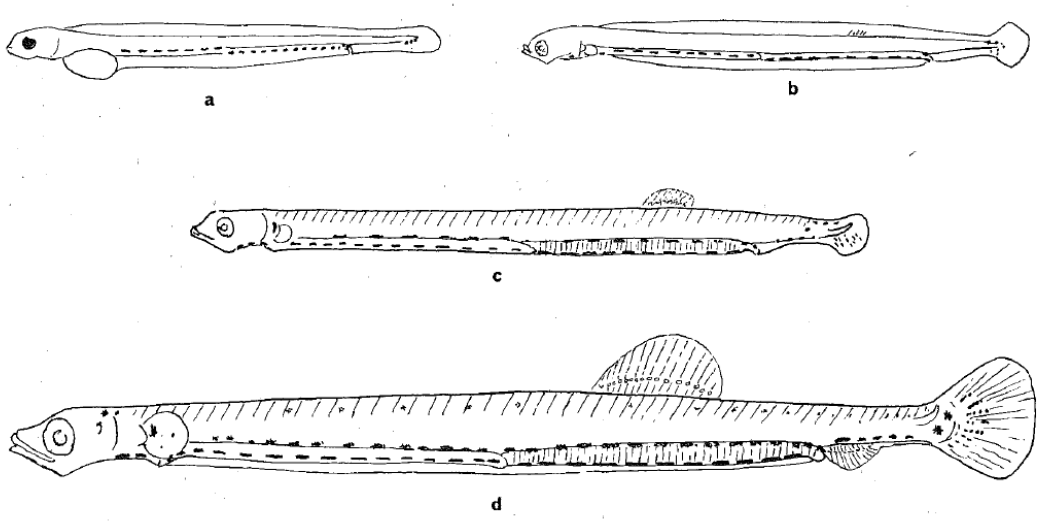


Figure 1.5: Myotome and fin configuration of a 26.2 mm (SL) herring larvae. Vivian Fischbach (2021)

Secondary characteristics:

- until development of pelvic fins, herring larvae are always larger at any developmental stage than other clupeoid species
- eyes are fully pigmented at hatching, other clupeoid larvae hatch with unpigmented eyes
- yolk sac is totally absorbed at a body length of 9-12 mm depending on stock
- 19 fin rays in the dorsal fin (17-21 fin rays)
- 17 fin rays in the anal fin (15-19 variation) – from 18mm there are always 15-17 fin rays for Baltic herring, for sprat from 18mm there are always 18-22 (pers. comm. Andrejs Makarčuks)
- the anal fin originates 7-8 myotomes behind the last ray of the dorsal fin
- the hindmost fin ray in the dorsal fin is formed at 18-19 mm

Primary and secondary characteristics of herring larvae are exemplified in the figures 1.5 – 1.11



Clupea harengus.
(a) Larva, newly hatched, 9·2 mm, Clyde Sea Area, 15.iii.72.
(b), (c) and (d) Postlarva 11 mm, 14·5 mm and 22 mm, reared at Dunstaffnage Marine Research Laboratory, 23.iii.71, 6.iv.71 and 1.v.71.

Figure 1.6 Developmental stages of herring larvae (from Russell, 1976)

Figure 1.7 Herring yolk sack larva, 9.0 mm TL

Figure 1.8 Herring larva, 10.7 mm TL

Figure 1.9 Herring larva, 13.4 mm TL

Figure 1.10 Herring larva, 15.4 mm TL

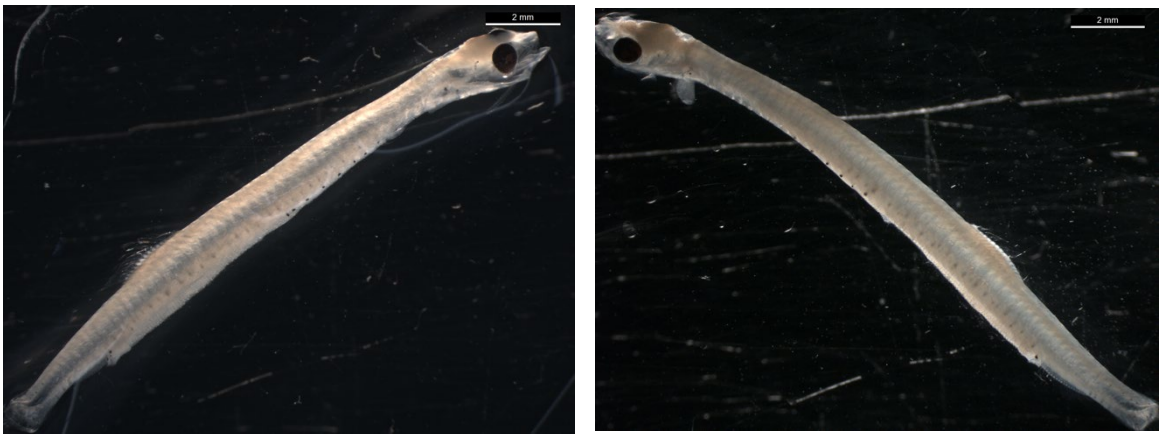


Figure 1.11 Herring larva, 21 mm (left) and 24 mm TL (right). Note the starting development of the pelvic fin in the larger larva.

Sprat *Sprattus sprattus*

Distribution of adults

The European sprat is a small planktivorous pelagic clupeoid species with a wide distribution on the shelf areas of the Northeast Atlantic, covering the coasts of Norway, the North Sea, Irish Sea, Bay of Biscay, the western coast of the Iberian Peninsula down to Morocco, the northern parts of the Mediterranean, the Black Sea, and the Baltic Sea (see Haslob, 2011 and references therein). Sprat is able to tolerate salinities as low as 4 psu and especially juveniles are known to enter estuaries (Whitehead, 1984a). In the Baltic Sea, sprat is located at its northern limit of geographic distribution (Muus and Nielsen, 1999). It is distributed throughout the western and eastern parts of the Baltic, up to the Gulf of Finland in the north.

Spawning

As many other clupeoid fish, sprat is an indeterminate batch spawner, i.e. it has indeterminate oocyte recruitment and is releasing several batches of pelagic eggs over a prolonged spawning season, and intra- and interannual variability is expected in spawning season length, batch fecundity, and batch frequency in all regions (Heidrich, 1925; Alheit, 1988). Based upon the timing of spawning at different latitudes, spawning occurs between 6 and 15 °C (Peck *et al*., 2012). In northern European waters (North and Baltic Seas), spawning occurs from January to August with peaks in spring and early summer when water temperatures are *commonly* between 8 and 15 °C. In southern European waters (Adriatic Sea), sprat generally spawns during the cooler time of the year (October-April) with peak spawning in winter (November to December) at water temperatures between 9 and 14 °C (Dulčić, 1998). However, in all regions the onset and duration of spawning may vary due to temperature and feeding conditions. See table 1.5 and annex 5 for a more detailed overview of spawning times in different areas.

Sprat spawns pelagic eggs that are buoyant at different water depths in different systems due to salinity effects on ambient density. In marine waters such as the North and Mediterranean Seas, eggs remain in surface layers but in the Baltic, eggs sink below the low salinity (5–7 psu) surface waters through the thermocline to the halocline (6–15 psu) located at intermediate water depths of 30–60 m (Wieland and Zuzarte, 1991). Due to this particular hydrographic situation in the Baltic with strong vertical stratification of salinity and temperature, the main spawning areas are located in the deeper areas of the central Baltic, i.e. the Arkona Basin, Bornholm Basin, the Gdańsk Deep and the Gotland Basin (e.g. Aro, 1989; Parmanne *et al*., 1994; Ojaveer and Kalejs, 2010; Köster *et al*., 2003). However, spawning is also observed in the Western Baltic, e.g. in the Kiel Bight, but a detailed mapping of spawning areas in this region is lacking (Haslob, pers.

comm.; Heidrich, 1925). In the most northern parts of the Baltic, sprat spawning occurs and sprat eggs can be found in the plankton, but no larvae (Sjöblom and Parmanne, 1980). In the central Baltic, spawning has been observed from February to August with a peak in spring, but differences in spawning time are possible due to temperature, salinity and potentially feeding conditions for adults (e.g. Haslob *et al.*, 2012; Voss *et al.*, 2011; Ojaveer and Kalejs, 2010; Wahl and Alheit, 1988; Petrova, 1960). In 2002, a second spawning event was observed in autumn, which was explained by the inflow of unusual warm water masses into the central Baltic (Kraus *et al.*, 2003).

In other areas outside the Baltic, sprat eggs can be observed in almost all areas where adult sprat are distributed (Milligan, 1986), but areas with high concentrations of spawning adults are e.g. found in the inner German Bight, the English Channel, the southern North Sea, northeast of England, north and west of Scotland, as well as in Skagerrak and Kattegat (Knijn *et al.*, 1993; Bailey and Braes, 1976; Torstensen and Gjøsæter, 1995; Worsøe *et al.*, 2002; Warnar *et al.*, 2011).

Note: information on specific spawning areas and seasons in other regions missing: Irish Sea, Bay of Biscay, the western coast of the Iberian Peninsula down to Morocco, the northern parts of the Mediterranean, and the Black Sea

Eggs and larvae

Egg characteristics for sprat are given in Russell (1976), who describes the eggs as pelagic, spherical, 0.8-1.3 mm in diameter without large perivitelline space, segmented yolk without oil globule, which makes them immediately recognizable among other fish eggs of comparable size.

Ré and Meneses (2009) provide the following information on sprat larvae: Hatching length - 3.0-3.6 mm; Yolk-sac absorption - 5.0-6.0 mm; Flexion length - 11 mm; Transformation length - 32-41 mm; Pigmentation - yolk-sac: small scattered melanophores in head and dorsal region (visible in the embryo).

Diagnostic features - newly hatched larva tube-like (typical clupeid form). Prominent sense organs (6) on each side of the body. Pigmented eyes at the end of yolk-sac absorption. Dorsal fin formation (28th myomere) at 8 mm. Formation of pelvic fins 4 to 5 myomeres behind pylorus at 17-20 mm (figure 1.12). Number of preanal myomeres 35-37. Tail length less the six times into total length.

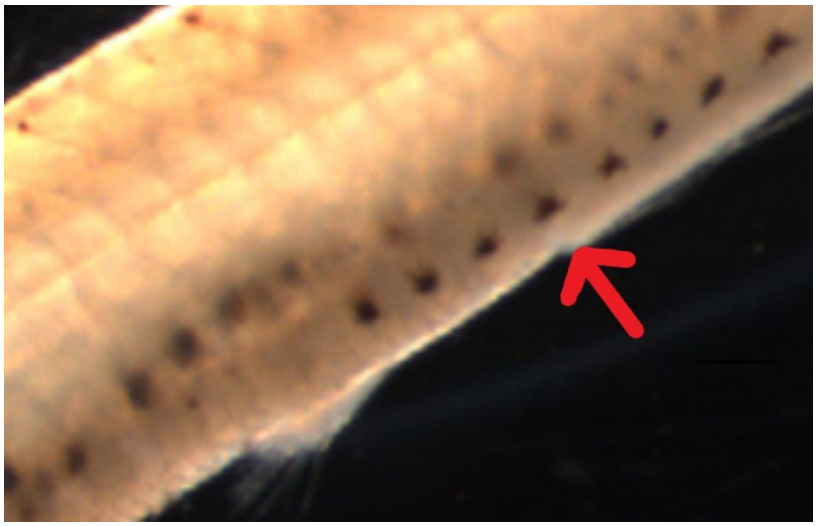


Figure 1.12: Position of pylorus (red arrow) in relation to the pelvic fin in *Sprattus sprattus*.

Ehrenbaum (1936) reports a size at hatching of ca. 4 mm, with larvae being less developed than herring, and expressing weak pigmentation and no pigmented eyes at hatch. He further reports the period until yolk-sac absorption to be ca. 8 days, during which the larva grows to ca. 5 mm. At 13-15 mm all fins are developed except for the pelvic fins, which develop at ca. 18 mm. Metamorphosis (silvery appearance) occurs at 25 mm.

Examples of sprat larvae can be seen in the drawings of figure 1.13 and images of figures 1.16 – 1.20.

Laboratory studies on Baltic sprat revealed notochord or standard length (SL) at-hatch was 3.3–3.5 mm and relatively similar at all temperatures < 17 °C as was the SL at yolk sac absorption (4.9–5.6 mm SL) (Alshut, 1988; Kanstinger, 2007; Petereit *et al*., 2008). Depending upon water temperature, sprat eye pigmentation occurs between 3 and 16 d post-hatch (dph) and jaw development and mouth opening occur ca. 48 and 72 h later (Nissling, 2004; Kanstinger, 2007). At constant temperatures between 5 and 13°C, the combined data of four studies on eggs and yolk sac larvae (Thompson *et al*., 1981; Nissling, 2004; Kanstinger, 2007; Petereit *et al*., 2008) indicated that the duration of the endogenous feeding period is 135 ± 3 degree-days (°C d) after which the larva is ca. 5.5 mm SL and must initiate feeding. For Baltic Sea sprat, Peck *et al*. (2012) defined six life stages or life-history events that occur after the egg and yolk-sac larval phases, based on changes in growth allocation between mass and length and inferences from field observations: (i) exogenously feeding but non-schooling larvae from 5 to 14 mm SL, (ii) likely onset of schooling behavior from 14 to 18 mm SL, (iii) a ''transitional-larval'' life stage from 18 to 35 mm SL, (iv) a period of late-larval/juvenile metamorphosis occurring at 35 to 55 mm SL, (v) a juvenile growth phase from 55 to 90 mm SL, and (vi) adult fish that exhibit seasonal energy allocation to somatic and gonadal growth starting at 100 mm SL. See table 1.6 for an overview of larval characteristics.

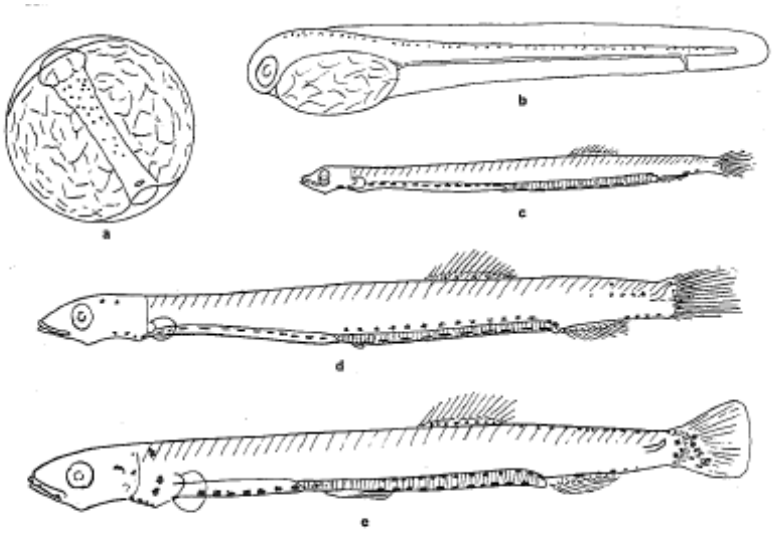


Fig. 9. *Sprattus sprattus*.
(a) Egg, 1·05 mm in diameter, Plymouth, 7.ii.24.
(b) Larva, 4·08 mm, 2–3 days old, after Ehrenbaum, 1897, Pl. IV, Fig. 16.
(c), (d) and (e) Postlarva, 14·2 mm and 22 mm, Plymouth, 15.iv.66 and 22 mm west coast of Scotland, 2.viii.70.

Figure 1.13 Developmental stages of sprat larvae (from Russell 1976)

During the WKIDCLUP2 video conference in 2020 a characteristic, which is not explicitly described in the literature, was presented to differentiate sprat larvae from similar sized sardine larvae. The hypothesis is that sprat larvae do not develop supra-intestinal hindgut pigmentation early in their development while herring and sardine do (see figure 1.14).

At the 2021 WKIDCLUP2 there was still no referenced study available that investigated this observation. It is reported (H. Ch. John, pers. comm.) that until the differentiation of the actinotrychs into pterygiophore and ray in the dorsal fin and the start of the formation of the anal fin, no rows of supra-intestinal pigment spots are developed in sprat. The presence of this pigmentation, might be confounded, however, if larvae were stored for a long time in formaldehyde and were kept at too light or warm conditions.

Another feature, which might help distinguishing large (20-25 mm TL) sprat from sardine larvae was presented during the 2021 WKIDCLUP2 meeting. The operculum of sprat larvae (20-25 mm TL) shows a distinctive pigmentation consisting of a four-point pattern, which is supposedly not present in sardine of the same size (see figure 1.15).

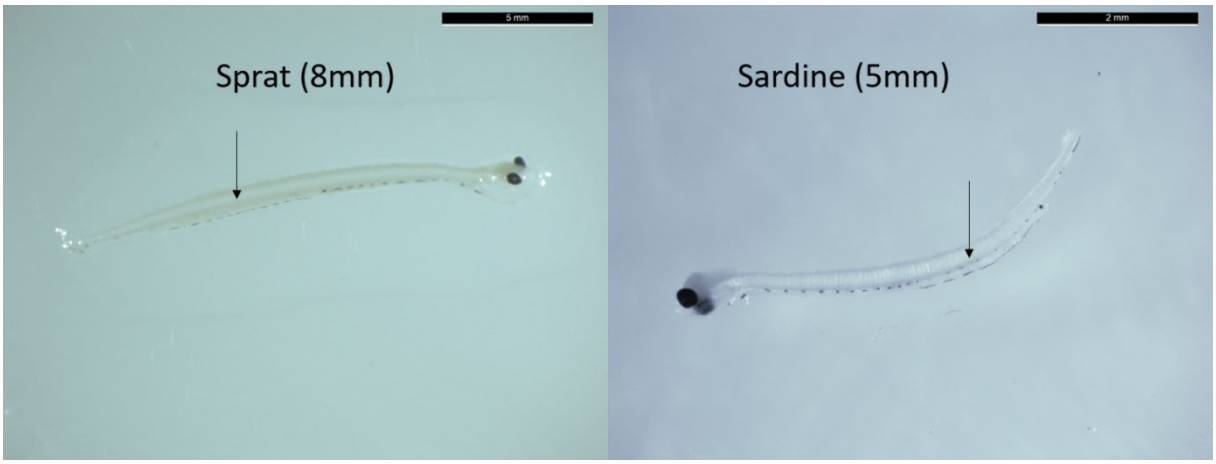
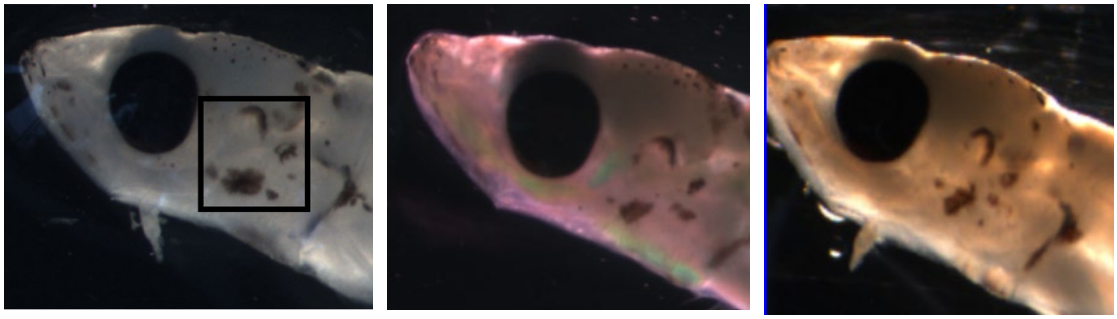


Figure 1.14 Comparison of sprat and sardine supra-intestinal hindgut pigmentation as viewed under the microscope in top light illumination and against a white background.



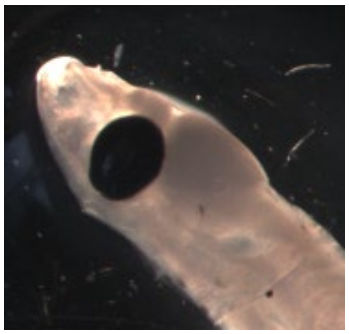


Figure 1.15 *Sprattus sprattus* pigmentation pattern on operculum (top row highlighted in box in LH image), and an image of the head of a 21 mm *Sardina pilchardus* larva for comparison (left)

Table 1.5 Spawning season of various sprat stocks (for references see table in annex 5; grey = spawning season, black is peak of spawning).

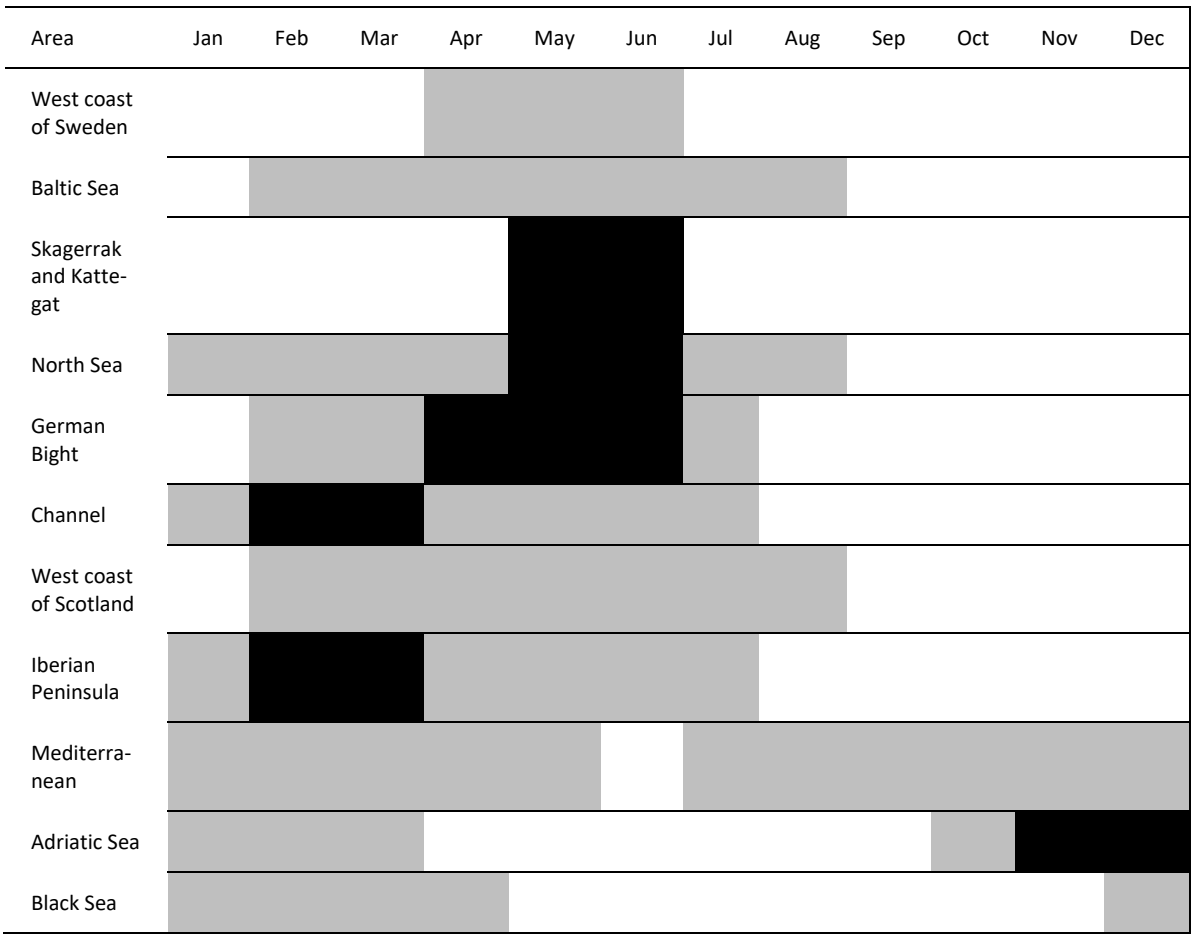


Table 1.6 Sprat egg and larvae characteristics.

EGGS								
Reference	**Size**	**Egg characteristics:**			**Comments**			
Russell (1976)	0.8-1.3 mm in diameter	eggs pelagic, spherical, small perivitelline space, segmented yolk without oil globule			Sprat eggs come within the size range of many other fish, but are immediately recognizable by the segmented yolk			
Kazanova (1954)	Baltic area: 1.2 - 1.6 mm				Key for identification of pelagic fish eggs and larvae in the Baltic Sea.			
LARVAE								
Reference	**size at hatching**	**size**	**# myotomes in trunk**	**position of pelvic fin**	**total # myotomes**	**flexion length**	**appearance of fins**	**comments**
Russel (1976) Munk and Nielsen (2005)	3.0 - 3.6 mm	< 10 mm	37	not yet appeared	46 - 48		dorsal: 8 mm	Eyes pigmented at 4.5 - 5 mm (end of yolk-sac absorption)
Ré and Meneses (2009) Lebour (1921)		10 - 20 mm	35 - 37	appears at 17.5 - 20 mm, 4-5 myotomes behind the pylorus* pylorus at myotome 15		11 mm	anal fin: 11 mm	Presence of prominent sense organs (6) on each side of the body is a characteristic feature of newly hatched larvae
		20 - 40 mm	31 -35					Changes in body proportions take place during development. Up to metamorphosis the anus has moved forward over 4 or 5 vertebrae.

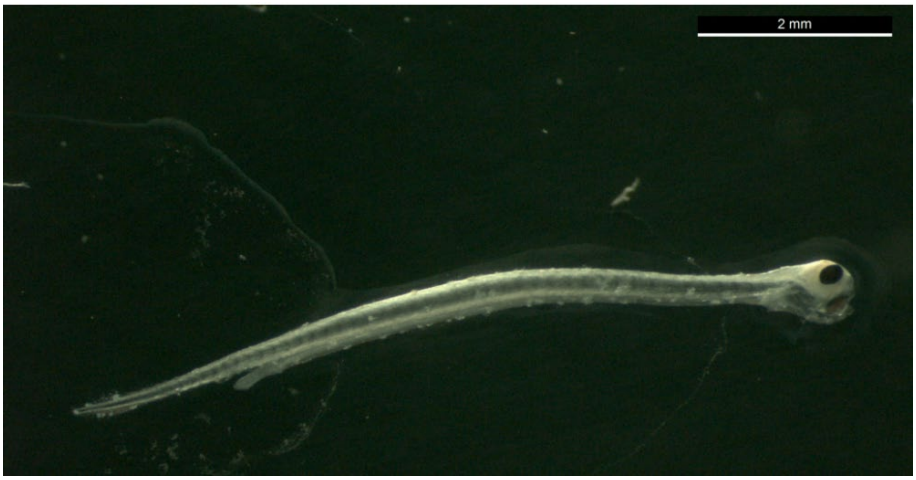


Figure 1.16: Sprat larva, 8 mm TL, dark field illumination

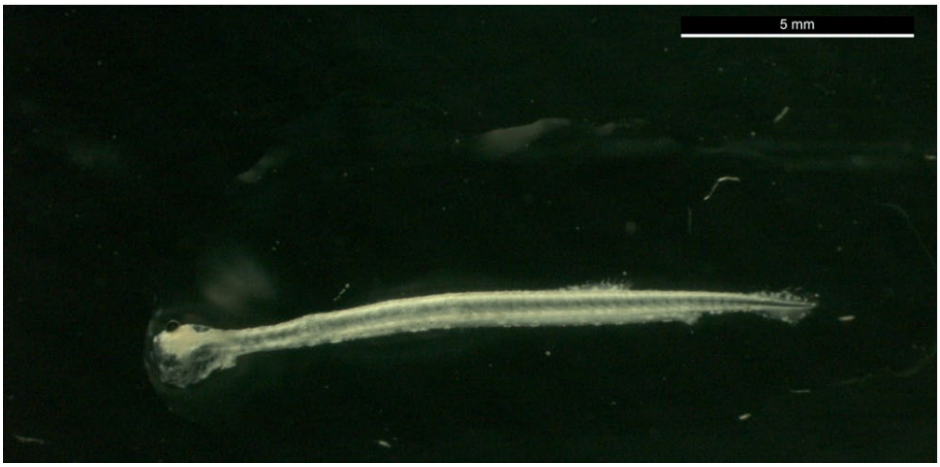


Figure 1.17: Sprat larva, 13.8 mm TL, dark field illumination

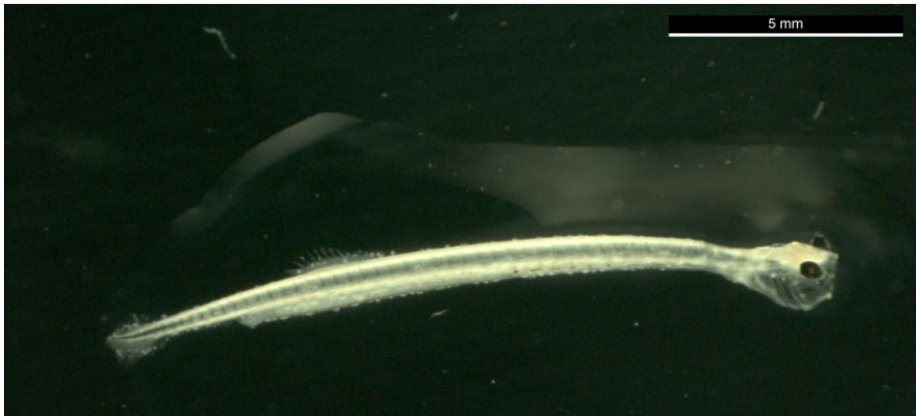


Figure 1.18: Sprat larva, 14.8 mm TL, dark field illumination

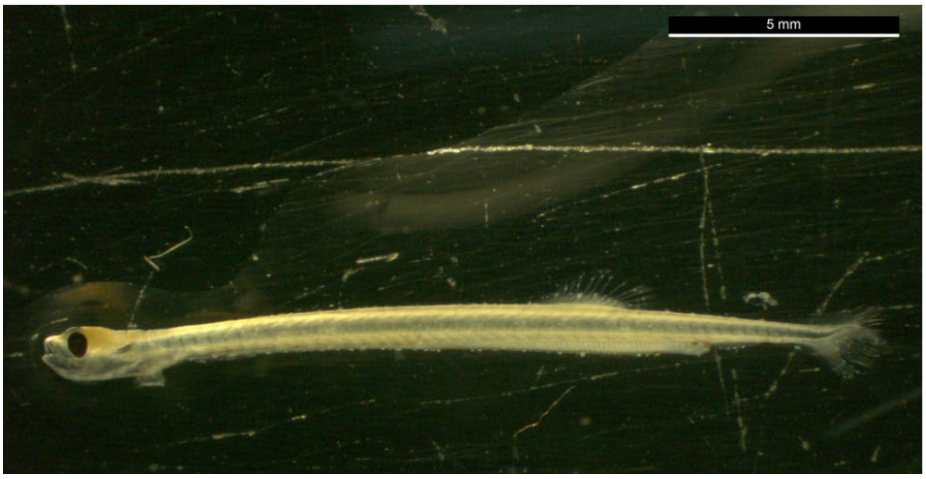


Figure 1.19: Sprat larva, 17.7 mm TL, dark field illumination

Figure 1.20: Sprat larva, 25 mm TL - myotomes and pigmentation visible

Sardine *Sardina pilchardus*

Adult characteristics and biology

Sardine is a small pelagic clupeoid characterized by an elongate and compressed body with large silvery scales of which about 30 can be counted in the lateral line. The mouth does not reach the posterior edge of the eye and the gill cover has radial striations. The pelvic fins are placed posterior to the origin of the dorsal fin.

Sardine lives in coastal waters, in large schools and feeds on phyto and zooplankton. It has an atlanto-mediterranean distribution pattern and ranges in the Northeast Atlantic from Iceland (where it is rare) south to Senegal. It is common in the Mediterranean and the Adriatic Sea, the

Sea of Marmara and the Black Sea (Parrish *et al.,* 1989). Sexual maturity is attained at 1 to 3 years of age and its lifespan could attain 15 years (Silva *et al*., 2006; Munk and Nielsen, 2005).

Life History

Sardine is a batch spawner with indeterminate fecundity, delivering batches of eggs during a relatively protracted spawning season (Ganias *et al.*, 2004). In the Eastern North Atlantic region, the main reproductive period exhibits a latitudinal gradient, being longer and with an earlier peak of spawning towards the south (table 1.7 and references therein). The pelagic egg stage could last from 2 to 5 days according to water temperature (e.g.. Miranda *et al.,* 1990; Bernal *et al*., 2008). The larval phase spans for around 5 to 7 weeks depending on environmental temperature and food availability (e.g. Dulĉić, 1995).

Table 1.7 The major spawning months for sardine in European waters, grey = spawning season, black = peak of spawning.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
North Sea[1]												
English Channel[2]												
Bay of Biscay[3]												
Iberia[4]												
Mediterranean[5]												

References: [1] Munk and Nielsen (2005);

[2] Southward *et al*. (1988); Coombs *et al.* (2005); Coombs *et al.* (2006); Stratoudakis *et al.* (2007)

[3] Solá *et al*. (1992); Coombs *et al.* (2006); Stratoudakis *et al.* (2007)

[4] Coombs *et al*., (2006); Stratoudakis *et al*., (2007); Nunes *et al*., (2011);

[5] Rodriguez *et al*., (2017)

Description of the larval stages

For a summary of primary characteristics through larval development see table 1.8 and figures 1.21 to 1.28.

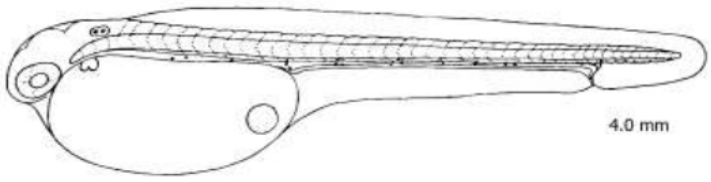


Figure 1.21 Yolk sack larva of *S. pilchardus*. From Ré and Meneses (2009).

Primary identifying characteristics in larvae less than 10mm

- At < 10 mm there are 41-42 pre-anal (back of head to anus) myotomes compared to 47 in herring and 37 in sprat.
- Hatching length 3.2 - 4.0 mm
- Newly hatched larva tube-like (typical clupeid form)
- Mouth and jaws undeveloped and unpigmented eyes at hatching
- Easily distinguishable from other clupeids by the presence of an oil globule in ventral posterior part of the yolk sac (Russel, 1976)
- Yolk-sac absorption at 4.0 - 5.5 mm
- Typical pigmentation develops around 5 - 6 mm
- Swimbladder formation at 10 mm

Primary identifying characteristics in larvae less than 20mm

- Between 10 – 20 mm there are still 41 – 42 myotomes in the trunk compared to 47 in herring and 35 - 37 in sprat.

Figure 1.22 Larva of *S. pilchardus*. From Ré and Meneses (2009).

Secondary (informative) characteristics in larvae less than 20 mm

- Notochord flexion starts at 11 - 12.5 mm
- Dorsal fin formation (at 31st myotome) 7.5 mm
- Typical larval pigmentation develops around 5 - 6 mm (see photos)

Primary Characteristics in larvae greater than 20mm

- Number of pre-anal myotomes: 41 reducing to 36 as larvae develops
- Formation of pelvic fins (level with pylorus) at 18-20 mm

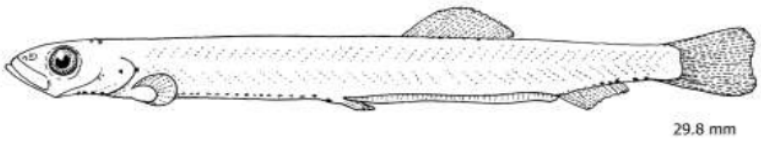


Figure 1.23 Late larva *of S. pilchardus*. From Ré and Meneses (2009).

Secondary characteristics (informative) in larvae greater than 20 mm

- Dorsal fin formation completed at 26 mm
- Anal fin formation completed at 28 mm
- Metamorphosis at lengths 40-50 mm

Table 1.8 Primary characteristics of sardine larvae

Development stage (total length)	Pilchard/Sardine
Yolk sac	Yolk segmented, oil globule in the yolk sac Hatching lenght 3.2 - 4.0 mm Yolk sac absorption at 4.0 - 5.5 mm Unpigmented eyes at hatching Mouth and jaws undeveloped
< 10 mm	
No. myotomes in trunk	41 - 42
Pigmentation	Typical pigmentation develops around 5 - 6 mm
Dorsal fin	appears at 7.5 mm in 31st myotome
10-20 mm	
No. myotomes in trunk	41 - 42
Position pelvic fin	appears at 18-20 mm, level with pylorus
Pigmentation	More pigmentation on dorsal side of hindgut than sprat
	Caudal and anal fins start developing at 11 mm
	Notochord flexion starts at 11 - 12.5 mm
20-40 mm	
No. myotomes in trunk	41 reducing to 36 as larva develops
Position pelvic fin	Level with pylorus
	Complete dorsal fin formation at 26 mm
	Complete anal fin formation at 28 mm
	Transformation length at 40 - 50mm

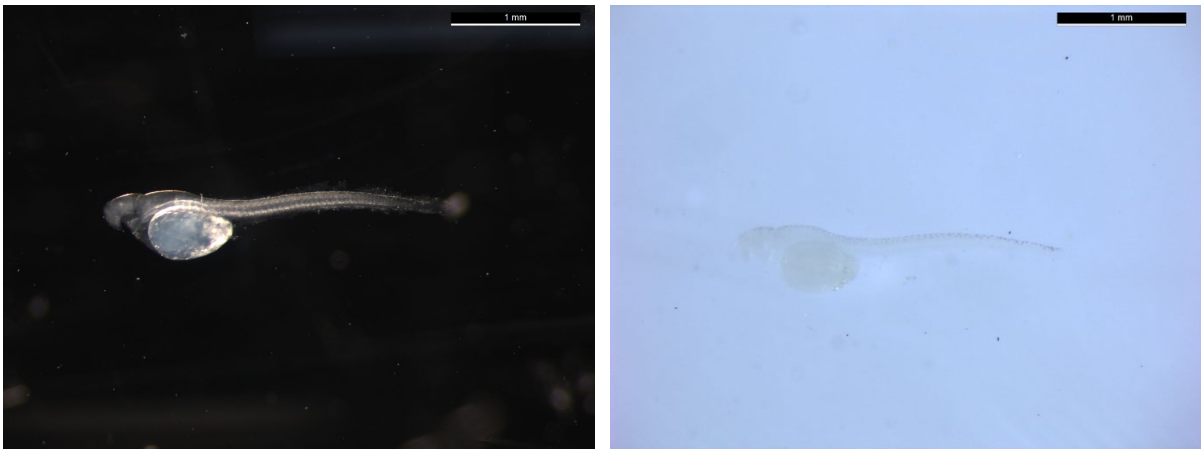


Figure 1.24: Two images of a sardine yolk sack larva, 2.5 mm TL, viewed at dark field (left) and top light (right) illumination.

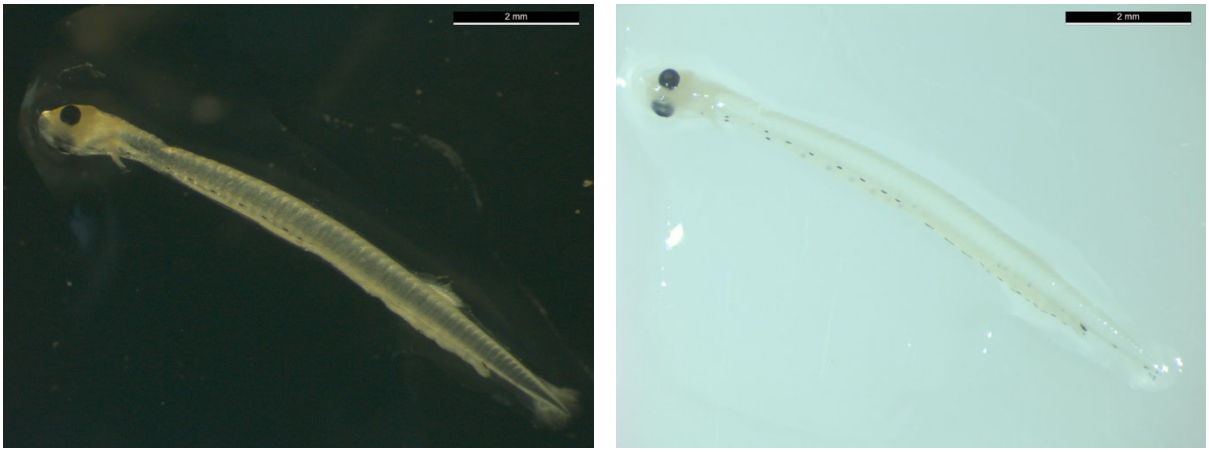


Figure 1.25: Two images of a sardine larva, 9.5 mm TL, from off the Iberian Peninsula, viewed at dark field (left) and top light (right) illumination. Note the supra intestinal pigmentation, which becomes visible in top light.

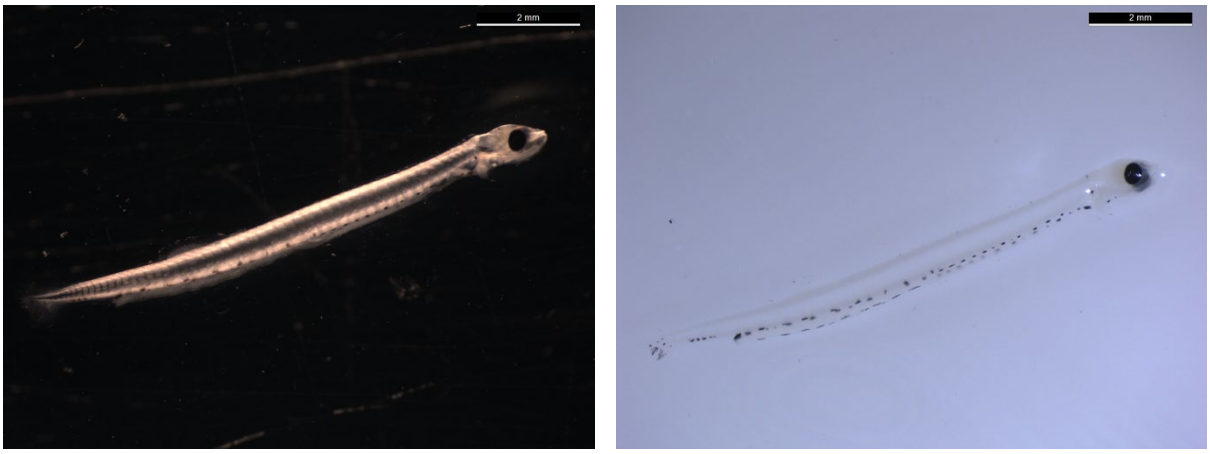


Figure 1.26: Two images of a sardine larva, 13 mm TL, from off the Iberian Peninsula, viewed at dark field (left) and top light (right) illumination. Note the supra intestinal pigmentation, which becomes visible in top light.

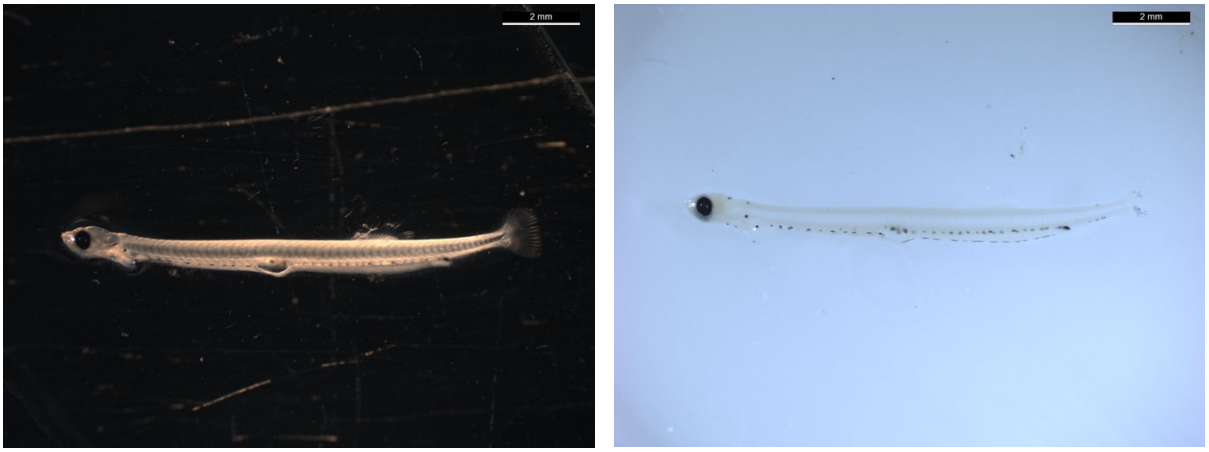


Figure 1.27: Two images of a sardine larva, 15 mm TL, from off the Iberian Peninsula, viewed at dark field (left) and top light (right) illumination. Note the supra intestinal pigmentation, which becomes visible in top light.

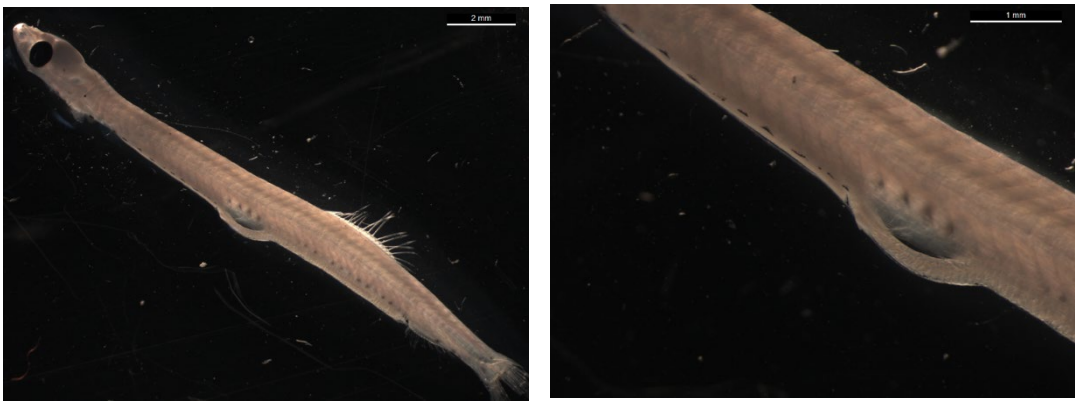


Figure 1.28: Sardine larva, 15 mm TL, from the North Sea, viewed at dark field illumination. Left: the whole animal, right: viewed at higher magnification showing the position of the developing pelvic fin level with pylorus.

Anchovy *Engraulis encrasicolus*

Adult characteristics and biology

The Anchovy (*Engraulis encrasicolus*) is a small coastal, euryhaline marine fish species that forms large schools and feeds predominantly on zooplankton (Whitehead, 1984b) which, from an ecosystem perspective, makes the link between planktonic production and higher trophic levels (Shannon *et al.*, 2009). It tolerates a large range of salinities (Fernández-Delgado et al., 2000; cited by Cosín, 2014) and in some areas enters estuaries and lagoons, especially during spawning.

Anchovies show a closed life cycle from spawning to larvae and juvenile phases until maturity within its first year of life (Uriarte, 2015). Adults may reach a maximum age of about 4/5 years (Pecquerie *et al.*, 2012; Cosín *et al*., 2015). One-year old anchovies become fully mature every year by May (Cort *et al*., 1976; Motos *et al.*, 1991; Lucio and Uriarte, 1990; Motos, 1996). The gonad maturation is correlated to the warming water masses in spring, when the water temperature increases from 12 °C, at the end of winter, to about 20 °C at the beginning of summer. In the Bay of Biscay, maturity peaks in May-June (Bay of Biscay) during the main spawning season and subsequently diminishes gradually during summer (Lucio and Uriarte, 1990; Sanz and Uriarte, 1989). Spawning locations are known to be located at river plumes or oceanic gyres (Motos *et al.*, 1996) as well as in shallow waters near the mouth of the Guadalquivir (Baldó *et al*., 2006).

Anchovies show indeterminate fecundity and short inter-spawning intervals (Uriarte, 2015). Relative fecundity of females per spawning batch ranges between 350 and 700 eggs per gram at a spawning frequency between once every 2 to 5 days, i.e. spawning fraction ranges between 0.2 and 0.5 (Motos, 1996; Uriarte *et al.*, 2012; Rodriguez-Roda, 1976; Cosín, 2014).

Growth is fast in the first two years of life (up to the age of 2) when it reaches most of its asymptotic growth (Uriarte and Astudillo, 1987; Vaz *et al.*, 2002; Hernandez *et al.*, 2009; Bellido *et al.*, 2000). The sharp decay of the abundance of the oldest age groups suggests a high natural mortality at those ages.

Geographical Distribution

Anchovy occurs in the eastern Atlantic between Bergen, Norway, and Angola. It also occurs in the Mediterranean, the Black and Azov Seas (Figure 1.29). European anchovy was also confirmed to occur in the Baltic, in the Bornholm Basin and the waters of the Gulf of Gdańsk (to the east of 18°20′E) (Draganik and Wyszynski, 2004). Anchovies were found even further east during the Baltic-Survey (BITS). Climate change has been attributed to the increasing occurrence of anchovy in the Baltic and North Seas, due to increasing water temperatures (Alheit *et al*., 2012; Montero-Serra *et al*., 2015).

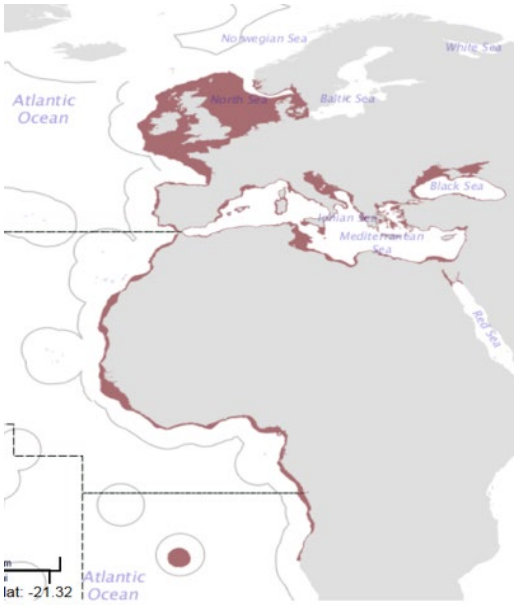


Figure 1.29: Geographical Distribution of adult Anchovy (FAO 2014).

Spawning season

The anchovy spawning period begins with the warming of surface waters and associated thermal stratification of the water column (Motos, 1996). The spawning season varies depending on the areas (table 1.9), but mainly occurs from March to November with peaks usually in summer. Eggs are mainly found at 17 – 23°C (Palomera *et al*., 2007). Anchovies are oviparous and have ellipsoid planktonic eggs.

Table 1.9 Spawning season and optimal temperatures for anchovy (*Engraulis encrasicolus*) in Mediterranean and Eastern Atlantic waters (modified from Zarrad *et al*., 2006).

Area	Temperature (ºC)	Spawning season	Spawning peak	Reference
Bay of Biscay	14-18	March-August	May-June	Motos *et al.* (1996)
Western Portugal	15.5-19.5	March-November	April	Ré (1996)
NW Mediterranean	15-20	April-October	May-July	Palomera (1992)
NW Mediterranean	15-22	April-October	June-August	García and Palomera (1996)
Alboran Sea	19-23	March-November	August	Rodríguez and Rubín (1986) Rodríguez (1990)
Ligurian and Tyrrherian Seas	-	May-September	July	Albertelli *et al.* (1988)
Adriatic Sea	17-22	April-October	May-August	Regner (1996)
Gulf of Tunis	16-25	February-October	April-August	Zarrad *et al.* (2006)
Gulf of Cadiz	-	March-November	July-August	Baldó *et al.* (2006)
North Sea	-	June-September		Munk and Nielsen (2005)

Description of eggs and larvae

Eggs

- Pelagic, ovoid shape – therefore anchovy eggs can be immediately told from other fish eggs
- Diameter: 1.2 – 1.9 mm x 0.5-1.2 mm (Rodríguez *et al.*, 2017)
- Smooth chorion and small perivitelline space.
- Segmented yolk with no oil globule.

Yolk sac larvae

- 3.3 – 4.0 mm (Rodríguez *et al.*, 2017).
- Elongated yolk sac which stretches almost to the anus (this characteristic and the absence of an oil globule discriminates from yolk–sac larvae of other clupeoids, e.g. *Sardina pilchardus* and *Sardinella aurita*).
- Unpigmented, non-functional eyes until yolk is absorbed at a length of about 5 mm.
- Unpigmented body

Preflexion stage

- Body elongate and slender.
- Gut is relatively shorter than that of other clupeoid species like *Sardina pilchardus*, *Clupea harengus, Sprattus sprattus.*
- Head length more than 20 % of total length and relatively larger than other clupeoid species.
- Dorsal fin above anus.
- The earliest stages of *E. encrasicolus* are distinguishable from clupeids (*S. pilchardus*, *S. sprattus*, *C. harengus* and *Alosa sp.)* by the conspicuously different pigmentation: a few groups of melanophores vs. rows of melanophores.

Post Flexion Stage

- The head has a characteristic rounded shape with inferior mouth.
- The length of the tail is one-third of total length, and the dorsal and anal fins are overlapping. There are 46-48 vertebrae.

Obvious distinguishing characters (especially vs. *Sardina pilchardus*)

- The posterior part of the dorsal fin overlaps the anal fin (figure 1.30).
- The long head, which at this stage is about one-fifth of the body length.
- The early appearance of the swimbladder (at around 11 mm TL).
- The shorter gut and longer tail relative to all other clupeoid larvae of the area.

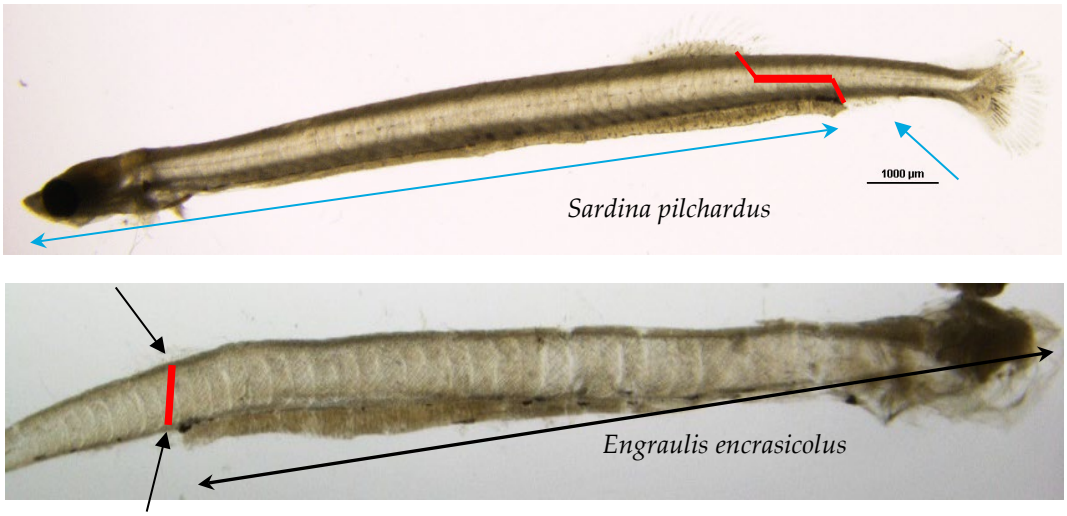


Figure 1.30: Pictures showing the position of dorsal fins and anal fins in sardine (*S. pilchardus*) and anchovy (*E. encrasicolus*) larvae.

The characteristic lengths at the different developmental stages of anchovy are given in Table 1.10.

Table 1.10 Typical total lengths (TL) of the various developmental stages of anchovy larvae.

Hatching length	3.0 - 4.0 mm
Yolk-sac absorption	5.0 mm
Dorsal fin starts development	6.0 mm
Notochord flexion	9.0 - 10.0 mm
Caudal and anal fin development starts	9.0 mm
Swimbladder develops	11 mm
Pelvic fin development level of pylorus	15 mm
Fully formed fins	20 mm
Transformation length	around 25 mm
Metamorphosis and scale formation	35.0 - 40.0 mm

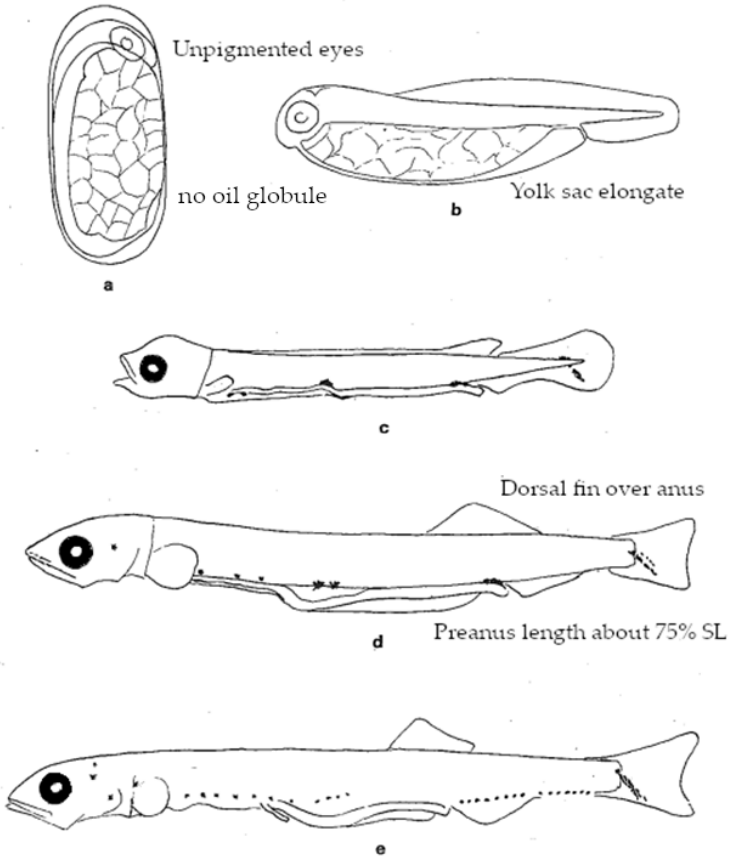


Figure 1.31 Egg and larval stages of anchovy (*Engraulis encrasicolus*) modified from Russell (1976); (a) Egg, 1.5 mm x 0.66 mm; (b) Larva, 3.2 mm; (c, d, e) Postlarva, 6.0, 11.0 mm, 19.0 mm

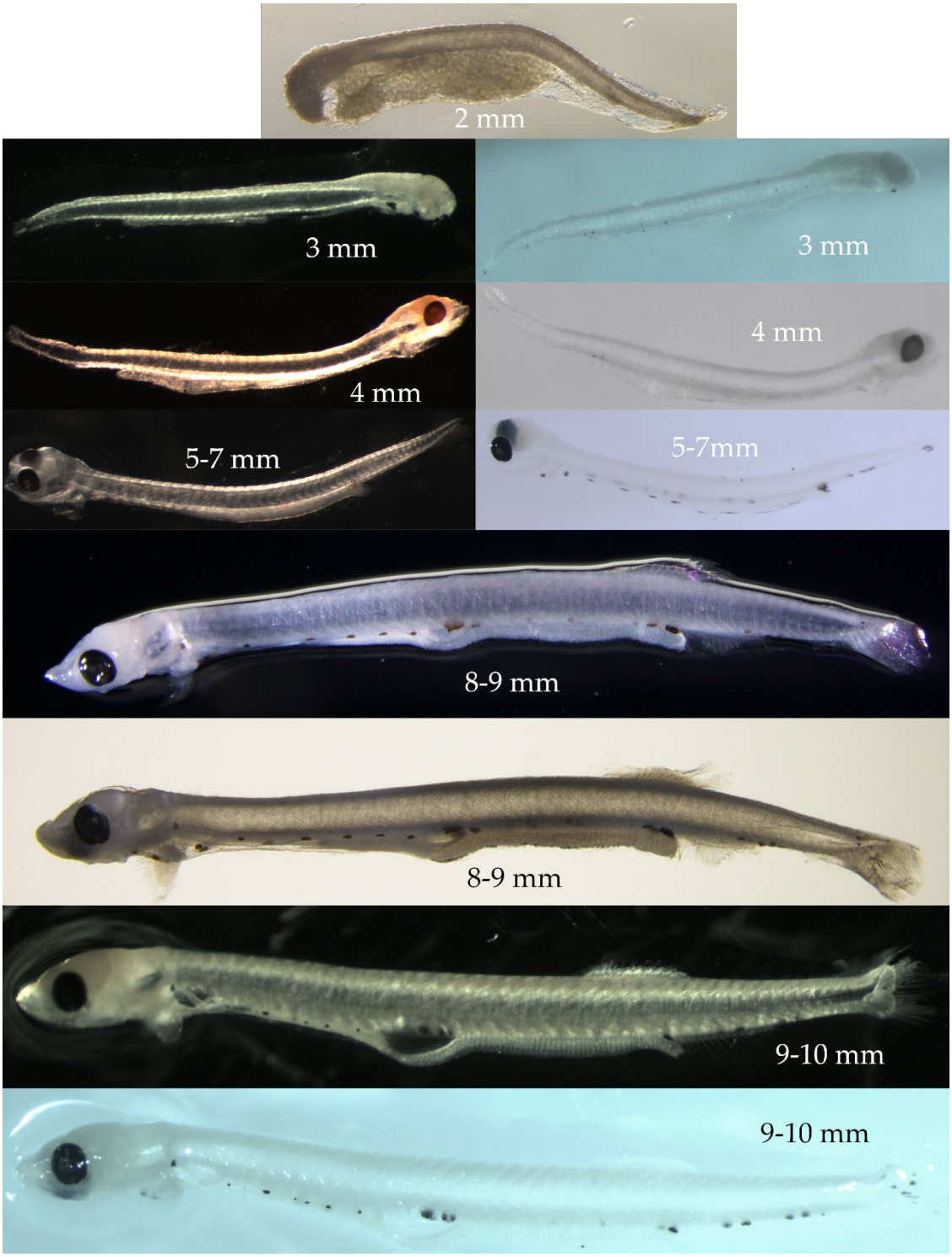


Figure 1.32 Images of larval stages of anchovy *(Engraulis encrasicolus*) with standard length given.

2 Clupeoid larvae identification trials using SmartDots (ToR a)

The SmartDots web application was originally designed by cooperation between ICES, ILVO, DTU-Aqua and IMR to aid maturity and age reading exchange, training and workshop events. Currently, its further development is facilitated through WGBIOP and WGSMART. When it became clear, that a video conference was planned to partly replace the 2020 physical WKIDCLUP2 meeting, it became desirable that SmartDots would be adapted to also aid ichthyoplankton identification events based on microscopic images of fish eggs and/or larvae. Scientists from DTU-Aqua, Denmark, WMR, the Netherlands, and in particular the ICES datacentre were involved to adapt SmartDots to the WKIDCLUP2 event. The following modifications were made to the application.

For the organizer of an event:

- The organizer was enabled to set a scale to each of the microscopic images enabling participants to undertake direct measurements on the larvae, e.g. of total length, standard length or head length

For the participants the following annotations were enabled

- Select the species name from a dropdown menu
- Counting myotomes of either the trunk or between pylorus and pelvic fin directly in an image by setting dots
- Measuring total, standard or head length of a larvae by creating poly-lines in an image.
- Making a comment

Based on feedback from participants in the first 2020 meeting, the SmartDots application was further developed, minor bugs eliminated and prepared for the postponed full meeting in August/September 2021.

2.1 Setting up for the 2020 WebEx meeting

Prior to the first 2020 meeting, 131 images of 60 larvae were uploaded to the SmartDots server and a scale was set to each of the images.

Species composition of the 60 larval samples was as follows

Herring, *Clupea harengus*: 13 larvae

Sprat, *Sprattus sprattus*: 12 larvae

Sardine, *Sardina pilchardus*: 14 larvae

Anchovy, *Engraulis encrasicolus*: 14 larvae

Other species with similar appearance to clupeid larvae, 7 specimens: 1 lesser Argentine, *Argentina sphyraena*, 1 Crystal goby, *Crystallogobius linearis*, 5 Sandeel, Ammodytidae gen. sp.

2.2 Setting up for the 2021 meeting

For the 2021 meeting, two SmartDots events were created in order to emulate the approved trial, analysis, retrial method, which is normally utilized during such identification events examining real samples under microscopes.

For both trials, 120 larvae were chosen with up to 3 images per each individual larva, each of the image either taken at different illumination (transmitted, dark-field, polarized or top-light illumination) or magnification, depending on which aided successful identification best. This resulted in 265 for the first and 306 images for the second round to be uploaded to SmartDots.

For both trials there were

Herring, *Clupea hare*ngus: 26 larvae

Sprat, *Sprattus sprattus*: 26 larvae

Sardine, *Sardina pilchard*us: 26 larvae

Anchovy, *Engraulis encrasicolu*s: 26 larvae

In addition to these, 16 larvae of non-clupeoid species with a similar appearance as clupeoids were used. These non-clupeoids were for the first trial: 5 Crystal gobies, 2 lesser argentines, 3 Ammodytidae gen. sp., 4 Greater Sandeel, *Hyperoplus lanceolatus* and 2 Sandeel, *Ammodytes tobianus*. For the second trial the 16 non-clupeoid consisted of: 5 Crystal gobies, 1 lesser argentine, 4 Ammodytidae gen. sp., 4 Greater Sandeel, and 2 Sandeel.

With the images, information was given on time and area of catch of the larvae for all 3 events.

All participants had at least to make an annotation in the species identification field, i.e. determine the species, either specifically one of the 4 clupeoid species or “other” for non-clupeoid larvae.

See also Annex 4 for a short report on the SmartDots beta for ichthyoplankton identification.

3 Larvae identification results (ToR a)

3.1 Results of the larvae identification trial on SmartDots.

Once the results were available from every participant from the SmartDots site, these were down-loaded and analysed. The original assessment of species identification for each larva, by each partic-ipant, was entered into a primary result table (not presented here) and compared with the validated (identifications done by experts) species.

The summaries of the results from each round on clupeoid larval species determination are presented in Tables 3.1, 3.2 and 3.3. The tables are divided into four sub-tables labelled A-D, where the performance of each participant is judged against the actual correct species identification.

Sub-table A shows the number of larvae of each species that were assessed by each participant (i.e. the number of larvae which the participant should actually have found per species). The numbers of each species will therefore be the same for all participants that read all the larvae.

Sub-table B shows the numbers of larvae of each species as actually annotated by each participant to the different larval image samples.

Sub-table C shows the over- or underestimation of each participant per species.

Sub-table D shows the percentage agreement in species identification between the assessment of each participant and the actual species.

After each identification trial, the results were presented to the participants and the features which aided clupeoid larvae identification were discussed. From the SmartDots Server, images of larvae of all clupeoid species were shown on the shared screen and identification characteristics were dis-cussed.

Larval features change with size and after the discussion as well as the analysis of the SmartDots results, it became clear that only very few of the participants were measuring the larval length. Al-so, not all did myotome counts, even though this is a crucial technique for identification in many specimens of clupeoid larvae. Both, measuring and myotome counting, was possible with SmartDots, and participants were instructed how to do this on their screens.

Results of the 2020 SmartDots event

The agreement among all participants for all species was 81.7 %, which was an increase of more the 25 %-points compared to 2014. The agreement for herring larvae was 86 %, for sprat 80 %, for sar-dine 86 % and for anchovy 71 %. All values are higher than observed in 2014. Except for two partic-ipants, all readers achieved agreement rates of more than 70 % with the actual species. Agreement rates of at least 90 % was reached by six participants, contrasting to the 2014 workshop, where none of the participants reached 80 % agreement.

In only 3 specimens, less than 50 % of readers misidentified the species. Two thirds (40 larvae, 66.7 %) of all specimens were correctly identified by at least 80 % of all readers. Since no self-evaluation was done by the different readers on whether they were experienced or unexperienced, results were not analysed with respect to this characteristic.

Table 3.1 Species identification round during the add-on event in 2020, 60 fish larvae. The species compositions based on actual species reflecting the best esti-mates based on only those larvae that were used for species identification by the participant (A), the species compositions as obtained per participant (B), the percent-age over- or underestimation (C) and the percentages agreement with actual species (D) are shown per species by participant and for the whole group that took part in the species identification exercise on fish larvae. A weighted mean percent agreement is given by participant and all participants combined.

A **Species compositions using actual, validated species (second last column input table)**

actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	TOTAL
Herring	1	13	13	13	11	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	297
Sprat	2	12	10	12	5	12	12	12	12	11	12	12	12	10	12	12	12	12	12	12	12	12	12	12	264
Sardine	3	14	14	14	7	14	13	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	314
Achovy	4	14	13	14	7	14	13	14	14	13	14	14	14	8	14	14	14	14	14	14	14	14	14	14	306
Other	5	7	6	7	3	7	5	7	7	7	7	7	7	7	7	7	7	7	7	6	7	7	7	7	153
Total		60	56	60	33	60	56	60	60	58	60	60	60	52	60	60	60	60	60	59	60	60	60	60	1334

B **Species compositions as estimated per participant and whole group**

Species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	TOTAL
Herring	1	11	13	15	12	18	14	14	14	12	13	10	12	11	16	13	12	11	12	13	13	14	11	11	295
Sprat	2	8	9	15	8	4	10	11	12	10	16	12	10	14	9	11	16	14	15	13	12	11	10	15	265
Sardine	3	15	16	13	7	15	19	18	17	16	15	13	16	13	14	13	11	15	11	18	13	12	16	12	328
Anchovy	4	12	14	10	5	12	8	11	9	12	8	13	13	5	14	11	15	13	6	10	14	15	14	6	250
Other	5	14	4	7	1	11	5	6	8	8	8	12	9	9	7	12	6	7	16	5	8	8	9	16	196
Total	1-5	60	56	60	33	60	56	60	60	58	60	60	60	52	60	60	60	60	60	59	60	60	60	60	1334

C **Percentage overestimation / underestimation**

actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	ALL
Herring	1	-15%	0%	15%	9%	38%	8%	8%	8%	-8%	0%	-23%	-8%	-15%	23%	0%	-8%	-15%	-8%	0%	0%	8%	-15%	-15%	-1%
Sprat	2	-33%	-10%	25%	60%	-67%	-17%	-8%	0%	-9%	33%	0%	-17%	40%	-25%	-8%	33%	17%	25%	8%	0%	-8%	-17%	25%	0%
Sardine	3	7%	14%	-7%	0%	7%	46%	29%	21%	14%	7%	-7%	14%	-7%	0%	-7%	-21%	7%	-21%	29%	-7%	-14%	14%	-14%	4%
Anchovy	4	-14%	8%	-29%	-29%	-14%	-38%	-21%	-36%	-8%	-43%	-7%	-7%	-38%	0%	-21%	7%	-7%	-57%	-29%	0%	7%	0%	-57%	-18%
Other	5	100%	-33%	0%	-67%	57%	0%	-14%	14%	14%	14%	71%	29%	29%	0%	71%	-14%	0%	129%	-17%	14%	14%	29%	129%	28%

D **Percentage agreement in species identification per species**

actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	ALL
Herring	1	69%	92%	92%	55%	85%	100%	100%	85%	77%	92%	77%	92%	77%	92%	100%	85%	85%	77%	77%	92%	100%	85%	77%	86%
Sprat	2	50%	80%	92%	40%	25%	67%	83%	67%	73%	92%	83%	83%	90%	75%	92%	100%	100%	92%	75%	100%	83%	83%	83%	80%
Sardine	3	79%	93%	79%	43%	86%	100%	100%	100%	79%	100%	71%	100%	79%	86%	86%	71%	100%	71%	93%	86%	86%	100%	64%	86%
Anchovy	4	57%	92%	71%	0%	71%	62%	71%	64%	85%	50%	57%	93%	50%	100%	64%	86%	93%	43%	57%	93%	93%	93%	43%	71%
Other	5	100%	67%	100%	0%	100%	100%	86%	100%	100%	71%	86%	100%	100%	100%	100%	86%	100%	100%	50%	100%	100%	100%	100%	92%
Weighted mean	1-5	68.3%	87.5%	85.0%	33.3%	71.7%	83.9%	88.3%	81.7%	81.0%	81.7%	73.3%	93.3%	78.8%	90.0%	86.7%	85.0%	95.0%	73.3%	72.9%	93.3%	91.7%	91.7%	70.0%	81.7%
RANKING		22	8	10	23	20	12	7	13	15	13	17	2	16	6	9	10	1	17	19	2	4	4	21	

Table 3.2 Species identification round 1 during WKIDCLUP 2 in 2021, 120 fish larvae. The species compositions based on actual species reflecting the best esti-mates based on only those larvae that were used for species identification by the participant (A), the species compositions as obtained per participant (B), the percent-age over- or underestimation (C) and the percentages agreement with actual species (D) are shown per species by participant and for the whole group that took part in the species identification exercise on fish larvae. A weighted mean percent agreement is given by participant and all participants combined.

A

Species compositions using modal/actual species (second last column input table)

actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	TOTAL
Herring	1	7	13	10	5	26	25	26	25	21	26	17	24	26	2	26	6	22	26	26	1	14	26	26	5	**431**
Sprat	2	9	13	9	6	22	21	23	22	21	22	24	21	26	1	22	6	19	22	23	5	13	22	22	9	**403**
Sardine	3	8	13	11	4	24	23	25	20	22	24	22	25	26	-	25	5	19	25	24	2	12	24	25	6	**414**
Anchovy	4	3	10	18	-	26	9	26	23	23	26	17	22	26	-	26	-	13	26	26	-	15	26	26	2	**389**
Other	5	5	10	14	2	16	16	16	16	16	16	16	16	16	-	16	3	15	16	16	3	10	16	16	6	**292**
Total	1-5	**32**	**59**	**62**	**17**	**114**	**94**	**116**	**106**	**103**	**114**	**96**	**108**	**120**	**3**	**115**	**20**	**88**	**115**	**115**	**11**	**64**	**114**	**115**	**28**	**1929**

B

Species compositions as estimated per participant and whole group

	Species	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	TOTAL
Herring	1	6	11	12	4	17	36	39	28	32	34	24	17	29	2	33	4	22	23	37	1	17	29	26	5	**488**
Sprat	2	6	10	5	4	26	17	14	14	25	21	22	17	20	-	21	9	14	25	25	3	9	14	17	6	**344**
Sardine	3	9	11	11	7	29	24	24	24	13	30	21	34	32	1	26	3	21	26	21	4	12	21	25	7	**436**
Anchovy	4	-	12	19	1	26	-	19	21	24	20	13	23	18	-	19	2	8	27	4	-	13	34	28	3	**334**
Other	5	11	15	15	1	16	17	20	19	9	9	16	17	21	-	16	2	23	14	28	3	13	16	19	7	**327**
Total	1-5	**32**	**59**	**62**	**17**	**114**	**94**	**116**	**106**	**103**	**114**	**96**	**108**	**120**	**3**	**115**	**20**	**88**	**115**	**115**	**11**	**64**	**114**	**115**	**28**	**1929**

C

Percentage overestimation / underestimation

actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	ALL
Herring	1	-14%	-15%	20%	-20%	-35%	44%	50%	12%	52%	31%	41%	-29%	12%	0%	27%	-33%	0%	-12%	42%	0%	21%	12%	0%	0%	**13%**
Sprat	2	-33%	-23%	-44%	-33%	18%	-19%	-39%	-36%	19%	-5%	-8%	-19%	-23%	-	-5%	50%	-26%	14%	9%	-40%	-31%	-36%	-23%	-33%	**-15%**
Sardine	3	13%	-15%	0%	75%	21%	4%	-4%	20%	-41%	25%	-5%	36%	23%	-	4%	-40%	11%	4%	-13%	100%	0%	-13%	0%	17%	**5%**
Anchovy	4	-	20%	6%	-	0%	-	-27%	-9%	4%	-23%	-24%	5%	-31%	-	-27%	-	-38%	4%	-85%	-	-13%	31%	8%	50%	**-14%**
Other	5	120%	50%	7%	-50%	0%	6%	25%	19%	-44%	-44%	0%	6%	31%	-	0%	-33%	53%	-13%	75%	0%	30%	0%	19%	17%	**12%**

D

Percentage agreement in species identification per species

actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	Reader 23	Reader 24	ALL
Herring	1	71%	62%	80%	80%	58%	88%	92%	88%	90%	77%	88%	67%	81%	100%	88%	67%	82%	69%	88%	100%	100%	92%	77%	80%	**81%**
Sprat	2	56%	23%	44%	67%	86%	48%	52%	55%	67%	27%	67%	52%	62%	0%	41%	100%	58%	82%	48%	60%	62%	50%	59%	33%	**56%**
Sardine	3	88%	15%	73%	100%	83%	65%	76%	80%	36%	67%	73%	88%	85%	-	64%	60%	63%	68%	58%	100%	67%	42%	76%	50%	**67%**
Anchovy	4	0%	60%	89%	-	92%	0%	73%	87%	61%	54%	71%	86%	69%	-	65%	-	46%	96%	15%	-	80%	100%	96%	100%	**72%**
Other	5	100%	100%	100%	50%	94%	100%	100%	94%	56%	50%	94%	100%	100%	-	88%	33%	100%	88%	94%	100%	100%	100%	100%	100%	**91%**
Weighted mean	1-5	**68.8%**	**49.2%**	**80.6%**	**76.5%**	**81.6%**	**67.0%**	**77.6%**	**80.2%**	**62.1%**	**56.1%**	**77.1%**	**77.8%**	**77.5%**	**66.7%**	**68.7%**	**70.0%**	**70.5%**	**80.0%**	**58.3%**	**81.8%**	**81.3%**	**76.3%**	**80.9%**	**64.3%**	**72.5%**
	RANKING	**16**	**24**	**5**	**12**	**2**	**18**	**9**	**6**	**21**	**23**	**11**	**8**	**10**	**19**	**17**	**15**	**14**	**7**	**22**	**1**	**3**	**13**	**4**	**20**	

Table 3.3 Species identification round 2 during WKIDCLUP 2 in 2021, 120 fish larvae. The species compositions based on actual species reflecting the best esti-mates based on only those larvae that were used for species identification by the participant (A), the species compositions as obtained per participant (B), the percent-age over- or underestimation (C) and the percentages agreement with actual species (D) are shown per species by participant and for the whole group that took part in the species identification exercise on fish larvae. A weighted mean percent agreement is given by participant and all participants combined.

A **Species compositions using modal/actual species (second last column input table)**

actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	TOTAL
Herring	1	26	23	15	26	26	26	19	26	24	26	26	21	26	10	22	26	26	15	26	26	26	26	**513**
Sprat	2	24	21	13	26	26	26	19	26	25	26	26	23	26	8	21	26	26	13	26	26	26	26	**505**
Sardine	3	23	22	15	26	26	26	22	26	25	26	26	23	26	12	23	26	26	14	26	26	26	26	**517**
Anchovy	4	26	22	1	26	24	26	14	26	25	26	26	19	26	7	23	26	26	8	26	26	26	26	**481**
Other	5	16	12	4	16	16	16	15	16	16	16	16	12	16	7	16	16	16	16	16	16	16	16	**322**
Total	1-5	**115**	**100**	**48**	**120**	**118**	**120**	**89**	**120**	**115**	**120**	**120**	**98**	**120**	**44**	**105**	**120**	**120**	**66**	**120**	**120**	**120**	**120**	**2338**

B **Species compositions as estimated per participant and whole group**

Actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	TOTAL
Herring	1	24	24	15	28	25	23	18	32	23	22	27	18	24	10	17	24	27	13	30	24	25	24	**497**
Sprat	2	23	23	18	26	26	29	23	33	27	26	35	29	32	9	32	29	26	19	33	30	31	34	**593**
Sardine	3	21	23	10	23	31	24	21	27	27	32	18	25	22	13	22	21	25	10	19	13	21	19	**467**
Anchovy	4	25	12	2	26	10	27	13	18	20	23	22	22	26	6	15	29	23	8	22	37	27	27	**440**
Other	5	22	18	3	17	26	17	14	10	18	17	18	4	16	6	19	17	19	16	16	16	16	16	**341**
Total	1-5	**115**	**100**	**48**	**120**	**118**	**120**	**89**	**120**	**115**	**120**	**120**	**98**	**120**	**44**	**105**	**120**	**120**	**66**	**120**	**120**	**120**	**120**	**2338**

C **Percentage overestimation / underestimation**

actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	ALL
Herring	1	-8%	4%	0%	8%	-4%	-12%	-5%	23%	-4%	-15%	4%	-14%	-8%	0%	-23%	-8%	4%	-13%	15%	-8%	-4%	-8%	**-3%**
Sprat	2	-4%	10%	38%	0%	0%	12%	21%	27%	8%	0%	35%	26%	23%	13%	52%	12%	0%	46%	27%	15%	19%	31%	**17%**
Sardine	3	-9%	5%	-33%	-12%	19%	-8%	-5%	4%	8%	23%	-31%	9%	-15%	8%	-4%	-19%	-4%	-29%	-27%	-50%	-19%	-27%	**-10%**
Anchovy	4	-4%	-45%	100%	0%	-58%	4%	-7%	-31%	-20%	-12%	-15%	16%	0%	-14%	-35%	12%	-12%	0%	-15%	42%	4%	4%	**-9%**
Other	5	38%	50%	-25%	6%	63%	6%	-7%	-38%	13%	6%	13%	-67%	0%	-14%	19%	6%	19%	0%	0%	0%	0%	0%	**6%**

D **Percentage agreement in species identification per species**

actual species		Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15	Reader 16	Reader 17	Reader 18	Reader 19	Reader 20	Reader 21	Reader 22	ALL
Herring	1	88%	78%	100%	92%	77%	88%	84%	73%	92%	85%	100%	38%	81%	90%	77%	92%	77%	87%	92%	92%	96%	88%	**85%**
Sprat	2	79%	76%	100%	85%	54%	100%	84%	62%	92%	73%	100%	30%	92%	63%	90%	100%	62%	92%	85%	100%	100%	100%	**83%**
Sardine	3	65%	77%	67%	69%	69%	81%	68%	65%	84%	81%	69%	43%	81%	75%	70%	77%	50%	64%	73%	46%	77%	65%	**69%**
Anchovy	4	81%	55%	100%	100%	42%	96%	71%	54%	76%	88%	85%	47%	100%	86%	65%	100%	88%	88%	85%	100%	100%	96%	**82%**
Other	5	100%	100%	75%	100%	100%	100%	93%	63%	100%	100%	100%	17%	100%	86%	100%	100%	100%	100%	100%	100%	100%	100%	**94%**
Weighted mean	1-5	**81.7%**	**75.0%**	**87.5%**	**88.3%**	**66.1%**	**92.5%**	**79.8%**	**63.3%**	**87.8%**	**84.2%**	**90.0%**	**36.7%**	**90.0%**	**79.5%**	**79.0%**	**93.3%**	**73.3%**	**86.4%**	**85.8%**	**86.7%**	**94.2%**	**89.2%**	**81.7%**
	RANKING	**14**	**18**	**9**	**7**	**20**	**3**	**15**	**21**	**8**	**13**	**4**	**22**	**4**	**16**	**17**	**2**	**19**	**11**	**12**	**10**	**1**	**6**	

Results of the first SmartDots event in 2021 – WKIDCLUP2 trial 1

The agreement among all participants for all species for the first identification round in 2021 was 72.5 %, which was lower than 2020 but still better than compared to 2014. The agreement for herring larvae was 81 %, for sprat 56 %, for sardine 67 % and for anchovy 72 %. All values are higher than observed in 2014. Except for two participants, all readers achieved agreement rates of more than 70 % with the actual species. Agreement rates of at least 80 % was reached by seven participants

In 28 specimens (23.3 %), more than 50 % of readers misidentified the species. 46% (55 larvae) of all specimens were correctly identified by at least 80 % of all readers.

Results of the second SmartDots event in 2021 – WKIDCLUP2 trial 2

The 22 readers participating in the second 2021 trial reached an overall agreement of 81.7 %, which is an improvement compared to the first round, equalling the result of the pilot event in 2020 but at a doubled number of samples. In all species, agreement with the correct identification was improved compared to round 1 and reached 85 % in herring, 83 % in sprat, 69 % in sardine, 82 % in anchovy and 94 % in non-clupeoids. Only 4 specimens (3.3 %) were misidentified by more than 50 % of the readers and a much as 79 specimens (65.8 %) were identified correctly by at least 80 % of the readers.

4 Sources of misidentification (ToR c)

SmartDots allows for extracting not only identification results, but also of results of measuring and counting. Especially the myotome counting results show that this is a prominent source of misidentification particularly in clupeid (herring, sprat or sardine) larvae (Figures 4.1, 4.2 and 4.3). In engraulids, the myotome count is not relevant because correct species identification relies on other characteristics such as position of anal and dorsal fins relative to each other or size of head, or pigmentation.

Those specimens of clupeid larvae with some of the lowest agreements in correct identification also had the highest variability of myotome counts.

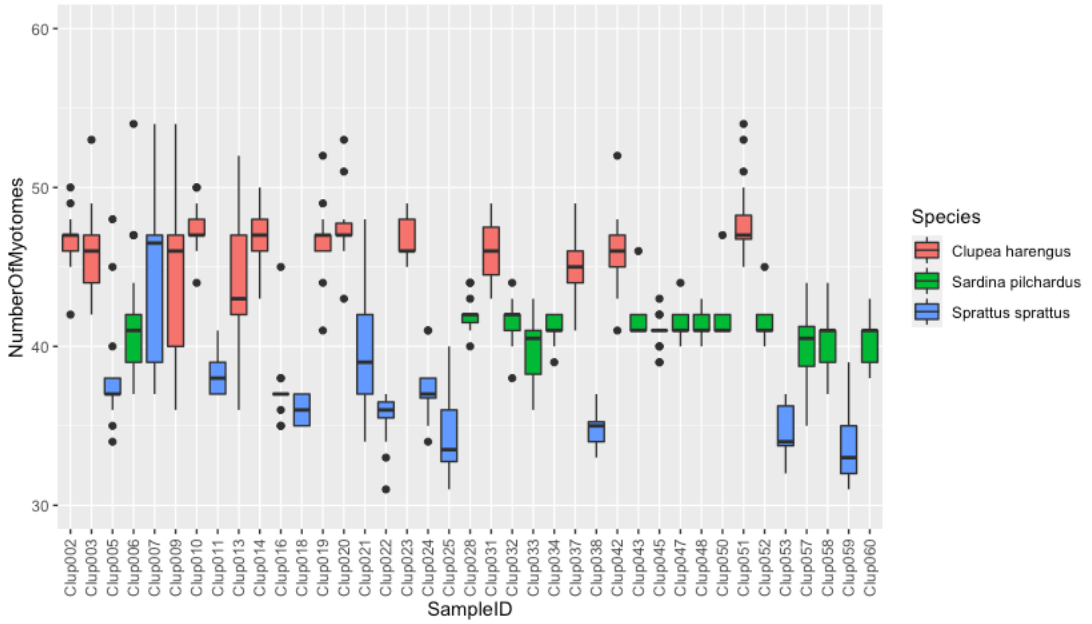


Figure 4.1 Box Plot on the variability of myotome counts in the different specimens of clupeid (herring, sprat and sardine) larvae by validated species determined during the add-on even of WKIDCLUP2 in 2020. Anchovy and other larvae were excluded from the analysis.

In the single event of 2020, the specimens with the IDs Clup007, Clup009, Clup013 and Clup021 had myotome count results between 34 and > 50, which would match meristics of all possible clupeid species (Figure 4.1). Consequently, annotations by participants contained all 3 possible clupeid species, herring, sprat and sardine, as well as anchovy. Herring (for Clup009 and Clup013) and sprat (Clup007 and Clup021) would have been the 2 only correct results for the 4 specimens.

In the first event of the 2021 workshop, the confusion was mostly between sprat and sardine, where either the participants counted too low for sardine or too high for sprat (Figure 4.2). These results clearly illustrate the major confounding issues in mytome counting, which could lead to erroneous identification results: where to start and end counting, and how to discriminate between true and false myosepts in order to distinguish between two adjoining myotomes. These issues were discussed thoroughly after the first trial in 2021 and participants were made aware of what was to be considered for myotome counting.

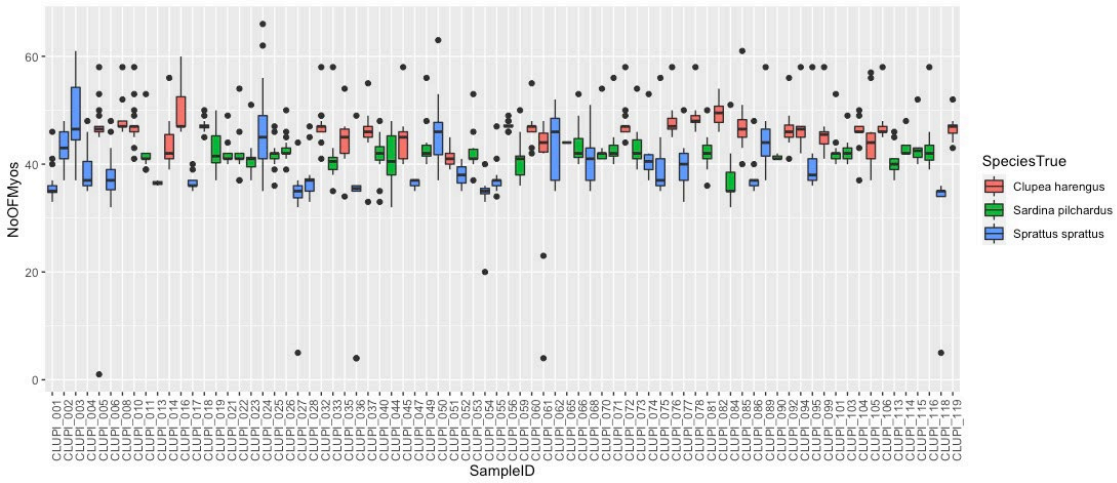


Figure 4.2 Box Plot on the variability of myotome counts in the different specimens of clupeid (herring, sprat and sardine) larvae by validated species, determined during round 1 of WKIDCLUP 2 in 2021. Anchovy and other larvae were excluded from the analysis.

Consequently, during the second round, when also agreement in correct species identification was increased, erroneous counts for the different valid species were much less evident than during the first round (Figure 4.3).

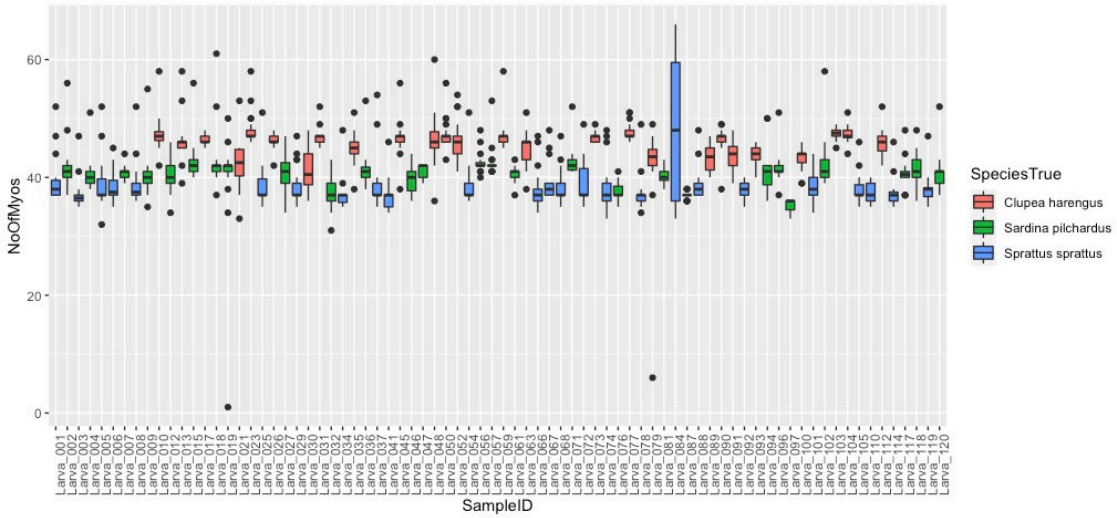


Figure 4.3 Box Plot on the variability of myotome counts in the different specimens of clupeid (herring, sprat and sardine) larvae by validated species, determined during round 2 of WKIDCLUP 2 in 2021. Anchovy and other larvae were excluded from the analysis.

During such an online event, when participants are only able to conduct species identification on images instead of real samples under a microscope, image quality will, of course, be another error source in correct species identification. Also, apart from choosing between a limited number of images taken at different illuminations and magnifications, participants had no chance to play with focus, lighting and magnification of the samples as they usually do. These facts may potentially confound correct discrimination of myotomes and, consequently, their correct enumeration.

5 Larvae identification error matrix (ToR c)

Uncertainty in clupeoid larvae identification can be quantified by an error matrix (EM). The elements of an EM are the probabilities that a sampled larva of a validated species *a* is assigned to one of the 4 clupeoid target species. For the majority of the larvae in this workshop the validated species was the visual identification from the individual providing the larvae (or larvae image) for this workshop. Before adding the larvae to the exercise, the species was checked by the organisers of the workshop. A few herring larvae came from fertilization experiments. 'True species' can be gained from fertilization experiments, but these are time and cost consuming. Also, it can be difficult to fertilize eggs and keep them alive until larvae hatch and after that it needs an expert to keep the larvae alive through the first feeding stage and onto metamorphosis.

5.1 Data on larvae identification uncertainty

During the full 2021 workshop, 120 images of larvae were available for both identification rounds. For the clupeoids, 26 images of each species were available (Table 5.1). For the group of other species 16 images were available. For various reasons, not all readers were able to identify all larvae.

Table 5.1. Number of images per species for each identification exercise.

Species	N images 1st round	N images 2nd round
Herring	26	26
Sprat	26	26
Sardine	26	26
Anchovy	26	26
Other	16	16

Of the 24 participants in the workshop not all readers provide larvae data for the assessments. The participants were divided in a group of experts, that provide data for assessments, and other participants. Of the 24 participants 8 were expert readers.

5.2 Matrix

For the construction of the error matrices only experts' readings were included. The only species, however, for which larvae data are used in the assessment, is currently only herring.

Table 5.2. Larvae identification error matrix based on the first identification exercise.

	Observed species				
Actual species	**Herring**	**Sprat**	**Sardine**	**Anchovy**	**Other**
Herring	0.80	0.04	0.16	0.00	0.00
Sprat	0.17	0.58	0.18	0.03	0.04
Sardine	0.11	0.12	0.70	0.05	0.02
Anchovy	0.01	0.14	0.05	0.69	0.11
Other	0.01	0.00	0.00	0.03	0.96

During the first round, apart from "other" species, the only species with a satisfactory identification fidelity was herring – 80 % of all validated herring larvae were identified as herring, while 4 % were identified as sprat, and 16 % as sardine. Only 70 % of all validated sardine larvae were correctly identified, while 11 % were called herring, 12 % sprat, 5 % anchovy, and 2 % something else. Of all validated anchovy, 69 % were correctly identified as anchovy. The worst results were achieved in sprat, where only 58 % of the individuals were correctly identified, 17 % were named herring, 18 % sardine, 3 % anchovy and 4 % other, non-clupeoid species.

Table 5.3: Larvae identification error matrix based on the second identification exercise.

	Observed species				
Actual species	**Herring**	**Sprat**	**Sardine**	**Anchovy**	**Other**
Herring	0.87	0.04	0.07	0.00	0.00
Sprat	0.02	0.87	0.10	0.00	0.00
Sardine	0.05	0.19	0.69	0.05	0.01
Anchovy	0.00	0.05	0.03	0.88	0.03
Other	0.00	0.00	0.00	0.00	1.00

The second round brought, after the major distinguishing features and how to identify them were discussed, improvement in most of the species. Only in sardine, which was in most cases confused with the very similar sprat, the results remained almost unchanged.

It has to be noted that the only species, however, for which larvae data are currently used in the assessment, is herring. Two of those surveys are conducted in the North Sea, the third in the Baltic. For the Baltic survey – The Rügen Herring Larvae Survey – the error probability can be considered as negligible. This survey is carried out in a rather confined area, where the occurrence of other clupeoid larvae can be ruled out almost completely.

In the North Sea, the International Herring Larvae Survey aims at recently hatched herring larvae in order to provide abundance indices for the autumn and winter spawning stock components of North Sea herring. Also, here a biased result can be ruled out almost completely, since sprat and sardine larvae of the same size will be very rare and will also be in a further developed stage. In the MIK survey, which provides an abundance index for autumn spawned pre-recruits of

North Sea herring, the recent years have shown that the occurrence of large sardine larvae in the catches could potentially provide a substantial source of error in the index estimation. However, only 5 % of the actual, validated sardine larvae were misidentified as herring, while 7 % of validated herring larvae were misidentified as sardine.

6 Standardization of sample processing and data reporting for clupeoid larvae (ToR d)

At the beginning of the workshop, all participants were asked to fill in a table on sample processing methods. Participants were requested to provide information on the following subjects:

Subjects and instructions for filling in the table

Country: name of country of the survey participant

Institute: name or acronym of participating institute

Survey: name and/or acronym of the survey

ICES area: area code

Target species: the name(s) of the target species of the survey

Non-target clupeoids: name(s) of any clupeoid species for which data are generated and which is/are not target of the survey

Survey purpose: the purpose of the survey w.r.t. the target species

Assessment group; relevant survey output: The ICES assessment group and the provided survey output (index) for assessment

Gear: the acronym of the gear used for catching the larvae. Preferably using ICES vocabulary

Gear deployment: mode of deployment of gear (e.g. vertical, horizontal, double-oblique) Preferably using ICES vocabulary

Mesh (μm): The mesh width of the net used for the catches in μm

Codend mesh (μm): mesh width of the codend if different from the latter

Location of clupeoid larvae sorting and identification: where samples are sorted and larvae identified – on board or in the lab

Fish larvae sorting and processing (fresh/preserved): is sorting and processing of larval sample done on fresh or preserved samples

Subsampling and method: is subsampling regularly applied and which method is chosen (e.g. Folsom splitter, other type of splitter, subsampling by weight, numbers). Some free text is allowed here

Identification of larvae: visual or molecular/genetic

Identification method: visual methods: micro- (with) or macroscopic (without a microscope) on the real sample of on an image of the sample maybe aided by image analysis. Genetic: barcoding, metabarcoding, MALDI-TOF…

Measurements: Counts, Weights, Lengths

Measured lengths (TL,SL,NL), smallest unit: total, standard or notochord length, smallest unit e.g. 1 mm, 0.1 mm, 0.5 mm…

Method of measurement: micro- (with) or macroscopic (without a microscope) aided by eye-piece graticule, ruler, image analysis….

Clupeid larvae samples kept (y/n): Are larvae kept/stored (y) or discarded (n) after analysis

Preservation of clupeid larvae: preservation fluid and concentration (%)

Buffer for clupeid larvae: name of the buffering agent if applicable

Other fish larvae kept (y/n): Are larvae kept/stored (y) or discarded (n) after analysis

Preservation of other fish larvae: preservation fluid and concentration (%)

Buffer for other fish larvae: name of the buffering agent if applicable

Remainder of plankton sample kept (y/n): Is remainder kept/stored (y) or discarded (n) after analysis

Preservation of remainder: preservation fluid and concentration (%)

Buffer for remainder: name of the buffering agent if applicable

Comments, suggestions for future methods: your thoughts and comments

From the table entries (the overview table is presented in Annex 6) it became apparent that while sampling procedures appear to be well standardized through the different survey manuals, work up of samples is done differently among the different institutes and/or nations. Major differences include whether samples are processed fresh or preserved on either ship or land, and the utilization of image-based systems for larvae identification and measuring. Some participants use sub-sampling in their sample analysis. However, while the minimum amount of counted and measured individuals per target species are often defined in survey manuals (e.g. ICES, 2017, 2019), methods on how these numbers shall be achieved are neither described in manuals, nor documented in survey protocols.

Formalin at 4 % concentrations still appears to be the major preservation fluid to be used, while some institutes and/or nations have switched to ethanol for safety reasons. France uses – as the only nation – the Battaglia solution for sample preservation and storage. In particular where fresh and preserved sample work-up as well as the differing preservation methods – prior to sample analysis and measurement of larvae – are used, measures should be taken to assure data comparability. For buffering formaldehyde, two chemicals are chiefly used. While borax has been the recommended buffering agent for fish larvae for decades (Ahlstrom, 1976), some nations apparently prefer sodium acetate. The benefits of the latter over the former appear unclear. Borax (Sodium tetraborate) tends to raise pH of formaldehyde solutions to more alkaline values around 8 and may produce white, crystalline precipitate inside or outside plankters if used in access (Steedman, 1976). Sodium acetate, on the contrary, produces pH values in the more neutral range around 7, which may be more suitable for preserving the pigmentation for a longer time than with Borax (Steedman, 1976).

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Annex 1: List of participants

Participants WKIDCLUP2 – short video workshop in 2020

Name	Institute	Country (of institute)	Email
Carolina Giraldo	IFREMER	France	Carolina.Giraldo@ifremer.fr
Isabel Riveiro	IEO	Spain	Isabel.Riveiro@ieo.es
Malin Werner	SLU	Sweden	Malin.Werner@slu.se
Maik Tiedmann	HI	Norway	Maik.Tiedemann@hi.no
Andrejs Makarčuks	BIOR	Latvia	Andrejs.Makarcuks@bior.lv
Bastian Huwer	DTU-aqua	Denmark	bhu@aqua.dtu.dk
Svend-Erik Levinsky	DTU-aqua	Denmark	sel@aqua.dtu.dk
Louise Scherffenberg Lundgaard	DTU-aqua	Denmark	lslu@aqua.dtu.dk
Alexander Neil Holdgate	DTU-aqua	Denmark	s190061@student.dtu.dk
Paula Alvarez	AZTI	Spain	palvarez@azti.es
Cindy van Damme	WUR	TheNetherlands	cindy.vandamme@wur.nl
Ewout Blom	WUR	TheNetherlands	ewout.blom@wur.nl
Erika Koelemij	WUR	TheNetherlands	erika.koelemij@wur.nl
Maria Manuel Angelico	IPMA	Portugal	mmangelico@ipma.pt
Elisabete Henriques	IPMA	Portugal	ehenriques@ipma.pt
Birgit Suer	TISF	Germany	birgit.suer@thuenen.de
Matthias Kloppmann (chair)	TISF	Germany	matthias.kloppmann@thuenen.de
Isabel Lee-Elliott		UK	isabellee_elliott@hotmail.com
Daniela Carriço	IPMA	Portugal	danielacarrico22@gmail.com
Anne Georgi	TIOF	Germany	anne.georgi@thuenen.de
Annegret Finke	TIOF	Germany	annegret.finke@thuenen.de
Lina Livdane	TIOF	Germany	lina.livdane@thuenen.de
Dagmar Stephan	TIOF	Gemany	dagmar.stephan@thuenen.de
Hannah Holah	Marine Scotland	Scotland	Hannah.Holah@gov.scot
Norbert Rohlf	TISF	Germany	norbert.rohlf@thuenen.de

Patrick Polte	TIOF	Germany	patrick.polte@thuenen.de
Hermann Neumann	TISF	Germany	hermann.neumann@thuenen.de
James Pettigrew*	CEFAS	England	james.pettigrew@cefas.co.uk
Nevena Almeida*	CEFAS	England	nevena.almeida@cefas.co.uk
Hayden Close*	CEFAS	England	hayden.close@cefas.co.uk

* participation in SmartDots event, only.

Participants WKIDCLUP2 – full workshop in 2021

Name	Institute	Country (of institute)	Email
Matthias Kloppmann (chair)	TISF	Germany	matthias.kloppmann@thuenen.de
Vivian Fischbach	TIOF	Germany	vivian.fischbach@googlemail.com
Valerie Lefebvre	IFREMER	France	Valerie.Lefebvre@ifremer.fr
Samantha Barnett	CEFAS	England	samantha.barnett@cefas.co.uk
Paula Alvarez	AZTI	Spain	palvarez@azti.es
Nevena Almeida	CEFAS	England	nevena.almeida@cefas.co.uk
Maria Manuel Angelico	IPMA	Portugal	mmangelico@ipma.pt
Malin Werner	SLU	Sweden	Malin.Werner@slu.se
Maik Tiedmann	HI	Norway	Maik.Tiedemann@hi.no
Luisa Igleasias	IEO	Spain	luisa.iglesias@ieo.es
Josselin Caboche	IFREMER	France	Josselin.Caboche@ifremer.fr
James Pettigrew	CEFAS	England	james.pettigrew@cefas.co.uk
Isabel Riveiro	IEO	Spain	Isabel.Riveiro@ieo.es
Isabel Lee-Elliott		England	isabellee_elliott@hotmail.com
Ineke Pennock	WUR	The Netherlands	ineke.pennock@wur.nl
Hannah Holah	Marine Scotland	Scotland	Hannah.Holah@gov.scot
Ewout Blom	WUR	The Netherlands	ewout.blom@wur.nl
Erika Koelemij	WUR	The Netherlands	erika.koelemij@wur.nl
Elisabete Henriques	IPMA	Portugal	ehenriques@ipma.pt
Cindy van Damme	WUR	The Netherlands	cindy.vandamme@wur.nl
Birgit Suer	TISF	Germany	birgit.suer@thuenen.de

Annegret Finke	TIOF	Germany	annegret.finke@thuenen.de
Anne Georgi	TIOF	Germany	anne.georgi@thuenen.de
Andrejs Makarčuks	BIOR	Latvia	Andrejs.Makarcuks@bior.lv
Amy Larter	CEFAS	England	amy.larter@cefas.co.uk

Annex 2: Resolutions

WKIDCLUP2 - Workshop 2 on the identification of clupeid larvae

2019/WK/EOSG05 The **Workshop 2 on the identification of clupeid larvae** (WKIDCLUP2), chaired by Matthias Kloppmann, Germany, will meet online, 1 – 2 September 2020 and 30 August – 03 September 2021 By correspondence/online meeting to:

Conduct comparative identification trials focusing on clupeid and clupeid-like larvae evaluating suitable criteria for the identification using the trial – analysis – retrial methodology (Science Plan codes: 3.1, 3.2);

a) Review available information on the identification of clupeoid larvae on the Northeast Atlantic Shelf, with special consideration of the larval appearance and morphology through development (Science Plan codes 3.1, 3.2);
b) Identify and evaluate sources of misidentification of larvae by preparing an uncertainty matrix of clupeid larvae identification (Science Plan codes: 3.1, 3.2);
c) Standardize sample processing and data reporting of clupeid larvae surveys (Science Plan codes: 3.1, 3.2).

WKIDCLUP2 will report by 8 October 2021 for the attention of EOSG, SCICOM, WGSINS, WGALES, WGBIOP and HAWG.

Supporting Information

Priority	Different clupeid larvae surveys, e.g. IHLS and MIK are carried out on the Northeast Atlantic Shelf and provide essential data for the assessment of fish stocks in the North Sea, Irish Sea and the Baltic.
Scientific justification	Larvae surveys are carried out by different countries and the result of these surveys are of direct importance for the assessment. In recent years other clupeids besides herring are occurring in the survey samples in increasing numbers. Since clupeid larvae can easily be mixed up, effective quality control and proper larvae identification is essential to reliable survey results. The overall agreement on clupeid larvae identification between participants at the 2014 WKIDCLUP workshop was 66%. It is necessary to repeat these identification workshops regularly in order to keep the level of identification for experienced and train and improve the skills of new survey participants.
Resource requirements	None.
Participants	Mainly scientists and technicians (approximately 12 - 15) involved in the surveys.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to advisory committees	SCICOM, ACOM

Linkages to other committees or groups	HAWG, WGSINS, WGALES, IBTSWG, WGBIOP
Linkages to other organizations	None.

Annex 3: Agenda

The short supplementary meeting in 2020 to test the SmartDots environment

(all times given in CEST)

Tuesday, 01 September 2020

10:00 – 10:15 Short Introduction to the video conference

10:15 – 11:00 Looking at several specimens of larvae of the target species, herring, sprat, sardine and anchovy in plenary. Discuss the several characteristic criteria to discriminate between the different species.

11:00 – 12:00 Presentation: Introduction in species identification of marine Northeast Atlantic clupeid larvae.

Introduction into using SmartDots for identification trials on fish larvae.

Lunch break

In the afternoon: Identification trials using SmartDots

Wednesday, 02 September 2020

09:00 – 11:00 continue with identification trials on SmartDots and filling in of feedback file on the use of SmartDots

14:00 – 16:00 Discussion on results of identification trials, looking at single specimens from the tri-als. Discussion on the use of SmartDots. Meeting dates 2021

End of meeting

The full WebEx meeting in 2021 (all times given in CEST)

Monday 30 August
10:00 Start of meeting – Welcome and general announcements
Introduction round
10:30 Presentation on workshop rationales and history
11:00 Introduction to clupeid larvae identification
12:00 Lunch
13:00 1st individual larvae identification trial using SmartDots at https://smartdots.ices.dk/manage/ViewLarvaeEvent?tblEventID=360
17:00 End of the day

Tuesday 31 August
09:00 Continue 1st individual larvae identification trial
12:00 Closing of 1st round, Lunch
13:00 Review available information on clupeid larvae identification, updates on descriptions from 2014 report (break out groups)
16:00 End of the day

Wednesday 1 September
10:00 Review available information on clupeid larvae identification (presentations of groups)
11:00 Presentation and discussion of results of 1st identification round
12:00 Lunch
13:00 Start 2nd individual larvae identification trial at https://smartdots.ices.dk/manage/ViewLarvaeEvent?tblEventID=361
17:30 End of the day

Thursday 2 September
09:00 Continue 2nd individual larvae identification trial
12:00 Closing of 2nd round, Lunch
13:00 Establish and agree on a clupeid larvae identification key (break out groups)
15:00 Break
15:15 Presentation of break out groups on identification key
15:45 Presentation and discussion of results of 2nd identification round
17:30 End of the day

Friday 3 September
10:00 Compile overview of methods of clupeid larvae sampling and sample processing, preservation used and agree on an overview of suggested future methods for different survey demands
11:00 Break
11:15 Report writing: discussion, conclusions, recommendations and future, e.g. creating an image database on ELH stages of marine fish
12:00 Final discussions
12:30 End of the workshop

Annex 4: Report on SmartDots during WKIDCLUP2 2020 and 2021

SmartDots during WKIDCLUP2 2020

The ICES Workshop 2 on the Identification of Clupeid Larvae was scheduled to take place as a physical meeting 31 August – 4 September 2020 in Bremerhaven, Germany. Following several national measurements to fight the Covid19 pandemic including restrictions on larger group meetings and international travel, the workshop had to be postponed to 2021. However, because of the importance of the subject – the correct identification of clupeid larvae in the light of increasing overlap in spatial and temporal overlap of the different species – to have at least a small video conference to give potential participants the opportunity to sharpen their expertise.

The original ToRs for WKIDCLUP2 were, for the purpose of the shortened meeting, stripped down to one identification trial and to a quick plenary round on determining sources of identification errors. For the identification trial it was suggested to use the SmartDots WebApi, which was set up originally by collaboration of ICES, DTU-Aqua, ILVO and IMAR for otolith reading and sex and maturity de-termination in fish based on images. For ichthyoplankton identification, SmartDots had to be adapted, which was done prior to the event by collaboration of ICES, DTU-Aqua and WUR, and the event coordinator during several video sessions. The overall aim was not only to assist this workshop (WKIDCLUP2) but to also prepare SmartDots for other ichthyoplankton identification and staging events, e.g. the fish egg identification and staging workshop which is held prior to each mackerel and horse mackerel egg survey. It is hoped that the adaptation of SmartDots to ichthyoplankton work would enable the scientific community to better harmonize their ichthyoplankton survey work both, nationally and internationally.

For the WKIDCLUP2 meeting, a beta version of SmartDots for ichthyoplankton was launched, a sample file and the respective images uploaded to the SmartDots site and an event created. All workshop participants were invited to use the website and try to identify the fish larvae, which were displayed in the images. Apart from the mandatory naming of the species, in the annotation window, all participants were enabled to measure different features of the larvae as well as to count myotomes. Because of the novelty of the application to most of the participants, it was decided to leave the event open until a week after the official end of the workshop on 2 September.

A first results sheet was submitted to the coordinator of the event in the morning of 2 September. The results could be easily extracted and copied to the original WKIDCLUP evaluation sheet for an over-view of the results. It was also possible to extract length measurements, which had been transformed from pixels to mm, and myotome counts, analysis of which enabling for a better identification of sources of misidentification of the species.

Overall, the WebApi SmartDots proved to be very useful for holding such events like WKIDCLUP2. Once all images of larvae were available, it was rather easy to upload them to the SmartDots server. During the workshop, I never had the impression that anyone was having serious problems nor problems at all with annotating the images. Support through ICES and the SmartDots support team was excellent.

SmartDots during WKIDCLUP2 – 30 August - 3 September 2021

The ICES Workshop 2 on the Identification of Clupeid Larvae was scheduled to take place as a physical meeting 31 August – 4 September 2020 in Bremerhaven, Germany. Following several national measurements to fight the Covid19 pandemic including restrictions on larger group meetings and international travel, the workshop had to be postponed to be held 30 August – 3 September in 2021.

Instead of the physical meeting in 2020 and as an add-on to the postponed workshop, it was decided to test an online format for such an event utilizing SmartDots. SmartDots, previously only used for otolith reading and maturity staging events, was quickly adapted for fish larvae identification during the first event in 2020, when participants had to annotate 60 samples of individual fish larvae during one identification event. All participants were pleased with the application and, based on their feedback, SmartDots was further developed and adapted for use with coming fish egg and larvae identification and staging events. In 2021 those events are the postponed WKIDCLUP2 and WKMACHIS (Workshop on Mackerel, Horse Mackerel and Hake Egg Identification and Staging, 11-15 October). Already in spring 2021 it became apparent that because of the ongoing pandemic, both meetings had to be scheduled as online events. Postponement, this time, was no option.

For the WKIDCLUP2 event in 2021, two SmartDots events were created, representing the 2 planned identification rounds of the meeting, in order to follow the approved trial, analysis, retrial exercises of previous ichthyoplankton identification workshops. During both of these rounds, participants had to annotate 120 samples – including 266 and 306 images in round 1 and 2, respectively – of individual fish larvae within 24 hours.

Apart from the mandatory naming of the species in the annotation window, all participants were enabled to measure different features, such as total and standard lengths, of the larvae as well as to count myotomes. The annotations, in particular those for counting myotomes, were subsequently used for identifying sources of identification errors. After closing of each event/round, participants' annotations were downloaded by the event manager/WKIDCLUP2 chair and analysed.

Particularly after the first round, inspection of the downloaded myotome counts by species helped to analyse these counts as the major source of error in discriminating between sprat and sardine. Aided by box-plots, which summarized the myotome counts by each individual sample and clupeid species (Figure 1), single sample IDs corresponding to problematic individuals could be identified, their images displayed and discussed in plenary. This helped to improve agreement in species identification among all participants by almost 10 % from 72.5 % in the first round to 81.7 % in the second round, illustrated by the improved determination of the correct myotome counts in the individual larvae (Figure 2).

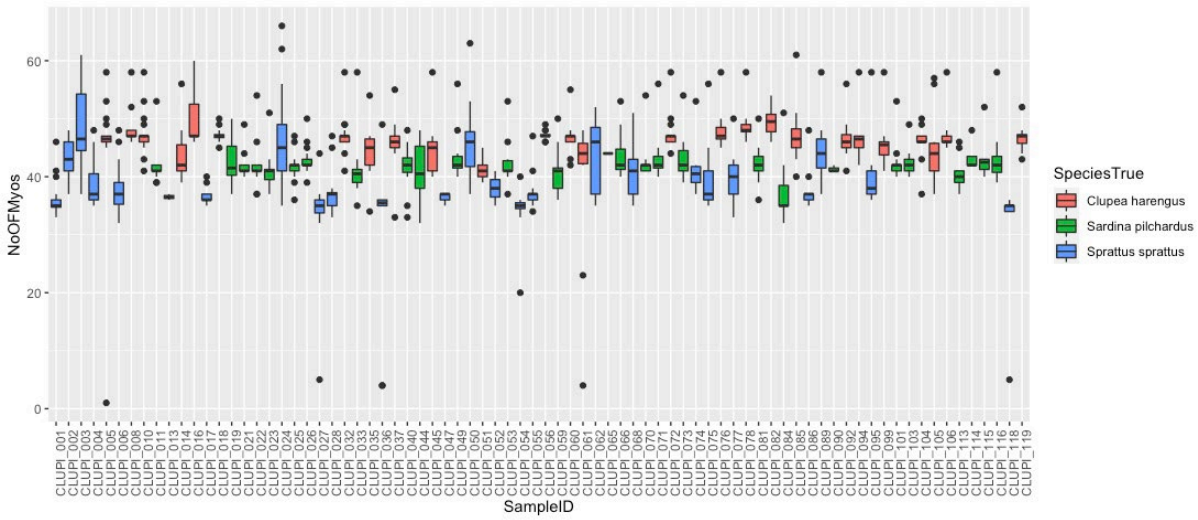

Figure 1: Boxplot of myotome counts per each individual sample and true (validated) species in round 1. Note that particularly in sprat (species-specific myotome count is 35-37) the numbers counted by the participants were often too high, while in sardine (species-specific count is 41-42) the numbers were sometimes too low, which led to erroneous identification, i.e. true sprat were identified as sardine and vice versa.

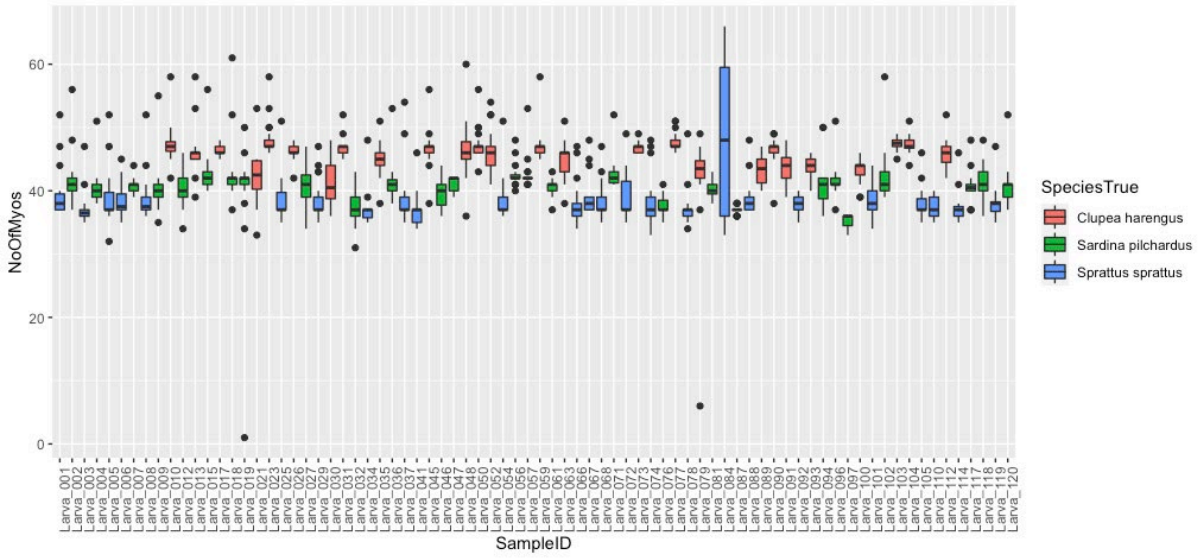

Figure 2: Boxplot of myotome counts per each individual sample and true (validated) species in round 2. Particularly in sprat (species-specific myotome count is 35-37) the numbers counted by the participants were now more within the realistic range for this species (apart for one specimen), while in sardine (species-specific count is 41-42) the numbers were still too low in some cases.

Again, SmartDots proved to be very useful for holding such events like WKIDCLUP2. Once all images of larvae were available, it was rather easy to upload them to the SmartDots server. Even with the larger numbers of images, some of them of a large size > 10 MB, uploading the images never lasted longer than 10 minutes. During the workshop, some participants had problems logging in but these could be solved with help of ICES data center. Also, during the 2 rounds some participants had problems with their log-ins being timed-out, thereby deleting their most recent annotations. Support through ICES and the SmartDots support team was excellent.

Annex 5: *Sprattus sprattus* spawning times

Reference	Investigation Area	Spawning time	Spawning month start	Spawning month end	Peak spawning	Spawning area	Additional comments
Swedish agency for Marine and Water management	Westcoast of Sweden	April-June	4	6		Both offshore and coastal. The spawning occurs between 10 - 40 meters.	
	Baltic	March-August	3	8			
Whitehead 1984a	Black Sea	July-May	7	5		either near to coast or up to 100 km out to sea	Some spawning almost throughout year
	Mediterranean Sea	December-April	12	4			
	Atlantic	April-August	4	8			
	Baltic	April-August	4	8			
	English Channel	as early as January					
Russell (1976)	Plymouth area	January-July	1	7	February-March		
De Silva (1973)	West coast of Scotland	February-August	2	8			Eggs shed in 7-8 batches

Reference	Investigation Area	Spawning time	Spawning month start	Spawning month end	Peak spawning	Spawning area	Additional comments
Ré & Meneses (2009) Early stages of marine fishes occurring in the Iberian peninsula	Iberian Peninsula	January-July	1	7	February-March		
Ehrenbaum 1905-1909, 1936	North Sea	January-August	1	8	May-June	Spawning not coastal but further offshore	
Munk & Nielsen (2005) Eggs and larvae of North Sea fishes	North Sea	January-July	1	7			Larval emergence will be 1-3 weeks later, depending on temperatures
Milligan (1986)	Southern English Channel	January-July	1	7	February-March		
Bailey & Braes (1976)	German Bight	February-August	2	8	April-June		
	North Sea						Highest concentrations of newly hatched larvae between May-September
Torstensen & Gjøsæter (1995)	Skagerrak & Kattegat				May-June		Spawning extending over several months with peak in May-June
Milligan 1986, Knijn et al. 1993, Bailey & Braes 1976, Torstensen & Gjøsæter 1995, Worsøe et al. 2002	North Sea, Skagerrak & Kattegat					Sprat eggs can be observed in almost all areas where adult sprat are distributed, but areas with high concentrations of spawning adults are the inner German Bight, English Channel, southern North Sea, northeast of England, north and west of Scotland, Skagerrak and Kattegat	

Reference	Investigation Area	Spawning time	Spawning month start	Spawning month end	Peak spawning	Spawning area	Additional comments
Aro 1989; Parmanne et al. 1994	Baltic	March-June	3	6		Important spawning areas of the Baltic sprat stock are located in the three central Basins of the Baltic, namely the Bornholm Basin, the Gdańsk Deep and the Gotland Basin	
Voss R., Hinrichsen H.-H., Stepputtis D., Bernreuther M., Huwer B., Neumann V., Schmidt J.O. (2011) Egg mortality: predation and hydrography in the central Baltic. ICES Journal of Marine Science 68(7): 1379-1390		February-August	2	8	spring		
Sjöblom and Parmanne, 1980	Northern Baltic					In the most northern parts of the Baltic, sprat spawning occurs and sprat eggs can be found in the plankton, but no larvae	
Wahl and Alheit, 1988; Petrova, 1960	Northern European waters (North and Baltic Sea)	spring and early summer			May-August		Spawning at water temperatures between 6 and 15 C
Dulčić 1998	Adriatic Sea	October-April	10	4	November-December		Spawning at water temperatures between 9 and 14 C

Reference	Investigation Area	Spawning time	Spawning month start	Spawning month end	Peak spawning	Spawning area	Additional comments
Haslob 2011, Ojaveer & Kalejs 2010	Baltic	February-August	2	8			Differences in spawning time possible due to temperature, salinity and potentially feeding conditions for adults. Requirements for spawning between 6-12C and at least 5-6 psu. In 2002, a second spawning event was observed in autumn, which was explained by the inflow of unusual warm water masses into the central Baltic (Kraus et al., 2003).
Ojaveer & Kalejs 2010, Baumann et al. 2006, Köster et al. 2003	Baltic Sea					Due to the hydrographic situation with strong vertical stratification and the buoyancy of the eggs, the main sprat spawning areas are found in the deep Baltic basins: Arkona Basin, Bornholm Basin, Gdańsk Deep, Gotland Basin as well as parts of the Gulf of Riga and Gulf of Finland.	
Haslob pers. komm., Heidrich 1925	Western Baltic					Sprat is spawning in the western Baltic, e.g. in the Kiel Bight, but a detailed mapping of spawning areas is lacking.	

Annex 6: OVERVIEW TABLE SAMPLING AND SAMPLE PROCESSING METHODS

Overview of methods of clupeid larvae sampling and sample processing

Country	Institute	Survey	ICES area	target species	non-target clupeoids	survey purpose	assessment group; relevant survey output	gear	gear deployment	mesh (µm)	codend mesh (µ)
Spain	AZTI	Bongo	27.8.a 27.8.b 27.8.c 27.7h 27.7j1 27.7j2	Anchovy, sardine, mackerel, horse-mackerel, hake, whiting, bluewhiting, pearlside	--	distribution, abundance of larvae	--	BONGO 40	dO	250	250
Spain	IEO, CSIC	CAREVA JUREVA	27.9.a 27.8.c 27.8.b 27.8.a	-	sardine, anchovy	distribution and abundance of mackerel and horse mackerel eggs	not used for assessment	BONGO	dO	250	250
Portugal	IPMA	DEPM	27.9.a	sardine	anchovy			CalVET	dV	150	
Netherlands	WMR	DRS	27.4.b/ 4.c	herring	sardine, sprat	distribution, abundance of larvae	HAWG; herring recruitment index	MRN2 (blue)	dO	1600	500
Germany	TISF	IHLS	27.4.a 27.4.b 27.4.c 27.7.d	herring	sardine, sprat	distribution, abundance of larvae	HAWG; stock component index	Nackthai	dO	280	280
Netherlands	WMR	IHLS	27.4.a/4.b/4.c/ 7.d	herring	sardine, sprat	distribution, abundance of larvae	HAWG; stock component index	Gulf7	dO	280	280
France	ifremer	MIK	27.4 a 27.4.b 27.7.d	herring	sardine, sprat	distribution, abundance of larvae	HAWG; herring recruitment index	MRN2	dO	1600	500
Germany	TISF	MIK	27.4.a 27.4.b	herring	sardine, sprat	distribution, abundance of larvae	HAWG; herring recruitment index	MRN2	dO	1600	500
Netherlands	WMR	MIK	27.4.b/ 4.c	herring	sardine, sprat	distribution, abundance of larvae	HAWG; herring recruitment index	MRN2 (black)	dO	1600	500
Scotland	MSS	MIK	27.4.a 27.4.b	herring	sardine, sprat	distribution, abundance of larvae	HAWG; herring recruitment index	MRN2	dO	1600	500
Sweden	SLU Aqua	MIK	27.3.a.20 27.3.a.21 27.4.a 27.4.b	herring	sardine, sprat	distribution, abundance of larvae	HAWG; herring recruitment index	MRN2	dO	1600	500
England	Cefas	Peltic	107d,e,f,g,h,	Sardine		Distribution, abundance of larvae		1 m ringnet	v	270	270
Scotland	MSS	Q3 MIK	27.4.a 27.4.b	spratt	sardine	distribution, abundance of larvae	possible future sprat recruitment index	MRN2	dO	1600	500
Germany	TIOF	RHLS	27.3.24	herring	sprat	abundance, growth	HAWG; N20	Bongo	dO	335	335
Latvia	BIOR		27.3. SD 26 and 28	Sprat, cod, flounder, herring	anchovy	distribution, abundance of eggs and larvae		IKS-80, IKS-80 circulation	V, H (on surface)	500	500
									dO - double oblique **H** - horizontal **OD** - oblique downward **OU** - oblique upward **sH** - stepwise or stepped horizontal **V** - vertical		

Overview of methods of clupeid larvae sampling and sample processing, continued

Country	Institute	location of clupeid larvae sorting and identification (ship/lab)	fish larvae sorting and processing (fresh/preserved)	subsampling (y/n) and method	identification of larvae (visual/genetics)	identification method	measurements	measured lengths (TL/SL/NL*), smallest unit	method of measurement	clupeoid larvae samples kept (y/n)
Spain	AZTI	lab	preserved	y, Folsom	visual	macroscopic, sample microscopic, sample	counts, lengths	Tl and SL, 1 mm below	macroscopic, on graded surface	y
Spain	IEO, CSIC	lab	preserved	n	visual	macroscopic, sample microscopic, sample	counts, lengths	TL, 1mm below	microscopic, based on images	
Portugal	IPMA	lab	preserved	n	visual	macroscopic, sample	counts, lengths	TL, 1 mm below	macroscopic, on graded surface	y
Netherlands	WMR	ship and lab	fresh and preserved	y, folsom splitter and counts	visual	microscopic, sample	counts, lengths	SL, 1mm below	macroscopic, on graded surface	y
Germany	TISF	lab	preserved	n	visual	microscopic, sample	lengths	SL, 1 mm below	image of larvae with ImageJ	y
Netherlands	WMR	lab	preserved	y, folsom splitter and counts	visual	microscopic, sample	counts, lengths	SL, 1mm below	macroscopic, on graded surface	Y
France	ifremer	lab	preserved	n	visual	macroscopic, sample microscopic, sample	counts, lengths	SL, 1 mm below	macroscopic, on graded surface	y
Germany	TISF	ship	fresh	n	visual	macroscopic, sample microscopic, sample	counts, lengths	SL, 1 mm below	macroscopic, on graded surface	y
Netherlands	WMR	ship and lab	preserved	y, folsom splitter and counts	visual	microscopic, sample	counts, lengths	SL, 1mm below	macroscopic, on graded surface	y
Scotland	MSS	sorting - ship, identification - lab	sorting fresh, identification preserved	n	visual	macroscopic, sample microscopic, sample	counts, lengths	TL, 1mm below	macroscopic, on graded surface	y
Sweden	SLU Aqua	ship	fresh	n	visual	macroscopic, sample microscopic, sample	counts, lengths	SL, 1 mm below	macroscopic, on graded surface	y
England	Cefas	ship	fresh/ presserved	y, Folsom	visual	microscope sample	counts, lengths	TL, 1mm below	macroscopic, on graded surface	y
Scotland	MSS	sorting - ship, identification - lab	sorting fresh, identification preserved	n	visual	macroscopic, sample microscopic, sample	counts, lengths	TL, 1mm below	macroscopic, on graded surface	y
Germany	TIOF	lab	preserved	n (abundance), y (size)	visual	macroscopic, sample microscopic, sample	counts, lengths	TL, 1 mm below	microscopic on labled slide	y
Latvia	BIOR	lab	preserved	n	visual	microscopic, sample	counts, lengths	TL, 0.5 mm to the nearest	microscopic, eyepiece graticule	n
						visual methods: macroscopic or microscopic from the sample; macroscopic or microscopic from an image		* TL - total length SL - standard length NL - notochord length		

Overview of methods of clupeid larvae sampling and sample processing, continued

Country	Institute	preservation of clupeoid larvae	buffer for clupeoid larvae	other fish larvae samples kept (y/n)	preservation of other fish larvae	buffer for other fish larvae	remainder of plankton sample kept (y/n)	preservation of remainder	buffer for remainder
Spain	AZTI	3.6% formalin	sodium acetate	y	3.6% formalin	sodium acetate	y	3.6% formalin	
Spain	IEO, CSIC	4% formalin	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	y	4% formaldehyde, buffered	sodium acetate
Portugal	IPMA	4% formalin	sodium acetate	y	96 % ethanol, 4% formalin	none; sodium acetate	y	96 % ethanol 4% formalin	none; sodium acetate
Netherlands	WMR	4% formaldehyde, buffered	sodium acetate trihydrate	y	4% formaldehyde, buffered	sodium acetate trihydrate	y (maximum 5 years)	4% formal-dehyde, buffered	sodium acetate trihydrate
Germany	TISF	4% formaldehyde, buffered	borax	y	4% formaldehyde, buffered	borax	y	4% formal-dehyde, buffered	borax
Netherlands	WMR	4% formaldehyde, buffered	sodium acetate trihydrate	y	4% formaldehyde, buffered	sodium acetate trihydrate	y (maximum 5 years)	4% formal-dehyde, buffered	sodium acetate trihydrate
France	ifremer	Battaglia	none	y	Battaglia	none	y	Battaglia	no
Germany	TISF	96% ethanol, pure	none	y	4% formaldehyde, buffered	borax	y	4% formal-dehyde, buffered	borax
Netherlands	WMR	4% formaldehyde, buffered	sodium acetate trihydrate	y	4% formaldehyde, buffered	sodium acetate trihydrate	y (maximum 5 years)	4% formal-dehyde, buffered	sodium acetate trihydrate
Scotland	MSS	3.6% formalin	sodium acetate	y	3.6% formalin	sodium acetate	y	3.6% formalin	sodium acetate
Sweden	SLU Aqua	96% ethanol, pure	none	y	96% ethanol, pure	none	N	-	-
England	Cefas	4% formaldehyde	sodium acetate	y	4% formaldehyde, buffered	sodium acetate	y	4% formaldehyde	sodium acetate
Scotland	MSS	3.6% formalin	sodium acetate	y	3.6% formalin	sodium acetate	y	3.6% formalin	sodium acetate
Germany	TIOF	4% formaldehyde, buffered	borax	y	4% formaldehyde, buffered	borax	y	4% formal-dehyde, buffered	borax
Latvia	BIOR	4% formaldehyde, buffered	sodium hydrocarbonate	n	4% formaldehyde, buffered	sodium hydrocarbonate	n		

Overview of methods of clupeid larvae sampling and sample processing, continued

Country	Institute	comments, suggestion for future methods
Spain	AZTI	
Spain	IEO, CSIC	The sorting of larvae is not a routine work in the framework of these CAREVA and JUREVA surveys, it is carried out occasionally in association with research or training projects.
Portugal	IPMA	
Netherlands	WMR	image processing to enhance quality assurance
Germany	TISF	
Netherlands	WMR	image processing to enhance quality assurance
France	ifremer	
Germany	TISF	image processing, metabarcoding
Netherlands	WMR	image processing to enhance quality assurance
Scotland	MSS	
Sweden	SLU Aqua	
England	Cefas	
Scotland	MSS	
Germany	TIOF	image processing, stageing
Latvia	BIOR	