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Published in:
Journal of Fish Biology

Link to article, DOI:
[10.1111/jfb.14745](https://doi.org/10.1111/jfb.14745)

Publication date:
2021

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Geffen, A. J., Albretsen, J., Huwer, B., & Nash, R. D. M. (2021). Lemon sole *Microstomus kitt* in the northern North Sea: a multidisciplinary approach to the early life history dynamics. *Journal of Fish Biology*, 99(2), 569-580. <https://doi.org/10.1111/jfb.14745>

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Lemon sole *Microstomus kitt* in the northern North Sea: a multidisciplinary approach to the early life history dynamics.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/jfb.14745](https://doi.org/10.1111/jfb.14745)

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Abstract

Lemon sole *Microstomus kitt* is a commercially valuable flatfish species that occurs in shelf waters around the northeast Atlantic. Only the most basic life history information is available for the North Sea. Spawning is generally assumed to occur between early May and October, with a peak between May and August. Lemon sole larvae have been found in water column in the northern North Sea in winter, during standard surveys. We analysed larvae captured in November/December 2016 and January/February 2017 using ICES standard 2m Midwater Ring Trawls (MIK) to gain a better understanding of the pelagic early life history stages of lemon sole especially in relation to the timing of spawning, and the dispersal of overwintering larvae. Larval age was estimated from sagittal otolith primary increment counts. The larvae caught in November/December ranged in nominal age from 4-45 days post-hatch which suggests spawning continues into late October and November. Most, but not all, of the larvae caught in January/February were post metamorphosis and the difference in age between the two sampling dates was consistent with the elapsed time between sampling. The estimated hatching dates confirm that lemon sole spawning extends into late autumn in the northern North Sea, with overwintering larvae in all developmental stages. Drift modelling of eggs and larvae released at historically documented spawning grounds in the northern North Sea suggests that these grounds are also the source for all of the larvae sampled during the 2016-2017 surveys.

KEYWORDS: Larval dispersal, Overwintering, Otolith microstructure analysis, Hatching date, Flatfish, North Sea

1. INTRODUCTION

Lemon sole *Microstomus kitt* (Walbaum, 1792) is a commercially valuable flatfish species widely distributed across shelf waters from the southern part of the Barents Sea southward to the Bay of Biscay and from Iceland eastward to the White Sea. It is generally fished in waters of 50 to 200m depth, but has also been found down to a maximum depth of 1100m (Goldsmith *et al.*, 2015). Despite a long history of exploitation in the North Sea and around Iceland and the Faroes, very little is known about the ecology of the early life history stages. Spawning is generally assumed to occur across the central North Sea, between 55° to 58° N and 0° to 6° E (Sundby *et al.*, 2017). The spawning period is mainly between May and August, but several studies have suggested that spawning can last through November (Rae, 1965; Russell, 1976; Rønnestad *et al.*, 1992). Russell (1930) sampled eggs and larvae to the southwest of the UK in May – July. Rae (1965) found *Microstomus kitt* larvae in the North Sea in deeper waters; they were most abundant between 50 – 100m depth. Taylor *et al.* (2007) indicated that the majority of the larvae found during more recent North Sea surveys in the spring occur off the east coast of Scotland, to the east of the Moray Firth.

Late larval stages of *Microstomus kitt* were sampled in the water column as late as November in the North Sea (Rae, 1953, 1965). Although Rae (1965) suggested that only a small proportion of any year class overwinters as larvae, there is little direct observational data in historical records. In contrast to the older literature, recent International Council for the Exploration of the Seas (ICES) egg and larval surveys in the North Sea regularly report catches of *Microstomus kitt* larvae well into the winter months (ICES WGEGGS2, 2018). It is not clear whether this represents a shift or an expansion of the spawning season, or a change in spawning

location, which may be linked to changing climate or temperature regime. The increased numbers of overwintering larvae could also be an artefact of sampling programmes which were not regularly undertaken during the winter in earlier years.

The majority of fish species in the North Sea tend to spawn in the spring or early summer months (see Munk & Nielsen, 2005), taking advantage of the annual production cycle which peaks in the spring or early summer (Houde, 2008).

However, there are several species which spawn in the autumn or early winter e.g. North Sea Autumn spawned herring (*Clupea harengus*) where the larvae or early juveniles successfully survive in the plankton during periods when there is very little prey available (Hufnagl *et al.*, 2015; Denis *et al.*, 2016; Bils *et al.*, 2019).

Overwintering as larvae should be a risky strategy, considering size dependent mortality processes, since as smaller individuals they should face a higher probability of mortality compared with larger individuals (Hurst, 2007). Climate change may also increase the risk for overwintering larvae in the North Sea, as any benefits of elevated temperatures are offset by unchanging feeding opportunities. Since day-length is not altered in any climate change scenario, the available feeding time for visual predators such as small juvenile and larval fish remains limited in autumn and winter. In addition, other aspects such as increased 'storminess' (Hinder *et al.*, 2012) may disrupt dispersal patterns (Lacroix *et al.*, 2018) and feeding (Utne-Palm, 2004; Oshima *et al.*, 2009).

In addition to temporal shifts, climate change may alter the spatial locations of spawning, nursery and/or feeding grounds and thus disrupt 'life history closure' and the viability of a fish population (see Rijnsdorp *et al.*, 2009; Petitgas *et al.*, 2013). Shifts in spawning location could also result from changing patterns of seabed use such as construction (van Damme *et al.*, 2011; Höffle *et al.*, 2017). It is generally

assumed that there is one spawning population of *Microstomus kitt* in the North Sea (Rae, 1965; Sundby *et al.*, 2017), but larvae sampled during the winter may have come from new North Sea spawning grounds or may have drifted from other areas.

This paper examines the early life history (larvae) of *Microstomus kitt* in the northern North Sea. Otolith microstructure is used to estimate the age of the larvae in the field and to estimate spawning times and growth rates, especially during winter months. Drift modelling is used to determine connectivity between sampling periods and to investigate spawning locations along with potential areas for settlement. The results are integrated with historical information to provide a more comprehensive understanding of pelagic stages of *Microstomus kitt* in the northern North Sea. A greater understanding of *Microstomus kitt* ecology from spawning times to settlement would improve the evidence available for the management of this stock. This is especially relevant to developing an ecosystem approach to fisheries management.

2. MATERIALS AND METHODS

2.1 Sample collection

Microstomus kitt larvae were caught in November/December 2016 during the Institute of Marine Research (Norway) northern North Sea survey (Figure 1a), and in January/February 2017 (Figure 1b) during the ICES Co-ordinated International Bottom Trawl Survey (1st quarter IBTS, ICES IBTSWG, 2017). Larvae for otolith analysis were made available for this study from corresponding areas sampled by IMR from the RV Kristine Bonnevie (KB) in the northern North Sea between the 26th November and 2nd December 2016 (Figure 2a) and DTU Aqua's portion of the IBTS between 2nd and 17th February 2017 (Figure 2b). The February samples were

collected by the RV DANA (DANA) from the northern part of the lemon sole distribution, although there were stations with higher abundance in the southwestern portion of the DANA survey. In both cases the larvae were caught using ICES standard 2m Midwater Ring trawls (MIK) with 1600 μ m body mesh and 500 μ m cod-end mesh. Stations were sampled following the IBTS Q1 protocols, with double oblique tows from surface to 2m off the bottom (or 100m maximum depth) at a nominal ship speed of 3 knots (ICES SISP, 2017).

Temperature data were collected during the surveys using calibrated Conductivity Temperature Depth (CTD) profilers. In the case of the IMR November survey a Seabird SBE-911 was used. In the case of the ICES International Bottom Trawl Survey a variety of different CTDs were used with all survey data being uploaded to the ICES Egg and Larvae database along with the data on abundances of lemon sole larvae. A standard depth of 10m was used to characterize the thermal water conditions during the time of sampling.

2.2 Sample processing and data collection

Microstomus kitt larvae in the MIK samples are easily identified. They show the typical dorso-ventrally heightened and laterally compressed body form of a flatfish larva with a very distinct pigmentation pattern (see Nichols, 1971, Russell, 1976; Munk & Nielsen, 2005). Conspicuous blotches of dark melanophores are distributed along the dorsal and ventral margins of the body as well as along the margins of the anal and dorsal fins. The left eye starts migrating at about 15 mm TL (Howell, 1972) and almost all stages of developing asymmetry were observed in the larger specimens in the samples.

Microstomus kitt larvae were sorted from the samples onboard and preserved in alcohol for otolith analysis. We selected stations that preliminary modelling suggested could retain larvae for a relatively long period of time, so that we would be able to re-sample the same group two months later. We analysed all the larvae from each of these selected stations (Figure 2, Table 1). A total of 62 larvae were sampled during the KB cruise, of which 48 were used for otolith analysis (77%). The total number of lemon sole larvae in the whole DANA cruise was 159, of which 36 (23%) were used for otolith analysis.

Although all lemon sole larvae were identified and counted onboard in both surveys, no lengths were measured. In the laboratory, the larvae were removed from alcohol into fresh water for softening. When flexible, they were photographed for length measurement, and the sagittal otoliths extracted and mounted in crystal bond. Standard length of pre-metamorphosed larvae was measured from the calibrated images with a segmented line following the path of the notochord. Late-stage larvae were photographed on a mm grid and total length measured from the calibrated images. Length measurements were rounded to the nearest 0.1 mm and no corrections were applied to account for alcohol shrinkage.

Otolith increments were counted to estimate individual age, assuming that daily increment formation begins at hatching and continues throughout the pelagic stage (Supporting Information Figure 1). Otoliths of larger larvae were polished lightly with 1 μ m lapping film to increase the visibility of early increments. All otoliths were examined and photographed at 400x and 1000x magnification. Increment counts were made directly at the microscope, and again, by a different reader, from contrast-enhanced images. Any discrepancies in the increment counts were resolved by repeated direct examination at the microscope and further checking of new

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images. Age estimates of 11 larvae (12% of total) were rejected because increment counts could not be resolved. Otolith radius, distance to first increment, and distance to the first accessory primordium were all measured from the calibrated images using ImageJ (Schneider *et al.*, 2012).

Estimated hatching dates were calculated for each individual by subtracting age (from increment counts) from date of capture. Spawning times were estimated by assuming an interval of 12 days elapsed time between spawning and hatching, based on ambient temperatures of between 7 and 8C in the lower water column and egg development times from laboratory studies by Howell (1972) and Rønnestad *et al.* (1992). Length-at-age data was extracted from the figures in these two published laboratory studies to provide growth data for qualitative comparison with the growth estimates from our field samples.

2.3 Ocean modelling and particle tracking

Oceanographic drift modelling was used to predict transport of pelagic *Microstomus kitt* eggs and larvae from the assumed spawning areas in the northern North Sea (Sundby *et al.*, 2017). The ocean currents used were provided from the hydrodynamic model described in detail in Lien *et al.* (2014). This model system applied the Regional Ocean Modelling System (ROMS, <http://myroms.org>, see e.g. Shchepetkin & McWilliams, 2005; Haidvogel *et al.*, 2008). ROMS is a state-of-the-art, three-dimensional, free-surface, primitive equation numerical model using a generalized terrain-following s-coordinate in the vertical. The model archive covered parts of the North Atlantic (from about 50-52 °N) and all the Nordic and Barents seas, and it was implemented with a horizontal grid resolution of 4 km. The output from ROMS contained velocity fields in 32 vertical levels and a temporal resolution of

24 h. The ocean current archive that was applied here corresponded in time with the sampling period (autumn 2016 and winter 2017) to reflect the ambient conditions experienced by the eggs and larvae.

The advection of particles in the horizontal plane was simulated from the hatch date distributions by using the Lagrangian Advection and Diffusion Model (LADIM, Ådlandsvik & Sundby, 1994; Myksvoll *et al.*, 2018) which applies a Runge-Kutta 4th order scheme. Particles representing *Microstomus kitt* eggs/larvae were released in all grid points (7,397 locations) within the assumed spawning area and at every meter between 20 and 30m depth. In addition, drift was initialized similarly every 5th day from October 20th to November 19th, 2016, so that the total number of particles moved was close to 570,000. Initializing at several dates was conducted to capture the variation in spawning times. The drift model did not apply any larval behaviour (diel vertical migration), and thus the particles were kept at the fixed depth set at initialization throughout the simulation. All egg and larvae particles were allowed to passively drift with the ambient currents, and the final particle density was then projected to a $1.0^{\circ} \times 0.5^{\circ}$ longitude/latitude regular grid at December 1st, 2016 and February 8th, 2017, corresponding to the KB and DANA cruises, respectively.

3. RESULTS

3.1 Thermal environment

The sampling in November/December 2016 did not cover the whole of the North Sea so the only information readily available on the thermal environment below the sea surface (in this case 10m) was obtained at the sampling stations. At this time the temperature ranged from 8.6 to 10.9°C with the warmer waters occurring to the west

and north of the sampled area (Fig 2a). A more comprehensive view was available for January/February 2017 and there was a similar pattern with the warmer water occurring in the north and west of the North Sea (Figure 2b). The subsurface sea temperatures at 10m depth were cooler, ranging from approximately 6.5 to 8.5°C.

3.2 Spatial Distributions

The sampling in November/December 2016 was not a comprehensive coverage of the northern North Sea, consisting of two east/west transects and a set of stations along the western edge of the northern North Sea. *Microstomus kitt* larvae were found off the Moray Firth (east of Scotland) and in the eastern and central parts of the Aberdeen to Hanstholm transect (see Figure 1a).

The area coverage in the January/February 2017 IBTSQ1 survey was more comprehensive. These samples indicated that the majority of the *Microstomus kitt* larvae were in the western North Sea, centered to the southwest of the Dogger Bank (see Figure 1b).

3.3 Hatching and spawning dates based on otolith increment counts

Sagittal increments in the lemon sole larvae were generally clear, ranging from 0.5µm to 1.3µm in width (Supporting Information Figure 1). Among the smallest larvae caught in November / December 2016, which were 4 – 5mm in length, the sagittal otoliths were ca. 35µm in diameter, with 6-10 increments. The smallest larvae caught in February 2017 were 8 – 9mm in length, and their sagittal otoliths were 27 - 34µm in diameter with 19-24 increments. Clear increments usually started at a diameter of 10 - 15µm, but there were earlier increments at ca. 7 - 9µm diameter visible in some individuals. Since very few of the larvae were still in the yolk-sac

stage, it was not possible to assign either of the innermost increments to hatching, mouth opening, or yolk-sac absorption.

The largest larva caught in December 2016 was 20.4mm, with a sagittal otolith diameter of 181 μ m and with 56 increments. The largest larva caught in February 2017 was 28.8mm, with a sagittal otolith diameter of 324 μ m and with 93 increments. The accessory primordia (AP) typical of juvenile flatfish (Nash & Geffen 2015) was observed in individuals in the later stages of metamorphosis (Figure 3), and usually appeared at 50 ± 12 (mean \pm sd) increments from the core, at a diameter of ca. 220 μ m. Only the largest larva in the November / December 2016 samples had reached this stage and one AP was beginning to form on both right and left sagittae. Almost all of the larvae longer than 16.9mm sampled in February 2017 had otoliths with one AP, and those longer than 23mm often had two.

Otolith increment counts ranged from 6 – 57 for larvae sampled in November / December, and 19 – 104 for larvae sampled in the following February (Figure 3). Over both survey periods the otolith primary increment data suggests that the main hatching period of these larvae was the 9 - 29th November 2016 (Figure 4a). Assuming 10 to 14 day incubation period for the eggs (see Howell, 1972) this suggests that these larvae were spawned in the last week in October or first week in November. The presence of very small larvae in the February sampling, with estimated hatching dates in early to mid-January, suggest that spawning may continue into late December and early January.

3.4 Growth

Larvae ranged in size from 4.1 – 20.4mm in November/December, and 8.3 – 29.2mm in February (Figure 4b, Table 1). Larval length increased with age as

estimated from increment counts (Figure 5). Approximately 60 days separated the midpoint dates of the two surveys and all larvae fell along a similar growth trajectory. (see Figure 5). There is a greater variability in length for larvae estimated to be older than 60 days post-hatching.

The length-at-age relationship of *Microstomus kitt* larvae sampled during the November / December 2016 survey was $SL \text{ (mm)} = 5.32 + 0.20 * \text{age}$, and during the February 2017 survey was $SL \text{ (mm)} = 6.75 + 0.21 * \text{age}$, where age was estimated from increment counts. This corresponds reasonably well with the growth rate of 0.23 mm d^{-1} estimated from the 15mm increase in modal lengths over the 63-day interval between midpoint dates of the surveys (Figure 4a). The estimated growth of the field caught larvae was compared qualitatively with length data from laboratory reared larvae (Howell, 1972 – Figure 2; Rønnestad *et al.*, 1992 – Figure 3). The length data extracted from the published figures indicate that lemon sole larval growth was faster in the autumn/winter of 2016/2017 in the northern North Sea (Figure 5).

3.5 Modelled dispersal

Particles representing newly-spawned eggs that were released between the 1st and 15th November 2016 had not drifted very far by the time of the first sampling survey (midpoint 1st December 2016) with the main distribution of particles (81%) resembling the original spawning distribution and location (Figure 6a). In addition, some modelled particles were displaced east into the Skagerrak and northward along the Norwegian coastal current. However, the 2016 survey stations were concentrated in the middle to northern sections of the assumed spawning area (see

Figure 1a, 6a), and there are no sampling surveys along the Norwegian coast that could verify the presence of *Microstomus kitt* larvae.

By February, the particles which were released in early November were more widely dispersed, with the centre of the distribution shifted to the south and west of the spawning area (Figure 6b). There were modelled particles drifting into the Skagerrak, the Kattegat and northward along the Norwegian west coast as a continuation of the eastward spread in early December. Less than half of the particles released remained inside the assumed spawning area. The modelled drift did not carry any particles to the northwest of the spawning area in this time period. The predicted distribution was consistent with high concentrations found in the IBTSQ1 survey south and west of the assumed spawning area (see Figure 1b). However, the higher concentrations sampled in the IBTSQ1 survey off the eastern coast of England (along the west side of the Dogger Bank) were further south and west than predicted from the particle tracking model (Figures 1b, 6b).

The modelled distributions suggest that the larvae sampled in the two surveys probably originated from the same spawning time period.

4. DISCUSSION

Many commercially exploited fish species are considered to be “data poor” fisheries, and little is known about their life histories or ecology. *Microstomus kitt* is one of these species. The International Council for the Exploration of the Seas (ICES) provides advice on catches for *Microstomus kitt* in the North Sea (ICES Advice, 2019) and considers it a data poor stock due to the lack of pertinent data and understanding of its ecology. Much of the general information on *Microstomus kitt*

was first published by Rae (1965), who also studied the abundance and distribution of the early life history stages in the North Sea (Rae, 1953). With little focused research on this species in the years since, information on the spawning area, nursery grounds, and even population dynamics is scarce. It is now clear that *Microstomus kitt* is a species with a particularly protracted spawning season which results in early life history stages overwintering in the plankton.

4.1 Temporal occurrence

Microstomus kitt larvae are commonly collected in North Sea ichthyoplankton surveys, especially since the establishment of regular winter surveys since 1992 (Munk *et al.*, 2014). Typical identification literature suggests that spawning does not extend to the later months of the year (Russell, 1976), but the presence of small larvae in winter surveys is clear evidence for late spawning. The estimates of hatching and spawning dates, based on our otolith analysis, concur.

We estimated hatching dates from otolith increment counts, assuming daily periodicity of formation beginning at hatching. Although non-daily increment formation has been documented in several pleuronectid species, this is generally due to very slow growth at low temperatures (Casas 1998, Joh *et al.*, 2005).

Likewise, primary increment formation does not always begin at hatching in pleuronectid species, and may start around 2-5 days after hatching or be linked to a physiological or developmental event such as yolk sac absorption or mouth-opening (see Nash & Geffen 2015)

The age of the *Microstomus kitt* larvae was estimated from primary increments in the sagittal otoliths, using light microscopy. In general, the rings on the otoliths of these young fish (<30mm standard length) were clear and distinct and only

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in the larger larvae was grinding or polishing necessary to count increments. It is known that in some species the number of rings does not equate with age especially where the environmental conditions can severely disrupt growth rates (Fox *et al.*, 2003). In this case we have assumed that the pelagic *Microstomus kitt* larvae deposit daily increments, and that these could be identified with our microscopy techniques. The estimated growth rates from the otolith increments were similar for larvae caught in November/December and February (0.21 and 0.20 mm.d⁻¹). The combined laboratory data from Howell (1972), and Rønnestad *et al.* (1992) gave a lower estimated growth rate (0.16 mm.d⁻¹) than was estimated for overwintering field caught larvae.

Microstomus kitt larvae are 3.5 to 5.5mm at hatching (see Russell, 1976). The estimated hatch length for the larvae we analysed was 5mm, based on the intercept of the larval length at increment number relationship. The “size-at-age” of all the larvae, from both surveys, fell along a common trajectory, or growth curve. With *Microstomus kitt* hatching at around 4-5mm SL, larvae at this size are not generally retained in the MIK net (1.6 mm mesh netting) so we do not know if smaller larvae were present in the area. In addition, there are no egg samples during this time period so there is no direct evidence of spawning. However, 10 to 15 mm TL *Microstomus kitt* larvae were caught in both November/December and February with the cruises being approximately 60 days apart. Even with a growth rate (and shift in the relationship between age and increment number) of about 0.1 mm.d⁻¹ the smallest larvae in February should still have hatched in late December 2016. Whilst the growth rate may have been severely impacted and/or increment rates reduced substantially we think that in this case it is unlikely. Size-selective mortality could also have resulted in lower apparent growth over the winter, but we do not have data

at sufficient temporal or spatial resolution to address questions of mortality or its effects. Howell (1972) observed a slow-down in larval growth after about 70 days post hatch (at around 15mm). Whilst this was not obvious in the field data, there was an increase in the variability in length at age after approximately 60 days post-hatch.

For an estimate of spawning date it was necessary to make some assumptions about the development rate of the eggs. Howell (1972) indicates that eggs hatch after 7 to 12 days over the temperature range 7-10°C. We do not have direct measurements of the water temperatures between October and December 2016, during egg incubation, however, larvae were caught in waters which were between 6 and 10.5°C, so it is reasonable to use a range of 10-14 days for egg development.

Russell (1976) gives 18mm for the size at completion of metamorphosis in lemon sole. Howell's larvae (Howell 1972) were 19.5mm and at Stage 5 (metamorphosed) at the end of their experiments at 105 days. He did not specify whether they were settled at this point or not, but observations of lemon sole larvae are that they remain active in the water column most of the time well past metamorphosis (A. Geffen, pers. obs.- opportunistic experiments at Port Erin Marine Laboratory, University of Liverpool, Isle of Man in the late 1990s). We caught individuals of up to 20.4mm in the plankton during the day in 2016, and most of the larvae caught in the plankton at night in 2017 were 20mm or longer. So the real age at settlement and the duration of the transition to the benthos is unknown. Rae (1965, p.42, text and Figure 4) assumed that fully metamorphosed lemon sole, at approximately 25.4mm, would "take to life on the sea bottom" – but this could be just an estimate based on the maximum sizes he obtained in the water column.

The observation of over wintering *Microstomus kitt* larvae is not novel (Rae 1965), however, our growth and hatch-date analyses are the first evidence of spawning so late into the winter. Other species do spawn in January and February in the North Sea, namely the Downs (southern) component of the North Sea herring stock (Dickey-Collas *et al.*, 2009). Mean spawning date is known to vary between years and is often associated with fluctuations in ambient annual thermal regimes (Lambert, 1987). Later spawning dates in autumn-spawning fish is generally coincident with warmer temperatures (R.D.M. Nash unpubl. data) and by implication can be a consequence of climate change (Simpson *et al.*, 2013). In the case of *Microstomus kitt* we have been able to document late autumn spawning and overwintering by estimating hatching dates from otolith analysis. However our samples are not sufficient to determine whether the spawning season has extended in response to climate change, or whether more sampling in the winter period has simply revealed the prevalence of overwintering lemon sole larvae.

Microstomus kitt larvae are more abundant in the plankton during late summer and early autumn in the northern North Sea (Rae, 1953; R.D.M. Nash unpubl. data), but the relative contribution of early or late larvae to recruitment is still unknown. Overwintering as pelagic larvae could cause problems with the identification of the first annual ring when some fish are aged. This was mentioned by Rae (1965) as a potential problem and is a recognized issue for ageing *Microstomus kitt* (Smith, 2014). Rae (1965) examined the otoliths and the scales of adult *Microstomus kitt*, and identified a varying proportion of individuals who overwintered as larvae based on a “missing” initial winter annulus. This does indicate that some late spawned larvae do survive to later life, despite the generally assumed higher mortality rates during winter (Hurst, 2007) .

The occurrence of very small flatfishes during the winter in the water column is not uncommon. Dab (*Limanda limanda*), long rough dab (*Hippoglossoides platessoides*) and witch (*Glyptocephalus cynoglossus*), ranging in lengths of between 30 and 50 mm, have been caught off the bottom during the February MIK sampling in the northern North Sea (R.D.M. Nash unpubl. data). All of the MIK sampling in February was during night so we cannot be certain whether larvae sampled in the water column were pre-settling individuals or post-settling individuals with a nocturnal vertical migration off the bottom. If these fish survive the winter period then, as with *Microstomus kitt*, the first winter ring in the otoliths will be very small and easily missed in age readings. The potential for parts of a cohort or year class overwintering at a very small size to affect age estimation should be investigated for other species in addition to *Microstomus kitt*.

4.2 Distributions and modelled dispersal

The modelled drift of *Microstomus kitt* eggs predicted that the bulk of the eggs spawned in November would remain near the spawning areas, where they were sampled by the transect surveys in November/December 2016. Continuing through the following months, the modelled distribution indicated a drift southwest from the spawning area, toward the east coast of England. *Microstomus kitt* larvae were sampled in February surveys in these areas but were not retained for otolith analysis. Thus, we cannot be certain that the larvae that were analysed, which came from more eastern survey stations, were from the same spawning events. However, the modelled drift did predict that a proportion of larvae would be in the area that was sampled, and the otolith increment counts are consistent with these larvae coming from the main spawning area and from the same time period.

The behaviour of the larvae was not considered in the model so the real spatial distribution may be different. The larvae sampled in February were generally larger and should have more advanced swimming abilities compared to the smaller larvae from November/December. These older larvae may also already show some kind of 'pre-settlement behaviour' and thus be in deeper water. This may be the cause of Rae's (1953) observations that larvae seem to be sparse at 0-30m but more abundant at 50-100 m. The overall distribution of *Microstomus kitt* larvae in February (see Figures 2 and 6) suggests that the larvae sampled in November/December had moved southwest as well as east and southeast. In that case the larvae analysed in February were most likely from the same spawning but had drifted from the locations sampled in November/December.

4.3 Exploring connectivity through particle tracking models

The scarcity of early life history data for *Microstomus kitt* emphasizes the advantages of using simulations to explore its larval ecology in the northern North Sea. However, there are also limitations to this approach, especially when real data are limited. The movement and location of *Microstomus kitt* eggs and larvae was simulated in a particle tracking model, specifically reflecting conditions in the study years and dates. The oceanography (currents) are the 'best case' scenario using the available historical information, meaning that these are the most likely conditions to have prevailed during the time period under investigation (Autumn/Winter 2016 to 2017). The modelled behaviour of eggs and larvae in the water column was simplified by selecting a constant intermediate depth, because there is no detailed information on any diel vertical migration of *Microstomus kitt* larvae. Rae (1965) speculated that the eggs are spawned at depth, rising to near surface waters during incubation, and then sinking prior to hatching. This speculation was based on the

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close taxonomic relationship between plaice *Pleuronectes platessa* and *Microstomus kitt*, although there are many examples of closely related species with significantly different spawning behavior and egg characteristics (e.g. *Gadus morhua* and *G. microcephalus*, Froese & Pauly, 2000). Newly hatched *Microstomus kitt* larvae have been found at depth (100m, Taylor *et al.*, 2007), while newly hatched plaice are generally found shallower than 30m in the southern North Sea (Coombs *et al.*, 1990). In the absence of any detailed information on vertical distributions, we opted to maintain the eggs at an intermediate depth, namely 20-30m below the surface with no variation in buoyancy during development. To compensate for different transport routes dependent on the vertical positioning, though, particles were distributed evenly between the most likely depths and pathways at all depths were given equal weight. At this time of the year the water column is well mixed so there is relatively little variation in density through the water column (Pohlmann & Puls, 1994). Buoyancy measurements of *Microstomus kitt* eggs, especially over the developmental period are needed for more realistic modelling of where these eggs are in the water column and hence their spatial trajectories (Coombs, 1981; Sundby & Kristiansen, 2015).

As with the egg stages, there is little detailed information on the vertical distribution of *Microstomus kitt* larvae. In the English Channel the majority of the early larvae were found between 50 and 100m depth (Russell, 1926, 1930; Rae, 1953) with (Rae, 1953) reporting very low abundances between 0 and 30m depth. In addition, there appears to be very limited diel vertical migration (DVM), generally encompassing a vertical excursion of only 10-20m (Rae, 1953). Larvae have been found as shallow as 10m depth at night (Russell, 1928). With no clear evidence for DVM we opted to maintain the particles representing between 20 and 30m during all

larvae stages. Using this shallower depth than that reported in the literature was a compromise to minimize the chances of the particles representing eggs or larvae encountering the bottom in the coastal and southern portion of the distributional area. Inclusion of individual behavior generally improves the output of particle tracking models in studies of larval dispersal, as seen in plaice in the Irish Sea (Fox *et al.*, 2006) and sole in the North Sea (Lacroix *et al.*, 2018). In the case of *Microstomus kitt*, the effects of adding incorrect behavior patterns may be worse than the assumption of an average depth distribution. However, by releasing the particles (eggs) across all depths in the upper water column, we hoped to capture most of the likely drift scenarios.

It is not known whether *Microstomus kitt* larvae undertake directed swimming, either in relation to horizontal directional movement or simply actively locating at selected depth in the water column. In some flatfish species e.g., plaice a selective behaviour for being deeper in the water column with age or size actively transports larvae into shallow water nursery areas (see Fox *et al.*, 2006). Likewise, flounder larvae utilize selective vertical behaviour for tidal transport into estuaries and rivers (Jager, 1999). Modelling which does not include these behaviours will not place metamorphosing larvae in the correct location for settlement at their preferred nursery areas. Unfortunately, real data on settlement and habitat preferences of juvenile lemon sole are limited. Rae (1965) suggests that *Microstomus kitt* will settle at around 27-28mm. The numerous surveys in shallow coastal areas (inshore sand/mud nursery areas) along the east coast of England and the Dutch and German Wadden Sea (e.g. Rogers *et al.*, 1998; Berghahn, 2001; H. W. van der Veer unpubl.data) have rarely found newly settled *Microstomus kitt* on these productive flatfish nursery grounds. In fact there are very few reports of newly settled 0-group

Microstomus kitt from sampling benthic habitats. A survey in summer 2019 in the northeastern North Sea covered 45 stations suitable for sampling with a 2m beam trawl. Only one 23mm juvenile was caught on 29th July 2019 at 57.23°N 1.48°W (39km east-northeast of Aberdeen, Scotland) at a depth of 96m (R. Wienerroither, unpubl.data). This location would fit with the comments by Rae (1965) and Jennings *et al.* (1993) that *Microstomus kitt* do not disperse far from their spawning grounds and that the nurseries or settlement areas are in deeper water. Both Rae (1965) and Jennings *et al.* (1993) suggest that the lack of very small *Microstomus kitt* in samples or surveys is that they tend to settle on the rougher ground in these deeper water areas. This could either suggest that *Microstomus kitt* do not settle in this area or that sampling tended to be on less rough ground and thus they were not sampled. In conclusion, the limited evidence here suggests that any further studies on settlement and nurseries for *Microstomus kitt* will indeed need to concentrate on deep water (50m+) rough substrata.

5. CONCLUSION

In summary, it appears that *Microstomus kitt* spawning extends further into the winter period than previously known. The consequence is that some portion of the population spends their early life history stages over-wintering in the water column. Whilst the observation of later spawning may be a consequence of extended sampling during the winter period, the possibility of extended spawning due to climate change cannot be ruled out. In addition, the modelling studies indicate that there is probably limited dispersal from spawning grounds and that the nurseries are indeed offshore. The lack of newly settled individuals in more frequently sampled areas supports the suggestion that settlement and early nursery areas are located on rougher terrain in deeper, offshore areas.

ACKNOWLEDGEMENTS

The authors are grateful for the invaluable assistance of the captains and crews of the research vessels, along with all the scientific personnel on Kristine Bonnevie (IMR, Norway) and on all the vessels participating in the North Sea IBTS Q1 in 2017. We are also grateful to Julie Skadal, Department of Biological Sciences, University of Bergen for her help with otolith preparation and photography, and to G. Berg, H. Nåvik and S. Tonheim for preliminary otolith analysis completed as part of the Early Life History of Fish (BIO 308) course at the University of Bergen. The research was supported by the IMR North Sea Programme and the Department of Biological Sciences of the University of Bergen.

AUTHOR CONTRIBUTIONS

R.D.M.N and A.J.G conceived the project. R.D.M.N and B.H. collected the field samples. A.J.G. undertook the otolith work and growth analyses, J.A. undertook the particle tracking work along with its analyses. All authors contributed to interpretation and writing.

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Figures

FIGURE 1. Survey stations and abundance of lemon sole larvae sampled from (a) RV Kristine Bonnevie (KB) between 26th November and 2nd December 2016, and (b) during the IBTS 1st Quarter in January to March 2017. Underlying contour maps shows depth contours and size of circles shows larval abundance

FIGURE 2. Temperature (°C) at 10m depth in the North Sea (a) at survey stations in November/December 2016 and (b) as contours in January-March 2017. Stars show location of samples that were selected for analysis of larvae.

FIGURE 3. Developmental series of lemon sole larvae and their sagittal otoliths. Developmental stage, larval lengths and number of otolith increments are given for each individual, along with sample source. Larval stages are based on Nichols (1971). Note different units for scale bars in each image.

FIGURE 4. (a) Estimated hatch dates (based on otolith increment counts) and (b) length frequency distributions of lemon sole (*Microstomus kitt*) larvae caught in the analysed samples from the northern North Sea in November/December 2016 (hatched/blue) and February 2017 (green).

FIGURE 5. Relationship between days after hatching and standard length (growth rate) of larval lemon (*Microstomus kitt*) sole in the northern North Sea during the autumn and early winter of 2016/2017. Larvae sampled in November/December 2016 (blue triangles) and February 2017 (green circles). Linear length-at-estimated age relationships are shown in blue (solid line) for autumn 2016 samples ($SL \text{ (mm)} = 5.32 + 0.20 * \text{age}$, $r = 0.83$, $p < 0.001$) and green (dashed line) for February 2017 samples ($SL \text{ (mm)} = 6.75 + 0.21 * \text{age}$, $r = 0.87$, $p < 0.001$). Comparative data from

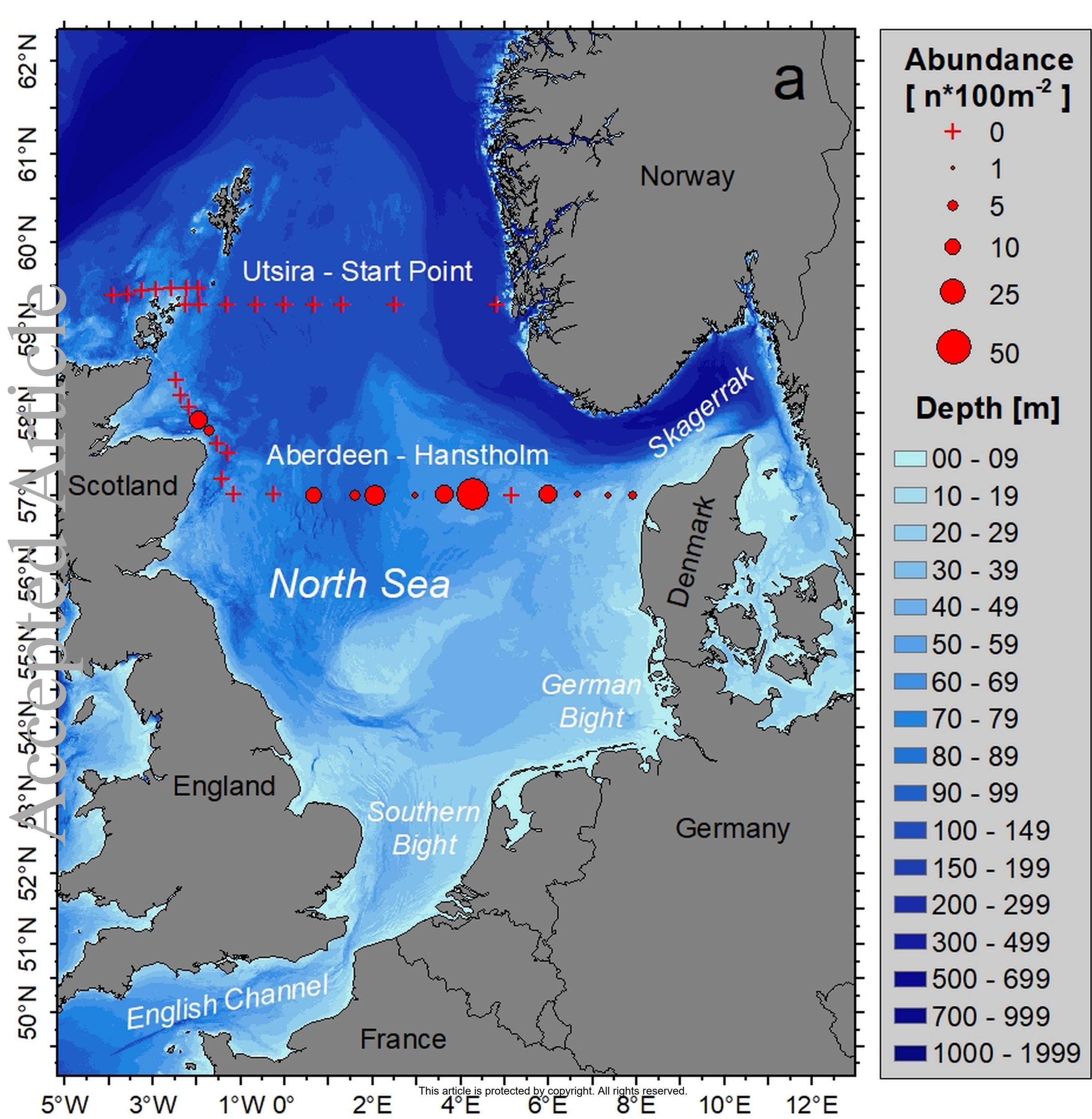
laboratory studies are shown with open black squares (Rønnestad *et al.*, 1992) and filled black squares (Howell, 1972).

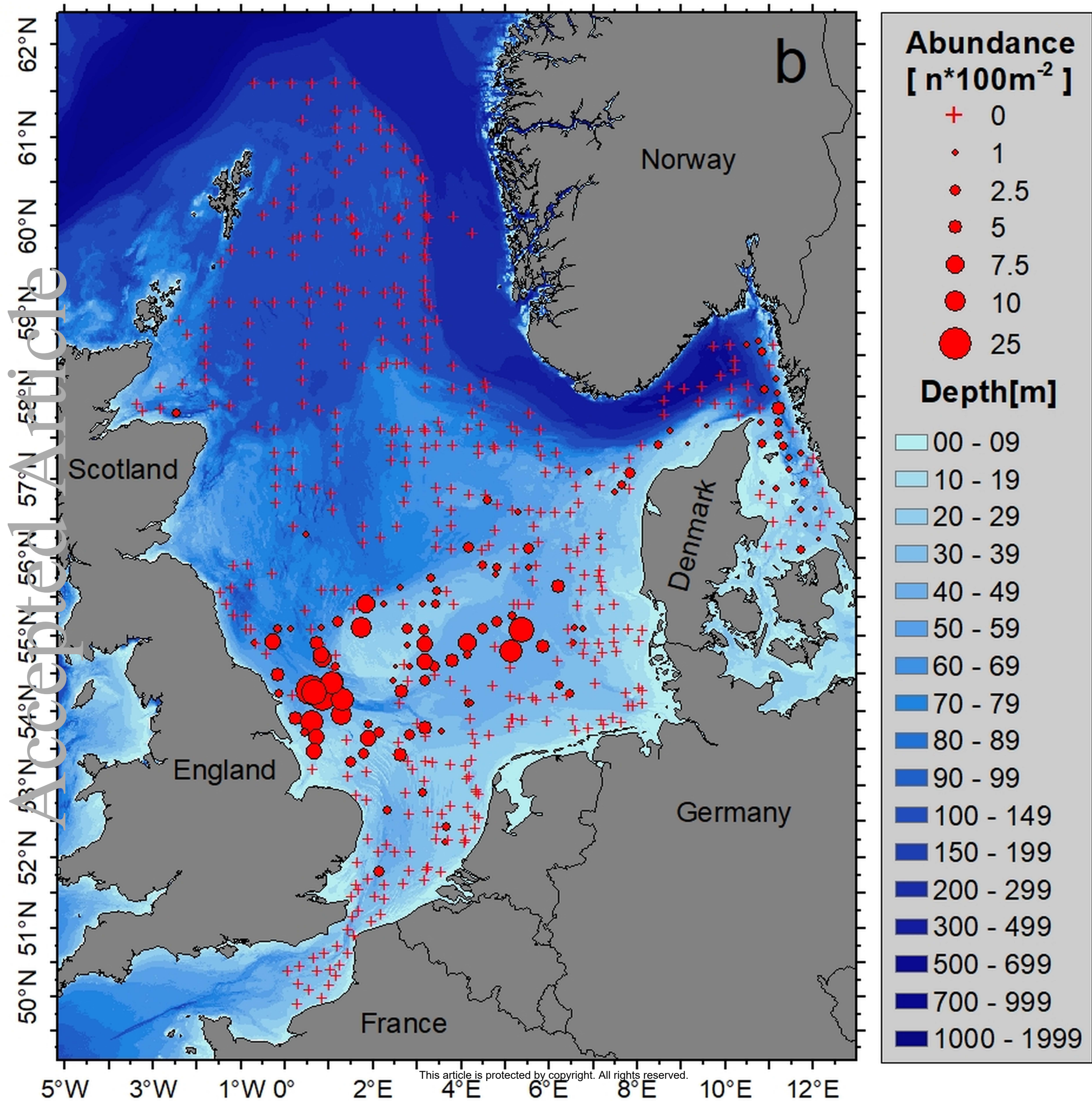
FIGURE 6. Predicted distribution of lemon sole (*Microstomus kitt*) larvae spawned between the 1st and 15th November 2016, from the known main spawning grounds (grey polygon, Sundby *et al.*, 2017) in the northern North Sea on (a) 1st December 2016 and (b) on the 8th February 2017.

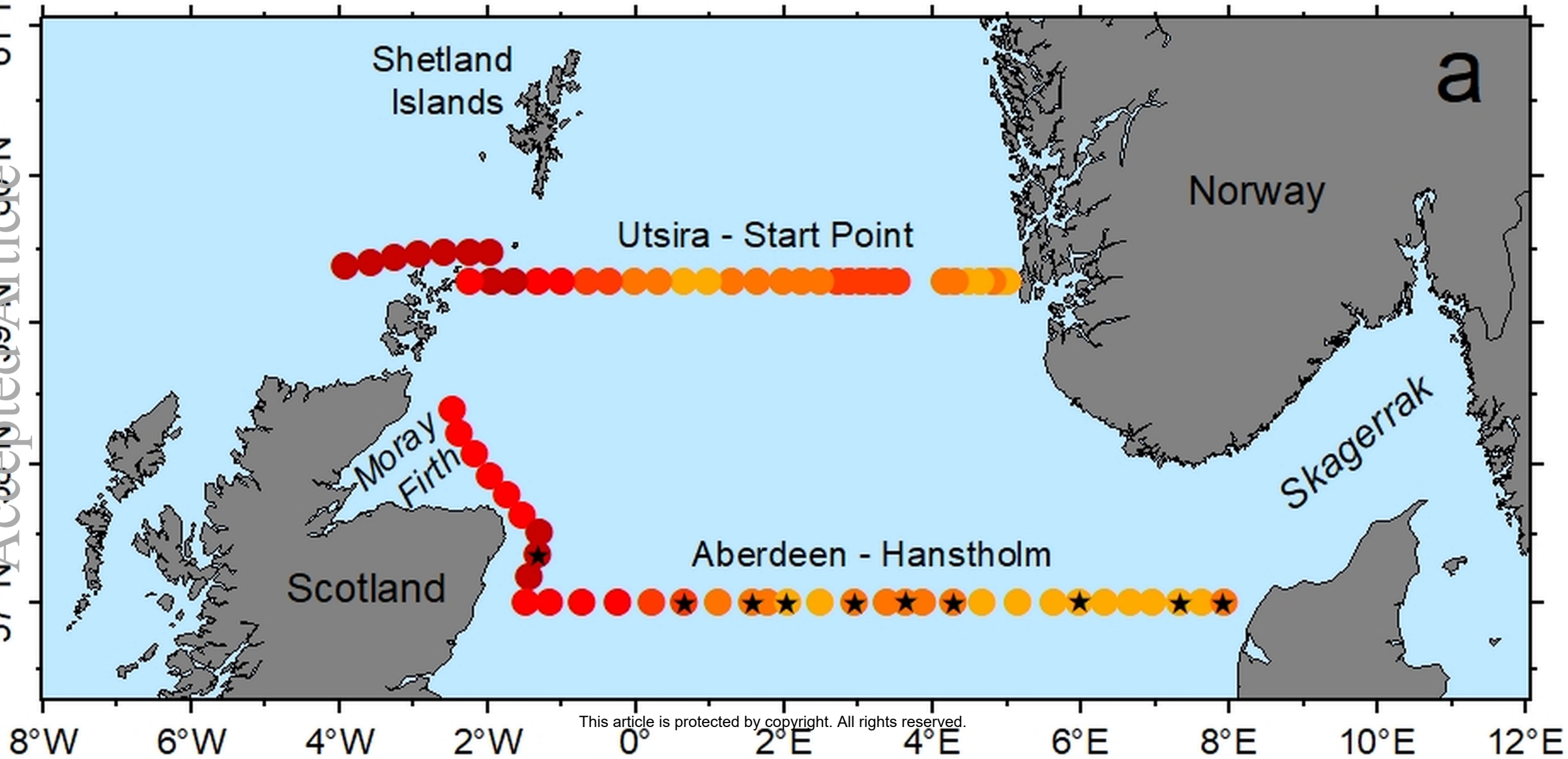
TABLE 1. Numbers and sizes of analysed lemon sole (*Microstomus kitt*) larvae collected at selected survey stations in late 2016 and early 2017. KB = RV Kristine Bonnevie, DANA = RV Dana. The total number of lemon sole larvae in the whole Dana cruise = 159, of which 36 (23%) were used for otolith analysis. From the KB cruise, a total of 62 larvae were sampled, of which 48 were used for otolith analysis (77%). All larvae from each of the selected stations were analysed.

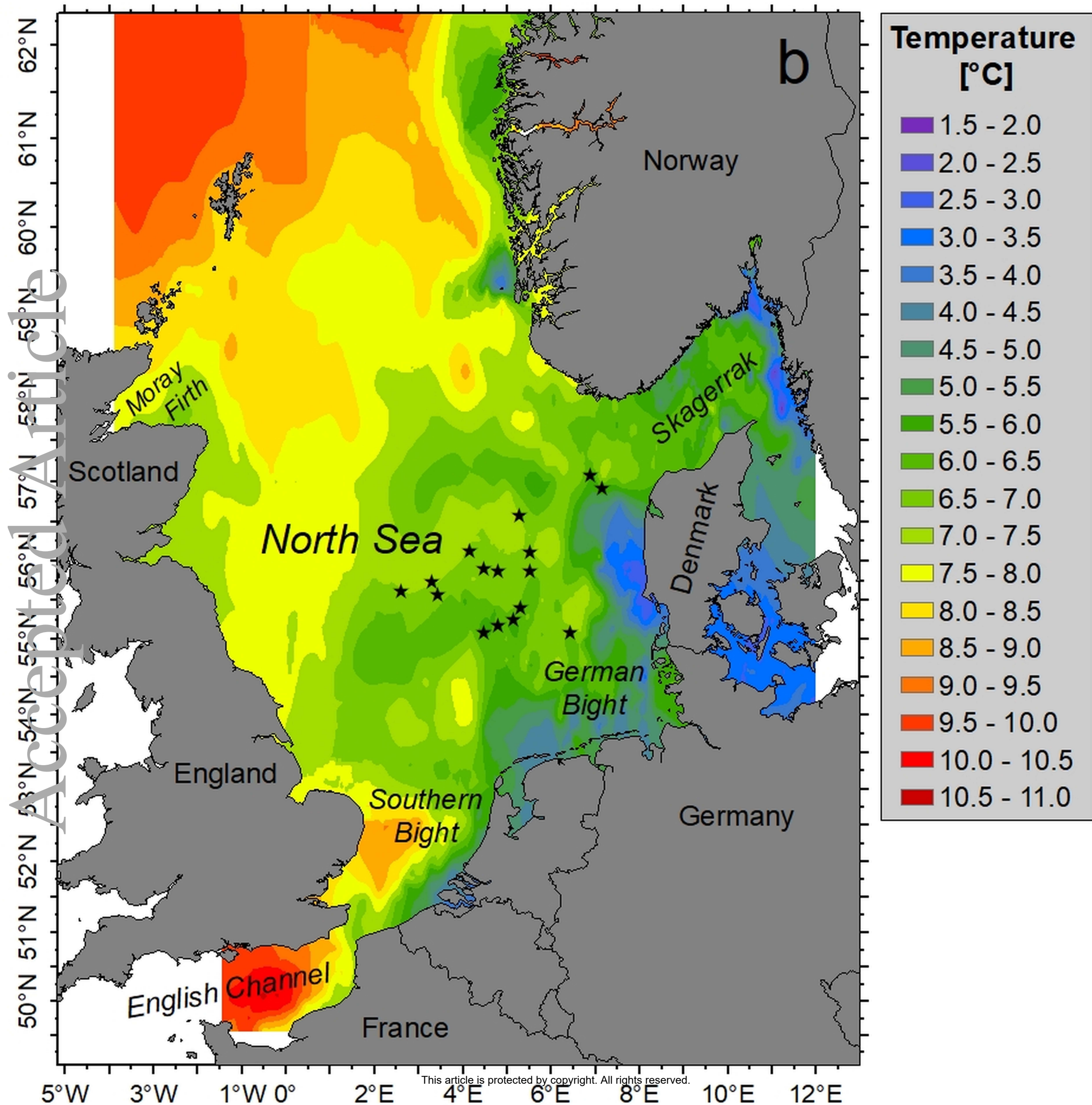
| Survey / Station (Location) | Number of Larvae (n) | Standard length (mm, mean) | Standard length (mm, range) |
|-----------------------------|----------------------|----------------------------|-----------------------------|
| KB (Nov/Dec 2016) | | | |
| St.No | Lat | Long | |
| 44 | 57.35 | -1.31 | 2 |
| | | | 8.8 |
| | | | (5.7 - 11.9) |
| 51 | 57.00 | 0.67 | 2 |
| | | | 9.7 |
| | | | (9.4 - 9.9) |
| 53 | 57.00 | 1.60 | 2 |
| | | | 7.4 |
| | | | (5.8 - 8.9) |
| 55 | 57.00 | 2.05 | 9 |
| | | | 8.5 |
| | | | (5.7 - 10.8) |
| 57 | 57.00 | 2.97 | 1 |
| | | | 4.1 |
| | | | |
| 59 | 57.01 | 3.65 | 4 |
| | | | 8.9 |
| | | | (5.2 - 11.7) |
| 61 | 57.00 | 4.30 | 13 |
| | | | 9.8 |
| | | | (8.5 - 11.4) |
| 65 | 57.01 | 6.00 | 10 |
| | | | 11.9 |
| | | | (7.8 - 20.4) |
| 69 | 57.00 | 7.36 | 1 |
| | | | 8.4 |
| | | | |
| 71 | 57.00 | 7.94 | 4 |
| | | | 9.5 |
| | | | (8.1 - 10.7) |
| Totals | | | 48 |
| | | | 10.3 |
| | | | (4.1 - 20.4) |
| DANA (Feb 2017) | | | |
| St. No | Lat | Long | |
| 3 | 57.08 | 6.93 | 1 |
| | | | 8.3 |
| 16 | 56.59 | 5.30 | 1 |
| | | | 12.0 |
| 28 | 56.11 | 5.54 | 3 |
| | | | 18.0 |
| | | | (16.1 - 21.1) |
| 29 | 55.89 | 5.54 | 2 |
| | | | 26.8 |
| | | | (26.6 - 27.0) |
| 31 | 55.88 | 4.83 | 2 |
| | | | 26.9 |
| | | | (26.0 - 27.7) |
| 32 | 55.90 | 4.51 | 3 |
| | | | 12.6 |
| | | | (9.7 - 17.3) |

| | | | | | |
|-------------|-------|------|----|------|---------------|
| 34 | 56.14 | 4.18 | 2 | 25.0 | (24.3 - 25.7) |
| 41 | 55.74 | 3.33 | 2 | 19.6 | (15.9 - 23.3) |
| 42 | 55.58 | 3.45 | 1 | 13.7 | |
| 49 | 55.62 | 2.62 | 1 | 25.8 | |
| 103 | 55.09 | 6.47 | 2 | 21.0 | (19.2 - 22.8) |
| 122 | 55.40 | 5.34 | 3 | 26.0 | (24.3 - 29.2) |
| 123 | 55.26 | 5.18 | 3 | 23.3 | (20.6 - 26.4) |
| 124 | 55.18 | 4.83 | 8 | 21.8 | (19.0 - 28.8) |
| 125 | 55.09 | 4.51 | 2 | 25.2 | (24.6 - 25.8) |
| Totals | | | 36 | 21.2 | (8.3 - 29.2) |
| Grand Total | | | 84 | 14.9 | (4.1 - 29.2) |



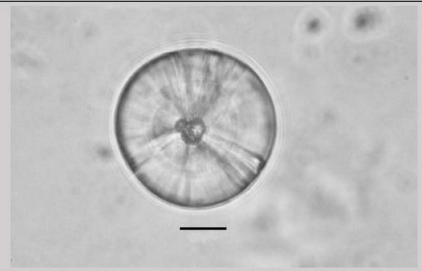






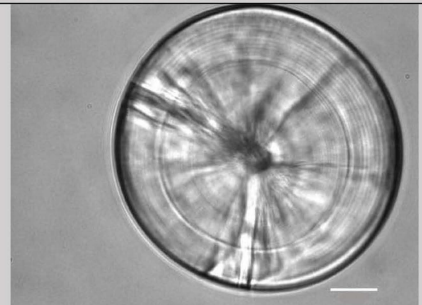
7.6 mm
13 incr
KB Dec 2016
Stage b

Scale Bars:
1mm | 10 μ m



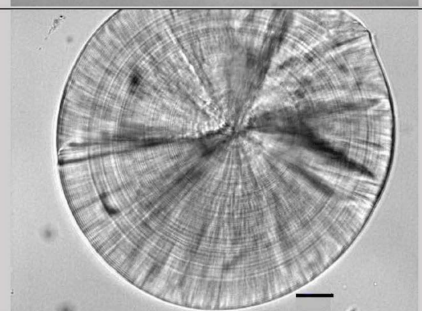
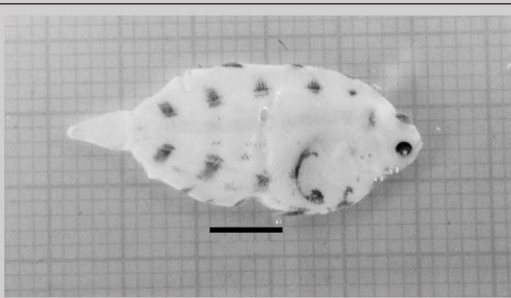
11.7 mm
30 incr
KB Dec 2016
Stage c

Scale Bars:
1mm | 10 μ m



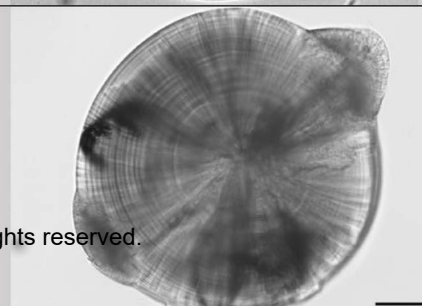
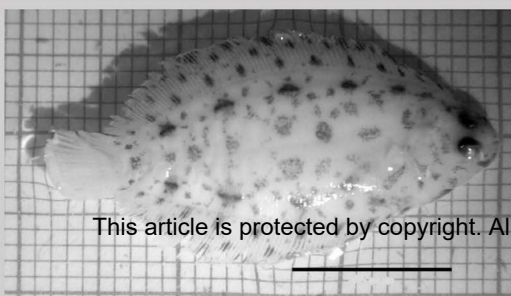
20.4mm
50 incr
KB Dec 2016
Stage e

Scale Bars:
5mm | 20 μ m

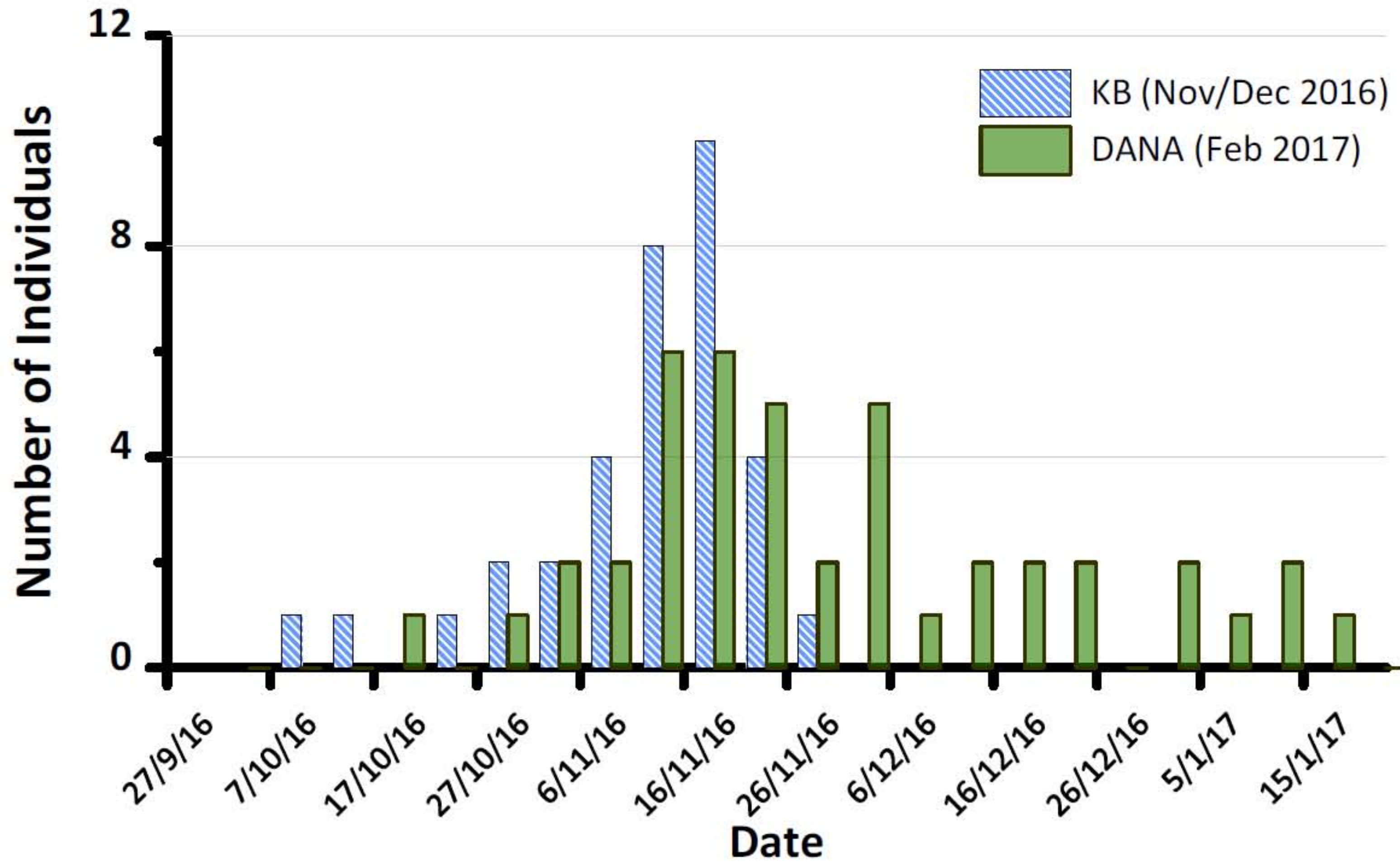


27.7mm
85incr
DANA Feb 2017
Stage h

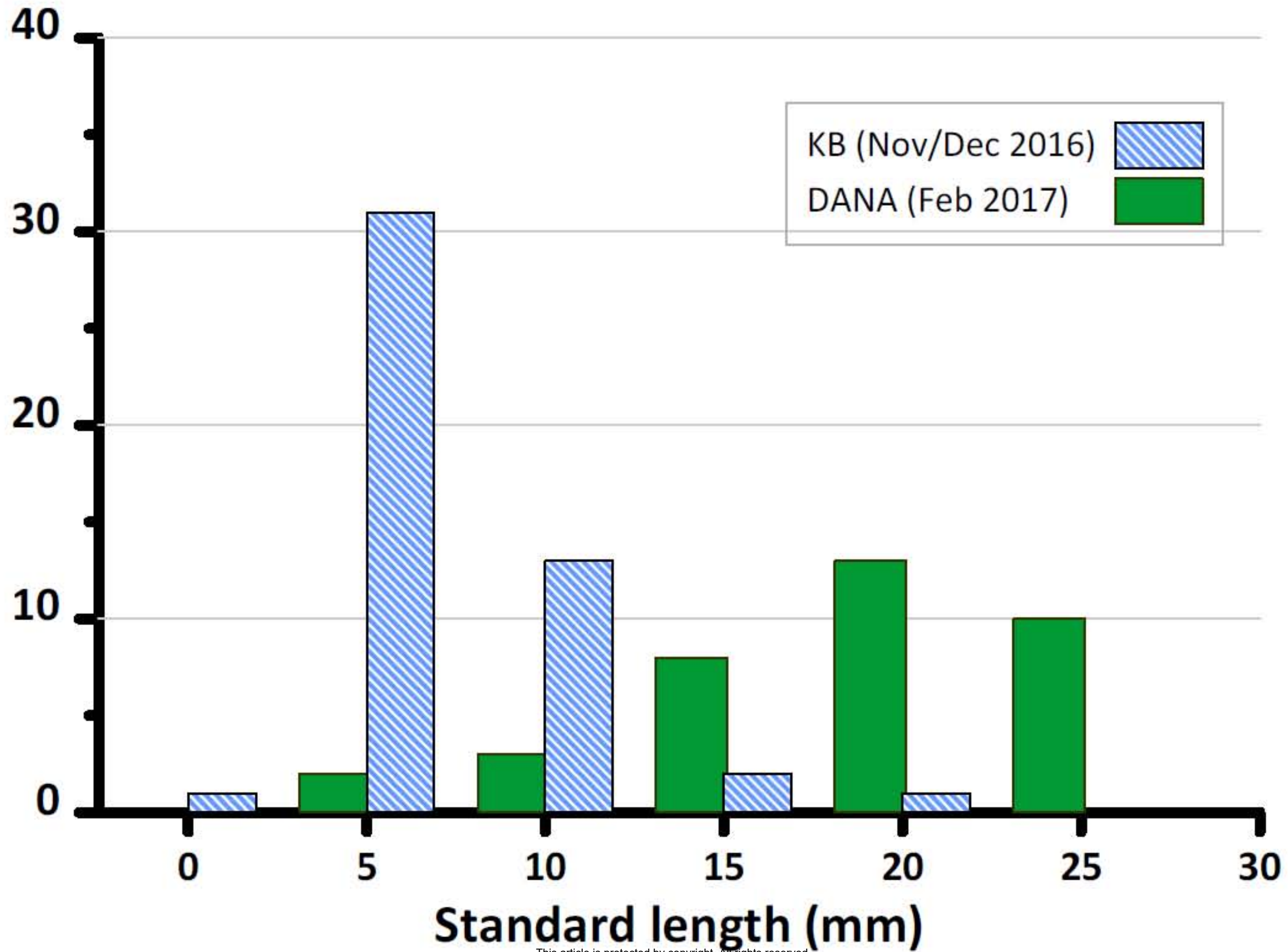
Scale Bars:
10mm | 50 μ m



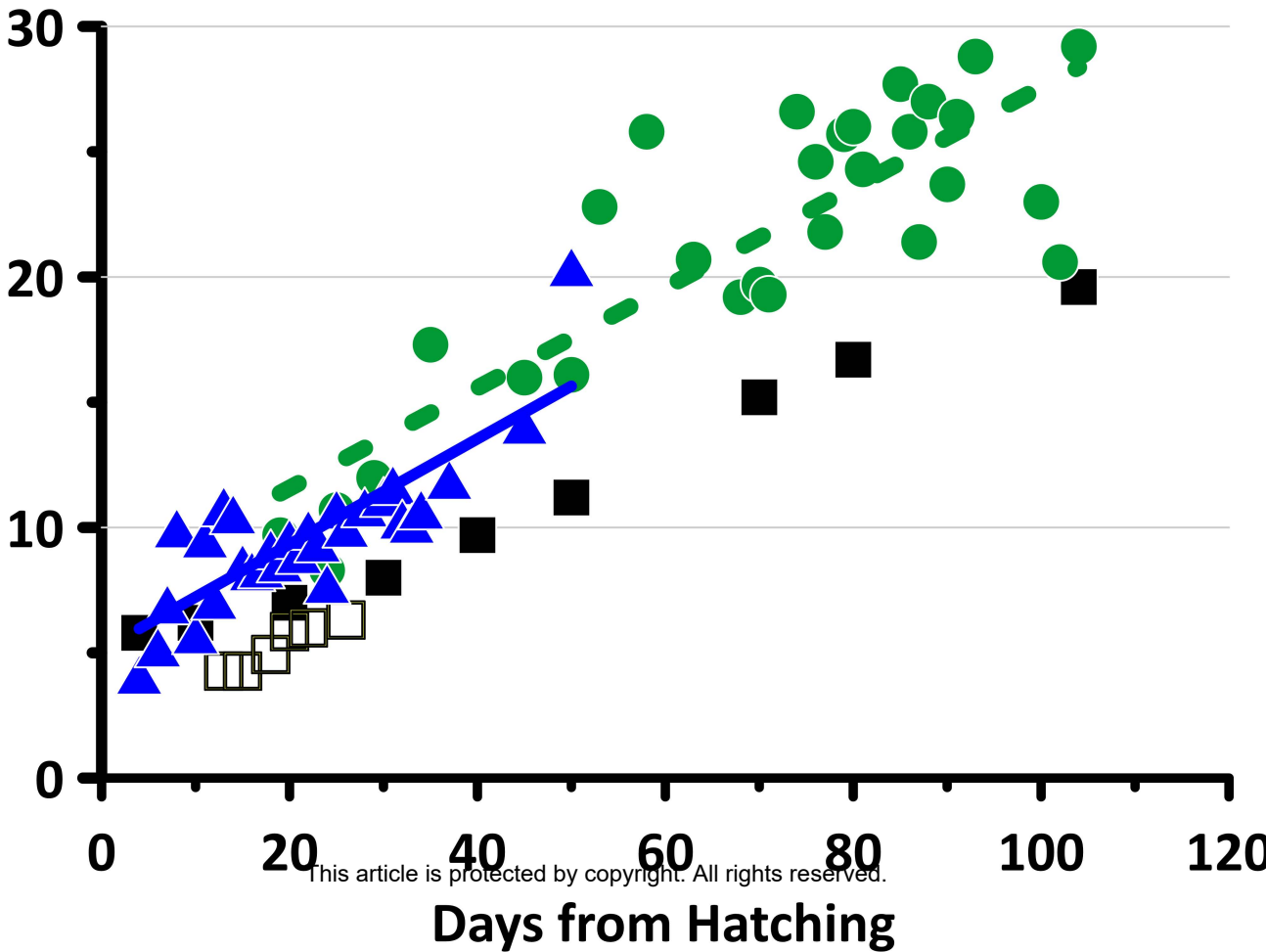
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Number of Individuals



Standard length (mm)



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