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# A staging system for Atlantic herring (*Clupea harengus*) larvae based on external morphology and skeletal development

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## Abstract

Atlantic herring (*Clupea harengus*) plays a key role within temperate marine food webs and is targeted by a significant over-regional fishery. Due to its high economic importance, dynamics in herring stock biomass and recruitment are closely monitored, forming the basis for fisheries management advice. As recruitment patterns translate into the adult stock biomass, early life stage ecology has been thoroughly addressed in fisheries research. Larval monitoring programs commonly focus on length measurements and abundance indices, rarely, information on larval developmental stages is given. As length is highly influenced by temperature, salinity and food availability, their size range can significantly vary between cohorts, populations, and ecotypes. Nowadays, a systematic staging system from the 1970s provides the standard guide for herring larval development, although it does not fully resolve important developmental stages. Here, we propose an improved staging system based on external morphology and skeletal development of herring larvae. The staging system has been developed and tested with herring larvae from different populations of the North and Baltic Sea to ensure applicability. The system comprises 15 stages (+substages) in 5 major developmental phases: the yolk sac phase, the dorsal fin development, the caudal fin development, the pelvic fin development, and the juvenile phase. This staging system aims to simplify herring larval staging to gain a more specific picture of early life dynamics. Because of the detailed description of the development, future studies are better equipped to identify stages which, for example, show high mortality rates and better link them to environmental circumstances.

The Atlantic herring *Clupea harengus* Linnaeus, 1758 constitutes a key species for temperate marine ecosystems (Blaxter and Hunter 1982) and plays a major role within marine food webs. Also, Atlantic herring is amongst the most important fish species in commercial fisheries, since it is the fourth most caught fish according to biomass landings worldwide (FAO 2022). Due to its economic importance, annual variability and long-term trends in stock biomass, recruitment success and fishing mortality are closely monitored, as baselines for fisheries management and catch advice (ICES 2021*a*,*b*, 2022).

In the early days of fishery science, it has been shown that herring recruitment is largely driven by the survival of the earliest life stages during particular phases in larval development which is reflected in the "critical period" hypothesis (Hjort 1914). Consequently, understanding the position and the nature of survival bottlenecks during herring early life history is a major task in fishery science and fish ecology. One method to estimate year class strength of recruits is observing the larval and juvenile development of a fish stock. As the response of a larva in a certain life stage to environmental (and anthropogenic) drivers is elementally linked to its stage-specific, morphological, and physiological traits, such as swimming speed (Moyano et al. 2016), gape size (Blaxter 1965), visual field of the larvae (Blaxter 1968, Klinkhardt 1996), and the development of the digestive system (Joly et al. 2021), it is

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essential to understand critical development steps in larval growth.

Most fishery surveys focus on the growth rate of fishes, which is depending on several abiotic and biotic factors (Dodson et al. 2019). Temperature, for example, can highly influence both growth and development but not necessarily at the same rate (Forster et al. 2011). For example, lower temperatures can decelerate the development, resulting in large changes in stage duration, while higher temperatures accelerate the stage duration (Houde 1989), which has already been shown to be true for herring larvae (Moyano et al. 2016). Although there are studies showing that in a stable temperature regime larval length can be used as an indicator for developmental progress (Fuiman 1998), natural habitats where herring larvae develop show high temperature fluctuations. This means that larval length measurements cannot implicitly provide information on developmental stages, especially when comparing herring larvae of different ecotypes or different stocks (Gamble et al. 1985).

Integrating over differing larval stages, for example, by using only length measurements, and therefore neglecting those stage-specific traits leads to precarious results in larval drift models by setting larval fish in general synonymously with passive particles (Bauer et al. 2013).

Also, in the Baltic Sea, North Sea, and the Atlantic Ocean, there are several herring stocks which are separated based on differing spawning grounds, spawning time, and temporal and spatial segregation (Popiel 1985; Cardinale et al. 2009; Bekkevold et al. 2023). According to the varying habitat conditions and spawning biology, herring stocks in the Northern hemisphere show differences in larval development. It has been shown that larvae from different spawning ecotypes such as autumn and spring spawner herring show differences in growth rates characterized by, for example, the length at a certain stage (Gamble et al. 1985; Johannessen et al. 2011). Gamble et al. (1985) found that larvae of a North Sea autumn spawning stock are substantially larger at the same developmental stage than larvae of a spring spawning ecotype. The same pattern has been observed in spring and autumn spawning larvae in the Baltic Sea. Therefore, length measurements from different herring stocks with different spawning biology cannot be transferred to other stocks.

Staging the embryonal, larval, and juvenile development of fish helps to understand the influence of environmental factors on the early development in various ecological studies, for example, the growth rates at specific temperatures (Moyano et al. 2016) or their age in dependence of their growth rate (Folkvord et al. 2000). Miller and Kendall (2009) reviewed different definitions of the larval and juvenile phase and concluded that larval development begins with hatching and ends when the complete fin ray count is attained furthermore stating that squamation begins, when the larva is transformed into a juvenile fish. Also, different generalized larval staging systems were proposed which try to apply to as many fish species as possible (*see* review by Miller and Kendall 2009). One way to stage the early development of fish can simply be to group larvae into embryonic, larval, and juvenile categories. Larvae that still carry a yolk sac can then be either grouped within the larval stage, for example, as pro-larvae or prelarvae or within the embryonic development, since the larvae do not feed exogenously yet (*see* review by Miller and Kendall 2009). One rather commonly accepted way to further stage marine fish larvae is the division in yolk sac, preflexion, flexion, postflexion, and transformation stages (*see* review by Miller and Kendall 2009). In general, this system can be applied to most species and, therefore, secures comparability of larval stages of different species. However, they do not work for all fish species: for example, members of the gadiforms, which possess a nearly symmetrical caudal skeleton with no apparent flexion of the notochord, are excluded (Miller and Kendall 2009; Moritz et al. in press). This system also emphasizes the development of the caudal fin as a main characteristic in larval development and the different stages vary greatly in their duration, since the flexion stage covers only a very short time period, which is seen as controversial (Penaz 2001). Studies for specific species also focus on different developmental events due to a specialization of the staging system to a specific research question and therefore discrepancies between the descriptions of larval development arise (Penaz 2001). In other staging systems (*see* review of Penaz 2001), characters such as the skeletal development, development of organs, or changes in behavior are included in the staging of the larvae, in addition to externally visible anatomical and morphological features to provide additional insight in the specific abilities and needs of larvae in different larval phases (Penaz 2001).

To date, Doyle (1977) provides the standard work on herring larvae staging and the system is widely used in ecological studies as well as in some herring larvae surveys. Doyle (1977) organized herring larvae in four stages, with substages in yolk sac, preflexion, flexion, and postflexion larvae due to external morphological features. The four stages provide a good overview on the general larval development and often studies using this system exclusively limit staging, accordingly, not further subdividing into substages (Polte et al. 2017; Dodson et al. 2019; Joly et al. 2021). When larval staging is done superficially, important information may be overseen and stages which show, for example, a higher vulnerability to external factors can be missed. Staging larvae on a more detailed level provides detail on the sensitivity to external factors, which are experienced differently as the larvae proceed in development and gain further traits. Furthermore, some of the stages proposed in the system by Doyle (1977) are not clearly separated and distinct morphological features are occasionally missing. This complicates the correct assignment of larvae to certain developmental stages. Also, not all phenotypes of herring larvae have been described, which was implied by Stenevik et al. (2021) and recognized in preliminary studies. Stenevik et al. (2021) tested first feeding success



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in herring larvae and introduced an additional "first feeding stage" to Doyle's system, which can be identified and subsequently serve as a proxy for feeding success. This and other missing stages seem to be equivalent to major changes in behavior, feeding habit, or overall biology and their (abundant) occurrence in survey data can therefore provide important information, for example, of the success of first feeding (Stenevik et al. 2021).

The present study aims to define distinct developmental stages throughout the herring early life history from yolk sac larvae to juvenile herring, based on the postcranial skeletal development (Fischbach et al. 2022) and the external morphology. We further assigned distinct larval stages to major developmental phases. The proposed staging system was prepared to serve as a technical guideline to facilitate and standardize the developmental staging of herring larvae. It also aims to provide the tool to conduct further detailed analyses and comparisons of the development of herring larvae in different environments. The provided detailed descriptions and high-resolution pictures of each developmental stage aim to result in a simplified and reproducible application of developmental staging in larval surveys and ecological studies. This approach considers the technical advances made since the previous staging systems for herring have been published (Blaxter 1962; Doyle 1977). Application of detailed staging systems results into further knowledge on how external factors and environmental effects affect different larval life stages during development.

## Materials and procedures

### Larval sampling

Herring larvae were sampled in and close to the Greifswalder Bodden, a major spawning area for Western Baltic spring spawning herring in the southern Baltic Sea (Moll et al. 2019). Samples were collected from February to September in 2020 and 2021 using a bongo net (60 cm diameter, mesh size 335 $\mu$m), CalCofi net (100 cm diameter, mesh size1550 $\mu$m), and a beach seine (6.9 m opening, mesh size 2 mm, 1.2 m wing height).

### Building the staging system

A total of 88 herring larvae and juveniles with sizes ranging from ~ 6 to ~ 80 mm were analyzed to determine larval stages based on characteristic external and internal features. External morphological features were documented for each larval stage in the formalin fixed larvae. In addition, information of the skeletal development gained by the cleared and stained (C&S) larvae was added (Fischbach et al. 2022). Afterwards, characteristic features of the different larval stages were analyzed and verified in another set of 315 larvae with a length distribution of 6–35 mm standard length (SL).

### Cross-validation

To evaluate the applicability of the staging system to other ecotypes and different herring populations, two additional sets of herring larvae were staged, and larval stage features were analyzed. First, we examined 150 herring larvae presumably belonging to the autumn spawning ecotype that were caught in the Greifswalder Bodden area in January–March in 2013 and 2022. Second, the staging system was applied to 335 larvae from the three different North Sea stocks, that is, the Downs herring, Buchan Bay stock, and herring of the Orkney–Shetlands, which were obtained by the International Herring-Larvae Survey and the International Bottom Trawl Survey—Midwater Ring Net Sampling (ICES 2021*b*; https://www.ices.dk/data/data-portals/Pages/Eggs-and-larvae.aspx).

### Documentation

Pictures of the herring larvae in different stages were taken with a single-lens reflex camera (Canon EOS 80D, lenses: Canon MP-E 65 mm, Sigma EX 105 mm) as well as stereomicroscope (Leica M165C) equipped with a dedicated Leica DFC425 camera using the software Leica Application Suite (Leica Microsystems, version: 4.9.0). The pictures were edited using Adobe Photoshop 2022. The final figures for each stage were compiled using Abode Illustrator 2022. Graphs were created using R Studio (4.0.5, 31 March 2021, "Shake and Throw"). SL (tip of snout–body-parallel distance - posterior margin of the hypurals or if no hypurals are present to the end of the notochord, respectively) or total length (TL, tip of snout–end of caudal fin) and body height (measured vertically at the insertion of the posterior-most dorsal fin ray, if present, in smaller larvae the measurement was taken at the point of the largest body height of the trunk) of each larva were measured under the microscope to the nearest 0.1 mm. For the ratio of body height to length, we used SL.

To further verify the staging system and analyze the length distribution of each stage specifically for the Western Baltic spring spawning stock, a total of 2546 larvae from the Western Baltic herring stock were measured and staged according to the proposed system. The larvae were provided by the Rügen herring larvae survey, mentioned above, which samples 36 stations in the Greifswald Bay area, weekly from February to June. For a good spatial and temporal resolution, larvae of 2010 from 7 stations all around the sampling area were selected and up to 50 larvae per station were staged so that the sample sized of each stage resembled the relative proportion of larvae caught in the whole survey. The year 2010 was chosen because since then the abundance of herring larvae has been steadily decreasing (ICES 2022). To estimate the length stage distribution for Western Batltic spring spawning herring, the TL and SL of the larvae for the size distribution were rounded to the nearest 0.5 mm.

## Results

The proposed staging system comprises five major developmental phases in which key elements (such as fins) are



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developing (Table 1): the yolk sac phase (Y), the dorsal fin development phase (D), the caudal fin development phase (C), the pelvic fin development phase (P), and the juvenile phase (J). Each phase is then further divided resulting in a total of 15 stages and 8 substages.

### The yolk sac phase (Y)

The yolk sac phase starts directly after hatching. During the yolk sac phase the larvae continuously deplete their yolk sac reservoirs and eventually start exogenous feeding. The yolk sac phase is therefore completed when the yolk sac is fully consumed. In addition, the caudal fin starts to develop.

#### *Stage 1 Y (Fig. 1)*

The head is tilted over the yolk sac, as it is still attached to it by connective tissue. The pectoral fins are yet immobile due to the size of the yolk sac. The yolk sac is largest, since the larvae just hatched, and generally measures about 1 mm in diameter, although this may vary according to the size of the larvae. The eyes are already fully pigmented, and the larval fin fold stretches around the body, except for the head. No cartilages or bones are developed in the postcranial skeleton.

#### *Stage 2 Y (Fig. 1)*

The body of the larvae is elongated, and the head is free from the yolk sac. The pectoral fins are mobile and no longer covered by the yolk sac. The latter is more depleted and noticeably flatter. No cartilages or bones are developed in the postcranial skeleton.

#### *Stage 3 Y (Fig. 1)*

The yolk sac is almost depleted but remains are still visible. No cartilages or bones are developed in the postcranial skeleton.

#### *Stage 4 Y (Fig. 1)*

The yolk sac is fully depleted, and no remains are visible. Elements of the dorsal fin are still completely absent. C&S revealed the first bones and cartilaginous precursors. Ventrally at the posterior end of the unflexed notochord, a mesenchymal condensation is present, hence, the caudal fin starts to differentiate. The cleithrum is formed by intramembranous ossification and the coracoscapular cartilage is also present.

### Dorsal-fin development phase (D)

During the dorsal-fin development, the dorsal fin forms as a mesenchymal condensation in the larval fin fold. As the development progresses, cartilaginous pterygiophores (= proximal and middle radials) and ossified dorsal-fin rays form. In addition, the development of the caudal fin progresses and cartilaginous elements such as the parhypural and the first hypurals appear. Further in the developmental process, in the posteroventral portion of the fin fold, the first trace of the anal fin differentiating as a mesenchymal condensation becomes visible.

#### *Stage 5 D (Fig. 2)*

On the posterodorsal side of the trunk, anterior to the level of the anus, a mesenchymal condensation is visible. The mesenchymal condensation lies within the larval fin fold. The

**Table 1.** Overview of the staging system for *Clupea harengus*.

| Phase | Stage | Substage | Description |
|---|---|---|---|
| Yolk sac (Y) | 1 | | Head connected to yolk sac |
| | 2 | | Yolk sac and head separate |
| | 3 | | Depleted yolk sac |
| | 4 | | Absence of yolk sac |
| Dorsal fin development (D) | 5 | | Mesenchymal condensation |
| | 6 | | Pterygiophores |
| | 7 | a | Lepidotrichia precursor |
| | | b | Fin rays |
| Caudal fin development (C) | 8 | | Begin notochord flexion |
| | 9 | a | Ongoing notochord flexion |
| | | b | Round caudal fin |
| | 10 | | Incised caudal fin |
| Pelvic fin development (P) | 11 | a | Mesenchymal condensation |
| | | b | Lepidotrichia precursor |
| | 12 | a | Fin rays |
| | | b | Transformation |
| Juvenile (J) | 13 | | Stating squamation |
| | 14 | | First keel scales |
| | 15 | | Subadult |



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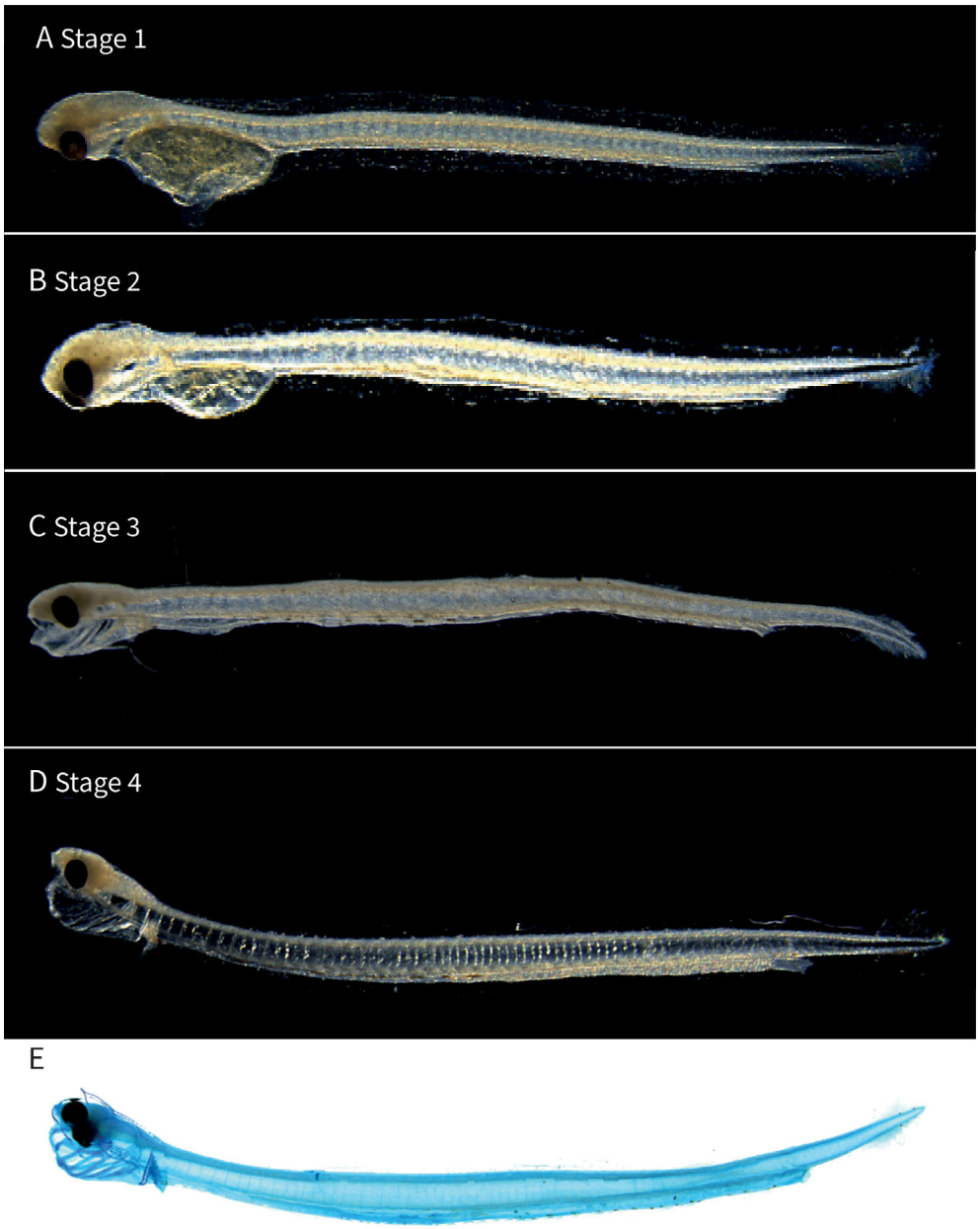


**Fig. 1.** Yolk sac stages of the herring *Clupea harengus*. Larval lengths: stage 1: 6.9 mm TL; stage 2: 7.2 mm TL; stage 3: 8 mm TL; stage 4: 8.9 mm TL.

mesenchymal condensation in the caudal fin has progressed. C&S specimens show that no pterygiophores or hypuralia are present yet and only mesenchymal condensations have formed.

***Stage 6 D (Fig. 2)***

The pterygiophores of the dorsal fin are present within the dorsal fin fold. In the caudal fin the parhypural and the fist hypural develop but are difficult to differentiate from the mesenchymal condensation in formalin preserved larvae. The dorsal fin is not yet separate from the larval fin fold. The anal fin starts to differentiate, since a mesenchymal condensation can be observed within the posteroventral part of the larval fin fold. C&S specimens show cartilaginous pterygiophores in the dorsal fin. In the caudal fin, the parhypural and the first hypural are present as cartilage.

***Stage 7 D***

The formation of lepidotrichia precursors from connective tissue and the subsequent ossification of the dorsal-fin rays occur. The outer morphology of this stage can differ; therefore, the stage is further divided into two substages: 7a



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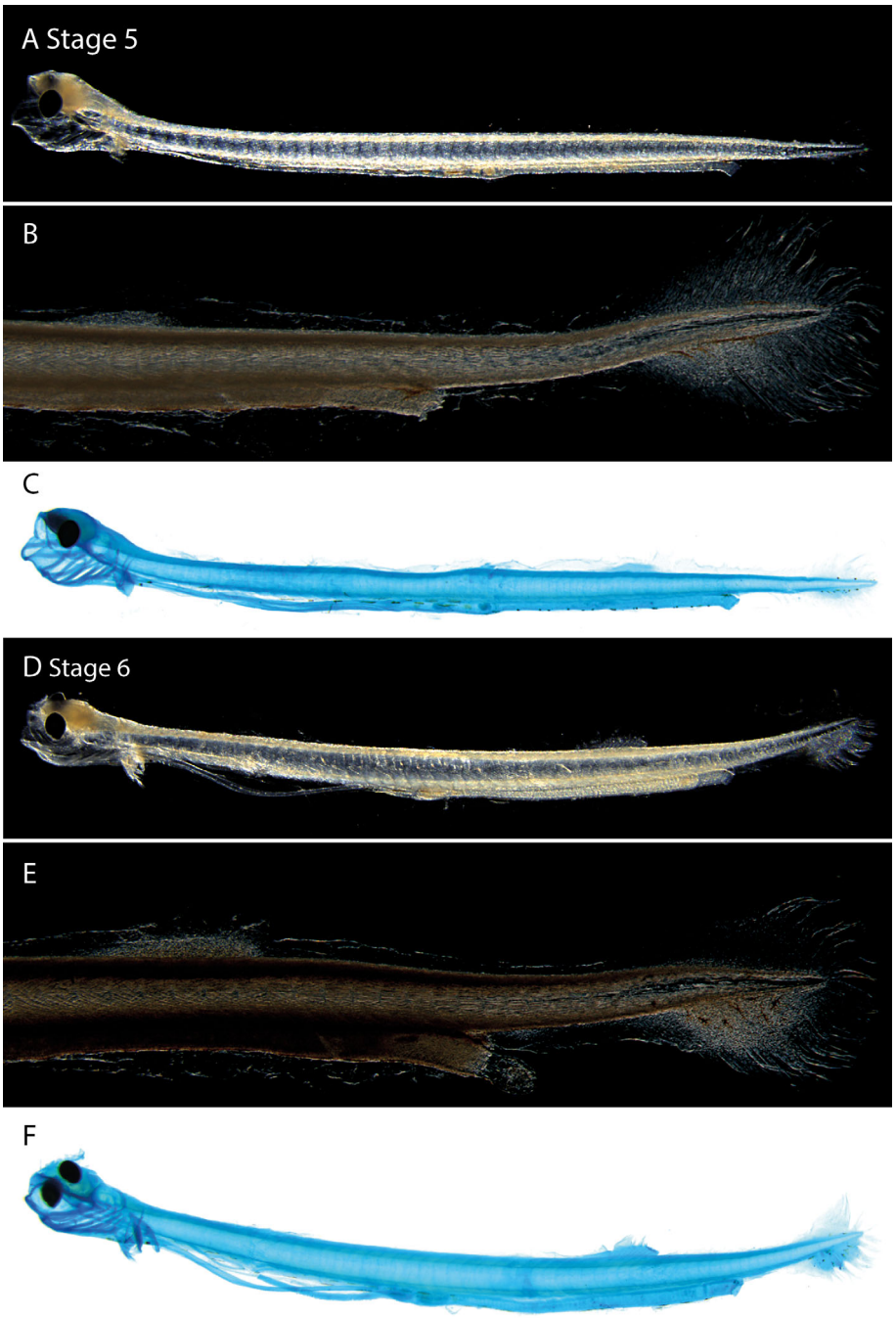


**Fig. 2.** Larval stages 5–6 of the herring *Clupea harengus*. Larval lengths: stage 5: 9.9 mm TL, stage 6: 14.5 mm TL.

(formation of lepidotrichia precursors) and 7b (ossification of fin rays).

***Stage 7a D (Fig. 3)***

Dorsally, the larval fin fold is frayed and the formation of lepidotrichia precursors from connective tissue becomes visible, but individual fin rays are not yet confined and ossified. The fraying of the fin fold probably is an artifact of the formalin fixation as well as handling and may not occur in live larvae or larvae which have been fixed differently. Yet, in all material studied in this investigation, this character was observable. In addition, the dorsal fin is still not fully separate from the larval fin fold in its posterior portion, but the transition is evident. In C&S specimens, it can be seen that the rays of the dorsal fin are not yet ossified, but condensed lepidotrichia precursors have formed and appear bluish. In the



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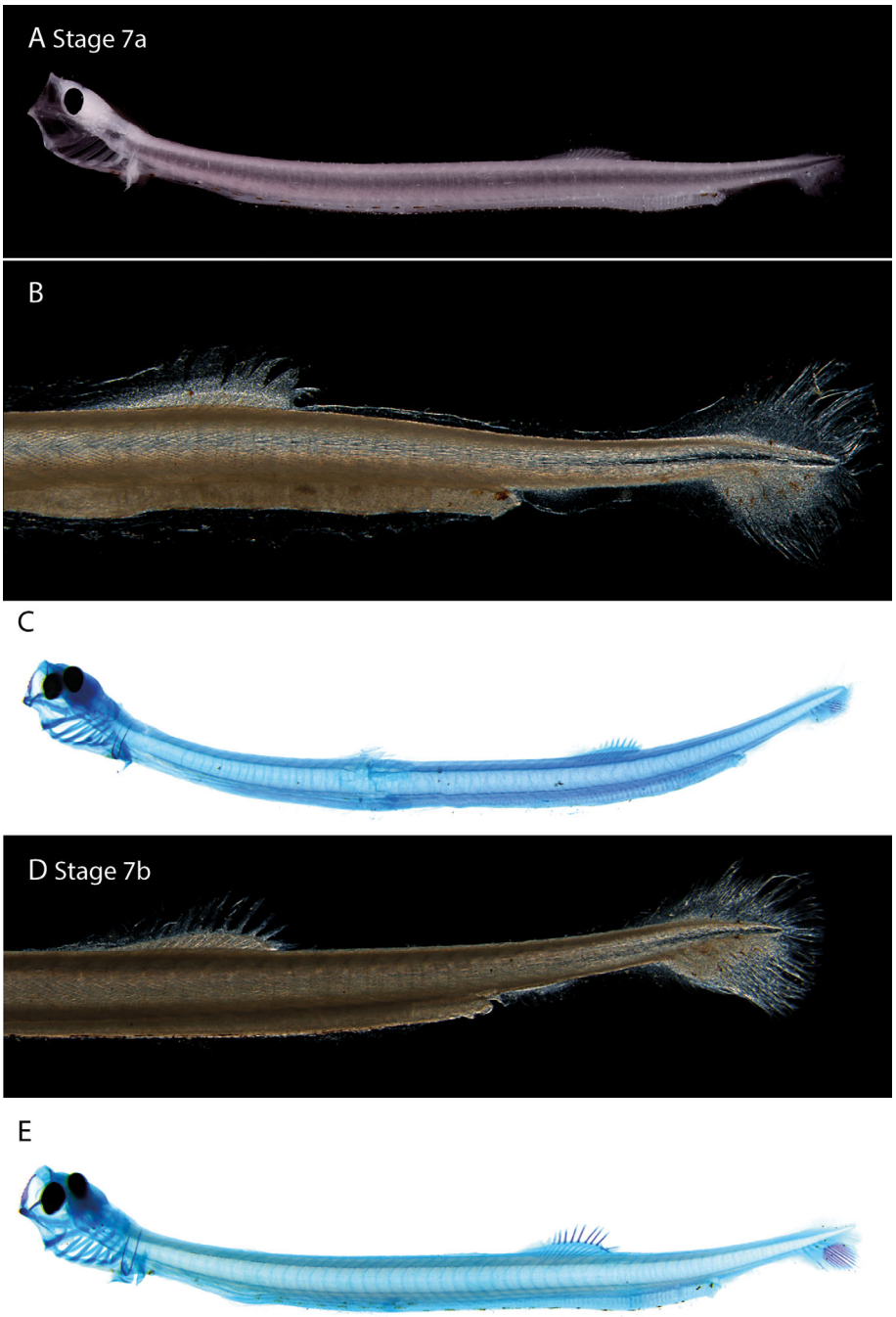


**Fig. 3.** Larval stages 7a–7b of the herring *Clupea harengus*. Larval lengths: stage 7a: 13.9 mm TL, stage 7b: 13 mm NL.

caudal fin, the first ossified rays are present and hypural 2 and 3 form in cartilage.

***Stage 7b D (Fig. 3)***

The dorsal fin develops further, and fin rays are clearly visible. The dorsal fin now protrudes significantly beyond the fin fold, which is present between dorsal and caudal fin and is fully separate from it posteriorly. The posterior end of the notochord is still straight; however, in some individuals, the end of the notochord can show the first slight signs of an upward bending. Yet, other features that are directly linked to the notochord flexion are not developed (e.g., enlargement of hypural 1; *see* stage 8a). The anal fin still shows only mesenchymal condensations and is mainly transparent and not separate from the larval fin fold posteriorly. In some larvae, however, pterygiophores are already visible, which most likely constitutes an intermediate step between



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stages 7b and 8. Since this intermediate step is only described by one feature, the development of pterygiophores in the anal fin, it has not been assigned its own stage and the main focus still remains of the beginning of the notochord flexion. The C&S specimens show that the dorsal fin rays are ossified. Also, the posterior-most hemal arch forms in cartilage.

**Caudal-fin development phase (C)**

During the caudal-fin development stages the tip of the notochord bends upwards, and the caudal fin develops further. When the complete flexion is attained, all hypurals and uroneural 1 are present. The anal fin also differentiates further and contains pterygiophores.

***Stage 8 C***

The initiation and progression of the notochord flexion together with the formation of the remaining hypurals occur. The progression of the notochord flexion can only be detected by the more upward flexion of the notochord in formalin preserved specimens, all other features do not change significantly and the formation of more hypurals is not clearly visible in specimens that have not been cleared and stained. To avoid confusion, the beginning and the progression of the stage were incorporated into one stage which is divided into 8a (beginning of flexion) and 8b (progression of flexion).

***Stage 8a C (Fig. 4)***

The flexion of the notochord begins. At this stage, at least four hypurals are present in cartilage. In addition, hypural 1 broadens at the anterior-most starting-point of the notochord flexion and forms a corner which is visible in the formalin fixed larvae. The pterygiophores in the anal fin are now clearly visible. The anal fin can also show fraying ventral to the pterygiophores where the lepidotrichia precursors form from condensed connective tissue. In the dorsal fin, cartilaginous distal radials develop and more hemal arches form in cartilage, which are visible in the C&S specimens. The pterygiophores in the anal fin are visible in cartilage.

***Stage 8b C (Fig. 4)***

The notochord flexion is in progress but not yet completed. The remaining hypurals 5 and 6 develop. The anal fin is still not separate from the fin fold posteriorly; however, the size of the fin fold has clearly decreased. C&S specimens show that in the dorsal fin, the fin stay develops and in the caudal fin, all six hypurals are now present as cartilages. In some specimens, uroneural 1 already forms by intramembranous ossification; however, the formation was mostly observed in specimens of stage 9.

***Stage 9 C (Fig. 4)***

The flexion of the notochord is complete. The posterior-most point of hypural 1 is now almost aligned with the posterior part of the notochord. The caudal fin is still round and only slightly incised. In the anal fin, no ossified rays have formed. In the C&S specimens, it is visible that in the caudal fin, uroneural 1 forms from intramembranous ossification. The first small cartilage precursor of the basipterygium of the pelvic fin is present but is not yet visible without C&S. Moreover, the posterior-most neural arches form in cartilage. In addition, within this stage, the shoulder gridle develops further and the fin plate forms in cartilage.

***Stage 10 C (Fig. 5)***

The caudal fin appears incised and clearly homocercal. The posterior parts of the hypurals are aligned and form a straight vertical line. Ossified fin rays in the anal fin are clearly visible. Through clearing and staining, the now still small and round basipterygial cartilage becomes visible about 7–8 myomeres anterior to the dorsal fin. The basipterygial cartilage lies about 24 myomeres anterior to the beginning of the anal fin (= location of the anus). C&S shows that the parhypural and hypurals begin toossify. Also, the rays in the anal fin ossify.

**Pelvic-fin development phase (P)**

In these stages, the pelvic-fin development is externally visible. In the stages prior, the basipterygial plate has already formed; however, now a mesenchymal condensation is visible ventrally. Eventually fin rays form and ossify. Later, the overall shape of the larva starts to change as the larva transform into the juvenile fish. Also, the shoulder girdle and the vertebral column develop further.

***Stage 11 P (Fig. 6)***

The basipterygium differentiates from a mesenchymal condensation (11a) to its cartilaginous precursor (11b). In the latter substage, also lepidotrichia precursors form at the posterior base of the cartilaginous basipterygium.

***Stage 11a P***

About 7–8 myomeres anterior to the beginning of the dorsal fin the anterior-most part of the basipterygium of the pelvic fin is visible as a mesenchymal condensation. The pelvic and anal fin are about 22–24 myomeres apart. C&S specimens show that in the caudal fin, ural centrum II ossifies as chordacentra and both epurals are present in cartilage. In addition, uroneural 2 forms of intramembranous ossification. In the shoulder girdle, the posttemporal and the supracleithrum appear as intramembranous ossification. In the anal fin, the distal radials and the fin stay appear in cartilage. Not shown in Fig. 6 is the formation of the first vertebrae, which can occur in stage 11a or b.

***Stage 11b P***

At the same position of the mesenchymal condensation described in stage 11a, the basipterygium is now visible as a cartilage precursor. It has not attained its final shape yet and is still roundish. Lepidotrichia precursors may already have formed at its posterior side but are small and at maximum of the same length as the basipterygium. This stage includes all phenotypes of the elongation of the basipterygium until the formation of fin rays.



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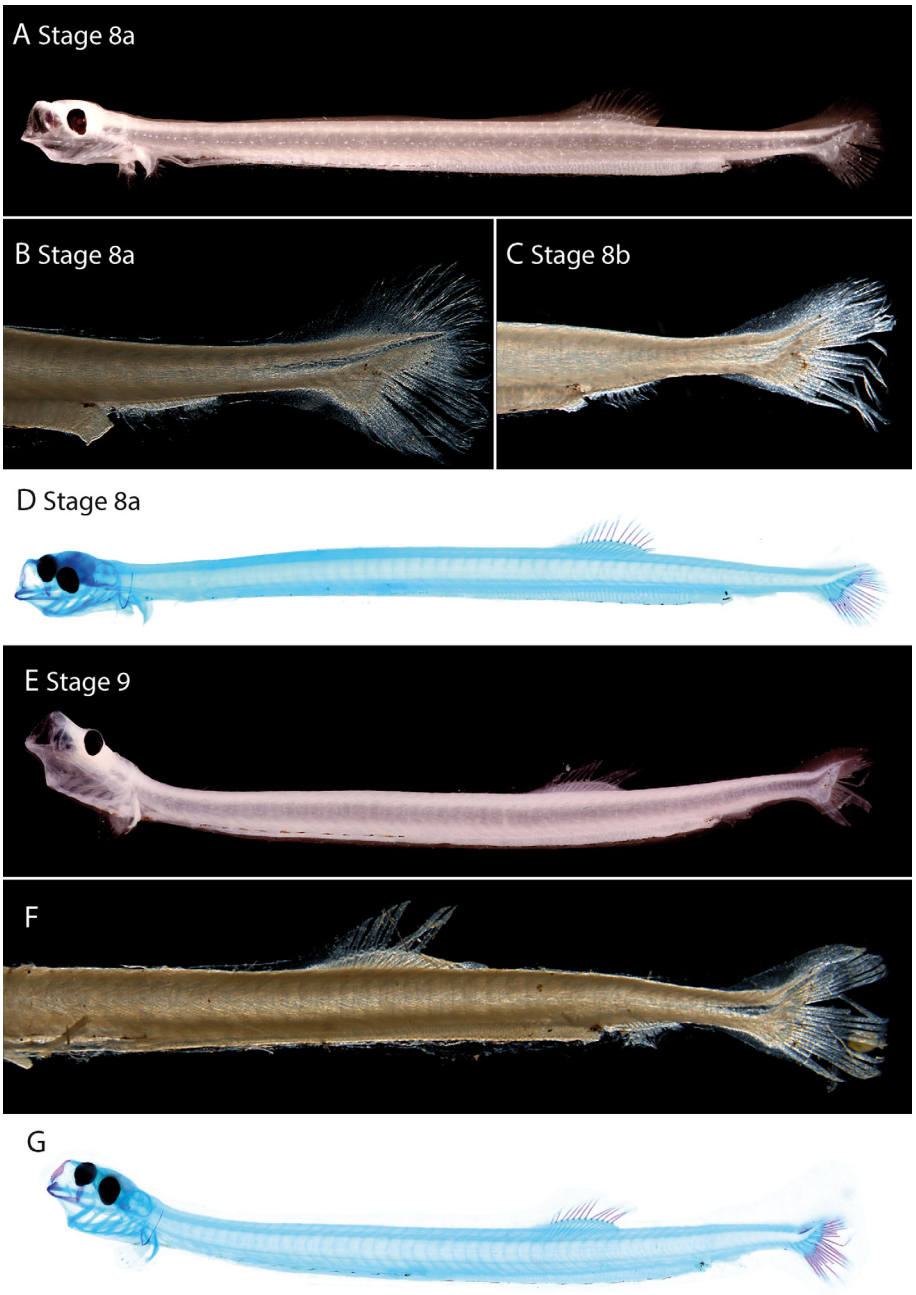


**Fig. 4.** Larval stages 8a–8b, nine of the herring *Clupea harengus*. Larval lengths: stage 8a: 15.2 mm TL, stage 8b: 18 mm NL, stage 9: 16.9 mm SL.

***Stage 12 P (Fig. 7)***

The pelvic fin develops further, and fin rays are now present as fully developed lepidotrichia (12a). The transformation of the larvae into a juvenile fish begins and the pelvic fin appears to move posteriorly. Also, the body height to length ratio increases (12b). Even though all fins are fully formed, which is a character of the end of the larval phase, but squamation has not yet started we propose to view stage 12 as an intermediate stage between larval and juvenile phase. This is further supported by the beginning of the metamorphosis from the larval body proportions to adult body proportions.

***Stage 12a P***

The larvae have a fully developed basipterygium with fin rays but the height to body length ratio has not increased yet and the larvae still appear elongated and thin. In larvae of the Western



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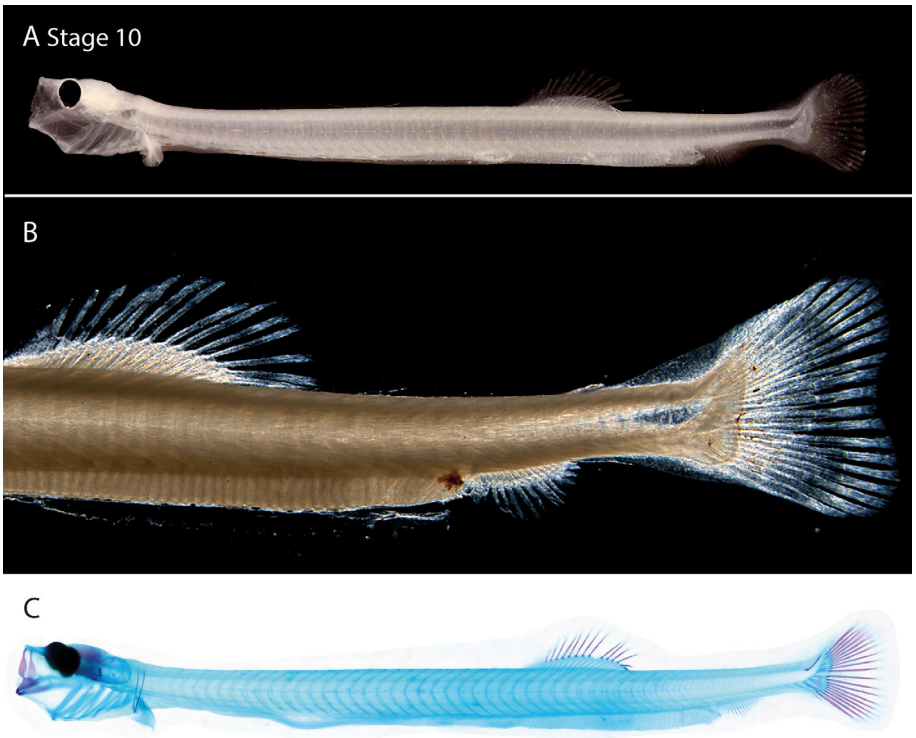


**Fig. 5.** Larval stage 10 of the herring *Clupea harengus*. Larval length: 20.2 mm SL.

Baltic autumn spawning ecotype, the mean ratio between body height and length was 0.075 (± 0.0055, $n = 10$). In some specimen, the fin rays are defined but appear shorter that the fin rays of larvae in stage 12b. This stage was found mainly in larvae of the Western Baltic autumn spawner population and in North Sea larvae, which were also spawned in autumn. It is possible that this stage occurs because of environmental factors such as prey availability and further development is delayed.

***Stage 12b P***

The basipterygium is fully developed and fin rays are present. The transformation of the larvae is in progress, the pelvic fin now still lies anterior to the beginning of the dorsal fin (about 8 myomeres anterior to the dorsal fin but only 19 myomeres anterior to the anal fin). The body height increases visibly, now the body is almost equally thick anterior and posterior of the dorsal fin. In larvae of the Western Baltic autumn spawning ecotype, the ratio increased to 0.10 (± 0.0081, $n = 10$). At this stage, C&S specimens show, that the basipterygium begins to ossify. In the caudal fin, uroneural 3 forms and uroneural 1 fuses to preural centrum I to form the pleurostyle. Also, ural centrum I forms as a chordacentra. Neural and hemal arches start to ossify and are equipped with spines which develop of intramembranous ossifications. Theribs start developing in cartilage. In the shoulder girdle, the radials and rays form.

***Remarks to Stage 12***

Since the larvae are still elongated and thin, they often break during catching at the point where the pelvic fins form. Also, the pelvic fin lies ventral to the gut, which is often ripped from the body, the pelvic fin can appear to be formed posterior to a bulge. The bulge, which forms as the result of a separate gut however is an artifact due to catching and handling the larvae and cannot be used as a criterion for identification.

**Juvenile phase (J)**

During the juvenile stage, the larvae have already attained typical adult characteristics. The body length to body height ratio has shifted further, squamation begins, and pigmentation is visible. The pigmentation of the larvae has been excluded as of now, since it is mostly limited to patches alongside the ventral ridge, the caudal peduncle, and later the head.

***Stage 13 J (Fig. 8)***

The larvae transform and now show adult characteristics which include shift in the overall body proportions. The dorsal fin is positioned more in the center and the pelvic fins now lie around 5 myomeres posterior to the beginning of the dorsal fin, counted from the anterior-most point of the visible basipterygium, and about 16 myomeres anterior to the anal



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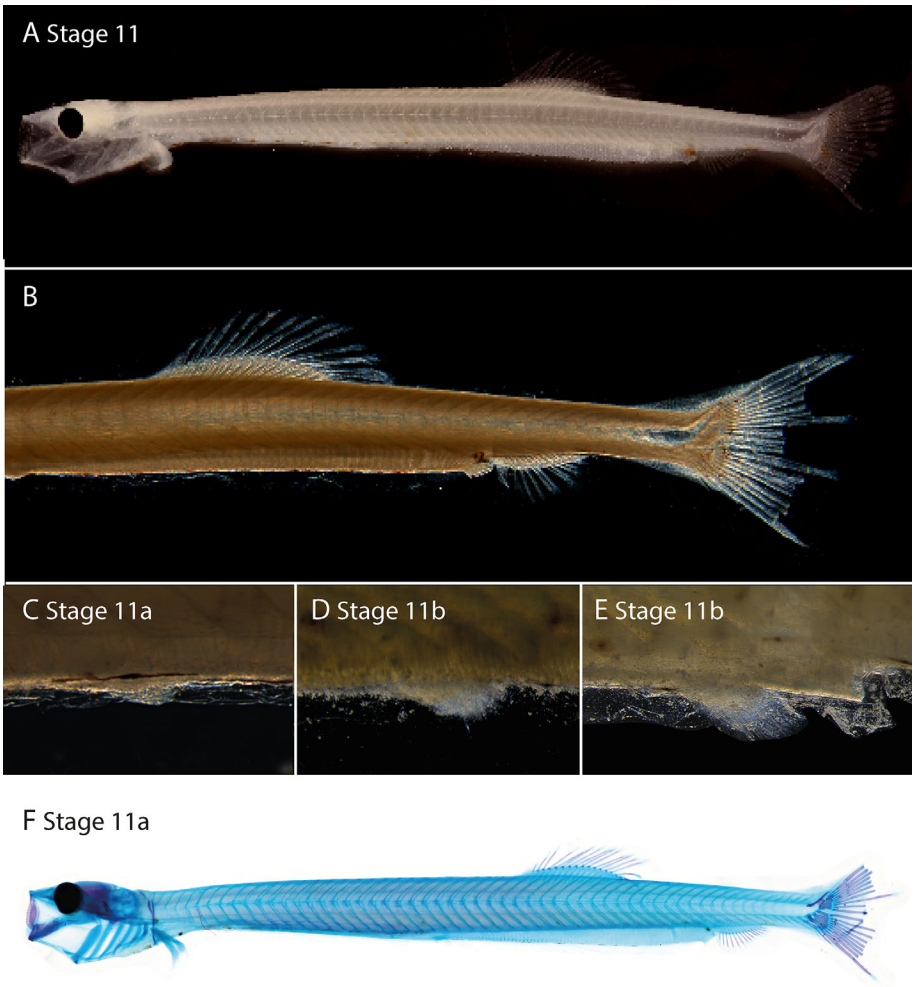


**Fig. 6.** Larval stage 11 of the herring *Clupea harengus*. Larval length: 22.2 mm SL.

fin. The body height increases further, and the shape of the adult fish is obtained. In addition, squamation begins. Also, the pigmentation of the larvae intensifies, and the dorsal ridge of the larvae is heavily pigmented. C&S specimens show that the two epurals in the caudal fin ossify. In addition, the ribs start to ossify. The basipterygia are ossified and supraneurals form as cartilage and ossify too. In the vertebral column, the ribs are ossified. In the shoulder girdle, the scapula, the coracoid, and the radials ossify; furthermore, two postcleithra form.

***Stage 14 J (Fig. 8)***

Pigmentation becomes more distinctive. A dark stripe appears at each flank and the top of the head is also pigmented. Finally, C&S shows that the pterygiophores of the dorsal fin begin to ossify. Keeled scales, also called scutes, are now present at the mid-ventral line.

***Stage 15 J (Fig. 8)***

The lateral stripe on each flank is no longer visible since it merged with the pigmentation on the dorsal ridge. Hence, the dorsal half of the herring is now darkly pigmented. The snout appears more pointed. All skeletal elements are now ossified, as for example the proximal part of the pterygiophores of the anal and dorsal fin.

## Length distribution per stage for Western Baltic spring spawning herring

The length per stage distribution shows that in general, with advancing stage, the herring larvae increase in size (Fig. 9). However, the size ranges for the stages overlap, in some developmental phases such as the yolk sac phase, quite substantially, although the average length per stage increases steadily (Table 2; means: stage 1: 6.29 mm, stage 2: 7.00 mm, stage 3: 7.46 mm, stage 4: 8.57 mm, stage 5: 10.40 mm, stage 6: 12.15 mm, stage 7a: 13.71 mm, stage 7b: 15.55 mm, stage 8a: 16.90 mm, stage 8b: 17.80 mm, stage 9: 19.07, stage 10: 20.57 mm, stage 11a: 21.81 mm, stage 11b: 22.81 mm, stage 12b: 22.69 mm). Since only spring spawned herring larvae were analyzed, stage 12a has not been found.



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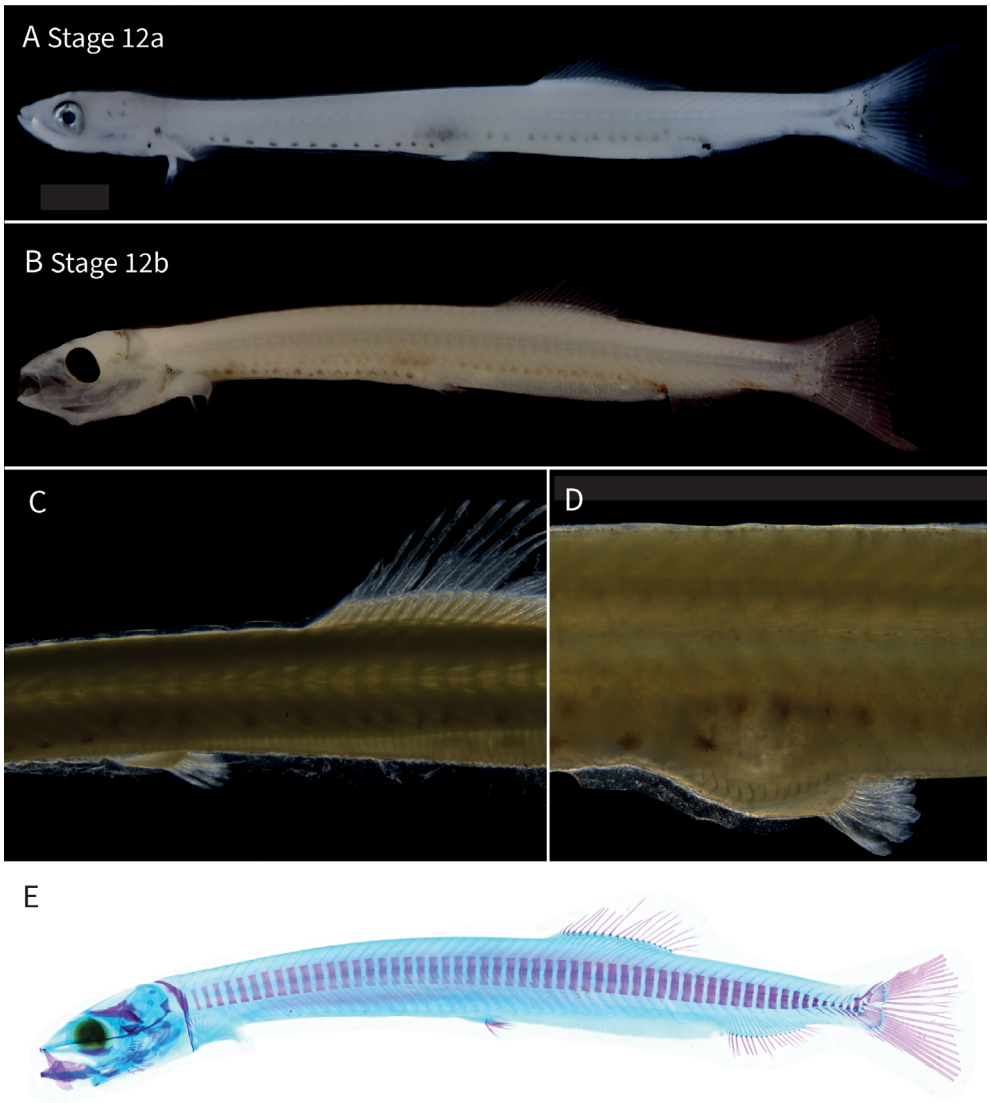


**Fig. 7.** Larval stage 12 of the herring *Clupea harengus*. Larval length: 26.2 mm SL.

## *Discussion*

The staging system proposed in this study consists of 15 developmental stages in 5 major developmental phases: yolk sac phase, dorsal fin development, caudal fin development, pelvic fin development and juvenile phase. Hence, at first sight it does not differ that much from the prior division in yolk sac, preflexion, flexion, and postflexion classification by Doyle (1977) (Table 3). However, on a more detailed level, the substages by Doyle (1977) are not entirely consistent with prior observations made by the authors of this study. For example, stage 2b (Doyle 1977) has not been recorded in this study, since the separation of the dorsal fin from the primordial fin fold first occurred in stage 7b. Also, in this study we found that the shortening of the gut and the movement of the ventral and dorsal fins occurs only in the later stages (12), the myomere counts in stages 4b–4d (Doyle) do not match, and the stages were matched based on the depictions. Furthermore, for some stages the descriptions are not entirely clear. As an example, and the phenotypes described in the limitations to stage 1c and the descriptions about the formation of the dorsal fin best correspond to stage 5; however, no larvae in stage 5 have been found which still carried remains of the yolk. Also, some substages described within this staging system have not been described by Doyle (1977) such as stage 4 or the later juvenile stages. Also, some (intermediate) stages are missing, as are stages 5, 7a, 10, 12a, 14, and 15. Some



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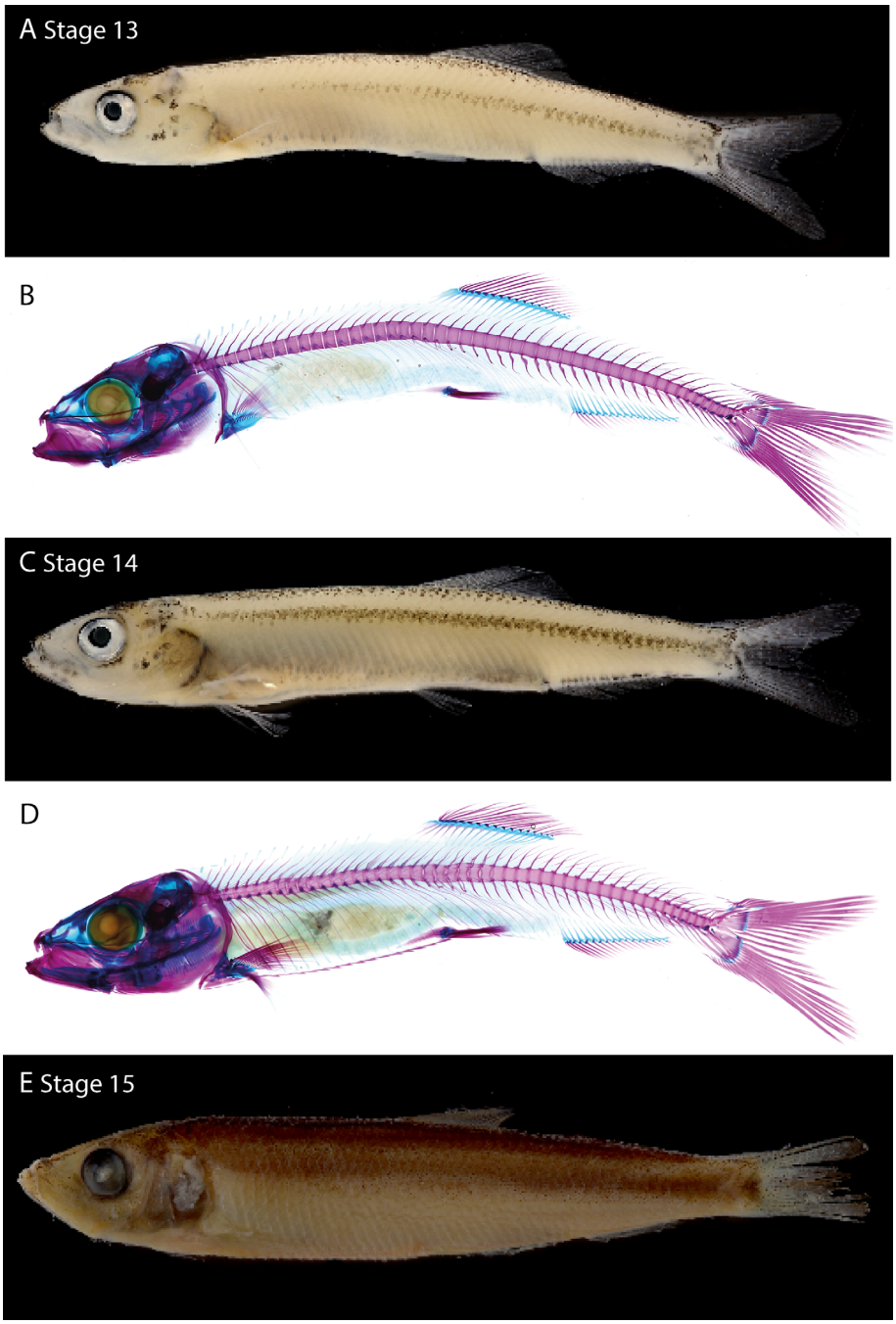


**Fig. 8.** Juvenile stages 13–15 of the herring *Clupea harengus*. Larval lengths: stage 13: 25.1 mm SL; stage 14: 29.2 mm SL and stage 15: 7.8 cm SL.

observations of Doyle (1977), which are quite detailed, only represent a short time interval in the total duration of the larval development but take up a major part of the entire staging system. For example, the flexion of the notochord that generally is a standard measurement for the development of most fish larvae, was measured in degrees during three substages. In herring larvae notochord flexion is completed in a relative short time period and skeletal elements formed during notochord flexion are only visible in C&S specimens (Fischbach et al. 2022). Furthermore, measuring the degree of notochord flexion is often unprecise, unpractical, and quickly becomes subjective. In this study, additional characteristics besides the progression of the flexion were described and flexion was linked to the development of cartilage precursors in the caudal fin which changed in shape and are clearly visible from external view in herring larvae. Hence, the need for a



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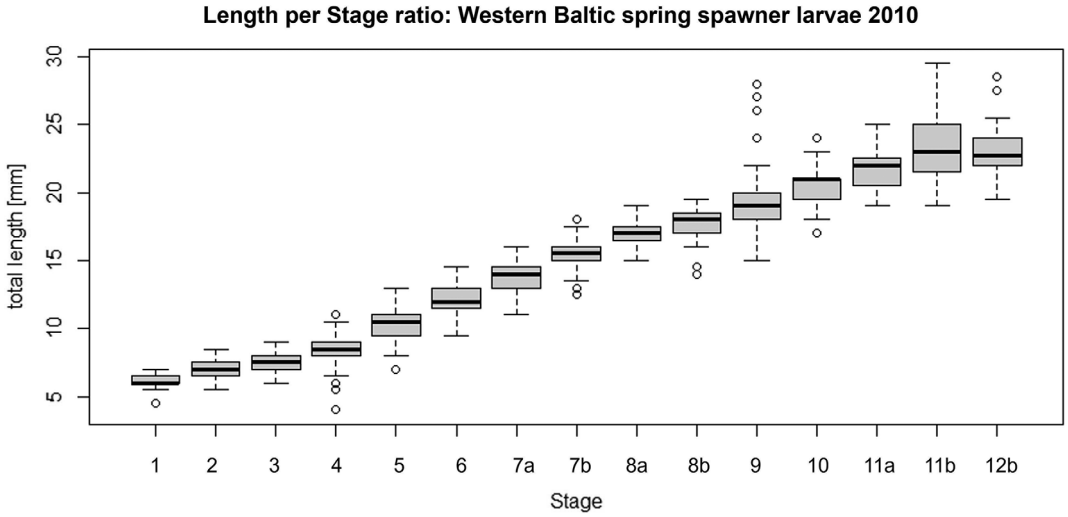


**Fig. 9.** Length per stage distribution for Western Baltic herring larvae in 2010.

new description of the developmental stages became clear. When staging larvae in detailed life stages, it is extremely important that the stages can be clearly identified by preferably more than one trait for cases where a single trait is not well recognizable, for example, due to handling procedures. The characters for each stage must be described so that the stages are correctly identified by different users. In this study, we present an easy-to-use guide for the identification of stages during the larval development of herring. For each proposed stage we included detailed descriptions of both the eidonomy and the skeletal morphology of the larval herring, based on samples from five different herring stocks with different spawning biology in the North and Baltic Sea. The outer morphology is coupled with the skeletal development to further indicate attributes the larvae has gained in this stage, for example, development of dorsal fin = more swimming stability (Grillner 1981). It can moreover be hypothesized that similar if not the same staging system can be applied to other clupeoid species, since the skeletal development and also the outer morphology of clupeoid larvae such as sprat and sardine are very similar (Fischbach et al. 2022).

Besides Doyle's (1977) study, herring larvae have been staged in other studies, mostly for specific purposes and not intended for general use (Ramanujam 1929; Blaxter 1962). Basic features of herring larvae such as myomere or fin ray count were also recorded in studies addressing the species discrimination from other clupeoid larvae (Russell 1976; Munk and Nielsen 2005) and can also be integrated into a staging system. Ramanujam (1929) proposed staging herring larvae based on a detailed description of the development of the vertebral column; however, the staging system is not applicable in larval surveys, since the stages cannot be identified by features of the internal morphology alone. Therefore, the staging system by Ramanujam (1929) is not comparable to the system proposed in this study. Blaxter and Hempel (1961) reared herring larvae of spring spawning herring from the Elbe estuary

**Table 2.** Sample size, average, median, and standard deviation of the length measurements of herring larvae in 2010.

| Stage | 1 | 2 | 3 | 4 | 5 | 6 | 7a | 7b |
|---|---|---|---|---|---|---|---|---|
| *n* | 40 | 135 | 166 | 695 | 316 | 262 | 182 | 202 |
| $\bar{X}$ | 6.29 | 7 | 7.46 | 8.57 | 10.4 | 12.15 | 13.71 | 15.55 |
| $\tilde{x}$ | 6 | 7 | 7.5 | 8.5 | 10 | 12 | 14 | 16 |
| $\sigma$ | 0.46 | 0.65 | 0.69 | 1.05 | 1.07 | 0.82 | 1.00 | 1.03 |

| Stage | 8a | 8b | 9 | 10 | 11a | 11b | 12b |
|---|---|---|---|---|---|---|---|
| *n* | 66 | 54 | 144 | 106 | 71 | 55 | 30 |
| $\bar{X}$ | 16.9 | 17.8 | 19.07 | 20.57 | 21.81 | 22.81 | 22.69 |
| $\tilde{x}$ | 17 | 18 | 19 | 21 | 22 | 22 | 23 |
| $\sigma$ | 0.88 | 1.11 | 2.15 | 1.36 | 1.35 | 2.1 | 1.25 |



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**Table 3.** Comparison of the staging systems for herring larvae by Doyle (1977), Blaxter (1962), and Blaxter and Hempel (1961) to the staging system proposed in this study.

| This study | Doyle (1977) | Blaxter (1962) | Blaxter and Hempel (1961) |
|---|---|---|---|
| 1 | 1a: Depth of yolk sac equal to, or exceeding, 2J times the depth of the myotomal musculature which lies immediately adjacent and dorsal to the sac. | Hatching, yolk sac present, lower jaw small, little pigment. | Hatching. |
| 2 | *1b: Depth of yolk sac about twice the depth of the myotomal musculature which lies immediately adjacent and dorsal to the sac. | | |
| 3 | 1c: Depth of yolk sac equal to or less than depth of the myotomal musculature which lies immediately adjacent and dorsal to the sac. | | |
| 4 | | Yolk sac absorbed, jaw grows forward, mouth becomes functional, pigment spots develop along the gut. | End of yolk sac stage. |
| 5 | | Dorsal fin develops as part of primordial fin (no fin rays). | |
| 6 | †2a: Dorsal fin not yet separated posteriorly from primordial fin. Yolk sac absent.<br>*2b: Division between the posterior edge of the dorsal fin and the posterior part of the primordial fin complete. Lobe of dorsal fin not protruding markedly beyond the posterior primordial fin nor growing along beside it. | | |
| 7a | | | Dorsal fin develops, hypurals are developed. |
| 7b | 2c: Dorsal fin protruding markedly beyond or beside the primordial fin and extending posteriorly above it. The dorsal fin is completely separate from the primordial fin posteriorly. The posterior tip of the notochord is still more or less straight, that is, making an angle of less than about 10–150 dorsally with the remainder of the notochord.<br>(This is sometimes difficult to determine when the whole notochord is contorted by fixation). | Dorsal fin rays develop (stainable with Alizarin). | |
| | | Anal fin develops (without rays) as part of primordial fin. Hypurals become stainable. | Anal fin develops. Hypurals become stainable. |
| 8a | 3a: Posterior tip of the notochord is turned dorsally less than 30–40° but more than 150 (angles estimated on the dorsal surface of the notochord). The anal fin is not yet separated posteriorly by a line from the ventral primordial fin. | Primordial fin disappears, tail begins to turn up. | Tip of tail bends upwards. |
| 8b | 3b: Posterior tip of the notochord is turned dorsally 40–500. The anal fin is separated posteriorly by a line from the ventral primordial fin. | | |
| 9 | 3c: Posterior tip of the notochord is turned dorsally more than 50°. | First uroneural becomes stainable, anal fin rays become stainable. | |

*(Continues)*



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**Table 3.** Continued

| This study | Doyle (1977) | Blaxter (1962) | Blaxter and Hempel (1961) |
|---|---|---|---|
| 10 | | | |
| 11a | 4a: At least 24 myomeres are present between the posterior point of insertion of the pelvic fin and the anus. | Last hemal arch becomes stainable. "Vertebral centra start become stainable (in center of body). | "Caudal hemal arches become stainable" "Vertebral centra in the middle of the vertebral column become stainable" |
| 11b | 4b: At least 21 such myomeres but less than 24. | First urostylar centrum becomes stainable. | First urostylar centrum becomes stainable. |
| 12a | | | |
| 12b | †4c: At least 18 such myomeres but less than 21. | | Start of metamorphosis. |
| 13 | †4d: At least 15 such myomeres but less than 18. | Caudal fin rays bifurcate, entire vertebral column becomes stainable. | End of metamorphosis. |
| 14 | | | |
| 15 | | | |

*Definitions that did not quite fit the definitions of the stages in this study.
†Party fits the defintions of the stages in this study.

(GER), the Kiel Fjord (GER), and the Firth of Clyde (UK) at different temperatures and salinities. They assigned different stages to different length groups according to Blaxter (1962), who had also used Alizarin staining to identify different larval stages based on the parts of the skeletal development. However, the staging did not differentiate between the more advanced larval stages and summed up stages 10–12 of the present study into one stage. The stages introduced by Blaxter (1962) mostly correspond to the observations made in this study (Table 3); however, the stages are less detailed and especially in the later stages, focus on the development and ossification of elements of the caudal fin and the vertebral column only, which are mostly visible by clearing and staining. Differences were observed in the development of the anal fin. In this study, the anal fin development was observed as cooccurring with the flexion of the notochord, Blaxter (1962) described the development to happen before the notochord flexion. Also, we observed that the hypurals only first started ossifying, and therefore being stainable, in stage 11a. The first uroneural occurred in stage 9, while anal fin rays were only stainable later in stage 10, when also the caudal fin rays developed their final shape, and the notochord flexion was completed.

The same applies to the staging system by Blaxter and Hempel (1961), although the stages were in overall conform with the findings of this study, the same complication occurred as described with the system by Blaxter (1962), since especially the later stages were very similar and used the same characters. However, it is noteworthy that the length frames of both studies varied even when describing the same character state. For example, in Blaxter (1962) the dorsal-fin development (without fin rays) and development of the hypurals starts at 12–13 mm, while in Blaxter and Hempel (1961) it begins between 10 and 11 mm. Juvenile stages are also not mentioned by Blaxter (1962).

Many other staging systems are based entirely on larval length at specific developmental steps and mostly describe the development of the fins, which is then recorded in relation to the TL of the larvae (Schnakenbeck 1929; Bridger 1956; Blaxter and Hempel 1961; Blaxter 1962). Schnakenbeck (1929) described the development of the dorsal fin in preserved larvae of North-Western herring at 9–10 mm TL and the development of the anal fin at 15–16 mm TL. Bridger (1956) divided herring larvae from the Flemish estuaries in five developmental stages. At 8–9 mm TL, the yolk sac is mostly absorbed, and fin rays are visible in the caudal fin, then the dorsal fin develops, later the anal fin and finally the pelvic fin. These descriptions were made for several herring populations as well as for herring larvae from the Baltic Sea (Heincke 1878; Meyer 1878a,*b*). Staging the larvae according to the development of different fins was also the approach in the present study, however, with the inclusion of the skeletal development the stages are more clearly defined, detailed, and provide the appropriate terminology.

Gamble et al. (1985) showed that the size distribution at the same developmental stage varies greatly between different herring ecotypes. Although it remained unclear if environmental, artificial, or genetic factors contributed to these differences, Moyano et al. (2016) were able to show that size differences within developmental stages are highly influenced by different temperature conditions. In this study, we showed that the length ranges overlapped between different stages and also varied within stages (Fig. 9). Since length measurements and stage information were collected from larvae sampled throughout the entire season in 2010 (from February to June), the larvae are subjected to different temperatures and



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belonged to different spawning cohorts. In this study, it could not be determined if the larval size was influenced mainly by environmental or genetic factors, nevertheless, it still shows that even larvae within a sampling season show variation in length per stage measurements. Data on developmental stages, however, are easily comparable among different ecotypes, populations, and larvae reared under different environmental conditions. Moreover, the stage duration between larvae of different cohorts and ecotypes is likely, among other factors, directly influenced by temperature and food availability, such as indicated by Stenevik et al. (2021). Therefore, an evaluation of the stage duration between different larval stages can serve as estimate for the condition of the respective herring stock and how well the herring larvae are coping with environmental conditions and anthropogenic influences. Different ecotypes could utilize different growth strategies, for example, autumn spawned herring larvae are hypothesized to remain longer within one stage and put more emphasis on growth than to pass through the next developmental stage. This would contradict the stage duration hypothesis and once more illustrates that pure length data are not well suited as proxy for development rates.

To evaluate long-term data series, for example, herring larvae surveys, which mostly only record length data, population, or stock specific size-per-stage ranges could be calculated as an approach to analyze the existing length data sets. Therefore, larvae from different years and, if possible, different temperature regimes should be analyzed regarding their stage–length relationship. Then probabilities can be calculated, which show how likely it is, that larvae of a specific size belong to a specific stage for a certain stock or population. With the calculated stock or population-specific probabilities, it would also be possible to analyze data within time series where staging of larvae is not possible anymore. These kinds of probabilities could also be calculated for ongoing herring larvae survey by staging a certain number of larvae per year. This ensures that length probabilities are accurate, and no major shifts have occurred, for example, due to mixture with another stock.

Information about developmental stages and early life stage succession (= stage duration) is also a valuable indicator for larval fitness, growth, and their potential to survive. As the larvae develop, they attain further traits which alter their ability to react to their surrounding environment. The different phases and stages are thus likely to also indicate altered vulnerability to certain environmental effects since the larvae inhabit a certain environment and further develop their feeding habits and abilities such as swimming speed. To create a larger overview of herring development, the new knowledge about the skeletal development of herring larvae can and should be aligned with previous research about the development of other functional units or abilities of herring larvae to increase the specification of the stages. However, in the past most abilities such as swimming speed have only been analyzed regarding size or age (Batty 1984; Hakala et al. 2003; Cresci et al. 2020). Therefore, the results depend on the population and external conditions, such as field or rearing temperature and can only be transferred to developmental stages to a certain degree. In other studies, the analyzed features were aligned according to Doyle's (1977) staging system or other developmental characteristics as described above and were independently documented (Gamble et al. 1985; Moyano et al. 2016; Joly et al. 2021). In these cases, the findings can be put in direct relation to the stages or developmental phases. Coastal nursery areas for herring larvae are particularly exposed to manifold climate change effects in synergy with eutrophication, habitat degradation, and other anthropogenic impacts (Moyano et al. 2016). Therefore, larval stages and stage duration provide an important parameter to identify potential survival bottlenecks as the most critical period in the early life stage history. From the literature, some stages described in this staging system can be hypothesized to be of greater importance when assessing larval herring mortality bottlenecks of different herring stocks. Stage 4 (Fig. 1) as mentioned above but also stage 10 (Fig. 6) and possibly 12b (Fig. 8) seem to be indicators for fortunate or unfortunate environmental conditions, which herring larvae are exposed to, since they inherit or do not inherit specific traits. Stage 10d describes the final flexion of the notochord and the completion of the caudal fin development. Since all elements of the caudal fin are now present, it is likely that this results in an increase of swimming speed, which is crucial for herring larvae survival. Moyano et al. (2016) stated that under different temperature regimes the critical swimming speed for larvae was achieved at 15–17 mm body length (at 7–15°C) when the notochord flexion was completed. Measurements of larvae from stage 10 also correspond with the length measured of the survivors for the main structuring bottleneck for Western Baltic spring spawning herring (Oeberst et al. 2009*a*,*b*). Therefore, this stage could be crucial especially for the estimation of mortality of the early stages in Western Baltic spring spawning herring and could also define the end of the main structuring bottleneck for herring larvae (Polte et al. 2014). Stage 12a describes a larva which progresses in development of the pelvic fin but does not start the transition to the juvenile fish yet. Stage 12a was mostly observed in Western Baltic autumn spawning larvae which are generally larger at the same stage than spring spawner, which has already been shown in North Sea autumn and spring spawners (Gamble et al. 1985). These larvae probably overwinter and transform into juveniles in the year following their hatch, as it might be possible that due to insufficient nutrition the larvae are not able to increase their body height to length ratio. This would correspond well with studies on other species which state that larvae in the later stages (flexion, postflexion) survive starvation longer that in the early preflexion stages (Powell and Chester 1985). Using energy for growth rather than development could also be an indication for a shift in survival strategies from a fast stage



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duration to "bigger is better" (Houde 1987; Anderson 1988; Leggett and Deblois 1994), which might be more beneficial to larvae in a specific environment. In stage 12b, an overall shift in the outer morphology of the larvae occurs as this is the transition into the juvenile fish.

However, these observations could be population specific and can likely not match with analysis of herring stocks other than the Western Baltic spring and autumn spawning herring. Further research and application of the staging system to long term data series is needed to further resolve the abilities and further traits associated which each larval stage proposed in the present study.

Staging herring larvae in this detail is more time consuming than staging larvae in only four categories or only recording length measurements; however, the advantages for larval studies are overbearing. Also, we experienced that the usage of the system becomes increasingly easier and faster, the more the experienced one gets. With the advances in microscope and photography technology, looking at smaller traits of the larvae is more easily today than some decades ago and the technological advances should be mirrored in the application of staging systems.

## Conclusions

This study provides a detailed, easy-to-use guide to the larval phases of Atlantic herring, introducing background information of its skeletal development. The staging system proposed in this study is a refined and easy to work with system in relation to Doyle (1977) and Blaxter (1962) who already proposed elaborate systems considering their technical equipment. Due to technical advances in microscopy, today more detailed analyses of morphological features are possible and should be integrated in the research about early life history of not only herring, but also other larval fishes. The additional information of the progress of ossification and development of the different skeletal elements introduces important background information when it comes to the determination of abilities and traits the larvae possess. Larval life stages have proven to be an adequate indication for ecological studies and larvae surveys because they ensure comparability between different populations reared or caught under varying environmental conditions and are not susceptible to environmental factors such as length data. Including stage data in fishery assessments and estimations of larval survival could help to identify critical stages which function as a bottleneck for larval survival. In conclusion, this staging system serves as a standardized framework to be applied in future ecological studies and fishery assessment models and lays the groundwork for further ecological studies regarding the ecological role of the proposed stages.

## Comments and recommendations

### How to use the staging system

The staging system presented in this study is intended to be used in herring larvae surveys as well as in ecological studies. An increased usage of staging also increases the transparency of herring development and possible implications of singular stages can be detected. Moreover, the staging system is supposed to increase the comparability of different studies, for example, of different stocks. The attached posters (Supporting Information Posters S1, S2) are meant to serve to give an overview on herring morphology as well as the staging system and hopefully simplify the application of the system in laboratory or field work. Before attempting to start staging the larvae, make sure to familiarize yourself with the terminology and the characteristics of all stages. Best results at staging herring larvae can be achieved by using a transmitting light microscope and choosing indirect light to get a dark background, so that the contrast between small transparent structures and the background is highest. It is also advised to attempt to flatten the herring larvae as much as possible without breaking them, in order to avoid missing important key features. The herring larvae should also lie upright to prevent misinterpretation of positional relations. It was attempted to describe each stage by several features, since generally, having only one character that defines a stage renders the system more susceptible to mistakes. For example, the yolk sac and the fin fold of herring larvae are quite delicate and can be easily damaged during sampling or repetitive handling.

### Data availability statement

The data that support this article will be available on request to the corresponding author.

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