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Effects of sediment type and light availability on the burying behaviour of small sandeel (Ammodytes tobianus)

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ABSTRACT
This study examines the sediment preferences of small sandeel (Ammodytes tobianus), an elongated forage fish common in marine and brackish environments of northern Europe. Sandeel have a high fidelity for sandy habitats and spend much of their lives buried, as an important part of both their diel behavioural cycles and overwintering behaviour. A series of independent choice laboratory assays were conducted using wild-caught schools of small sandeel, to: (1) determine their preferred substrate composition for burial; (2) identify the upper limits of sediment grain-sizes that may be utilised for burial, and; (3) investigate the effects of light intensity on burial behaviour. Initial experiments found a clear preference for burial within sediments composed of coarse sand and fine gravel (0.5–4.0 mm) but showed at least some utilisation of both coarser and finer sediment compositions. In further trials, burial was found to be almost entirely eliminated in sediments that contained significant gravel components (>4.0 mm). Light manipulation experiments also showed that light intensity was an important factor that influences their choice of burial area. This study is the first to experimentally investigate the sediment preferences and grain-size tolerance limits of small sandeel. These results specifically highlight how subtle differences in both sediment composition and light may influence the habitat usage of small sandeel, which may be valuable for understanding their distribution in the wild and to inform future management and conservation for the species.

1. Introduction

Burrowing behaviour has an important role in the evolution and ecology of many animals. As protection from predators and as a refuge from harsh environmental conditions, burrowing into or burying oneself within substrate has allowed animals such as the small sandeel (Ammodytes tobianus) to thrive in hostile or inhospitable conditions. This behaviour presents physical challenges that have shaped the characteristics of burrowing animals, such as the stout forearms of moles and armadillos, or the heavily reinforced skulls of limbless caecilian amphibians and dibamid lizards (Kleinteich et al., 2012; Rose et al., 2013). Adaptations for a fossorial lifestyle typically include elongation of the body, reduced eyes and limbs, and well-osseified fused bones in the head region (Das et al., 2022; Lee, 1998). While sandeels have some typical adaptations for burrowing such as an elongated, laterally flattened body, they also have large eyes and a skull that has no evident mineralisation for burrowing (Bizzarro et al., 2016). Nevertheless, individuals of this species are able to bury themselves rapidly and in a wide variety of sediments. Burrowing is observed in a diverse group of marine fish. Some fish, such as the Pacific sandfish, spend most of their life within the substrate after creating a tunnel that collapses behind them (Thedinga et al., 2006). Also, flatfishes and skates routinely cover themselves in substrate, while species of jawfish, tilefish and gobies commonly construct permanent burrows and often use their mouths for excavation (Able et al., 1982; Colin, 1973; Itani and Uchino, 2003).

Burying behaviour is a distinct behaviour common to all species of sandeels (Ammodiptidae, essentially translates into “sand burrowers”), which are a family of semi-pelagic fish also known as sand lances. Sandeel use a similar approach to terrestrial sand-swimming lizards, like the sandfish (Scincus scincus) and Uma iguanids, which use undulating...
movements and a flat stream-lined body to move through sandy substrates (Gidmark et al., 2011; Jayne and Daggy, 2000; Maladen et al., 2009). For example, the Pacific sand lance (A. personatus) dives headfirst into the sand, driving its head and anterior two-thirds of its body underground before undulating and drawing the remaining part of the fish beneath the sediment (Bizzarro et al., 2016; Gidmark et al., 2011). The burrowing strategy is also similar to the subterranean undulatory movements observed in some terrestrial snakes (Hu et al., 2009). This strategy is reflected in both the morphology, physiology (e.g. highly adapted musculature, scales and cells, Cané et al., 2020) and behaviour (O’Connell and Fives, 1995) of the small sandeel, with their sharp pointed jaw, slender elongate form, and occurrence within sediments suitable for burial (Tien et al., 2017). Sandeel burrows also lack openings for ventilation, therefore the physical composition of sediments can be an important factor in whether a habitat is suitable (Behrens et al., 2007, 2009). For example, extremely coarse substrates may be a physical barrier that prohibits burial, whereas particularly fine substrates (e.g., silts) can limit oxygen supply (Wright et al., 2000). Tidal currents, water velocity and water depth play critical roles in shaping a dynamic sedimentary environment and its aeration, which are essential for sandeel in their preferred dynamic bedform habitats (Greene et al., 2020; Tien et al., 2017).

Sandeels plays a crucial role in marine ecosystems around the world. Acting as an essential intermediate link between lower and higher trophic levels, sandeel are a primary source of food for a diverse range of predators, including seabirds, marine mammals, and predatory fish (Engelhard et al., 2013, 2014; Gunther et al., 2023; Staadinger et al., 2020). In the North Sea, sandeels have collectively been highlighted as the most important forage fish in the ecosystem (Furness, 2008; Sparholt, 1990). Sandeel also support one of the largest fisheries in the North Sea region, although high variation in their abundances and spatial distributions over time are challenges to their sustainable management (Dickey-Collas et al., 2014). Increased knowledge of the environmental factors that influence their distribution and habitat choices may therefore better inform management of small sandeel and the targeting of conservation efforts.

Sandy substrates are an important part of the life history of sandeel. Larvae settle onto sandy habitats after completing their development (Jensen, 2001; Jensen et al., 2011; Perrichon et al., 2023), after which they appear to show high site fidelity (Haynes et al., 2007; Wright et al., 2019). Their diel activity is strongly associated with day length, during which they form schools in the water column and feed on zooplankton (van der Kooij et al., 2008). The nocturnal burial is not only regulated by circadian rhythms and influenced by melatonin but is also concentrated at crepuscular periods, indicating a significant sensitivity to light levels during twilight, as highlighted in Baker et al. (2023) and Amiya et al. (2023), respectively. However, at night they bury themselves within the sand, which both provides a refuge from predation and acts as a strategy to conserve energy during dormant or non-feeding periods (Behrens et al., 2007; Freeman et al., 2004; van Deurs et al., 2010). Some sandeel species can also spend 6–8 months each year buried during an overwintering phase (Baker et al., 2019; van Deurs et al., 2010, 2011). After overwintering, fish emerge in early spring to initiate a feeding period that lasts until late summer, during which they are vulnerable to both predators and fisheries (Henriksen et al., 2021).

Because of this specialized lifestyle, several factors may influence their habitat choices, including preference for specific sediment compositions, physical conditions (e.g., water velocity and slope edges), and human factors (e.g., fishing intensity) (Langton et al., 2021; Rey, 1970; Tien et al., 2017). Studies have shown that sediment grain size is a particularly important factor, with several species preferring coarse to very coarse sandy sediments with grain size ranges between 0.5 and 2.0 mm (e.g., A. hexapterus (corrected to A. personatus), Pinto et al., 1984; A. marinus, Wright et al., 2000; A. japonicus, Endo et al., 2019; A. personatus, Bizzarro et al., 2016). Other sediment characteristics that can affect the burial behaviour include physical resistance (e.g., shear strength, Endo et al., 2019) and oxygen levels in the sediment (Behrens and Steffensen, 2007). Furthermore, the proportion of time spent buried can be mediated by the availability of light, food, and temperature (Winslade, 1974a, 1974b, 1974c). Previous studies have also identified a low tolerance threshold for small grain-size particles, where greater proportions of fine silt-clay within sediments can inhibit sandeel burial and presumably limit oxygen supply to burrows (Pinto et al., 1984; Wright et al., 2000). In contrast, little is known about the influence of larger grain-size particles (e.g., gravels of granule, pebble, and cobble fractions), which may facilitate oxygen supply into burrows but act as a physical barrier to their burial.

There are currently only a limited number of experimental studies that have assessed the burial behaviour and use of sediments for small sandeel (A. tobinius), and previous studies have primarily focused on the importance of oxygen availability (Behrens et al., 2007, 2009, 2010; Behrens and Steffensen, 2007). Therefore, this study further explored the habitat choices of small sandeel via a series of independent choice experiments. Our first aim was to experimentally investigate burial site preferences based on the grain-size composition of sediments, focusing on the size fractions that are utilised by the closely related sympatric sandeel, A. marinus (Wright et al., 2000). The second aim was to investigate how the proportion of large gravel fractions in sediments influences burial behaviour and identify whether there is an upper threshold that inhibits sandeel burial. Finally, light availability may also be a factor influencing habitat choice (Winslade, 1974c), and differences in depth (and therefore light intensity) appears to be an important factor influencing habitat utilisation by small sandeel and co-existing closely related sandeel species (Tien et al., 2017). Therefore, the third aim was to briefly explore the burial behaviour of small sandeel under varying light intensities in additional pilot experiments.

2. Materials and methods

2.1. Sampling and husbandry

Small sandeel were collected in the morning on 17–18 August 2020, off Bellevue Beach in the Øresund, Denmark (55.777291°N, 12.592927°E). A beach seine was deployed, and hand dragged across the sandy bottom at depths 0.5–1.5 m. >300 sandeel were roughly sorted out and selected from the catch, targeting larger individuals in order to avoid young post-settled individuals. The subsampled catch was transported to a fish holding facility at DTU Aqua, Kgs. Lyngby. Fish were transported in 0.5 × 1 m barrels filled with water from their sampling area and aerated with battery powered air stones. At DTU Aqua, the fish were kept in a 1 × 2 m (height x diameter) cylindrical holding tank with fully aerated flow-through recirculated water (10 ± 1°C and 12 ± 1 ppm salinity). The tank had a ~10–15 cm layer of medium to coarse composite sand at the bottom that was suitable for burial. The bottom sand was mixed from commercially purchased sediments but were not analysed further. Fish were fed frozen Arteria spp. and mysids every second day in their holding tank. Light levels followed a 12-h diel cycle (day: 07:00–19:00) with a crepuscular twilight period (dawn: 07:00–08:00, and dusk: 18:00–19:00) in the experimental facility room with light conditions being consistent ~45 lx, and thus no natural input of light was used for experimental work. Both the holding tank and the experimental tanks were present in the experimental room.

2.2. General experimental design

All experiments were conducted within a large black rectangular tank (160 × 40 × 100 cm in L × W × H, Fig. 1). The aquaculture facilities include a recirculation water system which was connected to experimental tanks and the holding tank, to ensure identical water quality conditions throughout the experimental period. Eight grey trays were filled with sediment and fully submerged within the experimental tank, where fish were able to freely swim among the trays. All experiments
were initiated by the fish being released simultaneously in the middle of
the tank at approximately midday. For each trial, fish were kept in the
experimental tank for several days (min. 3 days) to allow for acclimation
and natural behaviours such as daily emergence and burial, free swim-
ming, and interaction with conspecifics. This also gave fish a chance for
multiple burials before determining their choice. The fish were deprived
of food during the trials to encourage burrowing behaviour, with their
last feeding occurring the day before entering the experimental tank. At
the end of each trial, all trays were covered by lids to prevent escape, and
transferred to a separate tub to count buried individuals. Unburied in-
dividuals were not included in the analyses. All individuals were then
returned to the holding tank, excluding any dead or poor-condition
individuals that were removed/euthanized. For each experiment, posi-
tions of each sediment treatment in the experimental tank were initially
randomised (see Fig. 1), and trays where subsequently shifted one po-
sition clockwise after each experimental trial to limit the effects of any
replication issues (e.g. edge effects, position in relation to water inflow/
outflow etc.).

2.3. Experiment 1: sediment preference

The first experiment (Exp. 1) for small sandeel used sediment com-
positions close to the preferred sediment of *A. marinus* (coarse-very
coarse sand, 0.5–2.0 mm diameter, Wright et al., 2000), together with

![Fig. 1. Experimental tank with sediment trays used for choice experiments on small sandeel, (a) aerial view, and (b) side view. Sediment trays are filled to approxi-
mately 10 cm. Sediments are arranged in the initial positions used for a trial, using four sediment treatments (S1, S2, S3, and S4) with two trays per treatment.
For Exp. 1 and 2, the tank was lit by a fluorescent laboratory light positioned directly above the tank along the centre of the trays, whereas for Exp. 3, a fluores-
cent light source at one end produced a gradient of light intensity in sediment tubs along the length of the tank.](image-url)

### Table 1

Composition in percentage of sediment treatments used in choice experiments with small sandeel (*A. tobianus*). The sediment composition followed classification of
grade classes described by (Wentworth, 1922).

<table>
<thead>
<tr>
<th>Description</th>
<th>Grade (size fraction, mm)</th>
<th>Silt-clay (&lt;0.062)</th>
<th>Very fine sand (0.062–0.125)</th>
<th>Fine sand (0.125–0.25)</th>
<th>Medium sand (0.25–0.5)</th>
<th>Coarse sand (0.5–1.0)</th>
<th>Very coarse sand (1.0–2.0)</th>
<th>Fine granule gravel (2.0–4.0)</th>
<th>Medium pebble gravel (4.0–8.0)</th>
<th>Coarse cobble gravel (&gt;8.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium sand</td>
<td></td>
<td>0.01</td>
<td>0.25</td>
<td>11.41</td>
<td>84.32</td>
<td>3.43</td>
<td>0.52</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medium-coarse sand</td>
<td></td>
<td>0</td>
<td>0.02</td>
<td>0.38</td>
<td>19.6</td>
<td>79.81</td>
<td>0.33</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medium sand-fine gravel</td>
<td></td>
<td>0.03</td>
<td>0.06</td>
<td>3.07</td>
<td>15.97</td>
<td>20.59</td>
<td>26.02</td>
<td>33.97</td>
<td>0.33</td>
<td>0</td>
</tr>
<tr>
<td>Coarse-very coarse sand</td>
<td></td>
<td>0</td>
<td>0.03</td>
<td>0.12</td>
<td>1.01</td>
<td>47.6</td>
<td>51.17</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coarse-very coarse sand*</td>
<td></td>
<td>0.02</td>
<td>0.01</td>
<td>0.05</td>
<td>1.04</td>
<td>53.84</td>
<td>44.91</td>
<td>0.21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50% fine gravel</td>
<td></td>
<td>0</td>
<td>0.01</td>
<td>0.06</td>
<td>0.39</td>
<td>23.87</td>
<td>24.86</td>
<td>48.88</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>65% fine gravel</td>
<td></td>
<td>0</td>
<td>0.03</td>
<td>0.03</td>
<td>0.18</td>
<td>15.03</td>
<td>20.31</td>
<td>64.44</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>35% medium gravel</td>
<td></td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.36</td>
<td>27.66</td>
<td>26.5</td>
<td>7.93</td>
<td>34.63</td>
<td>2.93</td>
</tr>
</tbody>
</table>

* Used as reference for preferred sediment in Exp. 2 and Exp. 3
finer and coarser components that included fractions of fine sand (> 0.125 mm) and fine granule gravel (< 4.0 mm) (Tables 1 and 2). Two sets of four trays with four different sediment types (i.e., within-trial replicates) were placed inside the experimental tank; “medium sand”, “medium-coarse sand”, “coarse-very coarse sand” and “medium-sand-fine gravel” (Tables 1 and 2). Three trials each using 50 fish were conducted, each trial lasting 9–10 days. Fish were counted twice during each trial, on day 4, 5 or 6 and then again at the termination of each trial. Fish were then returned to the holding stock tank for re-use in later trials. To ensure that individuals were not run through two consecutive trials, the next group of 50 individuals were caught from the holding tank before experimental fish, kept in a bucket, were returned.

### 2.4. Experiment 2: effects of gravel on sediment preference

The second experiment (Exp. 2) tested whether the presence of larger grain sizes may deter small sandeel from using their preferred sediment (as determined in Exp. 1). Four different mixtures of gravel and their preferred sediment (i.e., coarse-very coarse sand) were used: 100% preferred sediment (i.e., control); 50:50, “50% fine gravel”; 65:35, “65% fine gravel”; and, 30:70, “30% medium gravel” (see Tables 1 and 2). The experimental set-up included eight trays where each sediment composition was replicated twice. Four trials using 49–52 fish per trial were run for five days each. After each trial all individuals in each tray were counted, and then were euthanized to record total length (TL, mm) for each fish.

### 2.5. Experiment 3: effect of light levels on sediment preference

The effect of light intensity (mimicking a possible depth gradient) on sediment preferences was investigated using the same sediment compositions as in Exp. 2 (Table 2). All experiments described in this study, followed a 12-h diel cycle with a crepuscular twilight period as noted above. Exp. 1 and Exp. 2 were primarily lit by a single fluorescent laboratory light (approx. 45 lx) positioned centrally above the tank, which provided consistent light conditions across the experimental tank.

An additional pilot experiment (Exp. 3) were set up and the light was covered by an opaque plastic sheet and a gradient of light levels was introduced along the length of the tank via a fluorescent light at one end of the tank (see Fig. 1). The experiment otherwise followed the same process as Exp. 2, including five trials with 27–50 fish per trial (Table 2). Light levels were measured with a basic light meter (Gossen Panlux Analog Electronic Luxmeter, Gossen, Germany, manufacture year of 1994) over each sediment tray five times during experimental days during hours of daylight set by the diel cycle in the room. The average light within each paired trays were 18.7, 23.8, 32.3 and 44.1 lx, from farthest to closest positions from the light source (Fig. 1). This pilot experiment, featuring a rudimentary and somewhat makeshift setup with inconsistently measured light conditions, inherently carries a degree of uncertainty. Thus, it is presented here as a secondary preliminary finding compared to other experiments (Exp.1 and 2) that needs more attention in the future.

### 2.6. Sediment composition analysis

The sediment compositions used in the holding tank and in each experiment were mixed from commercially purchased sands/gravels. The experimental compositions followed grade class classifications described by Wentworth (1922). These compositions were further characterised by sediment particle size analysis, based on mass loss from wet sieving with the aid of a mechanical shaker (3 replicates per tray, i.e., 6 per sediment per experiment).

Exp.1 contained a setup with four different sediments. The finest sediment of “medium sand” had a main composition of ~85% sand (grain fraction 0.25–0.5 mm) along with finer sands, whereas “medium-coarse sand” had coarser sands averaging ~80% of 0.5–1.0 mm and ~20% of 0.25–2.5 mm (Fig. 2). The sediment “coarse-very coarse sand” had an almost 1:1 ratio of ~48% and 51% sands of size fractions 0.5–1.0 mm and 1.0–2.0 mm, respectively. Lastly, “medium sand-fine gravel” was the most varied sediment mixture having averages of 15% to 26% of sands and gravels ranging over four size fractions (Fig. 2, but for details see Table 1).

The sediment analysis for Exp. 2 and Exp. 3 showed that the preferred sediment had a composition of ~54% and 45% coarser sands of size fraction 0.5–1.0 mm and 1.0–2.0 mm, respectively (Fig. 3). Two of the other mixed sediments contained more or less equal ratios of preferred sands in combination with increasing amounts of finer gravels from ~50% to ~65% granules. The mixture with the coarsest sediment included gravels ranging over three size fractions, 7% granules, ~35% pebbles and ~3% cobbles (Fig. 3 and Table 1).

### 2.7. Statistical analysis

Multinomial models were used to analyse sediment preferences in each of the three experiments. The approach has previously been used for similar choice experiments (Christoffersen et al., 2018). In the models, the vector of number of sandeel counted in each tray during each trial was assumed to follow a multinomial distribution. The goodness-of-fit was assessed by testing competing nested models via standard likelihood-ratio test. Model comparison was set up to assume

### Table 2 Summary of experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sediment compositions</th>
<th>Trials</th>
<th>Fish per trial</th>
<th>Mean length per trial (mm)</th>
<th>Other treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td>- Medium sand; - Medium-coarse sand; - Coarse-very coarse sand; - Medium-sand-fine gravel</td>
<td>6 (3 trials with two repeated measures)</td>
<td>50, 50, 50</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>- Coarse-very coarse sand +50% fine gravel</td>
<td>4</td>
<td>49, 51, 52</td>
<td>112, 110, 113</td>
<td>–</td>
</tr>
<tr>
<td>Exp. 3</td>
<td>- Coarse-very coarse sand +35% medium gravel</td>
<td>5</td>
<td>50, 26, 26</td>
<td>27, 27</td>
<td>Light level</td>
</tr>
</tbody>
</table>

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different couplings of the sediment preference probabilities and the best model was selected based on a likelihood ratio test comparing two competing models (e.g. a model with the same probability for all sediments versus a model with a separate probability for each sediment type).

As an example model, in the first choice experiment there were four sediment types ($i = S1, S2, S3$ and $S4$; see Table 1–2, Fig. 1) and six trials ($j = 1, \ldots, 6$). The multinomial model states that the vector of observed counts from each trial [i.e., $x_j$ where $x_j = (C_{S1,j}, C_{S2,j}, C_{S3,j}, C_{S4,j})$, and $C_{S1}$, $S4$ represent the counts within each sediment type in the $j$th trial] follows a multinomial distribution denoted $x_j \sim \text{multinomial} (N_j, P_j)$, where $N_j$ is the total number of sandeel in the $j$th trial and $P_j$ is a probability vector that describes the preferences for each sediment type in the $j$th trial. A separate probability vector was used for each possible combination of sediment and light level (e.g., $S1:L1$, $S1:L2$, $\ldots$, $S4:L4$) for Exp. 3. If variability between trials was greater than what could be explained by the multinomial distribution (overdispersion compared to multinomial), then the model was extended by trial-specific random effects. Technically, these are mean zero Gaussian additive deviations $z_{ij} \sim N(0, \sigma^2)$ at the transformed scale, such that:

![Fig. 2.](image1.png) Experiment 1 investigated burial behaviour of small sandeel (A. tobianus), comparing the probability of burial in four different sediment compositions. The left panel shows the observed proportions of burial in each trial (coloured dots) and predicted probability of burial (black lines with $\pm 95\%$ CI) in each sediment type. The size of dots is proportional to the number of fish in each sediment type per trial. The right panel shows the composition of grain fractions for each experimental sediment type (also see Tables 1 and 2).

![Fig. 3.](image2.png) Experiment 2 investigated burial behaviour of small sandeel (A. tobianus) comparing their burial in different mixtures of gravel and their preferred sediment (i.e., coarse-very coarse sand, established in Exp. 1). The left panel shows the observed proportions of burial in each trial (coloured dots) and predicted probability of burial (black lines with $\pm 95\%$ CI) in each sediment type. The size of dots is proportional to the number of fish in each sediment type per trial. The right panel shows the composition of grain fractions for each experimental sediment type (also see Tables 1 and 2).
This ensures that the $P_j$ is a probability vector and allows for additional variation between trials. Correlations between the normally distributed deviations were included in the model to effectively account for repeated measurements in Exp. 1 only. The estimated probability of burial in each sediment [i.e., $P_{1s} = \exp(\alpha_{s1})/\exp(\alpha_{s1} + \alpha_{s2} + \alpha_{s3} + \alpha_{s4})$] is used to infer the relative preference of sandeel for each sediment type within the experiments.

Furthermore for Exp. 3 linear regressions were fitted, and the deviance explained ($R^2$) were used to highlight the trends that were observed for this pilot experiment.

The analysis was conducted via the statistical software R (version 4.2.2, R Core Team, 2021) using the package TMB (version 1.9.5), which is a tool that can implement complex random effect models through simple C++ templates (Kristensen et al., 2016). The data, model and coding-script supporting this analysis are publicly available as supplementary material at DTU Data, which is a repository for publishing and indexing research data, https://doi.org/10.11583/DTU.25814107.

3. Results

3.1. Experiment 1, sediment preference for small sandeel

Comparing the fit of a model with no levels (i.e. random choice) to a model with eight levels, one for each tray (likelihood ratio test $P = 0.05$), did not improve the fit, but comparing to a model with four levels, one for each sediment (likelihood ratio test $P = 0.02$), revealed that four levels significantly improved the model fit. This suggests that the fish did not select trays and sediment randomly. Furthermore, having eight levels for each tray did not improve the fit compared to having four levels for each sediment (likelihood ratio test $P = 0.39$). As such, it could be concluded that there was no preference for specific trays (e.g. effects of position or edges) and a model with four levels for each sediment was selected. Therefore, all trays with the same sediment were pooled to get one probability for each sediment. The model estimated average probabilities with confidence intervals (CI) of $0.22$ [CI: 0.15, 0.28] and $0.23$ [CI: 0.16, 0.30] for burial in finer sediments of “medium sand” and “medium-coarse sand”, and $0.37$ [CI: 0.29, 0.46] and $0.18$ [CI: 0.12, 0.24] for coarser sands of “coarse-very coarse sand” and “medium-fine gravel”. Accounting for repeated measurements by pooling counts for each experimental trial only had a negligible impact on mean probability estimates, but increased confidence intervals. A model that estimated an individual probability for each sediment type did not improve the fit over a simpler model with only two levels for preference (likelihood ratio test $P = 0.28$). The simplest model estimated one probability for a preferred sediment of “coarse-very coarse” ($0.37$ [CI: 0.28, 0.47]) and one for all other sediment types ($0.21$ [CI: 0.18, 0.24]) indicating that the preference for other sediments were not different to each other. Overall, the results showed that small sandeel exhibited an almost two times greater preference for coarse to very coarse sand (~48% of size fractions 0.5–1.0 mm and ~51% of 1.0–2.0 mm, Table 1) compared to other sediments (Fig. 2).

3.2. Experiment 2, effects of gravel on sediment preference

Sandeel did not select trays/sediment randomly. Having models that assumed eight tray levels (likelihood ratio test $P < 0.001$) and four sediment levels (likelihood ratio test $P < 0.001$) improved the fit significantly. A model having eight tray levels did not improve the model fit compared to four sediment levels (likelihood ratio test $P = 0.93$), which indicated that sandeels did not have any preference for specific trays, and all trays with same sediment were pooled to get one probability for each sediment. The model estimated an average probability of $0.31$ [CI: 0.24, 0.38] that small sandeel were found in the preferred sediment “coarse-very coarse sand” (established as their preferred type in Exp. 1). Similar probabilities were estimated for sediments that had added finer granule gravel into the preferred sediment, where sandeel burial on average was $0.29$ [CI: 0.22, 0.36] in “50% fine gravel” and $0.33$ [CI: 0.26, 0.39] in “65 % fine gravel”. While only an average probability of $0.07$ [CI: 0.04, 0.11] were estimated for burial in “30% medium gravel”. Assuming four, three or no levels of preference did not improve the model (likelihood ratio test $P > 0.05$) and thus, the simplest model assumed two levels of preference, estimating a probability of $0.31$ [CI: 0.30, 0.32] for the preferred sediment and mixtures compositions with finer granule gravel, and a probability of $0.07$ [CI: 0.04, 0.11] for “30% medium gravel”. As such, small sandeel did not show any differences in preference between their preferred sediment (i.e., “coarse-very coarse sand” in Exp. 1) and sediment mixtures containing fine gravel (Fig. 3). These sediments contained size fractions from 0.5 mm to 0.4 mm. Thus, they exhibited an approximately four times greater preference for these mixtures than the coarser mixture containing medium and coarse gravels of pebbles (i.e., grain sizes >4 mm, see Table 1).

3.3. Experiment 3, effect of light intensity on sediment preference

Models with eight tray levels (likelihood ratio test $P < 0.01$), four sediment levels (likelihood ratio test $P < 0.01$), four light intensity levels (likelihood ratio test $P < 0.00$) and fourteen sediment-light levels (likelihood ratio test $P < 0.00$) resulted in a better fits to the data than the naive model that assumes no preference (i.e. common probabilities across all trays). Still, a model including fourteen levels for each combination of sediment and light resulted in a better fit to the data than all other models (likelihood ratio test $P < 0.00$) and thus, data were pooled for each level to get one probability for each sediment-light combination. A strong negative trend between preference for burial and increasing light intensity were observed for all sediments (same sediments as in Exp. 2), except for “30% medium gravel”. In fact, for all sediment types except medium gravel, light exposure explained a considerable amount of the variation in the preferences for burial with $R^2$ between 20 and 65% and 46–91% for observed proportions and predicted probabilities, respectively (Fig. 4). In summary, sandeels tended to avoid sediment trays in more well-lit areas, even if they contained their preferred sediment mixtures.

4. Discussion

The first experiment found a strong preference for coarse to very coarse sand, compared to other mixtures containing finer sand and gravel components. Overall, these results are very similar to findings obtained from several closely related sandeel species. For example, Wright et al. (2000) conducted choice experiments with different sediment mixtures and found that A. marinus preferred sediments with large amounts of coarse sands (0.5–2.0 mm) compared to finer grain mixtures. Similarly, Bizzarro et al. (2016) investigated the sediment preferences in A. personatus using more uniform sediments with sorted grain sizes as opposed to mixture sediments used in this study. They found that A. personatus specifically preferred coarse sand with a grain fraction of 0.5–1.0 mm. Specifically, based on experimental work presented here, an equivalent affinity for solely coarse sand as shown by Bizzarro et al.
(2016) seems to be less pronounced for small sandeel which chose sediments with lower amounts of coarse sand when presented with a more uniform sediment mixture (e.g., ~80% coarse sand). As such, it seemed that a mixed sediment was preferred compared to more uniform sediments. Mixed sediments have physical properties that differ from more uniform sediments, such as reduced compaction and therefore potentially increased oxygen penetration (Bizzarro et al., 2016; Endo et al., 2019), which may increase its suitability as a burial habitat.

An avoidance of finer grain sands has previously been found in experimental studies on Ammodytes spp. (Bizzarro et al., 2016; Wright et al., 2000). This is consistent with the lower utilisation of finer sediments in the first experiment of this study. This is likely driven by oxygen supply, as the permeability of finer sediments may allow oxygen penetration only within a few millimetres from the surface (Cook et al., 2007; de Beer et al., 2005). In contrast, early studies on sandeel burial behaviour found no difference in preferences between mixtures of fine-medium and coarse-very coarse sand (A. personatus, Pinto et al., 1984). Although this may have been influenced by their experimental design, which had continuous water flow into the benthos that provided oxygen supply into the sediments during the experiment. As such, these studies point towards a common preference for sediment grain sizes for Ammodytidae sandeel species. In addition, several field studies have reported coarser sands as an important component for many Ammodytidae species throughout the northern hemisphere (A. marinus, Holland et al., 2005; A. personatus; Baker et al., 2021; A. hexapterus, Haynes et al., 2007; A. japonicas, Endo et al., 2019). Sandeel appear to show a similar preference in this experiment.

Wright et al. (2000) has also found evidence that sediment preferences may be related to fish size in A. marinus, suggesting there may be an ontological shift at ~65 mm in length. Juvenile fish tended to prefer finer sands, whereas larger individuals tended to prefer coarser sands and gravels, which may be driven by increasing oxygen demands as well as increased burrowing ability in larger fish. Holland et al. (2005) proposed that sandeel may follow an ideal free distribution (Fretwell and Lucas, 1969), i.e., where the most favourable habitats are occupied preferentially. Thus, preferred habitat will remain occupied and more marginal habitat will become vacant when population size is reduced. If the preference for coarser sand is related to an ontogenetic shift with fish size, as suggested by Wright et al. (2000), one might expect a relationship where smaller-sized individuals, e.g. juveniles, are occupying more marginal habitat. Such shifts in distribution to more marginal habitat, particularly by A. marinus juveniles can be observed in the North Sea (Rindorf et al., 2019). Therefore, future experimental studies on Ammodytes spp. may benefit from including a wider size range compared to previous studies.

Fig. 4. Experiment 3 investigated how light levels influenced burial behaviour of small sandeel (A. tobianus) in various sediment mixtures. The upper panel shows observed proportional fish counts for each trial. The size of points were proportional to the number of fish in each condition. The lower panel shows the predicted probabilities of burial under each condition (i.e., sediment/light combination, ±95% CI). Linear regression lines have also been fitted, and the deviance explained ($R^2$) are given for all sediment/light combinations.
to this study, e.g. sizes that include juvenile to adults, to deal with questions of possible ontogenetic shifts in habitat preference. No effect of size were detectable in this study.

The lower limit for grain size tolerance for sandeel have been thoroughly investigated both in laboratory experiments (Bizzarro et al., 2016; Pinto et al., 1984) and in field studies (Haynes et al., 2007, 2008; Holland et al., 2005; Tien et al., 2017). Wright et al. (2000) found that A. marinus did not select sediments containing >10% silt-clay and would display avoidance behaviour at concentrations as low as 2%. Avoidance of finer more silty sediments is also consistent with field observations of small sandeel (Reay, 1970; Tien et al., 2017). Experimental studies have revealed that sandeels may lay passively on top of the sediment or only partially bury when presented with fine sediments (Bizzarro et al., 2016; Wright et al., 2000). Studies have also described some behavioural responses to low oxygen supply in sediments. For example, when subjected to decreasing oxygen levels from normoxia to anoxia, small sandeel will move closer to the surface layer, eventually exposing their heads to facilitate respiration (Behrens et al., 2007). Furthermore, swimming activity appears to become more frequent in lower oxygen conditions (Behrens et al., 2010).

There is less available data regarding the upper grain size tolerances for sandeel burial, and studies have either conducted experiments without including larger gravels (e.g., Bizzarro et al., 2016) or not analysed larger size fractions in detail within their sediments (e.g., Wright et al., 2000). Results from the second experiment seem to indicate that high amounts (48–64%) of fine gravel do not negatively affect sediment preference. Conversely, adding a component of medium gravels of pebbles sized 4.0–8.0 mm had a significant negative effect on sandeel burial. However, field reports have confirmed that habitat utilised by multiple Ammodytes species have sediment compositions with substantial amounts of gravel (Haynes et al., 2007, 2008). Holland et al. (2005) found that A. marinus around the Scottish coast could be found in 20–40% of all samples that contained 5–30% fine-medium gravel. A direct comparison to the findings here is difficult because field studies did not distinguish between granule and pebble gravels, which could have hidden any effects of larger grade classes of gravels. Nevertheless, the results from this work suggest that the upper limit in grain size for burial of small sandeel may be found in the transition between fine and medium gravel, such as pebbles >4mm.

Light availability appears to have a significant effect on sandeel burial behaviour (Freeman et al., 2004). Early experiments showed that light availability was positively correlated with the swimming activity (Winslade, 1974c). The stimulus for nocturnal burial can be strong enough to force this behaviour even under severe hypoxic conditions (Behrens et al., 2010). In Winslade (1974b), swimming activity reached maximum levels at approximately 100 lx, and there was an apparent threshold of ~20 lx where swimming activity was significantly reduced. The effect of light variation in this study (i.e., from 18.7 to 44.1 lx), is across a similar range and our effects are consistent with an increase in burial behaviour at ~20 lx. The light gradients used in our experiment may mimic a natural depth gradient, where the penetration of light is expected to decrease and supposedly act as better burial habitat with lower predation risk. Tien et al. (2017) found depth to be one of the main drivers of the distribution of three sandeel species in the field, where higher densities of small sandeel were found in shallower waters, whereas A. marinus were more abundant in deeper areas. Furthermore, the observed preference for burial in dimmer light conditions among sandeels in this study aligns with documented diet behaviors in related marine species. Specifically, Baker et al. (2023) noted a crepuscular movement pattern with increased activity at dawn and dusk, influenced by light thresholds necessary for initiating pelagic foraging. This crepuscular behavior was suggested critical for maximizing foraging efficiency while minimizing predation risk, which could parallel the increased burial activity of sandeels under lower light conditions. Such behaviors underscore the complex interaction between light availability and essential survival behaviors in sandeels and related species. Amiya et al. (2023) also revealed that light conditions and the circadian regulation via melatonin significantly affect the sand burrowing behaviors of Ammodytes spp. aligning with our observations that sandeels avoid well-lit areas for burrowing. Yet, due to the basic and somewhat improvised experimental pilot setup with inconsistent measurements of light conditions, as well as low treatment replication, lack of repeated trials and low sample sizes in each trial, our results regarding light effects should be interpreted cautiously. Nonetheless, the light effects reported in this study and the apparent importance of depth to sandeel distributions in the wild suggests that further experiments may be warranted focusing on the interactive effects of light variation across day-night cycles and depth.

Differences in experimental setups limit our ability to compare results among studies (e.g., duration of experiments, replication, composition of sediment treatment, holding tank conditions, size and species of animals etc.). Inconsistencies between outcomes may also be related to the different approaches used to analyse and interpret the data. There are various pitfalls from multiple testing (Ranganathan et al., 2016). However, the multinomial approach used in this study has been successfully applied in similar experiments (Christoffersen et al., 2018) and models presented here avoid multiple testing and are designed to correctly account for the uncertainty between trials (i.e. confidence intervals) and intertrial variability by modelling all levels simultaneously, and choosing the best model for fitting the data.

To the best of our knowledge, this is the first study to describe the preferred sediment for small sandeel (A. tobianus) through laboratory experimentation and to investigate the upper limit threshold of grain sizes for any sandeel species. The findings indicate that granule gravels (2.0–4.0 mm) serve as suitable habitat of similar quality as coarser sands for small sandeel, like many other sandeel species. Furthermore, sediments dominated by pebble or even cobble may represent an upper tolerance limit. The drivers for the upper and the lower limits for burial are likely related to a trade-off between the physical limitations of burial and the level of oxygen availability in different sediments. In addition, even small changes in light intensity had strong influences on burial behaviour, highlighting the importance of environmental conditions to burial behaviours. Further studies into the physical limitations, physiological requirements and environmental effects of burrowing may be valuable to understand their interactive effects in species of sandeel.

CRediT authorship contribution statement

Ole Henriksen: Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – review & editing, Writing – original draft. Nicholas P. Moran: Visualization, Writing – review & editing, Writing – original draft. Louis A. Veillex: Validation, Methodology, Formal analysis, Data curation, Conceptualization, Writing – review & editing, Writing – original draft. Jane W. Behrens: Validation, Supervision, Project administration, Conceptualization, Writing – review & editing, Writing – original draft. Anders Nielsen: Validation, Software, Methodology, Formal analysis, Writing – review & editing. Tobias K. Mildenberger: Validation, Software, Methodology, Writing – review & editing, Writing – original draft. Peter J. Wright: Visualization, Validation, Conceptualization, Writing – review & editing, Writing – original draft. Henrik Jensen: Conceptualization, Writing – original draft. Mikael van Deurs: Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization, Writing – original draft.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

Data, coding-script and model is available at https://doi.org/10.1185/DTU25814107 and on request.

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