Received: 15 November 2022 | Revised: 9 May 2023 | Accepted: 19 May 2023

DOI: 10.1002/dvdy.634

RESEARCH ARTICLE

Developmental Dynamics WILEY

Early ontogeny of the lesser sandeel (*Ammodytes marinus*)

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Funding information
Norwegian Institute of Marine Research, Equinor Energy, AkerBP, Lundin Energy Norway, Spirit Energy Norway, and DNO Norway, Grant/Award Number: Project no. 15781, KnowSandeel 1.0

Abstract

Background: Lesser sandeel (*Ammodytes marinus*) is widely distributed in North Sea ecosystems. Sandeel acts as a critical trophic link between zooplankton and top predators (fish, mammals, sea birds). Because they live buried in the sand, sandeel may be directly affected by the rapid expansion of anthropogenic activities linked to their habitat on the sea bottom (e.g., hydrocarbon extraction, offshore renewable energy, and subsea mining). It is, therefore, important to understand the impact of cumulative environmental and anthropogenic stressors on this species. A detailed description of the ontogenetic timeline and developmental staging for this species is lacking limiting the possibilities for comparative developmental studies assessing, e.g., the impact of various environmental stressors.

Results: A detailed description of the morphological development of lesser sandeel and their developmental trajectory, obtained through visual observations and microscopic techniques, is presented. Methods for gamete stripping and intensive culture of the early life stages are also provided.

Conclusion: This work provides a basis for future research to understand the effect of cumulative environmental and anthropogenic stressors on development in the early life stages of lesser sandeel.

KEYWORDS

development, early life stages, embryogenesis, fertilization, intensive culture, larvae, morphogenesis

1 | INTRODUCTION

Lesser sandeel (*Ammodytes marinus* [Raitt, 1934]) are very abundant and widely distributed in the North Atlantic and in the southwestern Baltic Sea.[1] In the North Sea, sandeel represents up to 15% of the total fish biomass and it is estimated that 90% of it is lesser sandeel (hereafter, sandeel).[2] The sandeel fishery developed in the 1950s and, with annual landings of up to 1.2 million tons,[3] the fishery has often been the largest single-species fishery in

the North Sea.[4] Due to this high abundance, and its lipid richness, it provides a critical trophic link between secondary producers (zooplankton) and a wide variety of top predators, including fish, mammals, and sea birds.[5-11] Changes in the abundance of sandeel have impacts on the food chain, from larger fish to seabirds, which display dramatically reduced breeding success when local sandeel stocks decrease.[12]

Sandeel juveniles and adults spend much of their time buried in oxygen-rich sand or gravel sediments with specific granulometry at depths from 10 to 150 m.[4,13,14] This lifestyle conserves energy and provides protection from predators.[10,15] Although they spend a considerable part of their time buried in the sediment (from September to March), sandeel emerge from the seabed and rise up into the water column to feed (during the spring) or spawn (December and January).[13,16-21] During the feeding season, a large number of individuals emerge from the seabed in large pelagic schools to prey upon zooplankton. These schools form bridging structures that likely play an important role in their ecology and prevent post-settled juveniles from being disconnected from suitable bottom substrate.[22] The strong selectivity for areas with specific granulometry represents a habitat limitation that increases the vulnerability of the sandeel to climate change, high fishing pressure, and anthropogenic activity that degrades or eliminates their habitat.[10,22-26]

These specific sandy habitats are also important for the early life stages of sandeel because they spawn a single batch of demersal eggs that adheres to the sand in an oxygen-rich environment.[24-26] After a long (1 month) embryonic period, larvae hatch and drift with the current in the deep layer. After metamorphosis, juveniles congregate and settle in the same areas where the adults are found.[27] Although they form large schools, post-settled juvenile and adult sandeel display high site fidelity.[28,29] During the winter (the spawning period), sandeels rarely emerge from the seabed to feed, subsisting on stored energy.

Considering the ecological and commercial importance of sandeel, risk assessments of the cumulative environmental and anthropogenic stressors impacting them are needed. Due to the rapidly expanding habitat-changing man-made structures and activity linked to the sea bottom, such as offshore renewable energy, oil and natural gas extraction platforms, and subsea mining, knowledge of the biology of sandeel is needed. Assessing any anthropogenic or climate-driven impacts on sandeel requires a better understanding of the sensitivity of their early life stages. To conduct any such study, it is essential to have a detailed table of all the developmental features and rearing methods that provide guidance for assessing fertility, hatchability, and larval survival and behavior. Although the main life stages in some sandeel species (*A. americanus*, *A. personatus*) have been described, a detailed systematic description of the morphological development of lesser sandeel is unavailable. The limited information that is available has been produced using different methods, at different times and stages of development, and intensive culture protocols have not been developed.[30-33]

The objective of this study is to provide a detailed description of the embryonic and larval biology of lesser sandeel and to outline their developmental trajectory. This study also describes methods for gamete stripping and intensive culture techniques.

2 | RESULTS AND DISCUSSION

2.1 | Spawning evaluation and comparison of fertilization substrates

Gamete (sperm and eggs) collection was successfully performed by hand-stripping the males and females. Excessive pressure applied along the abdomen was previously reported to rupture membranes holding the eggs, injuring the ovaries, and resulting in lower egg survival.[31] In this study, gentle pressure along the abdomen was sufficient to assist the natural flow of eggs. However, stripping often resulted in the death of the female during the days that followed. Therefore, we advise that females be euthanized after stripping. The weight of spawned eggs represented 16% of the female total weight (Figure 1A).

Egg fertilization was performed on different substrates (glass slide, filter mesh [500, 300, and 100 μm], and sand substrate). A comparison of substrates is summarized in Table 1. The fertilization rates and viability of embryos (48 hours post-fertilization, hpf) were 70%–80% for the egg batches placed in tubes on filter mesh regardless of mesh pore size (100, 300, and 500 μm). Fertilization from egg batches placed on microscope slides varied from 40% to 80%, whereas no fertilized eggs were found in the batches placed on natural sand. Fertilization and incubating sandeel embryos in natural sand was not a successful option, leading to a high number of inconveniences for experimental studies (no observations possible without disturbance, no control of egg quality and survival). This result was surprising since it is the natural egg substrate; the reason for it is unclear although possibilities include insufficient oxygenation of the sand or too rough handling of eggs. The glass microscope slide substrate was the most appropriate method for observing embryo development and sampling while introducing minimal disturbance to the embryos. Observations could be made on every slide and the whole egg batch could be

observed with minimal disturbance. Embryos could be efficiently sampled and dying individuals can be removed using this system. However, bacterial growth was observed on the slides, and hatching success was poor resulting in very few surviving larvae (<30%–40%). The hatching period on glass slides extended to over a month and many of the embryos did not hatch before they were mechanically stimulated with a flow of seawater. The prolonged embryonic period resulted in smaller larvae and higher mortality in hatched larvae and in unhatched embryos. The development of embryos and larvae from the glass slides also varied according to the number of egg layers on the slide (Figure 1B). Areas with more than three clustered embryos showed slower development compared to single embryos or embryos in smaller groups (Figure 1B), suggesting that the clustered eggs did not receive enough oxygenation or water flow to grow properly and were possibly also more susceptible to bacterial infection. The eggs placed in tubes on the filter mesh were more difficult to observe without physically removing them. Moving the filter system increased the chance of detaching the eggs from the substrate (due to water flow through the filter), increasing the chance of damaging and/or losing eggs in the rearing tank. The 500 μm filter mesh was too big, leading to the compression of some eggs trapped in the mesh opening. While the filter mesh systems (500, 300, and 100 μm) were more inconvenient for embryonic observations, embryos received more oxygenation and water flow compared to the batches placed on the glass slides, limiting the bacterial growth, and resulting in egg development being more consistent and synchronized (observations made from sampled embryos under the microscope). The hatching success was also high in the filter mesh systems; >70%–80% hatching and a shorter overall period for the batch to hatch. Hatching duration in the filter mesh systems was 8–9 days with a peak at 3 days after the first hatching had begun compared to over a month for the glass slide system. Synchronized hatching required good oxygenation and stimulation with sufficient water flow going over the eggs. Adult sandeel require well-flushed, tidally active areas that are oxygen-rich to survive and spawn.[4,13,14,34,35] Embryogenesis and survival of early life stages are influenced by temperature and oxygen concentration. Low temperature and oxygen concentration delay egg development in sandeel.[11,30,31] The literature on the embryonic development of sandeel is limited. Smigielski, Halavik, Buckley, Drew, and Laurence[31] reported an incubation and hatch duration for *A. americanus* of between 55 and 135 days at 10°C and 2°C, respectively. The authors suggested a lengthy hatching period for this species, although it is still uncertain if the hatching duration is as long in the natural environment. The rearing systems using a filter mesh size of 300 μm produced an outcome consistent with the hatching duration reported by Régnier, Gibb, and Wright[30] for *A. marinus*.

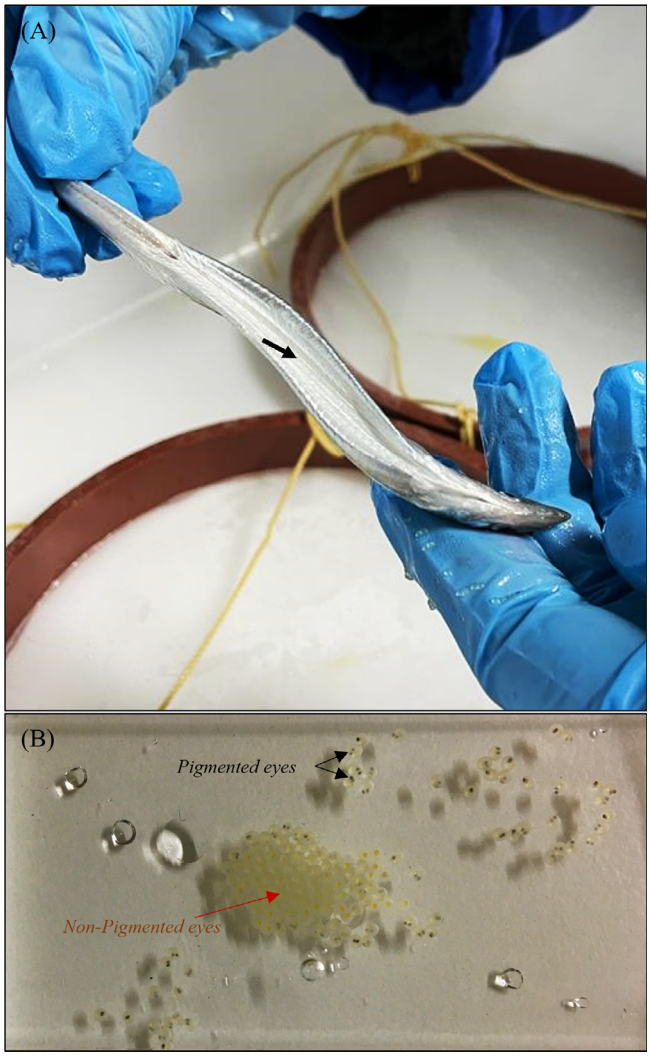

FIGURE 1 (A) Female lesser sandeel photographed after being stripped of eggs. The black arrow indicates the volume taken by the whole egg batch. (B) Egg batch placed on glass slide showing variation in developmental timing. The red arrow indicates grouped embryos with slower development (non-pigmented eyes) compared to a batch with a lower number of grouped embryos (pigmented eyes, black arrow). Photo credit: E. Sørhus.

2.2 | Early development

Embryonic and larval development in sandeel has only been partially described,[33] making it difficult to compare our results with those reported in earlier studies. From a developmental perspective, sandeel share numerous physiological and morphological features with many teleost fishes, particularly herring.[31,36,37] The most striking

TABLE 1 Comparison of fertilization substrates for lesser sandeel (*Ammodytes marinus*) and degree of ease.

	Glass slide	Tube mesh 500 µm	Tube Mesh 300 µm	Tube Mesh 100 µm	Natural sand
Fertilization and viability (24 h)	40%-80%	>70%-80% (estimation)	>80%	>70%-80%	0%
Holding method (ease)	2	3	3	3	1
Egg observation and manipulation	3	1	2	2	0
Control of eggs layers and dispersion	3	2	3	3	0
Egg sampling	3	0	3	3	0
No bacterial development	1	3	3	3	1
No oxygen limitation	1	3	3	2	?
Hatching success	1	3	3	3	0
Holding hatched larvae (*) and control of hatching number	0	1	3	3	?
	Rating scale:	*0* (*Bad*)	*1* (*Low*)	*2* (*Good*)	*3* (*Very good*)

*Applicable if tube height is high enough to retain the hatched larvae (depending on the tank size).

feature specific to sandeel and herring species is the long embryonic development (a month) with a long elongated pharyngula. A distinctive character in sandeel larvae is also the position of the anus; the ratio of pre-anal and post-anal body length is 4:3, compared 4:1 in herring.

Sandeel eggs are almost spherical with a diameter ranging from 0.85 to 1.23 mm (mean ± SD; 1.03 ± 0.07 mm; n = 1220 eggs). They have a semi-transparent adhesive layer and become brownish after fertilization, making observations of the embryo challenging. The eggs adhered immediately to the substrate after fertilization. Eggs have a clearly visible micropyle and the embryo contains a single oil globule measuring 0.021 ± 0.002 mm^3 (Figure 2A).

The developmental timing and characteristics were aggregated from data acquired at 7.8°C ± 0.5°C, a salinity of 34 ppt under a photoperiod of 12 h light/12 h dark. The developmental timings are expressed in degree days (DD) and normalized as hours or days post-fertilization/post-hatching (Table 2).

The cleavage, blastula and gastrula periods could be identified based on embryonic morphology. The first cleavage occurs a couple of hours after fertilization and results in two blastomeres of equivalent size, as observed in most teleost fishes. The second cleavage division (Figure 2A) occurs at 1.7 DD and cell division and migration continue thereafter. In sandeel, cleavage is meroblastic; a blastula appeared with a well-formed blastodisc and perivitelline space. The yolk sac syncytial layer is evident and a multilayered and highly domed blastoderm was formed at 10 DD (Figure 2B). The germ ring is then well defined at 15 DD, and the first epiboly movements begin. Gastrulation takes place with the appearance of the embryonic shield (Figure 2C). The cellular front reaches 50% epiboly at 18 DD (Figure 2C), 70% at 26 DD, and covers 100% at 35 DD. The end of the epiboly process indicates the beginning of the segmentation process with the formation of the first somites (38 DD; 10+ somites). The epiboly and segmentation steps were also successive in Pacific herring and in zebrafish.[38,39] The somites progressively increase in number, reaching 20–25 somites at 50 DD (Figure 2D–D′) and 54+ somites at 67 DD (Figure 2E–E′). At the same time, organogenesis begins. The optic placode is evident at 50 DD (Figure 2D–D′). The shape of the yolk extension continues to distinguish it from the anterior region. At 60–67 DD (SL = 2.3 ± 0.05 mm), the lens and otic vesicles are well developed, the heart tube is visible, and the end of the tail is detached from the yolk sac (Figures 2E–E′ and 3A). Brain differentiation is also well distinguished (Figures 2E′ and 3A). At this stage, the tissue is characterized by immaturity indicated by a high level of blue hematoxylin staining (Figure 3A). The cellular, tissue, and organ development accelerate at this point, and the sensory reflex begins with irregular peristaltic movements of the heart. The first muscle contractions of sandeel are observed at this point in development. At 76 DD, the finfold is present in its primary form and grows further with development. (Figure 2F–F′). Heart chambers are discernible in videos with contracting atrium and ventricle (HR = 48 ± 1 beat. min^{-1}; Figures 2F′ and 3B). Embryonic movement increases sharply when they are touched from 76 DD. Heart rate frequency increases with further development reaching 72 ± 2 $beat.min^{-1}$ at 174 DD.

A striking feature specific to sandeel and herring species is the long duration of the pharyngula stage with an elongated tail twisting around the yolk.[36] The tail twists around the yolk sac several times and the embryo

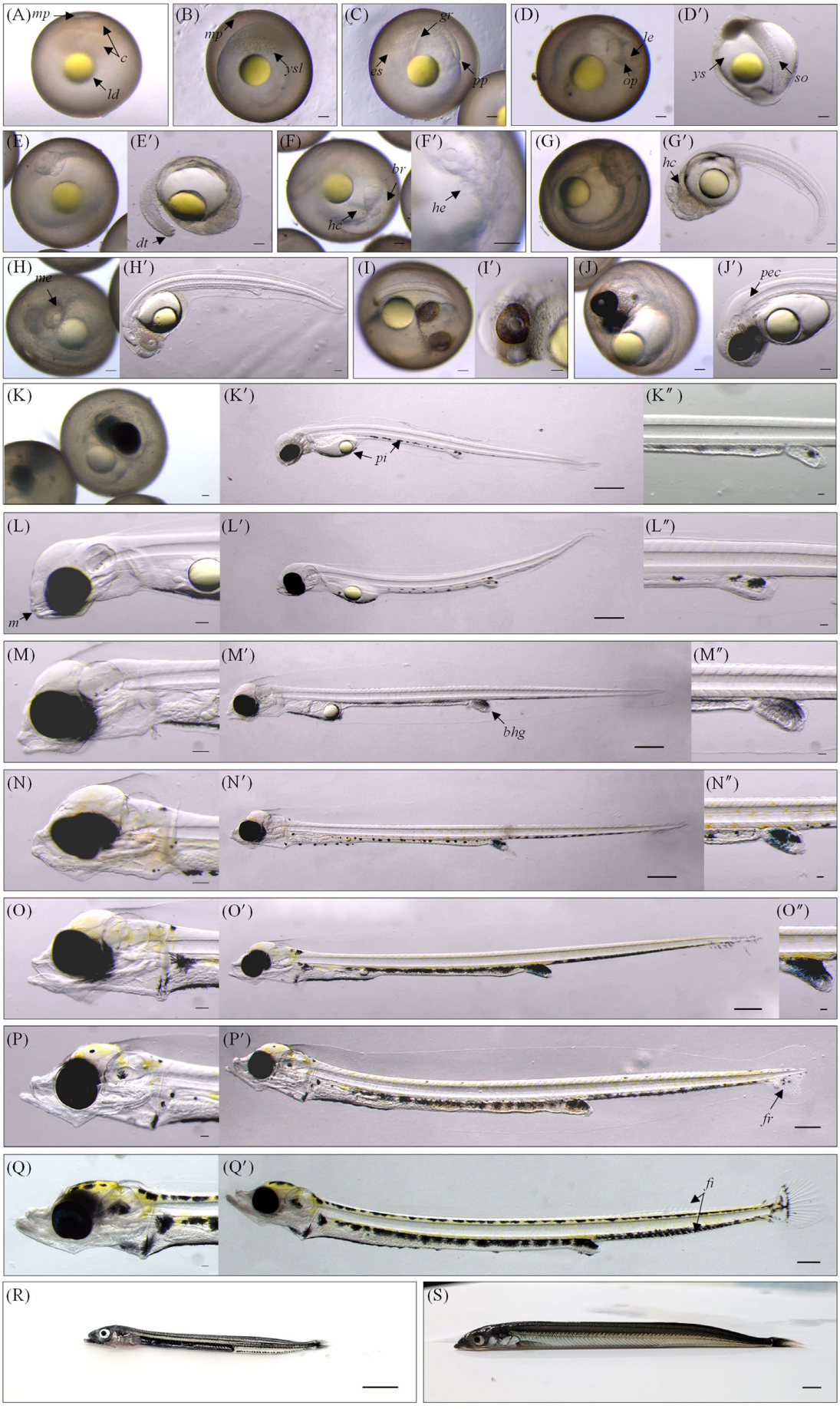

FIGURE 2 Legend on next page.

TABLE 2 Developmental timing of lesser sandeel at 7.8°C ± 0.5°C.

Figure 2 Reference	Developmental period	Description	Timing	
			DD	hpf/dpf/dph
A	Cleavage	4 cells, second division	1.7	5 hpf
B		Doming yolk syncytial	10	1 dpf
C	Gastrula/Segmentation	50% epiboly	18	2 dpf
D-D′		20+ somites	50	6 dpf
E-E′		54+ somites	67	8 dpf
F-F′		Heart chambers discernible; Embryonic movement; First hatching cells visible	76	9 dpf
G-G′	Pharyngula	Primary gut	92–100	11–12 dpf
H-H′		Head-trunk angle = 95–100°; melanophores	108–117	13–14 dpf
I-I′		Guanophores	121–133	16 dpf
J-J′		Head-trunk angle = 85–90°	150–158	18–19 dpf
K-K′-K″	Hatching period	Pre-hatching period; Head-trunk angle = 60°; tail twisting = 2.5-fold	190–198	23–24 dpf
L-L′-L″		Newly hatched larvae	210	27 dpf/1 dph
M-M′-M″	Post-hatching period/free-swimming larvae	Mouth opening stage	229	3 dph
N-N′-N″		First-feeding larvae	270–285	7–9 dph
O-O′-O″		Pre-flexion larvae	310	11 dph
P-P′		Early flexion larvae	400	22 dph
Q-Q′		Late flexion larvae		39 dph
R	Juvenile	Juvenile		4 months
S		Post-settled juvenile, young adult phase		8 months

Abbreviations: DD: degree days; dpf: days post-fertilization; dph: days post-hatching; hpf: hours post-fertilization.

exhibits very active convulsive movements the frequency of which increases until hatching. Early cells (granules) of the hatching gland are observed from 76 DD (Figure 2F) on the anterior part of the head region. The number of hatching cells greatly increases until hatching (Figure 2G′–K′). A primary gut and myomeres are clearly visible at 92–100 DD (SL = 3.07 ± 0.01 mm; Figure 2G–G′). At this point, the head-trunk angle increases as a consequence of straightening of the embryo into the chorion, reaching 95–100° at 108–117 DD (Figure 2H–H′), 85–90° at 150–158 DD (Figure 2J–J′) and 60° at hatching (190–198 DD; Figure 2K–K′). During the lengthening period, part of the head separates from the yolk sac, the eye pigmentation appears, and the pectoral buds increase in size. The first melanophores are seen on the eye at 108–117 DD (Figure 2H–H′). Guanophores appear at 121–133 DD (Figure 2I–I′) and the eyes are well structured (lens and iris) (Figure 3C) and fully pigmented at

FIGURE 2 Embryo-larval development of lesser sandeel, *Ammodytes marinus*. (A, B) Cleavage. (C–F′) Gastrula/segmentation. (G–J′) Pharyngula. (K–L″) Hatching period. (M–Q′) Post-hatching period/free-swimming larvae. (R, S) Juvenile. (A) Four cells (second division, 1.7 DD). (B) Doming yolk syncytial (10 DD). (C) 50% epiboly (18 DD). (D, D′) 20+ somites (50 DD). (E, E′) 54+ somites (67 DD). (F, F′) (76 DD). (G, G′) 92–100 DD. (H, H′) 108–117 DD. (I, I′) 121–133 DD. (J, J′) 150–158 DD. (K, K″) Pre-hatching period (190–198 DD). (L, L″) Newly hatched larvae/protruding mouth (1 dph/210 DD). (M, M″) mouth opening stage (2 dph/229). (N, N″) First-feeding larvae (7–9 dph/270–285 DD). (O, O″) pre-flexion larvae (11 dph/310 DD). (P, P′) Early flexion larvae (22 dph/400 DD). (Q, Q′) Late flexion larvae (39 dph). R, Juvenile (4 months). S, post-settled juvenile, young adult phase (8 months); bhg, blue hindgut; br, brain; C, cells; DD, degree days; dph, days post-hatching; dt, detached tail from yolk; es, embryonic shield; fi, dorsal and anal fins; fr, fin rays; gr, germ ring; hc, hatching cells; he, heart; ld, lipid droplet; le, lens; m, mouth; me, melanophores; mp, micropyle; op, optic primordium; pec, pectoral fin; pi, pigmentation; pp, posterior pole; so, somite; ys: yolk sac; ysl, yolk syncytial layer. Scale bars A-K, K″, L, L″, M, M″, N, N″, O, O″, P, P″, Q = 100 μm. Scale bars K′, L′, M′, N′, O′, P′, Q′ = 500 μm. Scale bars R, S = 0.5 cm. Photo credit: P. Perrichon.

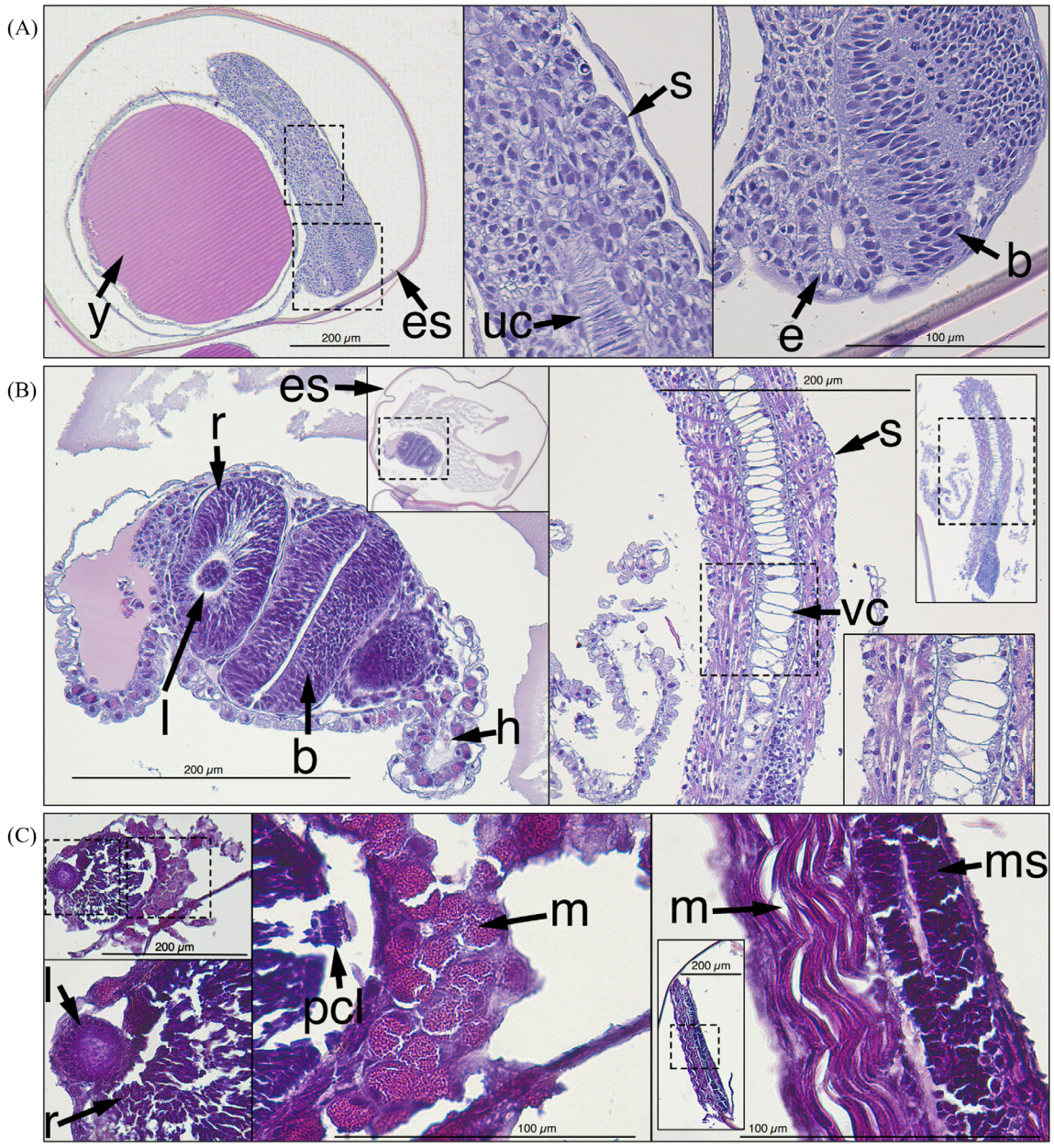

FIGURE 3 Light microscopic micrograph of lesser sandeel (*Ammodytes marinus*) embryo. (A) 60 DD/7 dpf; the tissue is characterized by immaturity indicated by high level of blue hematoxylin staining. Early eyes and brain were present. (B) 76 DD/9 dpf; the heart is present, and the somites contain both nuclei and striated muscle fibers. (C) 127 DD/16 dpf; rigid tissue structures made the sectioning challenging and result in a large degree of tearing tissue. Eyes structure with lens and iris are present. Long muscle fibers are visible along the trunk. b, brain; E, eye; h, heart; L, lens; M, muscle; ms, medulla spinalis; pcl, photoreceptor cell layer; r, retina; s, somite; uc, unvacuolated chordocytes, vc: vacuolated chordocytes, y: yolk.

150–158 DD (Figure 2J–J′). At 190–198 DD (SL = 5.53 ± 0.17 mm; Figure 2K–K″), the embryo wraps 2.5–times around the yolk. Melanophores are observed on the ventral side of the yolk and along the dorsomedial line of the gut. Pectoral fins are well-developed and continue to elongate. Close to hatching, the chorion of sandeel embryos loses stickiness and hardness.

On the tube mesh substrate, the hatching period lasted for several days (8–9 days; from 207 to 270 DD) with a peak hatching at 3 days from the start of hatching (229 DD). Newly hatched larvae measured 5.93 ± 0.41 mm (Figure 2L–L″), which is consistent with the size reported in the literature (ca. 5.5 mm at hatching[33]). The larvae are transparent, the head is straight along the body axis although the mouth is not yet fully open at hatching. The yolk sac is still present and still contains a lipid droplet. Pectoral fins and gill arches are well formed and movement of the pectoral fins is noted, along with

increased swimming. At the peak of hatching (229 DD; Figure 2M–M″), the mouth gape and the jaw expand. Exogenous feeding begins 3 days after hatching when sandeel larvae start to actively hunt prey. Similar to herring species, sandeel larvae may show evidence of enterohepatic recycling for taurine into the hindgut ("blue hindgut") between 2 dph/229 DD and 11–12 dph/310–320 DD (Figure 2M′–M″, N′–N″, O′–O″). Taurine is crucial in the development of some teleost larvae to maintain the antioxidant defense, redox homeostasis, and oxidative stress necessary for growth and metamorphosis.[40] Further analyses should be undertaken to confirm this physiological pathway. The first appearance of xanthophores is seen along the body at 7 dph/270 DD, along with a slight patch of melanophores appearing on the dorso-posterior side of the head and on the inferior part of the caudal fin (Figure 2N–O″). The intensity of pigmentation increases with further development. The yolk sac and lipid droplet were completely resorbed after 11 dph/310 DD. From 22 dph/400 DD, sandeel enters the flexion stage (Figure 2P–Q′). Flexion is defined as follows: (i) pre-flexion (notochord flexion 0–4°) with up to 10 fin rays is observed in 9.83 ± 0.39 mm larvae, (ii) early flexion (notochord flexion 10–25°) with greater than or equal to 10 fin rays is observed in 11.20 ± 0.49 mm larvae, and (iii) flexion/post-flexion (notochord flexion 30–45°) with greater than 13 fin rays is observed in 12.59 ± 0.58 mm larvae (39 dph; Figure 2Q–Q′). Dorsal and anal fins start to develop from the early flexion stage.

At this point, sandeel enters the flexion stage, and the musculature is evident throughout the body and the eyes and mouth are prominent and fully developed. Fin development progresses and displays the adult configuration (Figure 2R, S). The body coloration continues to progress, and the fish reaches 95–100 mg (SL = 3.3 cm, Figure 2R). At 5 months, body coloration is adult-like. At this stage, sand was offered to the fish for settlement. Settlement into sandy habitat occurs at a length of 40–50 mm in the wild.[1,29] In our rearing system, fish continued to grow, reaching 7.96 ± 0.90 cm after 8 months (Figure 2S). This body length is similar to our own measurements of wild juveniles, likely the same age, caught during a cruise carried out at the same time.

This study provides the foundation for further comparative developmental studies and a biological basis to better assess the condition of sandeel exposed to environmental and anthropogenic stressors. A detailed description of the developmental stages and key ontogenetic events from fertilization to post-flexion is also provided. Further studies should be undertaken to improve the quality of juvenile stages in intensive culture systems, including assessing the nutritional needs of sandeel larvae.

3 | EXPERIMENTAL PROCEDURES

3.1 | Ethics statement

The Austevoll Research Station, Institute of Marine Research, Storebø, Norway is a certified Research Animal Facility for fish of all developmental stages and has permits from the Norwegian Directorate of Fisheries to catch and maintain sandeel. All experimental protocols and procedures were performed in accordance with approved guidelines.

3.2 | Wild-caught broodstock

Lesser sandeel (*Ammodytes marinus*) were collected off the coast of Karmøy, Rogaland, Norway (59.255806° N latitude, 5.187222° E longitude) in December 2020 and 2021 using a sandeel dredge deployed from a 10-m long fishing vessel, the Åkrabuen. The individuals were transferred to a 120 L transport tank containing seawater at a salinity of 34 ppt at 8°C. The bottom of the transport tank was filled with sand from the sandeel habitat. The sex ratio of mature fish was three males to one female.

Once at the Austevoll Research Station, fish were transferred to a 1500 L tank. The conditions in the tank matched, as much as possible, those in the natural habitat where the sandeels were collected. A 10-cm layer of sand (0.8–1.25 mm granulometry) covered the bottom, a 12 h light/12 h dark photoperiod was applied and there was a continuous inflow of filtered seawater temperature at 8°C. Fish were not fed until spawning because they do not feed in the wild during the spawning season.[15,25] Starting 1 week before their natural spawning period (according to sandeel fishers during the second week of January), the sandeel were examined weekly to assess their reproductive status.

3.3 | Gamete collection and fertilization

Mature and ready-to-spawn individuals were selected from the broodstock tank. The average size of mature fish was 14.7 ± 1.4 cm. Maturity could be assessed as males had running milt and females released eggs when the abdomen was pressed gently. Pasteur pipettes were used to collect milt, and eggs were placed in different fertilization substrates prior to fertilization (see section 3.4. for a description of the different substrates). Each female exhibited one unique spawning event during the reproduction season, while the males had running milt throughout the period. As the eggs turn sticky shortly

after fertilization, particular care was taken, as much as possible, to ensure that eggs were evenly distributed in no more than 2–3 layers on substrates of different types (see section 2.4.). Fertilization was carried out by adding milt and seawater to the eggs placed on the substrates. The fertilization process lasted 45 minutes before rinsing, after which each substrate with fertilized eggs was transferred to a 50 L tank.

3.4 | Substrates for egg development

To establish the best incubation system for egg development, fertilization of the stripped eggs was attempted in five types of substrates (Figure 4): (i) glass microscope slides, 10 L cylindrical tubes fitted with filter mesh with pores sizes of (ii) 500 µm, (iii) 300 µm, (iv) 100 µm, and (v) a 10 L cylindrical tube filled with sand (similar to the substrate that was used in the broodstock holding tank). Eggs fertilized on glass microscope slides were spread out onto several slides to limit egg density. All of the different fertilization substrates were then placed into five 50 L tanks with the same water as that used in the broodstock tank (Figure 4). Tanks were covered with blinds to avoid direct exposure to light. The light delivered by the overhead lamps was measured using an Ocean Insight Flame-S spectrometer. The total irradiance (400–700 nm) measured over the water surface of the tanks was on average 200 µW/cm^2. Water flows through the tank was 10 L hour^{-1} during the embryonic period and was increased to 50 L hour^{-1} when the larvae reached the post-flexion stage. Water parameters were monitored daily to ensure stable rearing conditions. Oxygen was maintained at >80% of saturation, and the temperature was 7.8°C ± 0.5°C. The fertilization rate was calculated at 48 hours post-fertilization (hpf) by visual inspection of the different substrates under a microscope. Only a visual estimation of the fertilization rate was given for the eggs placed on the 500 µm filter mesh because moving or agitating this egg incubation substrate increased the chance of detaching eggs from the substrate and losing them in the rearing tank.

The efficacy of the different fertilization substrates was assessed using the following criteria: efficiency and ease of using the substrate, ease of observing and sampling embryos, bacterial growth, hatching success, and survival of hatched larvae.

3.5 | Larval rearing conditions

From 1-day post-hatch (dph), larvae were fed a mix of *Balanus crenatus* nauplii (CryoPlankton, Planktonic AS, Trondheim, Norway) and *Acartia* sp. nauplii (hatched from resting egg, CFEED AS, Trondheim, Norway). Live algae (*Rhodomonas* sp.) were added as a food supplement until 30 dph. The larvae were then fed with live *Artemia* nauplii (INVE aquaculture inc., Salt Lake City, UT, USA) and frozen *Artemia* nauplii from 130 dph. Live feed and algae were pumped into the culture tanks continuously

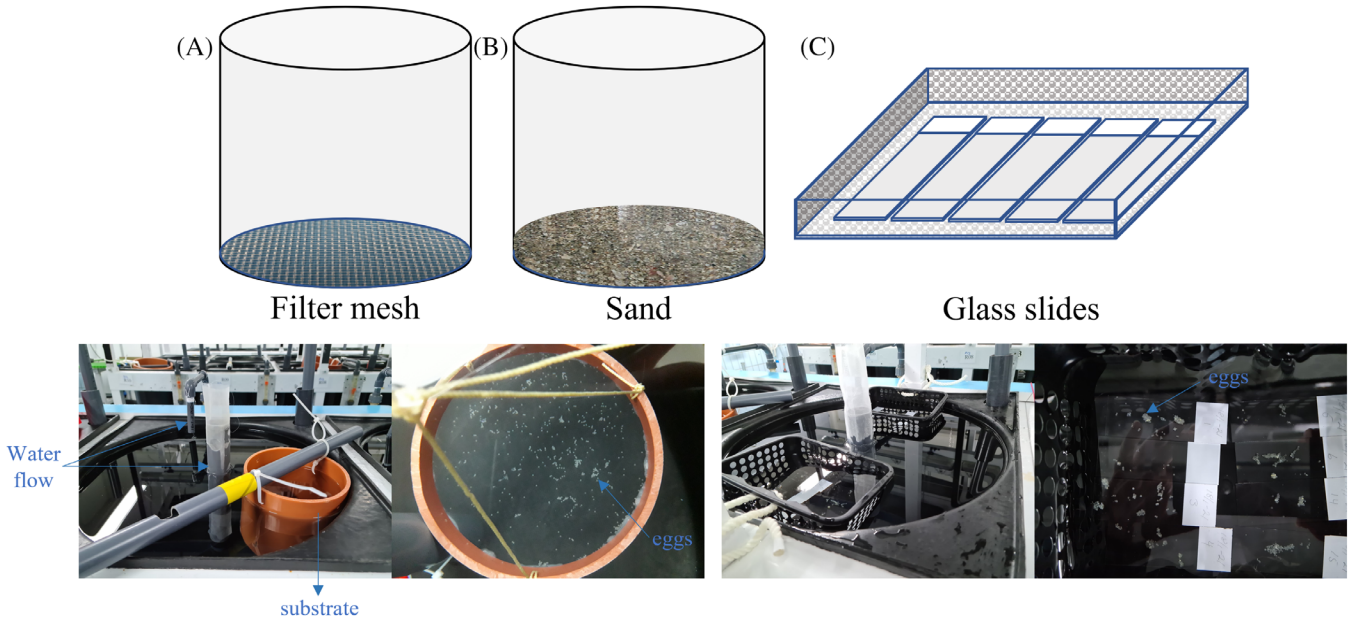

FIGURE 4 Fertilization substrates placed in 50 L tank for eggs incubation of lesser sandeel (*Ammodytes marinus*). (A) filter mesh with pore sizes of 100, 300, or 500 µm. (B) Meshed bottom covered with 5 cm sand. (C) Basket with glass microscope slides.

using peristaltic pumps to keep the concentration of prey above 3000 nauplii/L.

3.6 | Developmental timing and morphology of embryos and larvae

Embryogenesis was followed from the zygote stage to the free-swimming stage by examining embryos and larvae (n = 20–30 individuals per spawning from 8 females) under an Olympus SZX10 stereomicroscope coupled to a Moticam 1080 digital camera (Motic, Richmond, BC, Canada). The stereomicroscope was equipped with a thermally regulated microscope stage (Brook Industries, Lake Villa, IL, USA) set at 8°C, ensuring a constant temperature during observations. Major developmental landmarks and the morphology of specimens were observed. A small number of embryos were manually dechorionated to provide clear observations of the embryonic structures. Images and 20s videos were digitized using Motic Live Imaging Module. Heart rate (beat min^{-1}) was determined by manually counting the number of ventricular contractions in each 20s video collected throughout development (n = 5 embryos per stage). ImageJ software[41] was used to measure chorion diameter (mm), volume of lipid droplet (mm^3), and standard length (mm; SL).

All of the developmental landmarks and morphology were gathered from data on sandeel collected during 2021 and 2022 and are reported in degree days (DD) and in days post-fertilization/hatching. Degree days were calculated by adding the mean daily water temperature in degrees Celsius for the total number of days measured. The developmental stages and both metrics are presented in Table 2.

3.7 | Histology

Sandeel embryos and larvae were sampled and fixed in 4% PBS buffered paraformaldehyde and stored at 4.0°C until further use. A histological evaluation for morphological structures was performed on 20–30 embryos at 60, 76, and 127 DD. The samples were dehydrated and embedded in paraffin using Histokinette Leica TP 1020 (Leica Microsystems Inc., Buffalo Grove, IL, USA). Thick serial sections (3 μm) were performed through the entire embryo using a Leica RM 2255 rotary microtome (Leica Microsystems Inc., Buffalo Grove, IL, USA). Histological sections were dewaxed and stained with hematoxylin-erythrosin-safran (HES) following conventional histological procedures. Imaging was performed using a Leica DMRBE trinocular microscope (Leica Microsystems) connected to a SPOT insight CMOS camera (SPOT imaging, Sterling Heights, MI, USA).

AUTHOR CONTRIBUTIONS

Prescilla Perrichon: Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); supervision (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Reidun Bjelland:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); visualization (equal); writing – review and editing (equal). **Caroline M.-F. Durif:** Conceptualization (equal); investigation (equal); methodology (equal); resources (equal); validation (equal); writing – review and editing (equal). **Ingrid Uglenes Fiksdal:** Formal analysis (equal). **Espen Johnsen:** Funding acquisition (equal); investigation (equal); project administration (equal); resources (equal); validation (equal); writing – review and editing (equal). **Anne Berit Skiftesvik:** Conceptualization (equal); methodology (equal); writing – review and editing (equal). **Alessandro Cresci:** Methodology (equal); resources (equal); writing – review and editing (equal). **Howard I. Browman:** Methodology (equal); validation (equal); writing – review and editing (equal). **Elin Sørhus:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); supervision (equal); visualization (equal); writing – review and editing (equal).

ACKNOWLEDGEMENTS

We thank Geir Kenneth Eriksen for providing the fishing vessel Åkrabuen and essential support for capturing lesser sandeel adults. We thank the technical staff of the Institute of Marine Research, Austevoll Research Station for their assistance in maintaining the sandeel used in this study.

FUNDING INFORMATION

This work was financed by the Norwegian Institute of Marine Research, Equinor Energy, AkerBP, Lundin Energy Norway, Spirit Energy Norway, and DNO Norway under Project no. 15781, KnowSandeel 1.0, "Spatial structure and sensitivity of lesser sandeel to oil exploitation—filling knowledge gaps for improved risk assessment."

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a conflict of interest.

DATA AVAILABILITY STATEMENT

All data will be made available through the Norwegian Marine Data Centre.

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REFERENCES

1. Reay P. Synopsis of biological data on North Atlantic sandeels of the genus Ammodytes. *FAO Fish Synop*. 1970;82:1-50.
2. Sparholt H. An estimate of the total biomass of fish in the North Sea. *ICES J Mar Sci*. 1990;46:200-210. doi:10.1093/icesjms/46.2.200
3. Egan A, Rindorf A, Berges B, et al. Herring assessment working Group for the Area South of 62°N (HAWG). *ICES Sci Rep*. 2020;2(60):1151. doi:10.17895/ices.pub.6105
4. Wright P, Jensen H, Tuck I. The influence of sediment type on the distribution of the lesser sandeel, *Ammodytes marinus*. *J Sea Res*. 2000;44(3–4):243-256. doi:10.1016/S1385-1101(00)00050-2
5. Frederiksen M, Elston DA, Edwards M, Mann AD, Wanless S. Mechanisms of long-term decline in size of lesser sandeels in the North Sea explored using a growth and phenology model. *Mar Ecol Prog Ser*. 2011;432:137-147. doi:10.3354/meps09177
6. Haug T, Lindstrøm U, Nilssen KT, Røttingen I, Skaug HJ. Diet and food availability for northeast Atlantic minke whales *Balaenoptera acutorostrata*. 1995.
7. Pierce GJ, Thompson PM, Miller A, Diack J, Miller D, Boyle PR. Seasonal variation in the diet of common seals (*Phoca vitulina*) in the Moray firth area of Scotland. *J Zool*. 1991;223(4):641-652. doi:10.1111/j.1469-7998.1991.tb04393.x
8. Hislop J, Harris M, Smith J. Variation in the calorific value and total energy content of the lesser sandeel (*Ammodytes marinus*) and other fish preyed on by seabirds. *J Zool*. 1991;224(3):501-517. doi:10.1111/j.1469-7998.1991.tb06039.x
9. Santos M, Pierce GJ. The diet of harbour porpoise (*Phocoena phocoena*) in the Northeast Atlantic: a review. *Oceanogr Mar Biol*. 2003;41:363-369.
10. Bailey R. The interaction between sandeels and seabirds: a case history at Shetland. ICES. 1991.
11. Green E. A literature review of the lesser (Raitt's) sandeel Ammodytes marinus in European waters. The Royal Society for the Protection of Birds/Sandy (GBR), Project Number: LIFE14 NAT/UK00394 Roseate Tern, S; 37. 2017.
12. Furness RW, Tasker ML. Seabird-fishery interactions: quantifying the sensitivity of seabirds to reductions in sandeel abundance, and identification of key areas for sensitive seabirds in the North Sea. *Mar Ecol Prog Ser*. 2000;202:253-264. doi:10.3354/meps202253
13. Macer C. Sandeels (Ammodytidae) in the southwestern North Sea: their biology and fishery. MAFF Fishery Invest London Ser II 1966;24(6):1–55.
14. Holland GJ, Greenstreet SP, Gibb IM, Fraser HM, Robertson MR. Identifying sandeel *Ammodytes marinus* sediment habitat preferences in the marine environment. *Mar Ecol Prog Ser*. 2005;303:269-282. doi:10.3354/MEPS303269
15. van Deurs M, Hartvig M, Steffensen JF. Critical threshold size for overwintering sandeels (*Ammodytes marinus*). *Mar Biol*. 2011;158:2755-2764. doi:10.1007/s00227-011-1774-8
16. Johnsen E, Sørhus E, de Jong K, Lie KK, Grøsvik BE. Kunnskapsstatus for havsil i norsk sone av Nordsjøen. *Rapport Fra Havforskningen*. 2021;23:44.
17. Johnsen E, Pedersen R, Ona E. Size-dependent frequency response of sandeel schools. *ICES J Mar Sci*. 2009;66(6):1100-1105. doi:10.1093/icesjms/fsp091
18. Boulcott P, Wright PJ. Critical timing for reproductive allocation in a capital breeder: evidence from sandeels. *Aquat Biol*. 2008;3:31-40. doi:10.3354/ab00063
19. Høines ÅS, Bergstad O. Density of wintering sand eel in the sand recorded by grab catches. *Fish Res*. 2001;49(3):295-301. doi:10.1016/S0165-7836(00)00213-7
20. Bergstad OA, Høines ÅS, Krüger-Johnsen EM. Spawning time, age and size at maturity, and fecundity of sandeel, *Ammodytes marinus*, in the North-Eastern North Sea and in unfished coastal waters off Norway. *Aquat Living Resour*. 2001;14(5):293-301. doi:10.1016/S0990-7440(01)01134-2
21. van der Kooij J, Scott BE, Mackinson S. The effects of environmental factors on daytime sandeel distribution and abundance on the Dogger Bank. *J Sea Res*. 2008;60(3):201-209. doi:10.1016/J.SEARES.2008.07.003
22. Johnsen E, Rieucau G, Ona E, Skaret G. Collective structures anchor massive schools of lesser sandeel to the seabed, increasing vulnerability to fishery. *Mar Ecol Prog Ser*. 2017;573:229-236. doi:10.3354/meps12156
23. Heath MR, Rasmussen J, Bailey MC, et al. Larval mortality rates and population dynamics of lesser Sandeel (*Ammodytes marinus*) in the northwestern North Sea. *J Mar Syst*. 2012;93:47-57. doi:10.1016/J.JMARSYS.2011.08.010
24. van Deurs M, van Hal R, Tomczak MT, Jónasdóttir SH, Dolmer P. Recruitment of lesser sandeel *Ammodytes marinus* in relation to density dependence and zooplankton composition. *Mar Ecol Prog Ser*. 2009;381:249-258. doi:10.3354/MEPS07960
25. Eliasen K. Sandeel *Ammodytes spp*., as a link between climate and higher trophic levels on the Faroe shelf. *PhD Thesis*. 2013.
26. Henriksen O, Rindorf A, Brooks ME, Lindegren M, van Deurs M. Temperature and body size affect recruitment and survival of sandeel across the North Sea. *ICES J Mar Sci*. 2021;78(4):1409-1420. doi:10.1093/icesjms/fsa
27. Jensen H. *Settlement Dynamics in the Lesser Sandeel Ammodytes Marinus in the North Sea*. Doctoral dissertation, University of Aberdeen, Scotland; 2001.
28. Jensen H, Rindorf A, Wright PJ, Mosegaard H. Inferring the location and scale of mixing between habitat areas of lesser sandeel through information from the fishery. *ICES J Mar Sci*. 2011;68(1):43-51. doi:10.1093/icesjms/fsq154
29. Christensen A, Jensen H, Mosegaard H, St. John M, Schrum C. Sandeel (*Ammodytes marinus*) larval transport patterns in the North Sea from an individual-based hydrodynamic egg and larval model. *Can J Fish Aquat Sci*. 2008;65(7):1498-1511. doi:10.1139/f08-073
30. Régnier T, Gibb FM, Wright PJ. Temperature effects on egg development and larval condition in the lesser sandeel, *Ammodytes marinus*. *J Sea Res*. 2018;134:34-41. doi:10.1016/J.SEARES.2018.01.003
31. Smigielski A, Halavik T, Buckley L, Drew S, Laurence G. Spawning, embryo development and growth of the American sand lance *Ammodytes americanus* in the laboratory. *Mar Ecol Prog Ser*. 1984;14:287-292. doi:10.3354/meps014287
32. Tomiyama M, Yanagibashi S. Effect of temperature, age class, and growth on induction of aestivation in Japanese sandeel (*Ammodytes personatus*) in Ise Bay, Central Japan. *Fish Oceanogr*. 2004;13(2):81-90. doi:10.1046/j.1365-2419.2003.00272.x

33. Munk P, Nielsen JG. *Eggs and Larvae of North Sea Fishes*. Frederiksberg: Biofolia; 2005:215.
34. Pinto JM. Laboratory spawning of *Ammodytes hexapterus* from the Pacific coast of North America with a description of its eggs and early larvae. *Copeia*. 1984;1984(1):242-244. doi:10.2307/1445068
35. Pinto J, Pearson W, Anderson J. Sediment preferences and oil contamination in the Pacific sand lance *Ammodytes hexapterus*. *Mar Biol*. 1984;83:193-204. doi:10.1007/BF00394728
36. Kawakami T, Okouchi H, Aritaki M, Aoyama J, Tsukamoto K. Embryonic development and morphology of eggs and newly hatched larvae of Pacific herring *Clupea pallasii*. *Fish Sci*. 2011;77(2):183-190. doi:10.1007/S12562-010-0317-4
37. Blaxter J, Holliday F. The behaviour and physiology of herring and other clupeids. *Adv Mar Biol*. 1963;1:261-394. doi:10.1016/S0065-2881(08)60261-8
38. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Dev Dyn*. 1995;203(3):253-310. doi:10.1002/aja.1002030302
39. Hill J, Johnston IA. Photomicrographic atlas of Atlantic herring embryonic development. *J Fish Biol*. 1997;51(5):960-977. doi:10.1111/J.1095-8649.1997.TB01535.X
40. Pinto W, Ronnestad I, Dinis MT, Aragao C. Taurine and fish development: insights for the aquaculture industry. *Adv Exp Med Biol*. 2013;776:329-334. doi:10.1007/978-1-4614-6093-0_30
41. Schneider CA, Rasband WS, Eliceiri KW. NIH image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671-675. doi:10.1038/nmeth.2089

How to cite this article: Perrichon P, Bjelland R, Durif CM-F, et al. Early ontogeny of the lesser sandeel (*Ammodytes marinus*). *Developmental Dynamics*. 2023;252(10):1280-1291. doi:10.1002/dvdy.634