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New Finding of Eggs and Leptocephalus for *Muraenesox cinereus* off Jeju Island, Korea and Its Relation to Environmental Variables

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Abstract: The daggertooth pike conger, *Muraenesox cinereus*, is an important demersal fish species in East Asia but the catch amount has declined in recent years. Spawning areas of *M. cinereus* have not yet been determined; identifying these have serious implications for resource management. Ichthyoplankton surveys are an effective method of distinguishing fish spawning areas and periods. Fish eggs were sampled from the waters adjacent to South Korea in August 2020 and 2022 using a Multiple Opening/Closing Net and Environmental Sensing System or a bongo net. In 2021, *M. cinereus* eggs were not collected. Three unidentified eggs (2.0–2.2 mm in diameter) were collected from the southeastern sea of Jeju Island at a seawater depth of 20–30 m and temperature of 20–22 °C. *Muraenesox cinereus* preleptocephali were gathered at 10–20 m depths from Jeju Island's southernmost and eastern sea areas. The eggs and preleptocephali were identified as *M. cinereus* by their mitochondrial DNA 16S rRNA sequences. This is a new finding of eggs and leptocephalus of *Muraenesox cinereus* off Jeju Island, South Korea, which increases our understanding of the recruitment process of *M. cinereus* to facilitate resource management and species conservation.

Keywords: daggertooth pike conger; *Muraenesox cinereus*; eggs; preleptocephali; spawning area; Jeju Island

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1. Introduction

The daggertooth pike conger (*Muraenesox cinereus*), in the Muraenesocidae family, of the order, Anguilliformes, is widely distributed throughout South Korea, Japan, the Yellow Sea, East China Sea, and Indo-Western Pacific Ocean [1,2]. These fish live in soft sand at a water depth of less than 100 m and feed mainly on small benthic fish and crustaceans [3–5]. *Muraenesox cinereus* is an important commercial species mainly caught in trawl and longline fisheries in South Korea, Japan, and China [6]. Various studies have been conducted on *M. cinereus* age and growth [7–9], maturation and spawning [10], feeding [3,11], distribution and migration [12], reproductive ecology [13,14], resource amount, and management planning [15,16]. Through the collection of mature mothers, spawning areas of *M. cinereus* have been suggested in the seas around Jeju Island, Seto Island of Japan, and the coast of Zhejiang Province, China [10,13,17]. However, no studies have accurately identified *M. cinereus* spawning areas in natural conditions according to the presence of fish eggs and preleptocephali, that is, the stage immediately after hatching.

Similarly, identifying the spawning areas of two other commercial Anguilliformes species, *Anguilla japonica* and *Conger myriaster,* was a long-standing problem in marine science. However, following the collection of fish eggs and preleptocephali from the 1990s to the present, researchers discovered that these species spawn in the Mariana Trench in the Philippines [18–20]. In addition, spawning areas for *C. myriaster* have only been identified on the Kyushu-Palau Ridge and in the Mariana Trench based on the appearance of preleptocephali; eggs in the natural state have not yet been collected [20]. Umezawa et al. [21] conducted on egg development during the artificial breeding of Japanese *M. cinereus*. Further, Ji et al. [6] conducted an morphological characteristics and age analysis of the otoliths of *M. cinereus* leptocephali collected offshore of Jeju Island, which suggested that the spawning areas of *M. cinereus* may be located in the East China Sea, instead of coastal areas. A subsequent study of eggs recorded the presence of *M. cinereus* in the East China Sea close to northern Taiwan in summer [22]. However, no records currently exist of *M. cinereus* eggs in the waters adjacent to South Korea and Japan. Morphology-based identification methods used to estimate the spawning areas and life stages of fish include examining the gonads of mature fish and examining eggs and larvae fish [23]. For the latter, egg and larval samples are collected using an ichthyoplankton net at sea, followed by sorting and identification processes [24]. Previous studies based on these morphological identification methods have resulted in many cases of misidentification; however, the development of molecular identification technology has made it possible to accurately identify various eggs and juveniles [25]. The accurate identification of fish eggs using DNA analysis has enabled the discovery of spawning areas for different fish species [26,27]. In the current study, we aimed to identify the spawning areas of *M. cinereus* in the waters adjacent to South Korea. We identified *M. cinereus* eggs in their natural state for the first time off of the southeast coast of Jeju Island during a summertime (August) monitoring survey. The detailed morphological characteristics, distribution depths, and marine environment of *M. cinereus* eggs and leptocephali are reported. Additionally, for the first time, the vertical distribution of *M. cinereus* preleptocephali and leptocephali have been reported.

2. Materials and Methods

2.1. Sampling

Ichthyoplankton samples were collected from the sea offshore of the Jeju Island in 2020 and 2022 using Tamgu 22 (1458 tons) and Tamgu 23 (1670 tons) fishery resource research vessels operated by the National Institute of Fisheries Science (NIFS) (Figure 1). The Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS) was used to collect samples at 22 sampling stations, whereas bongo nets were used to collect samples from 81 sampling stations (Figure 1). Tamgu 22 and 23 operated simultaneously, and 14 days were required with a 24 h investigation per day. Samples were collected by nets for 5 min in each of the seven water layers (surface to 10 m, 10–20 m, 20–30 m, 30–40 m, 40–60 m, 60–80 m, 80–110 m) using a MOCNESS device (net area 1 m^2, mesh 330 µm). Oblique collection was performed from the bottom to the surface using a Bongo net (net area 80 cm, mesh 330 µm).

For species composition analysis on the collected ichthyoplankton sample, *M. cinereus* fish eggs and preleptocephali were separated. Three individual *M. cinereus* eggs (2.0–2.2 mm) were collected from the MOCNESS and bongo net at the southernmost end of Jeju Island in August, and eight individuals (11.5–34.4 mm total length; TL) of *M. cinereus* preleptocephali and leptocephali were collected from MOCNESS at the southernmost end or the eastern sea of Jeju Island in July (Table 1). *Muraenesox cinereus* preleptocephali (11.5–13.5 mm TL) were collected at 10–20 m depths from the eastern sea area of Jeju Island (Table 1). The flow meter attached to the MOCNESS was used to quantitatively analyze water filtered by the net. The number of individuals that appeared was converted to the number of individuals per unit volume (ind./1000 m^3).

To evaluate the marine environment of the study area, the temperature and salinity of each water layer were measured using a conductivity meter, temperature, and depth sensor (SBE 9plus; Sea-Bird Scientific, Bellevue, WA, USA). The collected samples were fixed on-site in 70% ethanol and then registered and stored in the Ichthyoplankton Laboratory of the Fisheries Resources Research Center, NIFS.

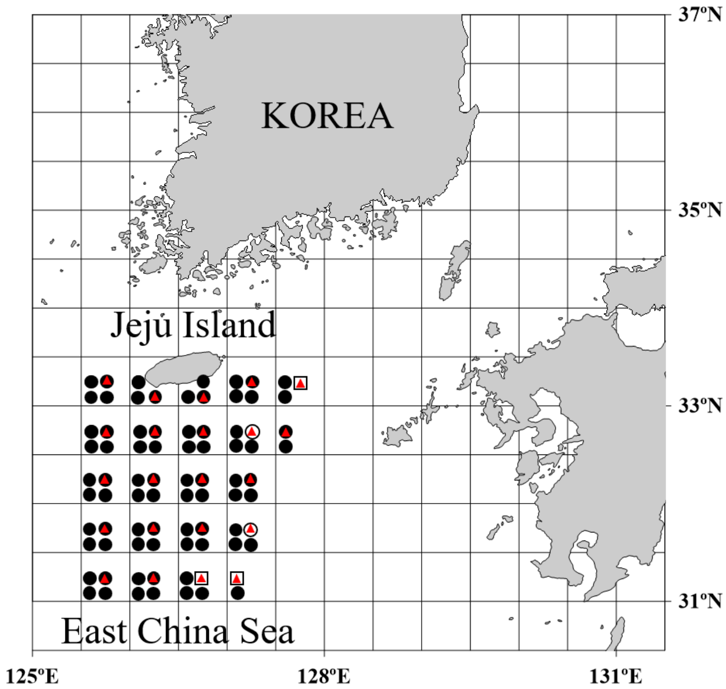

Figure 1. Location of sampling stations of *Muraenesox cinereus* eggs and preleptocephali in waters adjacent to Jeju Island in August 2020 and 2022. White dots indicate stations where *M. cinereus* eggs were collected. White squares indicate stations where *M. cinereus* preleptocephali and leptocephali were collected. Black dots show sampling stations using the bongo net. Red triangles show sampling stations using Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS).

Table 1. List of *Muraenesox cinereus* eggs and leptocephali collected from offshore of Jeju Island.

Collection Date	Latitude	Longitude	Sampling Gears	TL (mm)	Stage	Depth
19 August 2022	32°75′	127°25′	MOCNESS	2.0	Egg	20–30 m
17 August 2020	31°75′	127°25′	Bongo net	2.2	Egg	None
17 August 2020	31°75′	127°25′	Bongo net	2.1	Egg	None
15 July 2021	33°25′	127°75′	MOCNESS	11.5	preleptocephalus	10–20 m
15 July 2021	33°25′	127°75′	MOCNESS	13.5	preleptocephalus	10–20 m
15 July 2021	33°25′	127°75′	MOCNESS	20.9	leptocephalus	20–30 m
15 July 2021	33°25′	127°75′	MOCNESS	23.5	leptocephalus	30–40 m
15 July 2021	31°25′	127°08′34″	MOCNESS	34.4	leptocephalus	20–30 m
16 July 2021	31°25′	126°75′	MOCNESS	30.2	leptocephalus	10–20 m
16 July 2021	31°25′	126°75′	MOCNESS	31.5	leptocephalus	10–20 m
16 July 2021	31°25′	126°75′	MOCNESS	22.1	leptocephalus	20–30 m

2.2. Morphological Identification and Eggs Type Divide

The observation of the morphology and measurement of fish eggs and larvae were conducted using a stereomicroscope (SZX-16; Olympus, Tokyo, Japan). Fish eggs were classified into types by egg diameter, embryo development, and perivitelline space for measurement. At least five fish eggs per type for each station were subjected to molecular identification. Leptocephali were identified according to Tabeta and Mochioka [28] and Ji et al. (2011) [6]. Development of leptocephali was divided into two stages (preleptocephalus and leptocephalus), based on the criteria of Kurogi et al. [20].

2.3. Genomic DNA Extraction, Polymerase Chain Reaction (PCR), and Sequencing

Fish eggs fixed in ethanol were pierced with a needle, right eyeball of eight leptocephali were extracted using forceps, and DNA extraction was performed using the GeneAll Exgene™ Clinic SV DNA extraction kit (GeneAll, Seoul, Korea). To amplify the 16s rRNA genes of mitochondrial DNA, 16Sar (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16Sbr (5′-CCG GTC TGA ACT CAG ATC ATG T-3′) primers were used [29]. After adding 4 μL of genomic DNA to AccuPower® PCR Premix, tertiary distilled water was added to reach a volume of 20 μL. Subsequently, the PCR method was performed using the Thermal cycler (C1000™; Bio-Rad, Hercules, CA, USA) as follows: initial denaturation at 95 °C for 3 min; 37 cycles of PCR reaction (denaturation at 94 °C for 3 s, annealing at 52 °C for 30 s, extension at 72 °C for 1 min); final extension at 72 °C for 5 min.

After completion of the PCR, the PCR product was injected into 1.5% agarose gel, then electrophoresed for 25 min at 100 voltages in a submarine electrophoresis system (Mupid-2plus; Takara Bio Inc., Shiga, Japan) to determine. The final output was confirmed on a gel documentation system (Nippon genetics, Tokyo, Japan). Then, the PCR products were purified using ExoSAP-IT (ThermoFisher Scientific, Waltham, USA), and the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v 3.1 was used with an ABI 3730XL DNA Analyzer (Applied Biosystems Inc., Foster City, CA, USA) to obtain the nucleotide sequence by cycle sequencing under the following conditions: 35 cycles of PCR reaction (denaturation at 94 °C for 10 s, annealing at 56 °C for 10 s, extension at 60 °C for 3 min). The nucleotide sequence was aligned using the ClustalW program [30] (BioEdit version 7 [31]). The genetic distance between nucleotide sequences was calculated using the Kimura-2-parameter model [32] (Mega X [32]). The neighbor-joining tree was constructed using the Kimura-2-parameter model [33], with the confidence value assessed using 1000 bootstrap replications. Species identification was performed by comparing the genetic information registered in the National Center for Biotechnology Information.

3. Results

3.1. Molecular Identification Morphological Characteristics of M. cinereus Eggs and Preletocephalus

Details of the collected *M. cinereus* eggs and preleptocephali are presented in Table 1. After analyzing the sequence of 568 base pairs of mitochondrial DNA 16S rRNA, three eggs and eight preleptocephali collected from the southern sea and the southernmost part of Jeju Island were identified as *M. cinereus*, by having a 100% match with the nucleotide sequence of adult *M. cinereus. Muraenesox cinereus* eggs and preleptocephali were identical to those of *Muraenesox bagio* at a genetic distance of 95.3% (Table 2).

Table 2. Genetic distance among *Muraenesox cinereus* eggs, preleptocephalus, and six other Anguilliformes species.

	1	2	3	4	5	6	7	8	9	10
Muraenesox cinereus egg A (1)										
Muraenesox cinereus egg B (2)	0.000									
Muraenesox cinereus leptocephalus A (3)	0.000	0.000								
Muraenesox cinereus leptocephalus B (4)	0.000	0.000	0.000							
Muraenesox cinereus (5)	0.000	0.000	0.000	0.000						
Muraenesox bagio (6)	0.047	0.047	0.047	0.047	0.047					
Echelus uropterus (7)	0.151	0.151	0.151	0.151	0.151	0.137				
Ophisurus macrorhynchos (8)	0.172	0.172	0.172	0.172	0.172	0.156	0.086			
Gnathophis nystromi (9)	0.166	0.166	0.166	0.166	0.166	0.157	0.141	0.157		
Conger myriaster (10)	0.223	0.223	0.223	0.223	0.223	0207	0.191	0.212	0.178	
Anguilla japonica (11)	0.198	0.198	0.198	0.198	0.198	0.183	0.174	0.166	0.133	0.207

The neighbor-joining tree showed that *M. cinereus* eggs and preleptocephali were clustered perfectly with adult *M. cinereus* (Figure 2) and the next nearest neighbor was *M. bagio*.

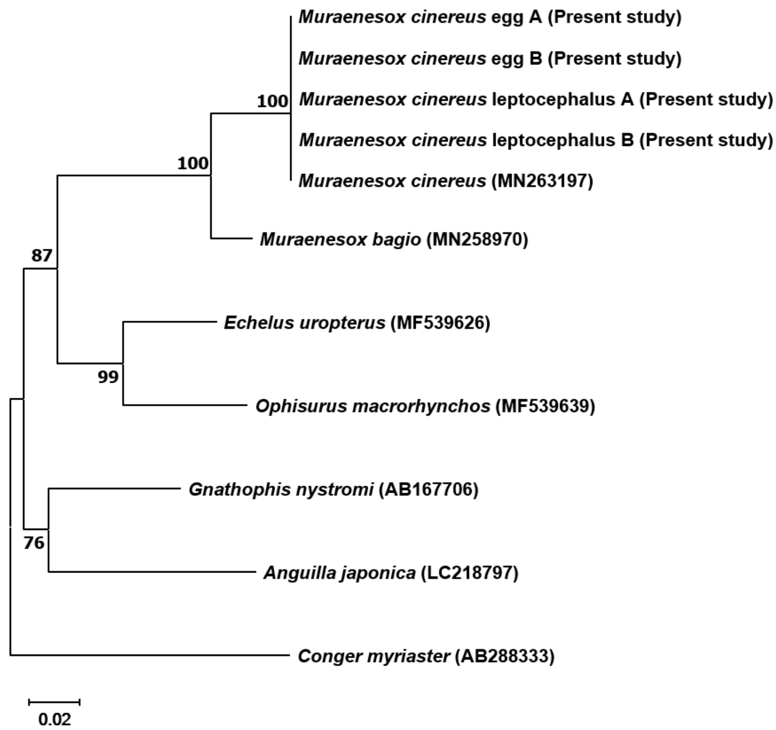

Figure 2. Neighbor-joining tree based on mtDNA 16S rRNA sequences, showing the relationships between two *M. cinereus* eggs and two preleptocephali from this study and six Anguilliformes species. The neighbor-joining tree was constructed using the Kimura 2-parameter distance model, with 1000 bootstrap replications. The scale bar indicates a genetic distance of 0.02.

The *M. cinereus* eggs analyzed in this study were colorless, transparent, spherical, and segregated paternal eggs with a diameter of 2.0–2.2 mm (Figure 3, Table 1). The collected *M. cinereus* eggs were in an early stage of embryogenesis before the pigmentation of vesicles on the eyeball. The size of the perivitelline space was narrow for the species, yolk was yellow and the embryo was elongated around the yolk in a round shape (Figure 3).

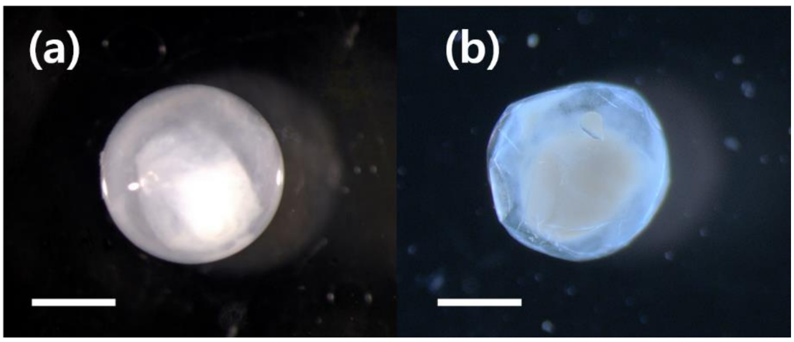

Figure 3. Photograph of an eggs of *M. cinereus* collected from waters adjacent to Jeju Island. (**a**) egg diameter = 2.1 mm, (**b**) egg diameter = 2.0 mm. Scale bar = 1.0 mm.

Developmental counts and measurement of *M. cinereus* preleptocephali and leptocephali are presented in Table 3. The TL of hatched *M. cinereus* preleptocephali and leptocephali was 11.5–13.5 and 20.9–34.4 mm, respectively, and their morphological features were as follows: total myomeres 145–153; preanal myomeres, 93–98; transparent and deeply compressed body; relatively large head; highly acute anterior end of snout; well-developed eyes; fang-like teeth on both jaws; two nostrils present in front of eyes; liver tissue located in anterior body; anus located in posterior body; branch-shaped melanophores present along the lateral surfaces of the head to behind the anus and extending to the caudal terminus; and branch-shaped melanophores present in the intestine (Figure 4). The upper jaw of 11.5 mm TL *M. cinereus* preleptocephalus was more protruded than the lower jaw (Figure 4a), and the length of both upper and lower jaws were identical in leptocephalus of 23.5 mm TL (Figure 4b). The tail fin of leptocephalus with 31.5–34.4 mm TL had begun to develop (Figure 4c). As they grew from 31.5 mm TL and above following hatching, their body depth became smaller and their myomeres became identical to those of an adult fish.

Table 3. Comparisons of measurements and counts for *Muraenesox cineleus* preleptocephali and leptocephali.

Development	Preleptocephalus	Leptocephalus
The number of specimens	2	6
Total length, TL (mm)	11.5–13.5	20.9–34.4
In % of total length		
Head length	13.3–14.7 (13.8 ± 0.5)	8.4–11.7 (9.8 ± 1.1)
Preanal length	75.8–80.0 (77.9 ± 2.1)	76.9–88.5 (81.2 ± 4.4)
Body depth	11.8–13.2 (12.5 ± 0.7)	9.0–11.1 (9.7 ± 0.7)
In % of Head length		
Eye diameters	22.8–23.6 (23.2 ± 0.4)	19.1–23.8 (20.7 ± 1.6)
Snout length	42.4–43.7 (43.0 ± 0.6)	42.0–45.7 (43.4 ± 1.2)
Upper jaw length	66.5–66.7 (66.6 ± 0.1)	56.2–62.5 (58.5 ±2.3)
Counts		
Total myomeres	>150	145–153
Preanal myomeres	95–104	93–98
Number of nostril	2	2
Dentition formula	$\frac{1+\text{III}-\text{IV}+1-2}{1+\text{III}-\text{IV}+1-2}$	$\frac{1+\text{III}-\text{VI}+2-3}{1+\text{III}-\text{V}+2-4}$

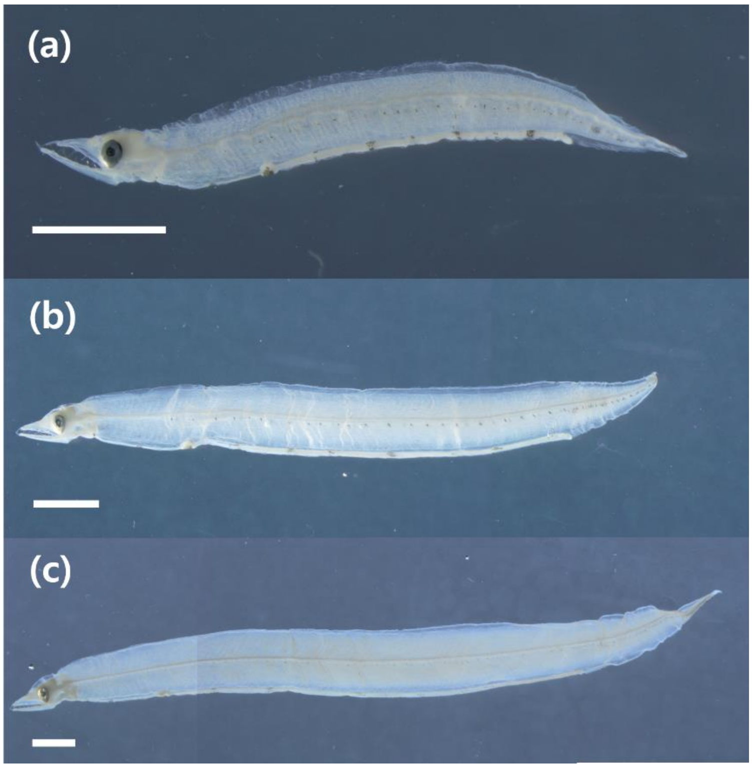

Figure 4. Photograph of preleptocephalus and leptocephali of *M. cinereus* collected from waters adjacent to Jeju Island: (**a**) preleptocephalus, total length (TL) = 11.5 mm, (**b**) leptocephalus, total length (TL) = 23.5 mm, (**c**) leptocephalus, total length (TL) = 34.4 mm Scale bars = 2.0 mm.

3.2. Spawning Area Characteristics of Muraenesox cinereus and Collection of Preleptocephalus

During the ichthyoplankton investigation performed in this study in the vast sea area of the southern sea of Jeju Island, *M. cinereus* fish eggs and preleptocephalus appeared only in Jeju Island's eastern and southern sea areas (Figure 1). *Muraenesox cinereus* fish eggs were collected at two sampling stations, covering the scope of latitude of 31°75′–32°75′ and longitude of 127°25′ in August 2020 and 2022, whereas the *M. cinereus* preleptocephalus and leptocephalus were collected from three sampling stations with the scope of the latitude of 31°25′–33°25′ and longitude of 126°75′–127°75′ in July (Table 1).

The spawning ground where *M. cinereus* fish eggs were gathered in August was at a water level of 120 m, surface level water temperature of 30 °C, and 100–120 m water temperature of 16 °C. The salinity was 31 Practical Salinity Unit (PSU) at the surface level and 34.5–34.7 PSU at 90–120 m. These spawning areas are affected by the warm Kuroshio Current in August. *Muraenesox cinereus* eggs were distributed over a depth of 20–30 m, water temperature range of 20–22 °C, and salinity range of 33.4–33.6 PSU (Figure 4). All *M. cinereus* fish eggs were at 20–30 m depths (19.3 ind./1000 m^3), with no eggs gathered at 0–10, 30–40, 40–60, 60–80, 80–110 m depths (Figure 5a). The summer southern area around Jeju Island where *M. cinereus* fish eggs were collected in this study had a strong thermocline with increasing water depth. The salinity tended to increase as the water depth increased; however, salinocline was not formed at 30–120 m depths. *Muraenesox cinereus* fish eggs were distributed close to the upper water layer of the thermocline. This is the first study to collect *M. cinereus* eggs in their natural state from the waters around Jeju Island, which indicates the existence of *M. cinereus* spawning areas in the waters near South Korea; specifically, in waters 50–100 km off the southeast coast of Jeju Island.

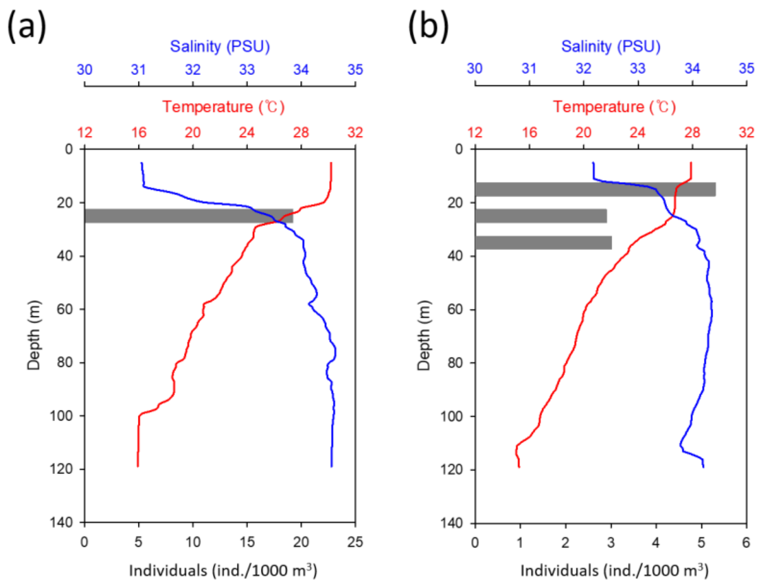

Figure 5. Water depth, salinity, and temperature distribution of *M. cinereus* eggs and preleptocephali collected in this study. (**a**) *M. cinereus* eggs, (**b**) *M. cinereus* preleptocephali and leptocephali.

Muraenesox cinereus preleptocephali and leptocephali were collected in July, and their vertical distribution was concentrated at 10–40 m depths. A total of 5.4 ind./1000 m^3 of *M. cinereus* leptocephali were collected at 10–20 m depths, 3.1–3.2 ind./1000 m^3 at 20–30 m and 30–40 m depths, and none were collected at surface layer–10 m, 40–60 m, 60–80 m, and 80–110 m depths (Figure 5b). The water temperature and salinity of their main distribution water depth was 21–27 °C and 33.3–34.2 PSU, respectively. *Muraenesox cinereus* preleptocephali were collected at 10–20 m depths. In our surveys, *M. cinereus* preleptocephali and leptocephali were abundant and widely distributed during July (Figure 1). Leptocephali with 11.5–13.5 mm TL were collected from the eastern sea areas of Jeju Island, whereas those with 22.1–34.2 mm TL were collected from the southernmost sea area of Jeju Island (Figure 1).

4. Discussion

In this study, *M. cinereus* eggs and preleptocephali from the southern sea area of Jeju Island (where mature *M. cinereus* females had appeared in the past) were collected to accurately investigate the spawning ground. The eggs analyzed were colorless, transparent, spherical, and segregated paternal eggs with a 2.0–2.2 mm diameter (Figure 3). A previous study on egg development during the artificial breeding of Japanese *M. cinereus* reported that the egg diameter ranges from 1.8 to 2.1 mm, which is slightly smaller than the size of *M. cinereus* in the wild [21]. Comparing the size of *M. cinereus* eggs with those of the Anguilliformes species, the former were larger than those of artificial breeding *C. myriaster* (0.86–1.06 mm), but smaller than those of Ophichthidae (2.20–3.83 mm) [34]. Under artificial breeding conditions, *M. cinereus* eggs hatch in 36 h at a water temperature of 25 °C [21]. The water temperature of the sea area where the *M. cinereus* eggs were collected was 23 °C, and the eggs were estimated to have been fertilized within approximately 30 h before collection. Previous studies on the spawning characteristics of *M. cinereus* based on the collection of mature fish suggested the existence of spawning areas in the waters surrounding Jeju Island, the continental coast of Zhejiang Province in China, and the coast of Seto Island in Japan [10,13,17]. However, spawning areas cannot be accurately estimated from the distribution of mature fish alone. In the case of sedentary fish species that do not migrate to spawn, it is possible to investigate spawning grounds by collecting the distribution of mature fish during the main spawning season, as well as by collecting fish eggs and yolk sac larvae. However, in the case of migratory fish species, it is only possible to accurately estimate spawning areas by collecting eggs or yolk sac larvae, because they migrate to a suitable environment for spawning according to factors such as water temperature, salinity, prey, etc. [19,20].

In mature, female *A. japonica* and *C. myriaster*, which migrate long distances for spawning, naturally-spawned fish eggs are rarely found, even after conducting intensive investigation into the estimated sea areas of spawning grounds [18]. Despite searching for young hatchlings and eggs in the sea, the spawning grounds of *A. japonica* remained a mystery for 100 years and were only recently discovered to be in the western sea area of Mariana Trench through the collection of naturally spawned eggs [18,19]. Collected preleptocephali of *C. myriaster* allowed its significant spawning ground to be estimated to be in the western sea area of Mariana Trench, with another spawning ground likely formed in Japan's southernmost end of Ryukyu Island [20]. However, *C. myriaster* eggs have yet to be collected in nature. The southern sea area of Jeju Island (the study area in this research) is dense in fishery resources as it is a nursery ground and spawning ground for fish that migrate along the Tsushima Warm Current from the East China Sea Kuroshio Current [35,36].

This was the first study to collect *M. cinereus* eggs in their natural state from the waters around Jeju Island, which indicated the existence of *M. cinereus* spawning areas in the waters near South Korea; specifically, in waters 50–100 km off the southeast coast of Jeju Island. This agreed with previous research that predicted the formation of a spawning ground near the sea areas of Jeju Island through the collection of mature *M. cinereus* females [10]. Therefore, we report that one of the principal spawning grounds of *M. cinereus* in East Asia is in the sea of Jeju Island in the East China Sea, in the route of the Kuroshio and Tsushima Warm Currents with high water temperatures. A spawning area was the east southern sea area of Jeju Island; however, no fish eggs or preleptocephali were collected here. This was attributed to the spawning ground of *M. cinereus* being impacted by the northward moving ocean current.

A previous study recorded the widespread presence of *M. cinereus* eggs in the East China Sea to the north of Taiwan during summer [22]. *Muraenesox cinereus* are also distributed in the waters of East Asian countries such as South Korea, China, and Japan; thus, their spawning areas are also presumed to include various sea areas [10,13,17]. This study was the first to reveal the marine environment and fish egg distribution water depth of a spawning ground of *M. cinereus* (Figure 4a). The water depth (20–30 m) where *M. cinereus* fish eggs were collected had a water temperature of 20–22 °C and salinity of 33–34 PSU. In comparison, the eggs of another Anguilliformes species (*A. japonica)* were collected at water depths of 150–180 m, the water temperature of 20–25 °C, and salinity of 34.4–35.5 PSU. While the water depth was different, the water temperature and salinity were similar to the spawning environment of *M. cinereus* [37]. The fish egg water layer distribution is different for each major commercial Anguilliformes species, and water depth was considered to be relevant to the spawning sea area. Moreover, the collection period of *M. cinereus* eggs in the current study coincided with the spawning season of *M. cinereus* (June–October) caught in the waters around South Korea and Seto Island, Japan [13,14]. *Muraenesox cinereus* preleptocephali (11.5–34.2 mm TL) were collected at the eastern and southernmost sea areas of Jeju Island in mid-July. Ji et al. [6] reported the daily ring of preleptocephali (16.6–29.0 mm TL) as 18–30 d and estimated that preleptocephali hatched between the end of June and early July. Thus, the primary spawning season of *M. cinereus* was estimated to be between June and July. Furthermore, Ji et al. [6] performed age analysis on the leptocephali of *M. cinereus* (16.6–29.0 mm in total length) and suggested spawning areas near Jeju Island or in the East China Sea. These findings are supported by the results of our study, which identified *M. cinereus* eggs in the waters around Jeju Island and its southernmost tip during August.

This research was the first to reveal the vertical distribution of *M. cinereus* leptocephali (Figure 4b). *Muraenesox cinereus* preleptocephali and leptocephali were distributed in water levels at 10–40 m depths, with a marked increase at 10–20 m, followed by 20–30 m. *Muraenesox cinereus* leptocephali were distributed at 10–20 m. The distribution depth of *M. cinereus* preleptocephali and *M. cinereus* eggs were identical; therefore, both fishes were determined to spawn, hatch, and grow at approximately 20 m depth. Furthermore, *A. japonica* and *C. myriaster* preleptocephali have been found to be distributed at 150–200 and 50–150 m depths [19,20]. Research on the vertical distribution of Anguilliformes leptocephali from sea areas near the North Equatorial Current have revealed that *Ariosoma*, *Avocettina*, *Nemichthys*, and Serrivomerdiae leptocephali are dominant at 30–50 m depths, where the surface water layers are mixed, whereas *Anguilla*, *Conger*, *Derichthys,* Chlopsidae, Muraenidae, and Ophichthidae leptocephali are distributed at the top layer of the 70–100 m thermocline [38]. Thus, the vertical distribution of preleptocephali could be distinguished by species, and *M. cinereus* leptocephali were determined to be distributed in a shallower layer than *A. japonica* leptocephali and *C. myriaster* preleptocephali.

Previous studies on near-sea *M. cinereus* leptocephali distribution are lacking; however, research has been conducted on the species' distribution in the sea area near Jeju Island in South Korea and *M. cinereus* leptocephali just before metamorphosis were collected in August from the coral reef at the coast of Ishigaki Island located east of Taiwan [39]. Ji et al. [6] reported that *M. cinereus* leptocephali before metamorphosis, collected from the south coast of South Korea in August, spawned near the south coast, migrated with the current after hatching, and spent their initial stage undergoing metamorphosis near the coast.

It is postulated that that *M. cinereus* distributed in the waters around South Korea spawn in the waters around Jeju Island during the primary spawning season, after which the hatched leptocephali grow up and move north with the oceanic current toward the coast. In the future, a more intensive survey of the spawning areas and period of *M. cinereus* could shed light on the migration or recruitment process of *M. cinereus* leptocephali with oceanic currents. Furthermore, future studies should be conducted to collect more detailed information on *M. cinereus* through continuous monitoring of their early life cycles, such as spawning characteristics and growth, which are essential for resource management.

5. Conclusions

This is a new finding of eggs and leptocephalus of *M. cinereus* in nature during August 2020 and 2022, as part of an analysis of eggs and larvae for major fishery resources in the waters adjacent to South Korea. The collected eggs appeared in the early stage of embryonic development at a depth of 20–30 m and temperature of 20–22 °C. The eggs were identified as *M. cinereus* using the mitochondrial DNA 16s rRNA region. *Muraenesox cinereus* leptocephali and preleptocephali were distributed between depths of 10–40 m and 10–20 m, respectively, with the depth distribution of leptocephali concurring with that of the fish eggs in the spawning ground. *Muraenesox cinereus* spawns in the southernmost end of Jeju Island and East China Sea between July and August, and the hatched preleptocephali migrate northward with the current for their initial stage of life. *Muraenesox cinereus* is an important commercial species in South Korea, Japan, and China, but the catch amount has been rapidly decreasing in recent years, requiring significant efforts for resource management. Our understanding of the recruitment process of *M. cinereus* and help facilitate resource management and species conservation.

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