Identification of benthic egg masses and spawning grounds in commercial squid in the English Channel and Celtic Sea: *Loligo vulgaris vs L. forbesii*.

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Identification of demersal egg masses and spawning grounds in commercial squid in the English Channel and Celtic Sea: *Loligo vulgaris* vs *L. forbesii*

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Abstract

Common squid, *Loligo vulga*ris and veined squid, *Loligo forbesi* have nearly coinciding distribution in the northeast Atlantic, a similar reproductive seasonality, and largely overlapping depth ranges of spawning grounds. There are no unambiguous criteria to distinguish between egg masses of both species. This pioneer study was focused on Celtic Sea and western part of the English Channel and combined both research survey data and observations by recreational divers ("citizen science"). *L,vulgari*s was found to reproduce there in late winter – spring; distribution of egg masses coincided with bottom temperature range of 8.5-10°C and bottom salinities of 35-35.5 psu. No *L.forbesii* egg masses was found across the studied area though they are known from literature from deeper areas further west. Based on the original materials and literature data we provide a guideline to distinguish between egg masses of both squids based on egg size and embryonic stage as a tool to map species-specific spawning grounds for improvement of understanding of population structure, migrations and development of fisheries management measures.

Key words: *Loligo vulgaris*, *Loligo forbesii*, reprodiction, egg masses, spawning grounds

1. Introduction

Two commercial squid species genus *Loligo*, *L. vulgaris* (European squid) and *L. forbesii* (veined squid), with nearly coinciding species ranges inhabit waters around Europe from the east Mediterranean to the North Sea (Jereb et al., 2015). Both species potentially reproduce all year round but with distinctive peaks, mostly in the cold season, and mature females of both species are often captured together in the same hauls. In certain parts of their range (Celtic Sea/English Channel/North Sea), there is possible overlap of spawning grounds, however information to permit unambiguous identification of egg masses in these areas is absent. Spawning is extended in both species, but seasonal migrations are little studied. It is assumed that there is an important temporal and spatial overlap in occurrence of egg masses of both species in these European waters (Martins, 1997), particularly in the Celtic Sea and English Channel, which are the most important areas for loliginid fisheries in Europe, accounting for about one third of annual landings in the Northeast Atlantic (Royer, 2002). *L. forbesii* and *L. vulgaris* are both annual species and spawn only once in their life and deposit their eggs on various substrates in relatively shallow waters where bottom fisheries (e.g. dredges and beam trawls) can also occur. As eggs

might be destroyed by bottom fisheries, more complete knowledge of spatial and seasonal distribution of loliginid spawning grounds is needed for their protection and management to support a successful reproduction.

Loligo vulgaris reproduces in the English Channel between November and April, peaking in February-March (Moreno et al., 2002) whereas *L. forbesii* lays eggs in this area in December-January and may continue well into the spring, with some mature animals found also in summer (Holme, 1974; Jereb et al., 2015). In the southern North Sea, *L. vulgaris* was found to lay eggs later in the year, from April to August with some mature animals being captured in winter (Tinbergen and Vervey, 1945; Oesterwind et al., 2010). *Loligo forbesii* reproduces around Scotland from December to June, peaking in March, but some mature specimens can be found there throughout the year, and egg masses are reported by fishermen up to September (Lum-Kong et al., 1992; Pierce et al., 1994, Oesterwind et al., 2010). It seems that more recently the summer breeding population of *L. forbesii* has declined and that the winter breeding population is dominant in Scottish waters (Pierce et al., 2005). In Irish waters spawning occurs mostly between November and April and developing egg masses were found in the north Celtic Sea in every month but August (Collins et al., 1995).

Loligo vulgaris lays eggs mostly at 20 - 70 m, and occasionally down to depths of >100 m (Moreno, Pereira, 1998; Jereb et al., 2015). *Loligo forbesii* spawns slightly further offshore - at 10-150 m (Jereb et al., 2015), with gradually increasing depth during the winter reproductive season from inshore waters to ~ 140 m (Smith et al., 2013). In fjord-like areas with extremely steep slopes this species may occasionally lay eggs as deep as deep as at >700 m (Salman and Laptikhovsky, 2002).

Despite this extensive knowledge, much of what we know about seasonal timings is historical and needs to be re-evaluated in the light of climate change and the known plasticity of squid life-history traits in response to environmental drivers (Doubleday et al., 2016).

Egg masses consist of numerous finger-like capsules ("strings") that females attach to the ground one by one (Fig. 1). If egg capsules are already present on the spawning site, loliginid females tend to attach their spawn to already existing masses. Thus, egg capsules of the same egg mass might be at different stages of embryonic development because they were laid by different females at different times (Drew, 1911; Arkhipkin et al., 2000). Because of this, some single large egg masses of *L. vulgaris* could contain as many as 39,760 eggs (568 capsules, mean egg count of 70) in the Mediterranean (Bohadsch, 1761) and ~42,000 in the English Channel (Lee, 1875).

Throughout its distribution range *L. vulgaris* lays from 50 to 160 eggs in capsules of 60-170 mm (Grimpe, 1925; Mangold – Wirz, 1963; Sen, 2004), although in Portugal a 140 mm egg capsule was reported to contain ~ 174 eggs (Moreno, 2008). The respective values for *L. forbesii* are 36-100 eggs and 80-200 mm (Grimpe, 1925; Segawa et al., 1988; Hanlon et al., 1989; Porteiro and Martins, 1992; Orsi Relini et al., 2009; Pham et al., 2009). Therefore, the evidence suggests that individual variation in egg numbers and capsule length is very high and is probably not useful for distinguishing species.

Loligo forbesii produce much larger eggs than *L. vulgaris* (Grimpe, 1925), but during embryonic development the egg diameter, along with individual capsule length and width, increases several times due to increasing egg volume (Boletzky, 1987; Martins, 1997; Moreno, 2008). Therefore, although several descriptions were published during the last century, there is still no unambiguous criterion to distinguish between spawn from *L. vulgaris* and *L. forbesii*.

This paper provides unambiguous criteria to distinguish between eggs of both species based on i) observation of egg masses of known species origin and ii) observation of genetically identified egg masses and iii) distribution and timing of mature females. The results will support the identification and mapping of *Loligo* spawning grounds in the NE Atlantic (Irish Sea, Celtic Sea, English Channel and North Sea). Results are provided in conjunction with the Cephalopod Citizen Science project (https://www.researchgate.net/project/The-Cephalopod-Citizen-Science-Project), which was launched

in December 2017 to gather information on wild cephalopods via observations by the recreational diving community. By enabling identification of egg masses to species level, we aim to support efforts to map the spatio-temporal variability of spawning grounds of both species and help to mitigate the impact of fisheries on squid spawning grounds to support a sustainable use of this fishery resource.

2. Materials and Methods

In total, 21 squid egg masses were collected and frozen during beam trawl surveys by *RV Cefas Endeavour* in March – April 2017-2019 in the English Channel and Celtic Sea (Fig. 2). Visual observations of 58 *Loligo* egg masses from this area were extracted from the web page of UK Cephalopod Reports (https://www.facebook.com/groups/1772714999700580/), which is a part of the Cephalopod Citizen Science Project. Another 26 egg masses were reported by French divers for Base pour l'inventaire des observations subaquatiques (https://bioobs.fr/les-especes/especes-recherchees/).

Collected egg masses were defrosted in the lab and studied under a binocular microscope. Three random egg capsules were measured with 1 mm accuracy, the numbers of eggs were counted, egg length and embryo length were measured along the major axis to 0.1 mm accuracy, and the stage of embryonic development assigned following Naef (1928). These data were combined with available data from the literature on egg size, size at embryonic stage, egg capsule length, and numbers of eggs per capsule in both species, for inclusion in the analysis.

Parts from seven egg capsules belonging to seven different egg masses collected in 2019 were transferred to 96% ethanol and stored for DNA sequencing. DNA was extracted using the Invitrogen™ PureLink™ Genomic DNA Mini Kit, following the Mammalian Tissue and Mouse/Rat Tail Lysate protocol. DNA was eluted in 100 µL Genomic Elution Buffer and stored at – 20 °C.

DNA barcoding, which targets the COI gene, was performed using forward primer LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and reverse primer HC02198: 5'-taaacttcagggtgaccaaaaaatca-3' (Folmer et al., 1994). Each PCR contained 12.5 µL of Thermo Scientific™ DreamTaq Green PCR Master Mix (2X), 0.5 µM of each primer, 2.5 µL of DNA and 9 µL H_20 resulting in final reaction volume of 25 µL. A negative control was also included to ensure cross-contamination did not occur. The PCR conditions were 94°C for 2 min, 35 cycles of 94°C for 40s, 50°C for 40s and 72°C for 90s, followed by 72°C for 10 mins (Allcock et al., 2007). PCR products were visualised on a 1% (w/v) agarose gel and bands of 650 bp were obtained. PCR products were cleaned using Invitrogen™ PureLink™ PCR Purification Kit according to the manufacturer's instructions. Purified PCR products were then standardized to 12 ng/µL in accordance with the DNA sequencing facility specifications. Samples were prepared for sequencing by adding 5 µL of each purified PCR product to 5 µM forward primer LCO1490 resulting in a 10 µL reaction volume. Samples were sent to Eurofins Genomics (Germany) for DNA Sequencing on an ABI 3730XL DNA Analyzer.

All sequences obtained were input into MEGA Software (Molecular Evolutionary Genetics Analysis) (Kumar et al., 2016) trimmed to 479 bp and aligned using the MUSCLE algorithm. New sequences were submitted to Genbank with Accession Numbers MW264497-MW264503. Additional loliginid sequences for the COI gene were obtained from Genbank [*L. vulgaris*, KM517926-KM517928; *L. forbesii*, KM517907, KM517911, KM517913 (Gebhardt & Knebelsberger, 2015); *Alloteuthis subulata* EU668098-EU668100; *A. media* EU668077, EU668083, EU668097 (Anderson et al., 2008)] and were included in the analysis. All sequences were analysed together by running a statistical parsimony network using TCS software (Clement et al., 2000).

Presence and absence of mature adult females were combined from the following surveys: Irish Groundfish survey (IGFS) 2018, Irish Anglerfish and Megrim Survey (IAMS) 2019 and 2020, the

South Western Beam Trawl Survey, Quarter 1 (Q1SWBEAM) in 2017 - 2019. Fishing activity was only performed during daylight for IGFS and Q1SWBEAM, whereas on IAMS fishing was conducted all day around (24 hours). Sampling gear on IGFS was the IBTS standard 'Grande Overture Verticale' demersal trawl (GOV 36/47) with mesh sizes ranging from 100 mm at the trawl opening to a 20 mm liner in a 25 mm codend. Trawl tows were standardised to roughly 30 mins at 4 knots, as far as possible. IAMS used a standard commercial-derived Jackson trawl with mesh size that varied from 200 mm in the wings gradually reducing to 100 mm in the codend and trawl tows were standardised to roughly 1 hour at 4 knots, when possible. The Q1SWBEAM was carried out by commercially-rigged 4 m steel beam trawl with extended codend supplied with 40 mm mesh size liner.

Females were considered mature if they had reached at least stage 3a of the ICES WGCEPH scale (ICES, 2010) which corresponds to ovary containing a high proportion of large turgid amber-coloured oocytes (>=2mm), with plenty of oocytes in the oviducts. Females with maturity stage 3b were also included. These stages correspond to stages 4 and 5 on Lipinski's (1979) maturity scale for matured and spent respectively. The data were organised by month of observation i.e. November, December, February, March and April (no surveys carried out in this area during the month of January). Data collection spanned four years, 2017-2020.

Corresponding Oceanographic data (March-April 2017-2019) were downloaded from ICES Dataset collections (http://www.ices.dk/data/dataset-collections/Pages/default.aspx).

3. Results

3.1 Identification of egg masses collected in the West English Channel and Celtic Sea

The sampled egg masses (Fig. 2) had a capsule length which varied from 51 to 134 mm, and the number of eggs per capsule ranged from 40 to 138, which fits well into the range of values known for *L. vulgaris* in the Mediterranean Sea (Fig 3). Precise identification of the species based on these two characters would be doubtful, as egg size increases over development and this was not considered. Particularly, there was an important overlap between egg capsules containing ~80-110 eggs which had a similar length (115-140 mm) at early stages of development in *L. forbesii* and at stages close to hatching in *L. vulgaris* (Fig. 3).

3.2 Increase in egg and embryo size during development

Analysis of available data from the literature combined with our observations on genetically identified egg masses allowed reconstruction of curves of increase of both egg size and embryo size during development in both squid species (Fig. 4). Unfortunately, due to some damaged embryos because of freezing we were not able to assign embryonic stages in some egg masses the development of which was somewhere between stage VIII to XII. Dimensions of these eggs are shown on the Fig. 4 as stage 10.

At all stages of embryonic development, eggs of *L. forbesii* were much larger and contained larger embryos that those of *L. vulgaris*. Egg size at embryonic stage in all egg masses sampled in the present study fit the curve derived for *L. vulgaris*.

Finally, a statistical parsimony network analysis resulted in all of the seven egg samples falling out in one network, together with *Loligo vulgaris* sequences obtained from GenBank [see above]. Genbank sequences of *L. forbesii*, *A. subulata*, and *A. media* formed three separate networks, one per species, so all seven egg samples were considered to be *L. vulgaris*. Of the egg masses that were not

genetically identified, egg and embryo size at the observed developmental stage fit well into information existing for the *L. vulgaris* of the West Mediterranean in all sampled capsules (Fig. 3).

3.4 Distribution of the Loligo *squid egg masses in the Irish and Celtic Sea and the English Channel*

Loliginid egg masses were reported across the entire Northeast Atlantic shores (Fig. 2) with the greatest number of observations around the English Channel. Despite intensive survey activity by Cefas in the Irish Sea by bottom trawls, no egg masses were collected there. Very few were found or seen in the central North Sea, and species identification of these masses remains uncertain.

All identified egg masses of *L. vulgaris* were collected during research surveys and were sampled at depths ranged from 12 to 115 m (mean depth of 66 m). Number of capsules varied from 8 to 64 (mean 37.7 mm). Capsule length varied from 51 to 134 (mean 85.1) mm, number of eggs - from 51 to 138 mm (mean 94.2). Distribution of egg masses coincided with bottom temperature range of 8.5-10°C and bottom salinities of 35-35.5 psu (Fig. 5, 6).

3.5 Distribution of mature females

With one exception, mature females in the northern part of the study area in November/December were identified as *L. forbesii*. These were restricted spatially to a few stations located offshore to the west (November) and south (December) of Ireland, whereas the southern part of the Celtic Sea and English Channel remained unsampled. No observations were available in January. By February the mature females fished around Cornish peninsula were dominated by *L. vulgaris* and in March, mature *L. forbesii* were mostly northwest of Ireland, where their occurrence was very high later in April. The absence of mature females of both species in April (Fig. 6) shows that spawning in the Celtic Sea and west English Channel is generally over at this time

4. Discussion

The results confirm the assumption that eggs and embryos of *L. vulgaris* are smaller than those of *L. forbesii*. While this has been suggested for individual developmental stages in the past, the present study adds a size comparison that is stage-specific, to allow eggs/embryos of the two species to be identified once the stage of development is known. Mature eggs in the oviduct of *L. vulgaris* from warm waters off Morocco and the Mediterranean are of 2.0-2.2 mm along their major axis (Mangold-Wirz, 1963; Laptikhovsky, 2000), and 1.6-2.7 mm off Portugal (Coelho et al., 1994). Recently laid eggs are just marginally larger: 2.3-2.8 mm along their major axis (Mangold-Wirz, 1963; Sen, 2004). Grimpe (1925) described freshly deposited eggs as being 3.5 mm in length and 3.1 mm in width, which is consistent with our observations from the English Channel (2.7-3.6 mm along the major axis). It may indicate that eggs in the northern periphery of the species range are larger as in many boreal fish and squid including the Channel population of common cuttlefish (Laptikhovsky. 2006; Laptikhovsky et al., 2020). By the end of embryonic development, egg size in *L. vulgaris* increases to some 5-7 mm (Jecklin, 1934; Boletzky, 1987; this study).

The size of unlaid eggs in *L. forbesii* is 2.6-3.2 mm from Bay of Biscay to Irish waters (Guerra, Rocha, 1994; Collins et al., 1995), becoming 3.0-3.1 mm when just laid, quickly increasing to ~4 mm at the stage when the blastoderm covers 2/3 of yolk, and attaining 8-12 mm at hatching (Boletzky, 1987; Segawa et al., 1988; Moreno, 2008). Therefore neither egg size on its own, nor a combination of egg cluster length with number of eggs, provides a definite tool for egg mass identification, which is only possible after embryonic stage has been determined and egg length (or embryo length)

measured along their major axis. The Fig. 4 of this paper might be used as a solution of this problem, which is necessary for mapping of the species-specific spawning grounds, and their temporal and spatial variation. Without exact knowledge of which species reproduces where and when is impossible to understand its foraging and reproductive migrations and to introduce necessary measures for fisheries management if such measures would be deemed necessary.

Measurement and counts of egg clusters collected in this study and their unambiguous genetic identification as belonging to *L. vulgaris* confirm previous doubts of Martins (1997) who found a description of egg masses of "*L. forbesii*" collected off Plymouth (Holme, 1974) more similar to *L. vulgaris* from Portugal than to *L. forbesii* from Scotland, and therefore suspected mis-identification. Such a mistake is explainable as both species occur off Plymouth from October to May with *L. forbesii* being more abundant with some females maturing or mature, and egg masses were collected in November - August (Holme, 1974). Therefore, these egg masses potentially could belong to either species. Historically this area might be a common spawning ground for both species, though nowadays only both mature females and egg/embryo masses of *L. vulgaris* were observed there in spring (Fig. 2, 5, 6). Thus, the spawning of *L. vulgaris* in the western English Channel seems to take place in late winter and to be completed by April as no mature females were seen by then (Fig 6). No indication of spawning by *L. forbesii* was found there recently: neither egg masses nor mature females apart from a single specimen off Brittany. This could be attributed to two alternatives.

The first is that *L. forbesii* spawning takes place in the eastern Celtic Sea/English Channel outside of our survey months (i.e. in summer/autumn), which is highly unlikely as no major reproduction occurs in these months (Collins et al. 1995). The second, more likely, alternative is that this species nowadays spawns outside the study area, although it might have used this area half a century ago, together with *L.vulgaris*.

Loligo forbesii was initially thought to spawn only inshore (Lum-Kong et al. 1992; Collins et al. 1995). Spawning of *Loligo*-type eggs, presumed to be *L. forbesii*, was subsequently shown offshore by Lordan and Casey (1999) in the western Celtic Sea, in the west of Ireland and north Biscay (all were some distance offshore). These eggs were identified as *L. forbesii* on the basis that eggs were co-incident in the trawl with mature males and females of this species, or, on the basis of identification to species of well-developed embryos (stage 28+) after Segawa et al., (1988). The eggs were obtained in water depths of 135-507 m, which is at the deeper end of the range of previous reports. They were captured in March and April west of Ireland and west of Brittany, which is consistent with our observations on occurrence of mature females. The authors point out that egg masses may have been dragged along for some distance in trawls, but such a distance might not be significant as these masses do not show unavoidable damage in a bottom trawl. Thus *L. forbesii* probably spawns to the west of our study area. In addition to offshore spawning, egg masses have also been reported inshore in the same area, on static fishing gear off the coast of county Cork (10 – 50 m depth) in the western Celtic Sea. These identifications seem assured as egg masses were cultured, hatched and identified as *L. forbesii* on the basis of Segawa et al. (1988) (reported in Collins et al. 1995). Most were seen between Nov-April with a dip in January. "*L. forbesii"* egg masses have also been reported by fishermen from the west coast of Ireland (county Kerry) in September and October, which was suggested to be the site of spawning for the Rockall population. To the north of the range, e.g. in Scotland, identification of *Loligo* egg masses is unambiguous because only *L. forbesii* is present there. Here, spawning females and eggs masses (inshore, attached to creels, but also in deeper waters) were present from December to June, but also in August and September (Lum-Kong et al., 1992). Overall, the spawning area of *L. forbesii* spans northern waters (Scottish coast, northern North Sea) and almost certainly further offshore in the Celtic Sea (Lordan, Casey, 1999) and western Celtic Sea (Collins et al. 1995). The precise extent and boundary of *L. forbesii* spawning grounds in the western Celtic Sea requires more work, ideally using genetic barcoding, and should also investigate possible annual variability. Meanwhile, although *L. vulgaris* is rare in the bottom trawl surveys in Quarter 1 in the

northern North Sea, some mature females are present occasionally (Oesterwind et al., 2010) so the possibility of spawning activity there cannot be entirely ruled-out, particularly taking into account recent climate change. This is supported by the historical observation on summer spawning grounds of *L. vulgaris* off Netherlands (Tinbergen, Verwey, 1945). Hence, like the western Celtic Sea, this is an area where further research identifying egg masses to species level is necessary to get a complete understanding of the life cycle, including possible climate-related shifts.

In conclusion, the importance of the eastern Celtic Sea / English Channel as a spawning ground for *L. vulgaris*, particularly in spring months, is shown. Existing data suggest that this area in spring is used by *L. vulgaris* as spawning grounds, while we could not provide any spawning evidence for *L. forbesii* in our study area during this season. Given a lack of egg masses, reproduction of either species in the Irish Sea is probably not very intensive. Further work is needed to discover the boundaries of important spawning grounds of *L. forbesii* off the Irish west coast, in the western and southern Celtic Sea.

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References

Allcock, A. L., Strugnell, J. M., Prodöhl, P., Piatkowski, U., Vecchione, M. 2007. A new species of Pareledone (Cephalopoda: Octopodidae) from Antarctic Peninsula Waters. Polar Biol. 30, 883-893.

Arkhipkin, A.I., Laptikhovsky V.V., Middleton, D.A.J. 2000. Adaptations for the cold water spawning in squid of the family Loliginidae: Loligo gahi around the Falkland Islands. J. Moll. Stud., 66, 551-564.Boletzky, S. von. 1987. On egg and capsule dimensions in *Loligo forbesi* (Mollusca: Cephalopoda): a note. Vie Milieu 37, 187–192.

Bohadsch I.B. 1761. De quibusdam anomalous marinis eorumque propietatibus. Dresden

Clement, M.D., Posada, D., Crandall, K.A. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657-1659.

Coelho, M.L., Quintela, J., Bettencourt, G., Olavo, G., Villam H. 1994. Population structure, maturation pattern and fecundity of the squid *Loligo vulgaris* from southern Portugal. Fish. Res. 21, 87-102.

Collins, M.A., Burnell, G.M., Rodhouse, P.G., 1995. Recruitment, maturation and spawning of *Loligo forbesi* Steenstrup (Cephalopoda: Loliginidae) in Irish waters. ICES J. Mar. Sci. 52, 127-137.

Doubleday, Z.A., Prowse, T.A.A., Arkhipkin, A., Pierce, G.J., Semmens, J., Steer, M., Leporati, S.C., Lourenço, S., Quetglas, A., Sauer, W., Gillanders, B.M., 2016. Global proliferation of cephalopods. Curr. Biol. 26, R406–R407.

Drew, D. G. 1911. Sexual activities of the squid *Loligo pealei* (Les.). I. Copulation, egg laying and fertilization. J. Morphol. 22, 327–359.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cyto- chrome c oxidase subunit from diverse metazoan invertebrates. Molec. Mar. Biol. Biotechnol. 3, 294–299.

317 Grimpe, G. 1925. Zur Kenntnis der Cephalopodenfauna der Nordsee. Wissenschaftliche
318 Meeresuntersuchungen Helgoland 16, 1–124 [in German].

319 Guerra A., Rocha F., 1994. The life history of *Loligo vulgaris* and *Loligo forbesi* (Cephalopoda:
320 Loliginidae) in Galician waters (NW Spain). Fish. Res. 21, 43-69.

321 Hanlon, R. T., Yang, W. T., Turk, P. E., Lee, P. G., and Hixon, R. F. 1989. Laboratory culture and
322 estimated life span of eastern Atlantic squid, *Loligo forbesi* Steenstrup, 1856 (Mollusca:
323 Cephalopoda). Aquaculture Res. 20, 15–34.

324 Holme, N. A. 1974. The biology of *Loligo forbesi* Steenstrup (Mollusca: Cephalopoda) in the
325 Plymouth area. J. Mar. Biol. Ass. U.K. 54, 481–503.

326 ICES. 2010. Report of the Workshop on Sexual Maturity Staging of Cephalopods, 8-11 November
327 2010, Livorno, Italy. ICES CM 2010/ACOM:49. 97 pp.

328 Jecklin, L. 1934. Beitrag zur Kenntnis der Laichgallerten und der Biologie der Embryonen decapoder
329 Cephalopoden. Rev. Suisse Zool. 41, 593-673.

330 Jereb, P., Allcock, A.L., Lefkaditou, E., Piatkowski, U., Hastie, L.C., and Pierce, G.J. (Eds.) 2015.
331 Cephalopod biology and fisheries in Europe: II. Species Accounts. ICES Cooperative Research
332 Report No. 325. 360 pp.

333 Kumar,S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis
334 version 7.0 for bigger datasets. Molec. Biol. Evolut. 33,1870-1874

335 Laptikhovsky V. 2000. Fecundity of the squid *Loligo vulgaris* Lamarck, 1798 (Myopsida,
336 Loliginidae) off Northwest Africa. Sci. Mar. 64, 275-278.

337 Laptikhovsky V. 2006. Latitudinal and bathymetric trends in egg size variation: a new look at
338 Thorson's and Rass's rules. Mar. Ecol. 27, 7-14.

339 Lee, H. 1875. The octopus or the "devil-fish" of fiction and of facts. London: Chapman and Hall.

340 Lipinski, M., 1979. Universal maturity scale for the commercially important squids
341 (Cephalopoda: Teuthoidea). The results of maturity classification of *Illex illecebrosus* (LeSueur
342 1821) population for years 1973-1977. ICNAF Report No 5364.

343 Lordan, C., Casey, J., 1999. The first evidence of offshore spawning in the squid species Loligo
344 forbesi. J. Mar. Biol. Assoc. U.K. 79, 379–381.

345 Lum-Kong, A., Pierce, G. J., Yau, C. 1992. Timing of spawning and recruitment in *Loligo forbesi*
346 Steenstrup (Cephalopoda: Loliginidae) in Scottish waters. J. Mar. Biol. Ass. U.K. 72, 301–311.

347 Lum-Kong, A. 1993. Oogenesis, fecundity and pattern of spawning in *Loligo forbesi* (Cephalopoda:
348 Loliginidae). Malacol. Rev. 28. 81-88.

349 Mangold-Wirz. K. 1963. Biologie des céphalopodes bentiques et nectoniques de la Mer Catalane. Vie
350 et Milieu., Suppl.13.:1 - 285.

351 Martins, M.C.P.R. 1997. Biology of Pre- and Post-Hatching Stages of *Loligo vulgaris* Lamarck, 1798
352 and Loligo forbesi Steenstrup, 1856 (Mollusca, Cephalopoda). Ph.D. Dissertation. University of
353 Aberdeen: Aberdeen, Scotland, UK.

354 Moreno, A. 2008. Geographic variation in cephalopod life history traits. Dissertação apresentada para
355 acesso à categoria de Investigador Auxiliar. Unidade de Recursos Marinhos e Sustentabilidade.
356 Instituto Nacional de Recursos Biológicos INRB, I.P. / L-IPIMAR. Olhão, Portugal.

Moreno, A., Pereira, J.M.F. 1998. Identification of cephalopod paralarvae from the NE Atlantic. ICES 1998 Annual Science Conference CM1998/M:42.

Moreno, A., Pereira, J., Arvanitidis, C., Robin, J-P., Koutsoubas, D., Perales-Raya, C., et al. 2002. Biological variation of *Loligo vulgaris* (Cephalopoda: Loliginidae) in the eastern Atlantic and Mediterranean.Bull. Mar. Sci. 71, 515–534.

Naef, A 1928. Cephalopoda Embryology. Fauna and Flora of the Gulf of Naples. Translated from German. Smithsonian Institution Libraries, Washington, DC. pp ix–xiii. 2000.

Oesterwind, D., ter Hofstede, R., Harley, B., Brendelberger, H., Piatkowski, U., 2010. Biology and meso-scale distribution patterns of North Sea cephalopods. Fish. Res. 106, 141–150.

Orsi-Relini, L., Mannini, A., Lanteri, L., Beccornia, E. 2009. First record of an egg mass of *Loligo forbesi* (Cephalopoda: Loliginidae) in the Ligurian Sea, with notes about egg laying. Bollettino Malacologico, 45(Suppl. 8): 27–33.

Pierce, G.J., Zuur, A.F., Smith, J.M., Begoña Santos, M., Bailey, N., Chen, C.-S., Boyle, P.R., 2005. Interannual variation in life-cycle characteristics of the veined squid (*Loligo forbesi*) in Scottish (UK) waters. Aquat. Living Resour. 18, 327–340.

Pham, C. K., Carreira, G. P., Porteiro, F. M., Gonçalves, J. M., Cardigos, F., and Martins, H. R. 2009. First description of spawning in a deep-water loliginid squid, *Loligo forbesi* (Cephalopoda: Myopsida). J. Mar. Biol. Ass. U.K. 89, 171–177.

Pierce, G. J., Boyle, P. R., Hastie, L. C., and Key, L. 1994. The life history of *Loligo forbesi* (Cephalopoda: Loliginidae) in Scottish waters. Fish. Res. 21, 17–41.

Porteiro, F. M., Martins, H. R. 1992. First finding of natural laid eggs from Loligo forbesi Steenstrup, 1856 (Mollusca: Cephalopoda) in the Azores. Arquipelago 10. 119–120.

Salman A., Laptikhovsky V. 2002. First occurrence of egg masses of *Loligo forbesi* (Cephalopoda: Loliginidae) in deep waters of Aegean Sea. J. Mar. Biol. Ass. U.K., 82, 925-926.

Segawa, S., Yang, W. T., Marthy, H. J., and Hanlon, R. T. 1988. Illustrated embryonic stages of the eastern Atlantic squid *Loligo forbesi*. Veliger, 30: 230–243.

Sen, H. 2004. A preliminary study on the effects of different light intensities on hatching of European squid (Loligo vulgaris Lamarck, 1798) eggs. Turkish J. Fish. Aquat. Sci. 4, 1-4.

Smith, J. M., Macleod, C. D., Valavanis, V., Hastie, L., Valinassab, T., Bailey, N., Santos, M. B., Pierce, G. J. 2013. Habitat and distribution of post-recruit life stages of the squid *Loligo forbesii*. Deep-Sea Res. II, 95, 145–159.

Stowasser, G., Bustamante, P., MacLeod, C. D., Wang, J., Pierce, G. J. 2005. Spawning areas and selected metal concentrations in squid (*Loligo forbesi*) in UK waters, with notes on metal concentrations in other squid species. Project Report Department of Trade and Industry, UK. 24 pp

Tinbergen, L., and Verwey, J. 1945. Zur Biologie von Loligo vulgaris Lamarck. Archives Néerlandaises Zoologie 7, 186–213

List of figures

Fig. 1 A large egg mass of *Loligo vulgaris* probably laid by several females, Portland, English Channel (photo courtesy Mike Markey)

Fig.2. Occurrence of egg masses of *Loligo* spp. Samples used for genetic analysis are marked by cross.

Fig. 3 Existing data on relationship between egg capsule length and the number of eggs with respect to developmental stage. Egg masses shortly after fertilisation are shown by small symbols, and close to hatching – by large symbols. Egg clusters of *L. vulgaris* from Portugal were identified "on the basis of species distribution and abundance" (Martins, 1997) so their identification requires validation. Identification of *L. forbesii* from NW Scotland is not in doubt as there is no reproduction of *L. vulgaris* there (our data).

Fig. 4 Increase of egg and embryo size during development of *L. vulgaris* and *L. forbesii*. Egg masses with uncertain stages between VIII and XII are shown as Stage 10.

Fig. 5. Averaged distribution of bottom temperature in March-April 2017-2019.

Fig. 6. Averaged distribution of bottom salinity in March-April 2017-2019.

Fig. 7 Occurrence of mature female *L.forbesii* (circles) and *L.vulgaris* (rectangles) in the studied area. Position of hauls where no mature squid were found are shown by crosses.

- The paper provides a pioneer tool to differentiate visually between egg masses of two sympatric commercial loliginid squids, *L.vulgaris* and *L.forbesi*
- Celtic Sea and western English Channel are spawning grounds of *L.vulgaris* in late winter – spring. No reproduction of *L.forbesii* was found there during this season.
- *L.forbesii* forages in Celtic Sea and English Channel but reproduces in deeper water further west, mostly west of Ireland.

Figure

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Figure

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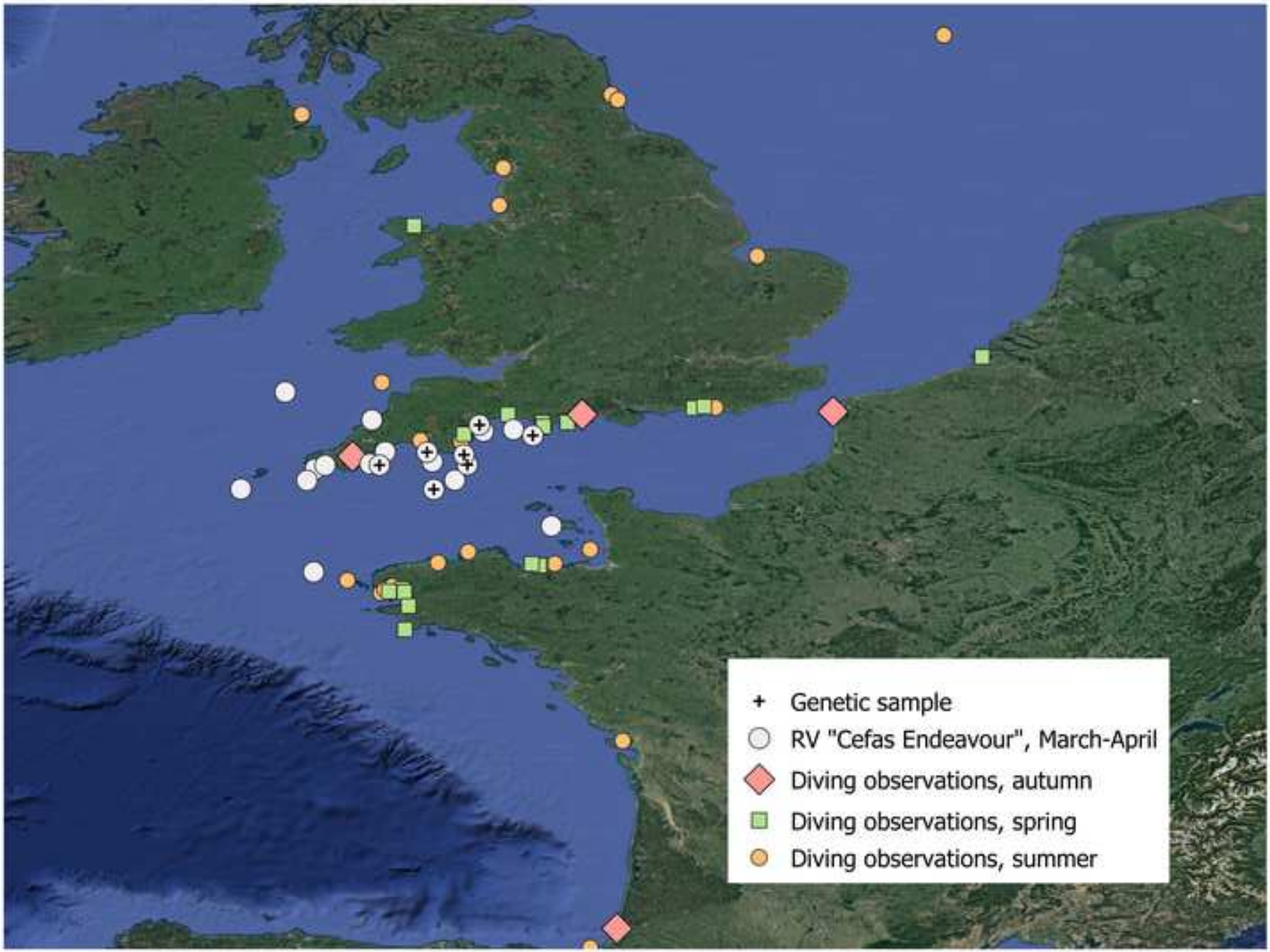

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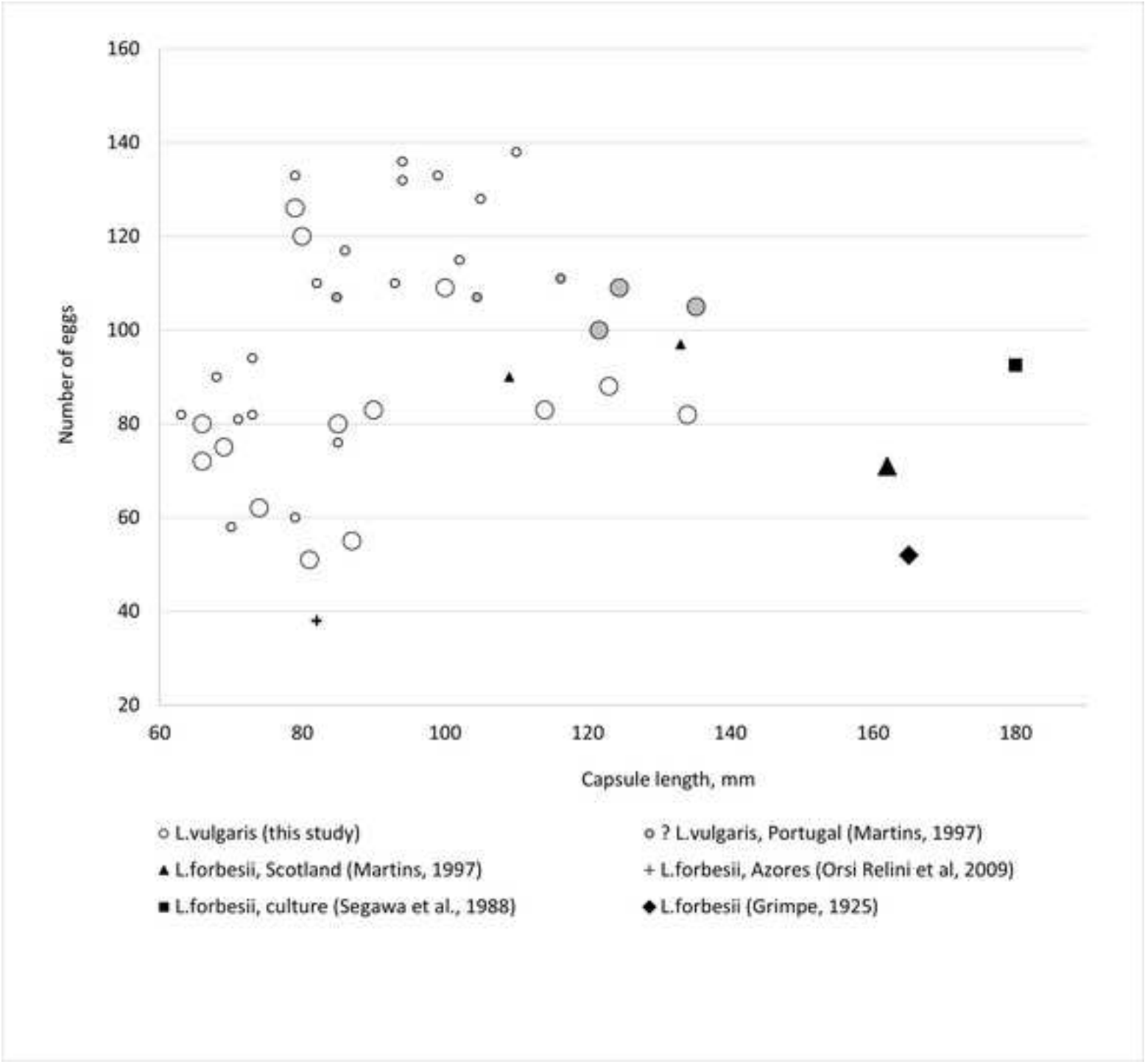

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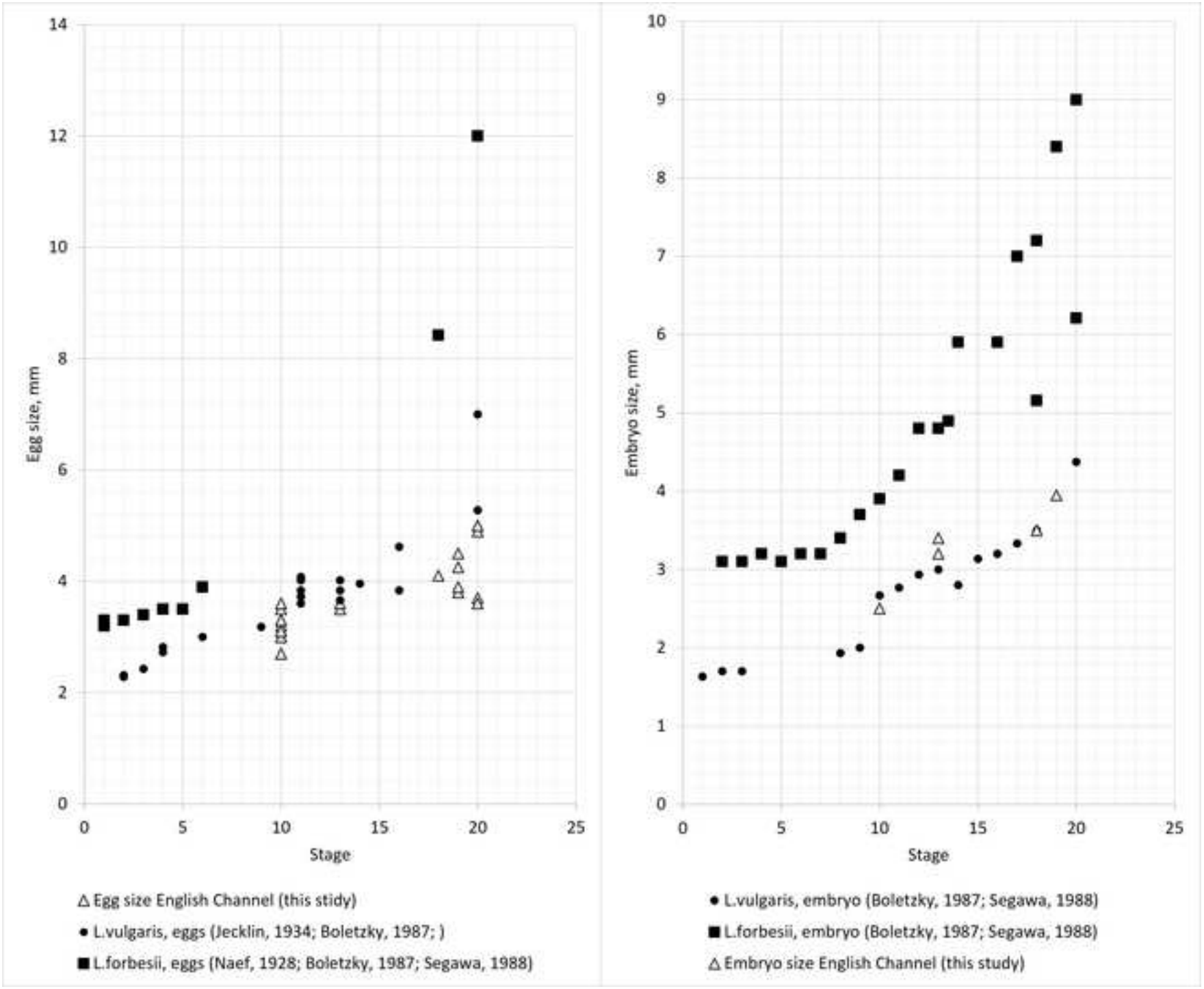

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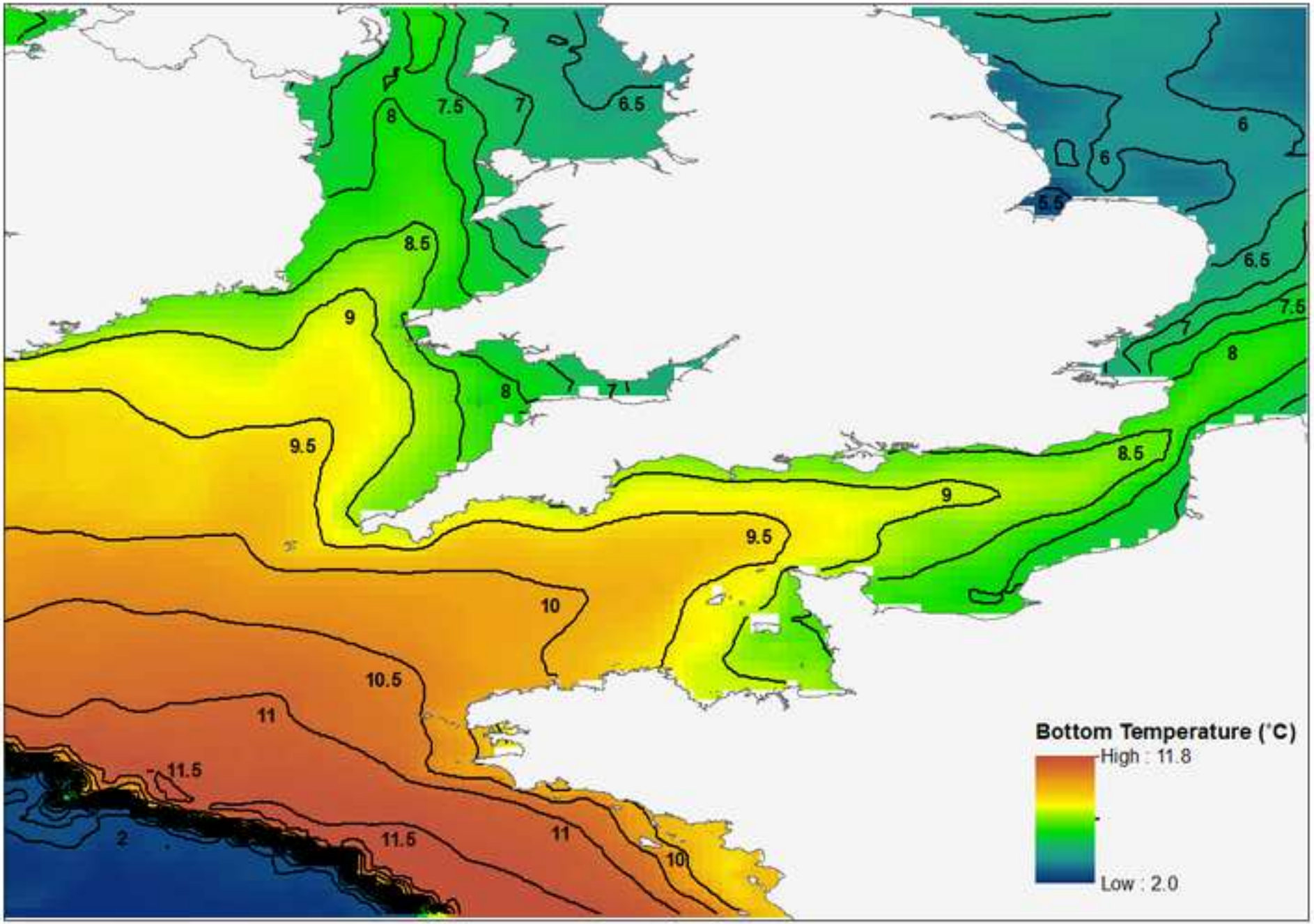

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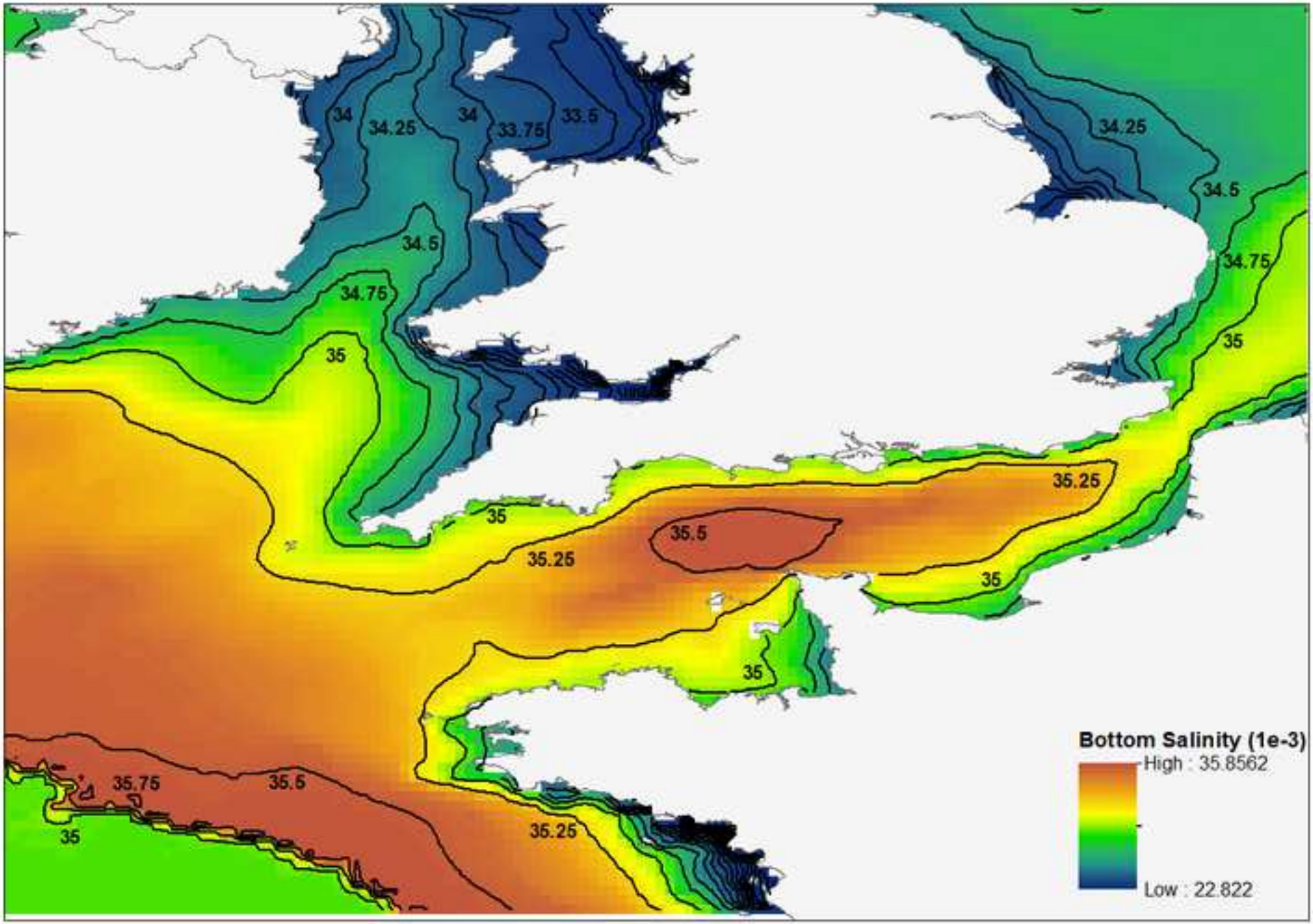

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