# A first glimpse of larval ecology of halibut species in the Gulf of St. Lawrence, Canada

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## Abstract

Knowledge on the larval ecology of winter-spawning fish from the Estuary and Gulf of St. Lawrence (EGSL), Canada, remains scarce due to the seasonal ice cover that prevents ichthyoplankton sampling using conventional methods. Two winter-spawning species, Atlantic halibut (*Hippoglossus hippoglossus*) and Greenland halibut (*Reinhardtius hippoglossoides*), support the most important groundfish fisheries of this area. In March 2020, we captured 10 halibut larvae ranging in size from 5 to 14 mm during an opportunistic survey in the GSL onboard an icebreaking vessel. Of these, eight were Atlantic halibut and two Greenland halibut. Judging by their very small size, the larvae were only a few days old, suggesting that the spawning grounds are close to the capture sites. This effort constitutes a first step in validating the putative spawning areas for these two important GSL stocks. This knowledge is important for the conservation and sustainable management of these fisheries.

**Keywords: Atlantic Halibut, Greenland Halibut, winter ecology, larval ecology, spawning ground, larval distribution**

Atlantic halibut (AH), *Hippoglossus hippoglossus*, and Greenland halibut (GH), *Reinhardtius hippoglossoides*, support the two most important groundfish fisheries in Atlantic Canada, representing 53% of overall groundfish landing value for the region in 2020 (DFO, 2022). The two flatfish species are characterized by discrete stocks in the Estuary and Gulf of St. Lawrence (EGSL) (DFO, 2021; Gauthier et al., 2021). Despite their high commercial importance, larval ecology remains poorly resolved for both species (Dominguez-Petit et al., 2013; Duffy-Anderson et al., 2013; Shackell et al., 2022), and most of the knowledge on early life stages comes from laboratory studies (AH: Blaxter et al. 1983; Pittman et al. 1990; Stickney and Liu 1993; Mangor-Jensen et al. 1997; Jonassen, et al. 1999; GH: Stene et al., 1998; Dominguez-Petit et al., 2013). In fact, only about 60 larvae captured at sea have been reported for AH over the whole species distribution (Haug, 1990; Bergstad and Gordon, 1993; Van Der Meeren et al., 2013). And while more observations exist for wild GH larvae, with several hundred larvae captured throughout the species' range (including preflexion, flexion and postflexion stages), observations of young preflexion larvae are scarce and account for less than one hundred in the literature (Simonsen et al., 2006; Sohn et al., 2010; Ouellet et al., 2011; Duffy-Anderson et al., 2013), including fifty individuals in a specific sector of the EGSL (Ouellet et al., 2011). This black box corresponding to a critical life stage needs to be investigated to understand natural larval mortality and its drivers and shed light on processes regulating recruitment.

In the EGSL, both halibut species reproduce in winter. Relying on the observation of spawning rises from geolocated pop-up satellite archival tag data, Gatti et al. (2020) revealed peak spawning activity in AH throughout the deep channels of the EGSL in

February. While telemetry data are not available to infer on spawning area of GH, historical occurrences of larvae in the EGSL summarized by Ouellet et al. (2011) point to a spawning area corresponding to the junction of the Laurentian and Esquiman channels, with peak spawning also occurring in February, or in early March.

At hatching, larvae measure around 6–7 mm in both species (Haug, 1990; Dominguez-Petit et al., 2013; Duffy-Anderson et al., 2013). The larvae are then bilateral and as their yolk reserves become depleted and they initiate exogenous feeding, they show positive phototaxis (Naas and Mangor-Jensen, 1990; Karlsen and Mangor-Jensen, 2001) and gradually rise towards the upper 100 to 200 m of the water column to feed on zooplankton (Haug, 1990; Simonsen et al., 2006; Ouellet et al., 2011). They passively drift with ocean currents and gradually reach the nursery areas (Riget and Boje, 1988; Albert et al., 2001; Bowering and Nedreaas, 2001; Sohn et al., 2010; Ouellet et al., 2011). Compared to the majority of boreal marine fishes, this drifting phase is relatively long in halibut species, lasting four to six months in GH according to observations in the natural environment (Sohn et al., 2010; Ouellet et al., 2011). Based on laboratory studies, Einarsdóttir et al. (2006) estimated of the duration of this phase would be 800 degree-days for AH, which would amount to four to six months for temperatures between 4 and 6°C.

In the EGSL, the spatial distribution and connectivity between spawning and potential nursery locations during halibut ontogeny remain unknown. Obtaining this information is critical because early life drift patterns can affect settlement success, larval survival, recruitment strength as well as population structure (Van der Veer et al., 1998, 2000; Sohn et al., 2010). In the present study, we relied on an opportunistic winter survey in the

EGSL in March 2020 onboard a Canadian Coast Guard icebreaker to target larval halibut for the first time during the larval drift season. From this first glimpse of larval halibut under the seasonal ice cover, we discuss potential spawning areas and larval drift patterns of halibut larvae in the EGSL.

Sampling took place in March 2020 aboard the CCGS *Amundsen* icebreaker during the "Odyssée Saint-Laurent" winter survey. One objective of this oceanographic survey was to characterize the winter zooplankton and ichthyoplankton communities in the EGSL. During the survey, 12 stations were sampled using a ring net (Ø = 1 m, mesh size 333 µm; Fig. 1) towed in an oblique pattern; vessel speed was ca. 2 kt to maintain a constant cable angle between 45°and 60°. The nets were lowered and raised at winch speed of 45 m $min^{-1}$ and 30 m $min^{-1}$, respectively, and maintained for one minute at their maximum depth, *i.e.,* ca. 15 m above the seabed. Except for one shallow station in the southern GSL (depth 85 m), water depths at the different stations in the Laurentian and Anticosti channels ranged from 177 to 386 m (average 293 ± 63 m). Sampling durations varied from 12 to 33 min (average 22 ± 06 min), and filtered water volumes varied from 188 to 471 $m^3$ (average 329 ± 91 $m^3$).

Sorted fresh fish larvae were photographed under a stereomicroscope, body width (BW; measured from the anus to the top of the back, excluding fins), and standard length (SL; from the lower jaw to the end of notochord, excluding caudal fin) were immediately measured given that larvae may shrink after death. One larva was damaged (no head) for which no standard length could be measured. Larvae were then stored in RNAlater and held at -20°C.

93 Macroscopic identification of these species was not possible due to the lack of knowledge
94 on the morphological characteristics of the early life stages, thus specimens were
95 identified using genetic barcoding (Hebert et al., 2003).

96 DNA from each larva was extracted using a DNeasy blood and tissue kit (Qiagen, Inc.,
97 Mississauga, ON, Canada). DNA purity, quality, concentration, and 260/280 absorbance
98 ratio were determined using SYBR Safe DNA Gel Stain 2% agarose gel electrophoresis
99 (ChemiDoc XRS+system, Biorad, CA, USA) and spectrophotometry (NanoVue Plus, GE
100 Healthcare, Pittsburgh, PA, USA). A region of 658 base pairs of the mitochondrial
101 cytochrome oxidase I (COI) gene was amplified with the FishCOI-F and FishCOI-R
102 primers (Table 1). The PCR reaction for each sample consisted of 6.25 µl of AccuStart
103 reagent (commercial ready-to-use kit, which includes taq polymerase, dNTPs, and
104 $MgCl_2$), 3.25 µl $H_2O$, 0.5 µl of each primer, and 2 µl of DNA, for a final reaction volume
105 of 12.5 µl. The following sequence was used for amplification: 1 min at 94°C, then 35
106 cycles of the series 30 sec at 94°C / 30 sec at 55°C / 45 sec at 72°C, and finally 5 min at
107 72°C.

108 The PCR products were then sequenced using the Sanger method on the genomic
109 analysis platform of IBIS (Institute of Integrative and Systems Biology) at Laval
110 University with the ABI Prism 3100® automated sequencer (Applied Biosystems).

111 The obtained sequences were edited using the Geneious software and compared to
112 reference sequences available in the BOLD database using the BOLD Identification
113 System (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) or GenBank's Basic
114 Local Alignment Search Tool (BLAST) (https://www.ncbi.nlm.nih.gov/genbank). A

sequence was considered correctly assigned to a species when the percentage of similarity was greater than or equal to 99%.

Ten halibut larvae were captured during the mission, ranging in size from 5.62 to 13.94 mm SL and 0.59 to 1.19 mm BW. Larvae were captured at three of the 12 survey stations, all of which were in the Laurentian Channel (Fig. 1). One station was near Cabot Strait (station A) and the other two further upstream, north of the Gaspé Peninsula (stations B and C).

Of these 10 larvae, eight were identified as AH and two as GH. AH larvae were present at all three stations (A, B, C), while GH were only found at station C.

AH larvae ranged from 5.62 to 13.94 mm SL (Fig. 2). The two larvae captured near Cabot Strait (station A) were the largest (SL: 12.71 and 13.94 mm; BW: 1.05 and 1.19 mm), while larvae from stations north of Gaspé Peninsula (B and C) were smaller (SL: 5.62 to 9.08 mm; BW: 0.68 and 0.92 mm). The two GH larvae measured 8.7 and 11.7 mm SL with 0.88 and 0.93 mm BW, respectively (Fig. 2).

This study reports the first mention of AH larvae captured in the EGSL, and they are the smallest wild-caught larvae that have been reported in the scientific literature for this species. Among the 60 larvae captured in the wild that have been described, the smallest was 9.1 mm long (Bergstad and Gordon, 1993). In the present study, five larvae were below this length, the smallest being 5.62 mm long.

The larvae captured at stations B and C were close to—or even below—hatching sizes estimated from laboratory studies (6–7 mm; Haug, 1990), which indicates that they should be just a few days old. We noted the presence of a yolk sac on a larva of about

137 8 mm (Fig. 3C) while it was absent in the rest of the larvae (e.g., Fig. 3A, B, D). Yolk
138 sacs may have been damaged during capture: studies under controlled conditions have
139 shown that yolk resorption occurs at the age of 50 d at temperatures ranging between 5.0
140 and 6.0°C, which corresponds to a larval size ranging between 11.5 and 13 mm (Blaxter
141 et al., 1983; Pittman et al., 1990). However, these larvae may also have reached the stage
142 of exogenous feeding. The two larvae captured at station A were larger than those
143 sampled at stations B and C. According to laboratory studies conducted at 6.0°C, larvae
144 exceeding 12.5 mm approach or exceed the age of 50 days post hatch (Pittman et al.,
145 1987; Haug, 1990; Karlsen et al., 1998). At this size, larvae have a functional mouth, the
146 yolk sac is resorbed, and exogenous feeding has already been initiated (Haug, 1990;
147 Harboe and Mangor-Jensen, 1998). These two larvae from station A also presented weak
148 pigmentation on their body (Table 2, Fig. 3.A). These characteristics indicate that these
149 two larvae have absorbed their yolk reserves and started their exogenous feeding (Table
150 2). Based on larval stage classifications from Haug (1990) and Duffy-Anderson et al.
151 (2013) for AH and GH, respectively, we consider that larvae captured at stations B and C
152 were at the yolk-sac stage given their morphological characteristics (Table 2) while the
153 two larvae captured at station A should be at the exogenous feeding stage. Even though
154 AH larval density was low and heterogenous, the presence of these post-hatch larvae in
155 the Laurentian Channel is in agreement and supports the estimated spawning area by
156 Gatti et al. (2020) from electronic tagging.

157 It should be noted that the surface (0–75 m) water temperature in March is below 0°C in
158 the EGSL, and temperatures above 4.0°C, which are favourable to larval AH survival, are
159 only found below 200 m depth (Galbraith et al., 2021). North of the Gaspé Peninsula,

eggs released at depth (> 200 m) experience temperatures varying between 4.5 and 5.5°C depending on the location, while the deeper waters at Cabot Strait (station A) are warmer, between 5.5 and 6.5°C. Although it is not possible to draw conclusions with the few larvae captured here, the difference in size between the individuals at stations A vs. B/C may reflect differences in embryonic and larval development rate under this gradient of temperature conditions, despite identical reproductive peaks in February (Gatti et al., 2020). The temperature stratification of the water column in March and the very young age of the larvae strongly suggest that all the larvae from stations B and C were captured at depth while the two larger larvae from station A had probably started their gradual ascent in the water column to feed on prey. Later in the spring, warming surface water, melting sea ice, and continental runoff lead to the formation of a warm surface layer under which the cold waters of the previous winter are isolated and then form the cold intermediate layer (CIL). This layer is located between 50 and 100 m in depth, with temperatures between 0 and 1°C, while the surface layer (≤50 m) gradually warms to temperatures near 6°C (Galbraith et al., 2021). Larvae must thus eventually cross the cold layer to feed and develop in the warmer surface waters, but the timing of this vertical migration is unknown.

GH larvae are hypothesized to follow the same pattern as previously described for AH larvae at stations B and C, *i.e.*, the eggs are released at depth (> 200 m) where they incubate at temperatures between 4.5 and 5.5°C. Subsequently, as their yolk reserves are depleted, the larvae migrate to feed in the surface water layer at a size of *ca.* 15-16 mm (Sohn et al., 2010; Ouellet et al., 2011). During fish surveys carried out in May and June from 2005 to 2009, Ouellet et al. (2011) captured fifty GH late larvae (14 to ~31 mm) in

183 the upper 150 m of the EGSL. These larvae were larger and more developed than those
184 captured in the present study, and had already completed their vertical migration in the
185 water column. These authors hypothesized that the main spawning area is located in the
186 portion of the Laurentian Channel facing southwest Newfoundland (Fig. 1). Given the
187 location of larvae captured in our study, we speculate that at least a part of the spawning
188 occurs more widely across the Laurentian Channel.

189 We report rare captures of Atlantic and Greenland halibut larvae in the EGSL. The
190 difficulty of capturing these very young larvae is largely due to the complex logistics of
191 working in this area during winter. The very small size of the larvae confirms local
192 reproduction in the EGSL, and the thermal stratification of the water column in March
193 confirms spawning at depth, at temperatures sufficient for embryonic development. The
194 effects of temperature on larval development remain largely unknown, which constitutes
195 a major knowledge gap for estimating age and growth from size, and facilitate larval
196 ecological studies in nature. Given the typical low larval halibut densities previously
197 reported (Ouellet et al., 2011) and observed in the present study, follow-up studies on the
198 ecology of halibut larvae in the EGSL will require higher sampling effort, which will
199 allow us to further our knowledge on larva distribution, as well as obtain new information
200 on diet composition, growth rate patterns, and other recruitment-relevant variables over
201 the distribution of these two stocks.

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204 winter survey onboard the Canadian icebreaker CCGS *Amundsen*. Special thanks are
205 extended to David Ouellet and Catherine Marcil for their invaluable assistance at sea.

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| Primer | 5 -3 Sequence |
|---|---|
| FishCOI-F | AAY CAY AAA GAY ATY GGY ACC CT |
| FishCOI-R | TAN ACT TCN GGR TGN CCR ZZG AAY CA |

| Station | Date (d/m/y) | Station depth (m) | Sampling depth (m) | Species | SL (mm) | BW (mm) | Larval stage | Larval characte |
|---|---|---|---|---|---|---|---|---|
| A | 09/03/2020 | 448 | 386 | AH | 12.71 | 1.05 | yolk-sac | Yolk absent, eyes fully pigmer some black melanophores |
| A | 09/03/2020 | 448 | 386 | AH | 13.94 | 1.19 | yolk-sac | Yolk absent, eyes fully pigmer black melanophores alc |
| B | 11/03/2020 | 323 | 283 | AH | 7.95 | 0.92 | yolk-sac | Yolk present, eyes pigmented, n pigmentatio |
| C | 12/03/2020 | 369 | 345 | GH | 8.7 | 0.93 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |
| C | 12/03/2020 | 369 | 345 | AH | 5.62 | 0.68 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |
| C | 12/03/2020 | 369 | 345 | AH | *NA* | 0.59 | yolk-sac | Head missing, yolk absent, no |
| C | 12/03/2020 | 369 | 345 | AH | 9.08 | 0.74 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |
| C | 12/03/2020 | 369 | 345 | GH | 11.7 | 0.88 | yolk-sac | Yolk absent, eyes pigmented, m pigmentatio |
| C | 12/03/2020 | 369 | 345 | AH | 7.61 | 0.81 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |
| C | 12/03/2020 | 369 | 345 | AH | 7.63 | 0.8 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |

| Station | Date (d/m/y) | Station depth (m) | Sampling depth (m) | Species | SL (mm) | BW (mm) | Larval stage | Larval characte |
|---|---|---|---|---|---|---|---|---|
| A | 09/03/2020 | 448 | 386 | AH | 12.71 | 1.05 | yolk-sac | Yolk absent, eyes fully pigmer some black melanophores |
| A | 09/03/2020 | 448 | 386 | AH | 13.94 | 1.19 | yolk-sac | Yolk absent, eyes fully pigmer black melanophores alc |
| B | 11/03/2020 | 323 | 283 | AH | 7.95 | 0.92 | yolk-sac | Yolk present, eyes pigmented, n pigmentatio |
| C | 12/03/2020 | 369 | 345 | GH | 8.7 | 0.93 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |
| C | 12/03/2020 | 369 | 345 | AH | 5.62 | 0.68 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |
| C | 12/03/2020 | 369 | 345 | AH | *NA* | 0.59 | yolk-sac | Head missing, yolk absent, no |
| C | 12/03/2020 | 369 | 345 | AH | 9.08 | 0.74 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |
| C | 12/03/2020 | 369 | 345 | GH | 11.7 | 0.88 | yolk-sac | Yolk absent, eyes pigmented, m pigmentatio |
| C | 12/03/2020 | 369 | 345 | AH | 7.61 | 0.81 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |
| C | 12/03/2020 | 369 | 345 | AH | 7.63 | 0.8 | yolk-sac | Yolk absent, eyes lightly pigme no body pigmen |

**Figure Caption**

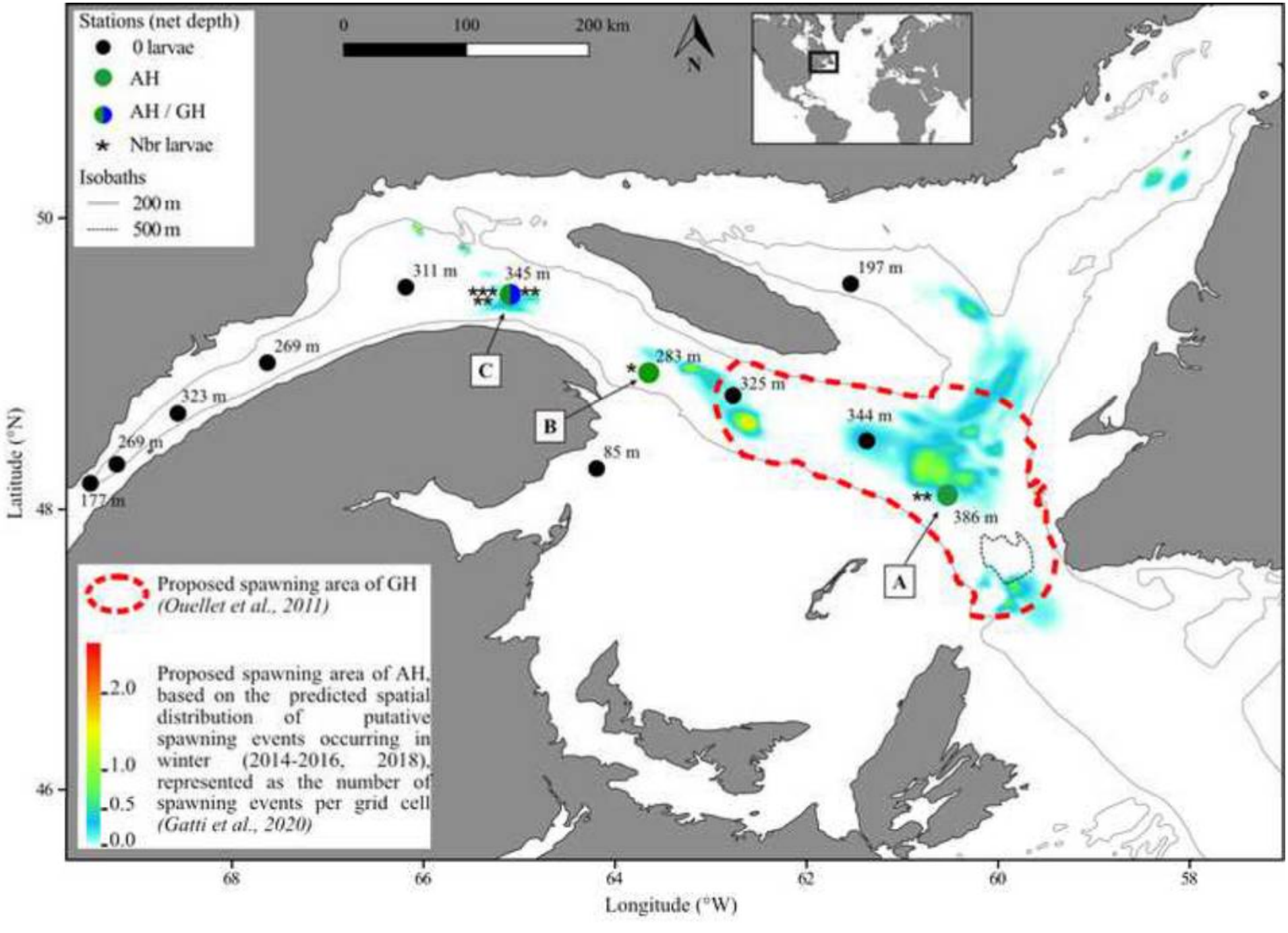
Figure 1: Map of winter ichthyoplankton stations sampled on board the CCGS *Amundsen*. Larvae were captured at stations A, B, and C; the species and numbers are indicated. The spawning areas proposed by Gatti et al. (2020) for Atlantic halibut (AH) and Ouellet et al. (2011) for Greenland halibut (GH) are also shown.

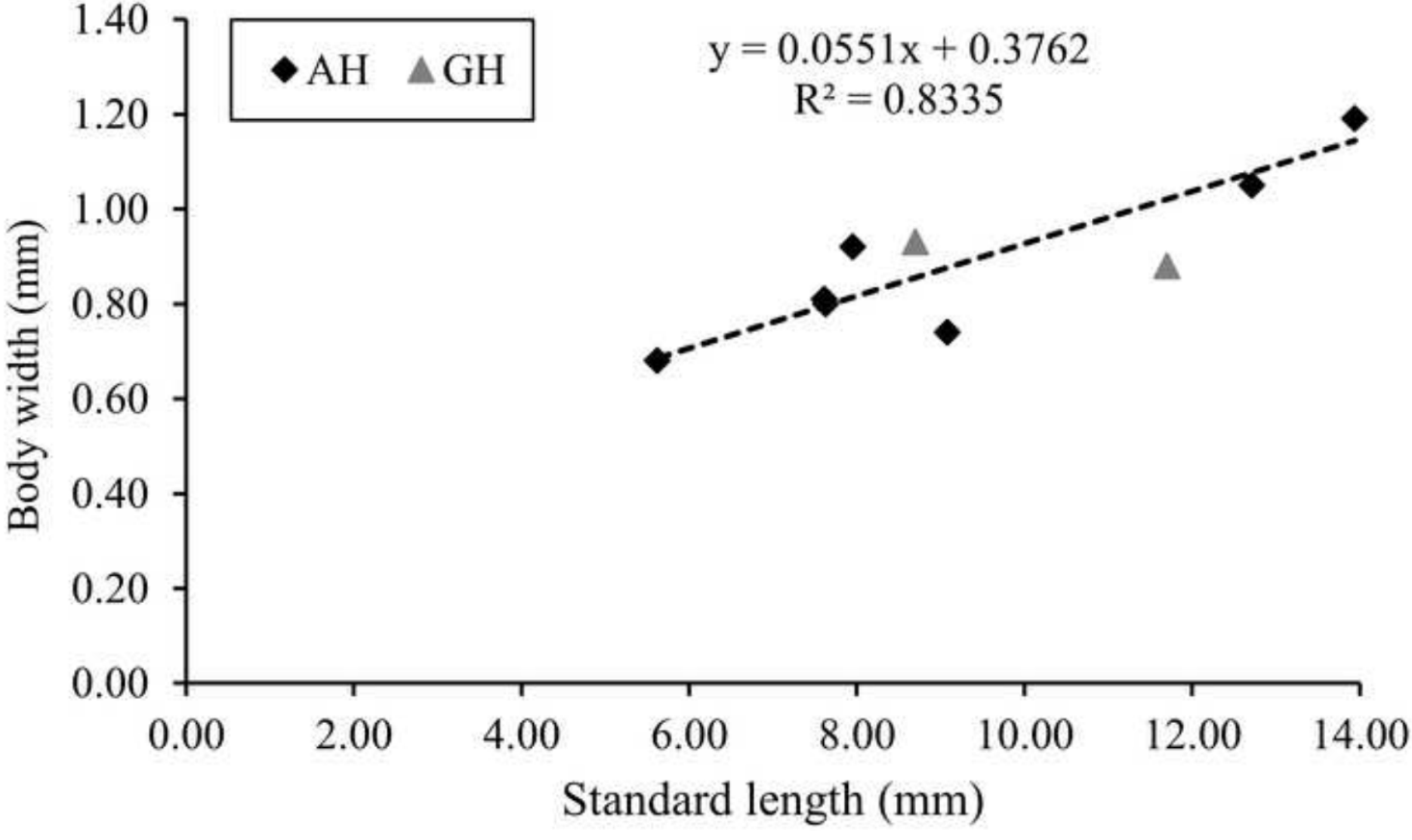
Figure 2: Linear regression of the standard length of the Atlantic halibut (AH) larvae as a function of their body width. Only AH larvae were used to establish the regression equation; Greenland halibut (GH) larvae are shown for comparison only. The damaged AH larva was not included in this analysis.

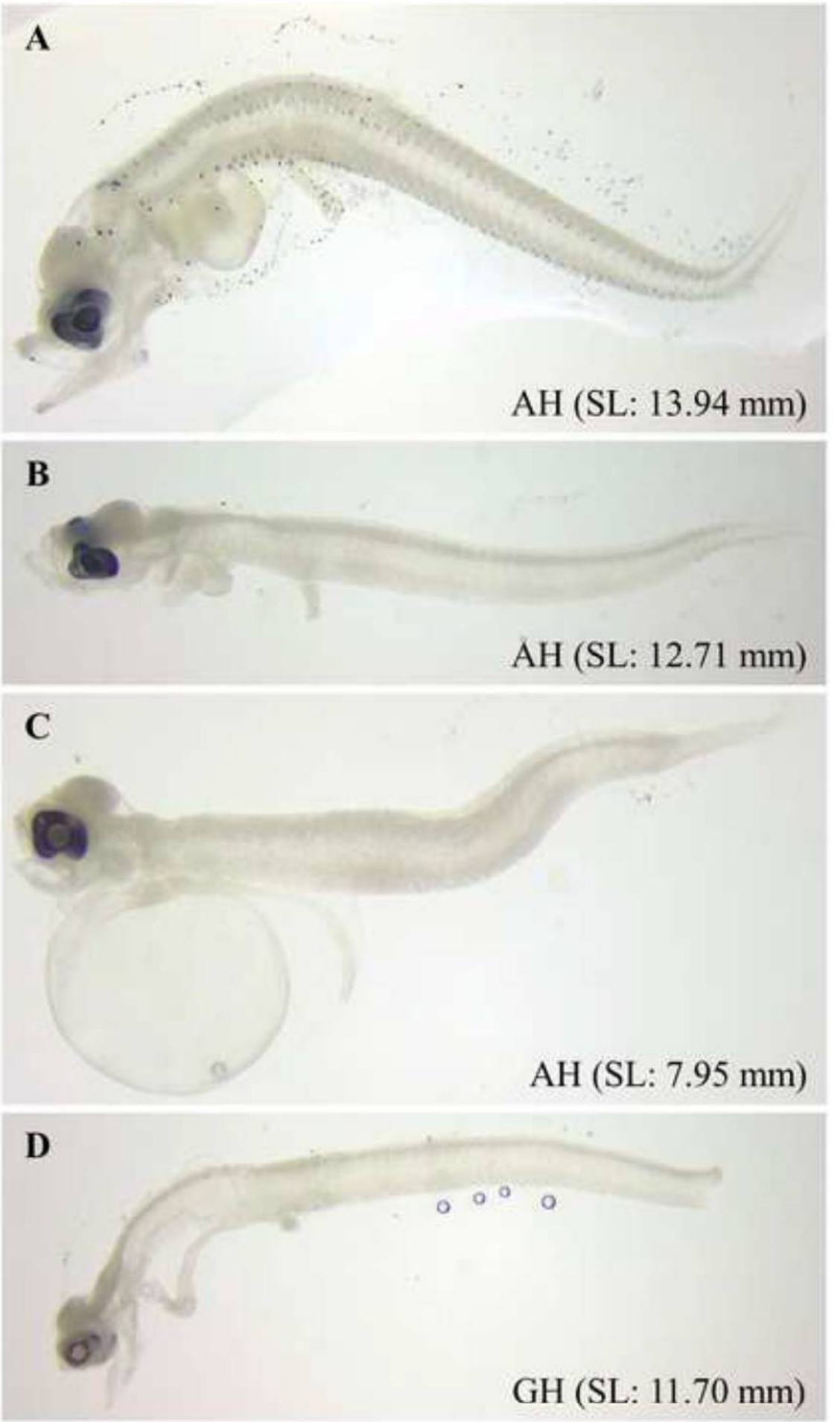
Figure 3: Stereomicroscope photographs of Atlantic halibut (AH; A, B, C) and Greenland halibut (GH; D) larvae.

Table 1: Sequences of primers used to amplify the cytochrome oxidase I (COI) gene from mitochondrial DNA.

Table 2: Information relative to capture stations (A: 48°05 29 0°32 22 ; B: 48°56 47 3°39 29 ; C: 49°28 34 65°04 56 , the morphology and the development stage of the larvae. SL: Standard length; BW: Body width.







## Highlights

- Ten young halibut larvae captured during a winter survey onboard an icebreaker
- First observation of Atlantic halibut larvae in the waters of the St. Lawrence
- Smallest larvae ever captured in the wild for both Atlantic and Greenland halibut
- The presence of these few days old larvae confirms local reproduction in the EGSL
- Water temperature stratification in March confirm an eggs incubation at depth