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Macroscopic and microscopic morphological characteristics of gelatinous egg masses (egg veils) of yellow
goosefish *Lophius litulon* (Lophiiformes; Lophiidae)

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Abstract

We recorded the morphological characteristics and effect of preservation methods on the structure of the egg veils of *Lophius litulon* found in field investigations. The egg veils were characterised as translucent sheet-shape with parallel opaque creases spaced approximately 2 cm apart. The egg veils were found to be composed of pentagonal or hexagonal chambers with rounded corners arranged in one layer, and each chamber enveloped one to three embryos. Cryopreservation is recommended to prevent structural changes in the egg veil rather than ethanol solution and neutral buffered formalin solution.

(89/90 words)

Key words: anglerfish, early life history, gelatinous egg mass, larvae; morphology

The genus *Lophius* (Lophiiformes; Lophiidae) is a demersal fish group composed of seven species distributed in the Atlantic Ocean, and Northwest Pacific (Fariña et al., 2008). Their reproduction is characterised by spawning ribbon-shaped floating gelatinous egg masses called “egg veils” (e.g. Agassiz, 1882; Lebour, 1925; Everly, 2002; Hoshino et al., 2006; Colmenero et al., 2015). The offspring remain in the egg veil until the stage of late yolk-sac larvae (Ishikawa et al., 2022b). However, it is still unclear what role the egg veils of *Lophius* spp. play in the growth and survival of the eggs and larvae during this period. One of the reasons for the current status is considered to be lack of information on the morphological characteristics of the egg veils to find them in the field. Therefore, it is important to provide details of criteria to recognize egg veils for further elucidation of the early life history of species of the *Lophius*.

Yellow goosefish *Lophius litulon* is distributed in temperate coastal areas of eastern Asia (Yoneda et al., 2001; Takeya et al., 2013). There are several previous studies about the early life history of the egg veils which were collected in the field as well as reared in laboratory (Kim, 1976; Nakaya et al., 2017; Gao et al., 2021; Ishikawa et al., 2022a,b). Regarding the appearance of the egg veils, Hoshino et al. (2006) reported that the length and width spawned in an aquarium were 750–1090 cm and 53–94 cm, respectively. The volume and length of the egg veils observed in the field (Kim, 1976; Nakaya et al., 2017) and some photographic evidence has also been provided (Hoshino et al., 2006; Nakaya et al., 2017; Ishikawa et al., 2022a,b). However, these studies focused on the ecology of *L. litulon* and did not provide sufficient morphological information on the characteristics of the egg veils to enable recognition in the sea. Furthermore, the methods how to preserve the structure of the egg veils have not been reported.

The objective of this study is to establish criteria to recognize the egg veils of *L. litulon* in field sampling, and to provide suitable preservation method of the egg veils. We investigated macroscopic and microscopic morphological characteristics, and changes in morphology of the egg veils before and after preservation by typical methods for planktonic investigation.

Egg veils of *L. litulon* were searched for and collected by visual observation and plankton net surveys. All of the egg veils were identified as *L. litulon* based on the morphology of eggs and larvae (Ishikawa et al., 2022b; Kim, 1976) in the field.

The egg veils were searched for and collected by visual observation in a coastal station in Aomori Prefecture, Japan (41°48'N, 141°00'E). The survey was conducted at least twice a day (06:30–07:30 and 14:30–15:30) between late May and late June in 2022 and 2023 (total 72 days) except for stormy days by walking along the shore. After the egg veils were found, their lengths were recorded whether > 2 m or not. The egg veils with > 2 m length were stretched and measured their largest length, and width in three parts directly with an accuracy of 10 cm and 5 cm by two researchers using a rod from the shore. Finally, parts of the egg veils (at most 20 cm square size) were collected using a scooping net for identification and microscopic observations.

The egg veils were searched by 4 researchers from the deck of the T/S *Ushio-maru* of Hokkaido University, Faculty of Fisheries (262 ton) off Aomori Prefecture on 6 June in 2023, in order to visually record of the egg veils offshore. The survey track followed a zig-zag pattern within 41°10'N–41°18'N, 140°40'E–140°75'E at 3.7 km h⁻¹. After the egg veils were found, the ship was moved close to the egg veils. Parts of the egg veils (approximately 20 cm square size) were collected using a scoop net.

Additional samples were collected by vertical tows of a ring net (80 cm diameter, 0.33 mm mesh and 2.7 m length) from 3 m above the sea floor to the surface at 2 stations off Aomori Prefecture (41°04'N, 140°58'E and 41°09'N, 140°44'E) in late-May and early-June, 2023. These samples were preserved in 5% neutral buffered formalin seawater.

Pieces of some egg veils (approximately 5 cm square size) were preserved in 5% formalin seawater and some were preserved in zipper bags in a freezer set at –30°C with 100 ml of seawater. Undiluted formalin solution (Formaldehyde solution; FUJIFILM Wako Pure Chemical Corporation, Japan) was neutralized with 20 g of hexamethylenetetramine (Hexamethylenetetramine; FUJIFILM Wako Pure Chemical Corporation, Japan) per 1 l.

73 Four egg veils each containing different developmental stages of the eggs or the larvae obtained in the field
74 surveys were cut with scissors into ca. 2 cm square. Each piece was preserved in a vial filled with 30 ml in 90%
75 ethanol solution (Pathological stain solvent ethanol 100 NP; Muto Pure Chemical Co. Ltd., Japan) or the 5% neutral
76 buffered formalin seawater. For each sampled egg veil, three pieces were fixed in each solution. The fixed pieces
77 were observed once every two weeks and recorded the date that the chamber structures collapsed. The vials were
78 kept at room temperature and were left to stand in a light-shielded box except for the time of observations.

79 All procedures and experiments were carried out in accordance with the experimental guidelines set by Hokkaido
80 University.

81 A total of thirty-four egg veils of *L. litulon* were obtained in this study. All of the egg veils were characterized to
82 have a translucent sheet-shape with parallel opaque creases (Fig. 1A&B). Two of the thirty-four egg veils were
83 considered to be intact. The opaqueness of the edge (Fig. 1A) and two roundish tips (Fig. 1A) were observed only
84 in these two egg veils. The length and width of one of the intact egg veils found at the coastal station was 720 cm
85 and 50–60 cm, respectively. The aspect rate of this egg veil (12–13:1) was similar to that of the egg veils spawn in
86 an aquarium (9.6–20.6:1; Hoshino et al. 2006). The other intact egg veil that was found and photographed in the
87 onboard survey was twisted and folded (Fig. 1A), implying that the egg veils do not always spread out and may
88 become convoluted in the sea. Two egg veils collected in the plankton net survey were the smallest (4 cm long at
89 the longest axis) in this study. Besides, the largest egg veil was found at the coastal station (1230 cm long), and
90 twenty-eight of the thirty egg veils were smaller than that of the five egg veils spawned in an aquarium in a previous
91 study (750–1090 cm; Hoshino et al. 2006). Therefore, many of the egg veils were considered to have been torn in
92 the sea, implying that the size of the egg veils would be unreliable to recognize in the ocean. Number of creases
93 were counted 22 and 26 from two egg veils that short axis were considered almost intact. The spaces between the
94 creases of these two egg veils were estimated 1.7 cm and 2.4 cm on average, respectively. Altogether, the egg veils
95 of this species were expressed as translucent flotsams showing parallel opaque creases spacing 2 cm apart in
96 macroscopic observation. Ovarian morphology of the genus *Lophius* is characterised as unique ribbon-shape
97 (Armstrong et al. 1992; Afonso-Dias and Hislop 1996; Yoneda et al. 2001). The detailed macroscopic observation
98 in this study would potentially also help to recognize egg veils of the other species of the genus *Lophius*.

99 The egg veils were composed of numerous chambers arranged in one layer (Fig. 1C, D). The chambers were

100 characterized as colourless, transparent and pentagonal or hexagonal prism shape with rounded corners. As a result
101 of observations of six egg veils, 1–3 individuals were enveloped per one chamber. The most frequent value of the
102 number of individuals per one chamber was 1 or 2 in the six egg veils (Table 2). These characteristics observed in
103 this study have also been confirmed in the photographic records and sketch of egg veils of the congeneric species
104 *Lophius americanus* (Agassiz 1882; Everly 2002) and *Lophius piscatrus* (Laurenson 2006; Colmenero et al. 2017),
105 as well as *Histrio histrio* and *Antennarius striatus* (Rasquin 1958) within the family Antennariidae of the order
106 Lophiiformes. Therefore, the consistence of the egg veil may be common within the order Lophiiformes. Gelatinous
107 egg masses were also spawned by Scorpaenidae, Ophidiidae and Carapidae species in marine teleosts. These egg
108 masses are hollow balloon-shape, and their eggs are embedded in gelatinous layer (reviewed by Ikeda et al. 2014),
109 that is, the polygonal chamber structures are only observed in Lophiiformes among the marine teleosts. The egg veils
110 are known to envelope early larval stage (Ishikawa et al. 2022b). Gelatinous egg masses of Ophidiidae also encased
111 early larvae (Greer et al. 2019). Besides, gelatinous egg matrix of Scorpaenidae egg masses dissolved and released
112 the eggs before hatching (Kimura et al. 1989; Sequeira et al. 2011). Duration of egg masses of marine teleosts may
113 be altered by their subjects to assemble the eggs rather than the differences in the microstructure of the egg masses.
114 However, further study is needed to reveal the role of gelatinous egg masses among the marine teleosts.

115 The egg veils went cloudy and shrunk immediately after being put in 90% ethanol solution (Fig. 2A). The egg
116 veils kept the chamber structures after fixation by the 5% neutral buffered formalin seawater for a while (Fig. 2B).
117 Thus, the 5% formalin solution is much suitable fixative to prevent enveloped embryos from damaging rather than
118 ethanol solution. However, the chamber structures collapsed and the eggs and larvae sank to the bottom of the vial
119 after 4–12 months after the formalin fixation (Table 2). The durations that the egg veils kept the chamber structures
120 in the 5% neutral buffered formalin seawater tended to be longer in the cases of the egg veils enveloping eggs than
121 the cases of the egg veils enveloping larvae. If the egg veils so small that it is hard to notice just after towing are
122 captured in a plankton net survey and are preserved in formalin solution, it is therefore necessary to note that eggs
123 and larvae of *L. litulon* may be misinterpreted as free buoyant eggs and larvae depending on the number of elapsed
124 days after the sampling. The egg veil which was defrosted 8 months after being frozen was confirmed to have kept
125 the chamber structure. Gelatinous egg masses of *Helicolenus dactylopterus dactylopterus* (Scorpaenidae) are
126 recommended to be cryopreserved because their morphology and colour are deteriorated by formalin fixation

127 (Sequeira et al. 2011). Although, the effect of preservation methods on changes in egg masses of teleosts could be
128 different among species, cryopreservation may be superior for preservation compared to other common fixatives,
129 in order to preserve the structure of the egg masses.

130

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140

141 **Author Contributions**

142 All authors contributed to the study conception and design. Sampling, and data collection were performed by
143 all authors. The first draft of the manuscript was written by Tomoya Ishikawa and all authors commented on
144 previous versions of the manuscript. All authors read and approved the final manuscript.

145

146 **Conflict of Interest Statement**

147 On behalf of all authors, the corresponding author states that there is no conflict of interest.

148

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Fig. 1 Egg veils of *Lophius litulon* found in the field. (A) an intact egg veil found and taken a photograph taken

210 from aboard of T/S *Ushio-maru*. Red arrows indicate both tips of the egg veil, (B) a torn egg veils found in the
211 investigation at a coastal station. White arrows indicate parallel opaque creases, (C-D) a photograph taken
212 under a stereomicroscope and a sketch of egg veil chambers and eggs of *Lophius litulon*. Scale bars represent
213 1 m (A), 10 cm (B) and 1 mm (C).

214 Fig. 2 Changes in appearance of pieces of an egg veil of *Lophius lituon* before and 5 days after fixation in 90%
215 ethanol solution (A1–2) and 5% neutral buffered formalin seawater (B1–2). Grid spacings are 5 mm.

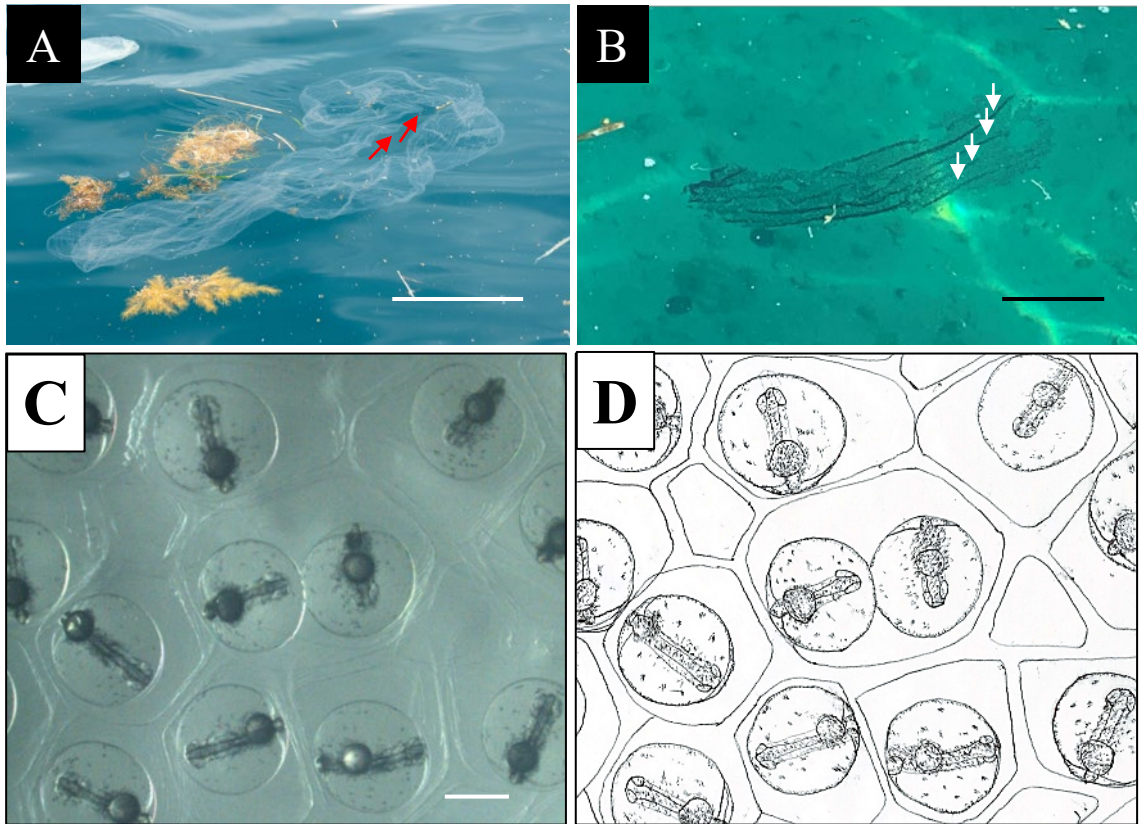


Fig. 1 Ishikawa et al.

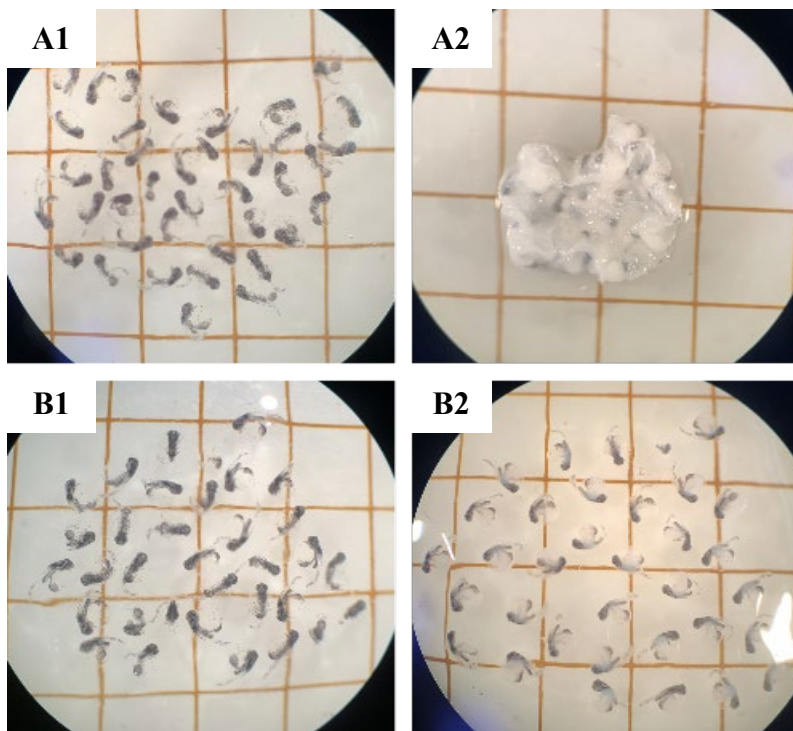


Fig. 2 Ishikawa et al.

Table 1 Frequency of number of embryos for each chamber of egg veils of *Lophius litulon*.

Ser. No. of the egg veil	Number of chambers examined	Number of embryos in the chambers (%)		
		1	2	3
1	44	70	27	2
2	44	32	55	14
3	46	87	13	0
4	78	100	0	0
5	50	90	10	0
6	37	97	3	0

Table 2 Summary of the duration that pieces of egg veils of *Lophius litulon* kept the chamber structure in 5% formalin solution. *Values are mean $\pm$ standard deviation.

Ser. No. of the egg veil	Number of embryos included in the egg veils examined	Developmental stages of embryos	Duration keeping the chamber structure (days)*
1	33, 33 and 47	Blastula stage of egg	361 $\pm$ 0.0
2	36, 38 and 40	Embryo stage of egg	305 $\pm$ 0.0
3	28, 30 and 32	Newly hatched larva	181 $\pm$ 0.0
4	28, 29 and 30	Late yolk-sac larva	112 $\pm$ 0.0