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メタデータ	言語: English 出版者: 公開日: 2024-07-25 キーワード (Ja): キーワード (En): 作成者: 渡井, 幹雄, 安田, 十也, 木下, 順二, 入路, 光雄, 長井, 敏, 高須賀, 明典, 米田, 道夫 メールアドレス: 所属: 水産研究・教育機構, 水産研究・教育機構, 水産研究・教育機構, 水産研究・教育機構, 水産研究・教育機構, 東京大学, 水産研究・教育機構
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Interspecific and intraspecific difference in egg size of two mackerel (*Scomber* spp.) species in relation to sea surface temperature in the western North Pacific: A new approach to species identification

Running title: Difference between two *Scomber* spp. eggs

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ABSTRACT

Chub mackerel (*Scomber japonicus*, Scombridae) and blue mackerel (*S. australasicus*) are two important fishery resources in the western North Pacific that spawn eggs during the same season. Although estimating the total egg production (TEP) is important for evaluating the spawning stock biomass of mackerel species, it is difficult to accurately identify formalin-preserved eggs collected during ichthyoplankton surveys. Hence, a new identification criterion that incorporates the effects of water temperature on egg size was developed in this study. The diameter of 37,304 mackerel eggs collected over 16 years (2006–2021) was measured, and frequency distributions of egg size across sea surface temperature (SST) ranges at 1 °C interval were constructed. The frequency distributions were classified into two groups using a Gaussian mixture model; based on the results of DNA analysis, the small group was chub mackerel, while the large group was blue mackerel. The SST at the sampling stations and the mean egg size of both groups were negatively correlated. The new identification criterion, incorporating the relationship between SST and egg size, provided reasonable estimates of the TEP of the two mackerel species compared with the conventional criterion. The new species identification approach is applicable to other fish taxa in the western North Pacific.

Keywords: blue mackerel, chub mackerel, egg diameter, total egg production, water temperature

1. INTRODUCTION

The distribution and abundance of fish eggs are important for a better understanding of fish population dynamics (Cowen & Sponaugle, 2009; Gallego & North, 2009; Hidalgo et al., 2012; Wright & Trippel, 2009) because such information indicates the location and time of spawning as well as the number of spawner fish (Furuichi et al., 2020), which affects the year–class strength (Houde, 1987; Leggett & Deblois, 1994). Ichthyoplankton surveys are usually conducted to study pelagic fish eggs. The species of fish eggs obtained through such surveys are often identified using the egg identification key, which is based on the morphological characteristics of eggs: shape, size, number of oil globules, yolk segmentation, and yolk diameter. However, morphological characteristics may be insufficient for accurate species identification (Miller & Kendall, 2009); thus, practical identification keys must be developed according to the characteristics of the target fish species.

Chub mackerel (*Scomber japonicus* Houttuyn 1782, Scombridae) and blue mackerel, also known as spotted mackerel (*S. australasicus* Cuvier 1832), are widely distributed in the western North Pacific and are important fishery resources in East Asia. Both species mature after reaching the age of 2 (Watanabe & Yatsu, 2006). Mature chub mackerel and blue mackerel spawn eggs from December to August and from January to July, respectively, in the coastal area of the Kuroshio Current region (Takasuka et al., 2012; Watai et al., 2019). Extensive ichthyoplankton surveys have been conducted for a long period in Japan to estimate the total egg production (TEP), which is an index of spawning stock biomass, of these two mackerel species (Yukami et al., 2022a; Yukami et al., 2022b). As the spawning of these two mackerel species in the

western North Pacific overlaps spatially and temporally (Watai et al., 2019; Watanabe, 1970; Watanabe et al., 1999), their eggs are collected together through net sampling. Hence, an accurate and convenient egg identification method should be developed to determine the spawning status of the two mackerel species and gain deep insight into their spawning ecology.

The eggs of chub mackerel and blue mackerel can be distinguished morphologically based on the presence of a yellow pigment plexus (Ikeda et al., 2014; Meguro et al., 2002); the eggs of blue mackerel exhibits a yellow pigment plexus on their tails, while those of chub mackerel lack such a structure. However, this identification method is not applicable to formalin-preserved specimens because the yellow pigment plexus becomes indistinct (Meguro et al., 2002). Similarly, DNA-based species identification might be inappropriate for formalin-preserved specimens because formaldehyde induces DNA degradation (Srinivasan et al., 2002). Since egg and larval samples are conventionally preserved in formalin immediately after collection (Ahlstrom, 1976; Smith & Richardson, 1977), a new species identification method applicable to historical formalin-preserved specimens obtained from ichthyoplankton surveys must be developed. Egg size is a distinguishable trait that may be included in the practical species identification key for these mackerel species. Previous studies have reported that a stable diameter is maintained in formalin-preserved mackerel eggs even after a long period of storage (Nyuji et al., 2022). Nishida et al. (2001) performed mitochondrial DNA analysis to identify 192 mackerel eggs collected around the Izu Islands, Japan, and found that the mean size of chub mackerel eggs was significantly smaller than that of blue mackerel eggs. The reproductive strategies of a fish species

88 may influence egg size (Laptikhovsky, 2006); however, the factors that cause the
89 difference in egg size between these mackerel species are poorly elucidated.
90 Nonetheless, based on egg size, Saito & Ikegami (2010) proposed that eggs with ≤ 1.1
91 mm diameter belong to chub mackerel, while those with > 1.1 mm diameter belong to
92 blue mackerel. This criterion has been referred to in estimating the annual TEP of these
93 two mackerel species (Oozeki et al., 2003). However, a recent stock assessment report
94 on blue mackerel noted that there was a non-negligible discrepancy between the TEP
95 and catch fluctuations, and this discrepancy might have been caused by species
96 misidentification due to ecological changes in egg size (Kanamori et al., 2021; Yukami
97 et al., 2022a). As the conventional identification criterion is based on limited data
98 (Nishida et al., 2001; Saito & Ikegami, 2010), egg size variability should be further
99 investigated using broad and long-term sampling data.

100 Previous studies have suggested that the egg size of fish can change under both
101 internal and external conditions (Chambers & Waiwood, 1996; Miller et al., 1995;
102 Morimoto, 1998) possibly due to specific nutritional factors influencing egg size and
103 number (Chambers & Leggett, 1996; McBride et al., 2015). In particular, changes in
104 fish egg size in response to water temperature indicate clear phenotypic plasticity; egg
105 size increases as temperature decreases regardless whether a fish family produces
106 smaller or larger eggs (Laptikhovsky, 2006). Previous studies have reported the
107 relationship between water temperature and the egg size of reared chub mackerel and
108 wild Atlantic mackerel (*S. scombrus*) (Ware, 1977; Yoneda et al., 2022). If the water
109 temperature at the spawning site affects the egg size of wild chub mackerel and blue

mackerel, the identification criteria for these species may be dramatically improved by expressing egg size as a function of water temperature.

In this study, we aimed to analyze the relationship between the egg size of two mackerel species and the water temperature in the Kuroshio Current system to develop a new criterion for distinguishing between chub and blue mackerel eggs. We estimated the annual TEP of these mackerel species using the new criterion and compared it with that using the conventional criterion. In addition, we evaluated the application of this new criterion for studying the spawning ecology of mackerel and classifying other marine fish species. This new species identification criterion not only contributes to the study of the spawning ecology of marine fishes by understanding egg size variability but also to the sustainable use of fishery resources through the reasonable estimation of TEPs.

2. MATERIALS AND METHODS

2.1. Field sampling and egg size measurement

Ichthyoplankton surveys were conducted monthly to collect mackerel eggs along the Pacific coast of Japan from 2006 to 2021 (Figure 1). The sampling locations were mostly consistent; however, some were changed for reasons such as sea conditions. The surveys were conducted by the Japan Fisheries Research and Education Agency and the fisheries research institutes in 18 prefectures in Japan using the same procedure. To obtain the eggs of small pelagic fish, plankton nets (Long NORPAC) were vertically towed from approximately 150 m to the surface or from the seafloor to the surface when

the water depth was less than 150 m. The diameter of the mouth of the plankton nets was 0.45 m, and the mesh size was 0.335 mm. Specimens were immediately fixed in 5% formalin after sampling. Mackerel eggs were extracted from the specimens based on egg diameter, presence or absence of oil globules, pigmentation, and structure of the enclosed egg cavity (Ikeda et al., 2014). The diameter of some eggs was measured using a micrometer with 0.025-mm increments. Approximately 100 eggs per station were obtained; 37,304 eggs were measured in total. A more detailed description of the surveys has been provided previously (Takasuka et al., 2008; Takasuka et al. 2017).

2.2. Dividing egg size distributions

We constructed a frequency distribution of egg size for sea surface temperature (SST) ranges at 1 °C interval. The egg size distribution data were then divided into one or two groups using a Gaussian mixture model (GMM). The mean values of the probability density distribution of the GMM were used as the mean egg size for each group. The mean between two groups was used as the boundary for the discriminant criterion for the two mackerel species. If the one-group model was better than the two-group model in terms of the Bayesian information criterion (BIC) or the two-group model did not estimate any parameters, the egg size distribution was considered as unimodal. These data were not used because we could not determine whether a single group consisted of chub mackerel or blue mackerel. The GMMs were applied to the data using the "mclust" package (Scrucca et al., 2023) in the R software (R Core Team, 2023). It should be noted that the best model in the "mclust" package is the one with the highest BIC among the fitted models (Scrucca et al., 2023).

2.3. Species identification of the genus *Scomber* using species-specific primers

DNA analysis was applied to some specimens to validate the results of the GMM analysis. Additional ichthyoplankton surveys were conducted in February and March between 2020 and 2021. The 99 mackerel eggs collected from 13 stations were immediately fixed in 5% formalin for 1–14 days. Afterwards, the diameter of the eggs was measured. The eggs were then individually stored in 2-mL tubes containing 99% ethanol for DNA analysis. DNA was extracted from the eggs using 5% Chelex buffer (Chelex 100 Molecular Biology Grade Resin; Bio-Rad Laboratories, Hercules, CA, USA). Each egg was placed in a 1.5-mL plastic tube containing 100 μL of Chelex buffer and then crushed using a pellet pestle motor (Kontes Glass, Vineland, NJ, USA) for 30 sec. The tube was heated at 97 °C for 20 min. Species identification was performed using species-specific primers, designed by Satoshi Nagai, that target mitochondrial DNA. The specific primers for *S. japonicus* were SJ-F1 (5′-CTCCACACAAGATCGTACTTA-3′) and SJ-R1 (5′-GAAAATGTACGACCTTGTCAAC-3′), while those for *S. australasicus* were SA-F1 (5′-CACACAAAATCGCACCCG-3′) and SA-R1-2 (5′-GAAAATGTACGACCTTGTGAGT-3′). PCR was performed using a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) in a reaction mixture (10 μL) containing 1.0 μL of template DNA, 3 μL of distilled water, 5.0 μL of GoTaq Green Master Mix (Promega, Madison, WI, USA), and 0.5 μL of each primer (10 μM). The PCR cycling conditions were as follows: initial denaturation at 95 °C for 3 min, 95 °C for 30 s in each cycle of 28–30 cycles, 56 °C (*S. japonicus*)/ 59 °C (*S. australasicus*) for

30 s, and 72 °C for 30 s. PCR amplification was verified by performing agarose gel electrophoresis.

2.4. Total egg production (TEP)

The number of eggs per unit water column area (m^2) for each sampling tow was calculated from the number of flow meter revolutions, flow meter revolutions per meter tow during calibration, wire length (m), area of the net opening (m^2), and wire angle (Takasuka et al., 2008; Takasuka et al. 2017; Takasuka et al. 2021). The average egg density at the 30° latitude and 30° longitude horizontal squares was determined and multiplied by the sea area of the horizontal square to calculate the monthly TEP for each square (Figure 1). The sum of the monthly TEP for all squares was used as the TEP for each year. We compared the TEP of chub and blue mackerel calculated using the conventional criterion (Saito & Ikegami, 2010) with that using the new criterion developed in this study.

2.5. Morphological traits of planktonic eggs

To assess the applicability of the new species identification key for chub and blue mackerel eggs to other fish taxa, the main morphological characteristics of the planktonic eggs of marine fishes were summarized using a comprehensively illustrated catalogue of eggs in the western North Pacific (Ikeda et al., 2014). Seven morphological characteristics (egg shape and size, yolk and chorion characteristics, perivitelline space, and number and size of oil globules) were included in the egg identification key.

Although the presence or absence of a segmented yolk cannot be determined in formalin-fixed eggs, useful information that can be derived from a yolk segment even after fixation can be used as a trait of yolk characteristics. However, the morphological characteristics of embryos at advanced stages of development were not included. The traits described by Kuroda et al. (1982), such as the oblique thin film that appears in the perivitelline space after fixation, were also added. Of the 375 planktonic eggs (including the eggs of unknown family, genus, and species of fish) presented by Ikeda et al. (2014), we only used the morphological information of 367 species, excluding 8 species with missing information for 4 morphological traits other than the yolk characteristics. The eggs were then typed according to the combination of morphological traits, and the overlap in egg size was examined to the nearest 0.1 mm for each type. The egg sizes of the two mackerel species were determined based on the results of this study.

3. RESULTS

3.1. Collection of eggs

The number of eggs collected from 2006 to 2016 was approximately 1,000; it increased from 2017 to 2020, with the highest number of eggs (6,326) recorded in 2019 (Figure 2a). The egg size of all samples ranged from 0.9 to 1.35 mm (Figure 2b), with a median of 1.05 mm and skewed toward 1.1 mm or less. The SST at the sampling stations ranged from 9.9 °C to 25.5 °C, with a median of 19.0 °C (Figure 2c). The mean annual water temperature ranged from 18.1 °C to 20.0 °C (Table 1).

3.2. Gaussian mixture model

The BICs were higher in the two-group model than in the one-group model in the 15 ℃–25 °C SST range (Table 2).The model diagnostics showed there were no major problems in the model fittings (Figures S1). We could not obtain estimates from the two-group model at less than 15 ℃ (i.e., this model did not converge). In the two-group model, the estimated mean egg size in the 15 ℃–25 °C SST range was 0.960–1.060 mm in the small group and 1.104–1.275 mm in the large group (Table 3). The mean egg size of the two groups decreased as the SST increased. The mean values between the two groups ranged from 1.032 to 1.167 mm.

The frequency distribution of egg size revealed that the number of eggs collected in the small group at a given temperature range was greater than that in the large group (Figure 3) except at 24 °C and 25 °C. There was a significant negative relationship between the mean egg size and mean SST in both the small and large groups (Figure 4). These relationships are expressed as linear function equations. A similar relationship was observed for the intermediate values between the two groups. Hereafter, the regression line of the intermediate value was used as the discrimination criterion for the two mackerel species examined in this study. In the range of SSTs below 15 °C, wherein no estimates were available from the model, mackerel eggs were discriminated into two species by extrapolating the regression line. The annual mean egg size of the small group during the sampling years was 1.007–1.042 mm, while that of the large group was 1.134–1.232 mm (Table 4).

3.3. DNA barcode sequence analysis

Of the 99 mackerel eggs successfully amplified through PCR, most eggs were identified as belonging to chub mackerel (Table 5). The egg size of chub mackerel was 0.975–1.125 mm, while that of blue mackerel was 1.175–1.300 mm. At a given temperature, the actual size of the chub mackerel eggs was smaller than that estimated from the discrimination criterion model (see the preceding paragraph), whereas the actual size of the blue mackerel eggs was larger than that estimated from the model (Figure 5).

3.4. Total egg production

The conventional and new criteria did not significantly differ in terms of the TEP estimates for the small group (Figure 6a). However, there was a significant difference between the two criteria in terms of the TEP estimate for the large group in 2018; the estimate from the conventional criterion was greater than that from the new criterion (Figure 6b).

3.5. Morphological traits of planktonic eggs

The egg identification key developed in this study was used to classify the planktonic eggs of 367 fish species, which are typically found in the western North Pacific, into 50 types (Figure 7; Table S1). Thirty types consisted of only one species; meanwhile, the remaining 20 types consisted of more than two species, and the range of the egg size of the species usually overlapped with those of other species, such as type-21 and type-*Scomber* spp. However, some types include species whose egg size ranges did not

overlap with those of other species; type-8 is an example. The egg sizes of 320 species overlapped with those of other species within each type, whereas the egg sizes of 47 species did not overlap with those of other species.

4. DISCUSSION

The GMM used in this study successfully divided the frequency distribution of the egg size of *Scomber* spp. into two groups (Table 3; Figure 3): the small group may pertain to chub mackerel, while the large group may pertain to blue mackerel, as previously reported (Nishida et al., 2001). The results of the DNA analysis supported the results obtained from the GMM (Figure 5). By summarizing the information on marine fish eggs currently available in the western North Pacific, it was proven that this approach is applicable to other fish taxa (Figure 7; Table S1). For example, three closely related species of *Hippoglossoides* spp. (type 21) exhibited a similar case with *Scomber* spp. in this study. As the range of the egg size of *Hippoglossoides* spp. also partially overlapped between species, quantitative division methods for egg size frequency distribution would be required for accurate species identification. The division method using GMM may be a novel and powerful approach for the species identification of marine fish eggs; this method is also applicable to fishes from other regions.

Incorporating the effects of water temperature on egg size is a novel approach for the species identification of marine fish eggs. The development of accurate and convenient species identification methods for planktonic marine fish eggs is required for estimating the TEP of several exploited fishes (Gonçalves et al., 2013). Although

287 several exhaustive atlases have been published for oceanic regions around the world as
288 references for the species identification of planktonic marine fish eggs (e.g., Fahay,
289 2007; Ikeda et al., 2014; Matarese et al., 1989; Moser, 1996; Richards, 2006), the
290 number of species listed in these atlases is far less than the actual number of fish species
291 (Kaschner et al., 2019). For example, there are at least 4,000 species of marine fishes in
292 the western North Pacific (Nakabo & Nakayama, 2013), several of which produce
293 planktonic eggs (Ikeda et al., 2014). However, as summarized in this study (Figure 7;
294 Table S1), only 384 species, including unidentified species, have known egg traits.
295 Furthermore, only 26 of these species have 'fish eggs that can be fixed and still be
296 identified to the species level'. Perhaps, there are several fish species whose egg traits
297 have not been documented yet in the eastern North Pacific (Matarese et al., 1989;
298 Moser, 1996) and western North Atlantic (Fahay, 2007; Richards, 2006). The species
299 identification of planktonic marine fish eggs is difficult because there are few egg
300 identification keys available. The results of this study revealed that *Scomber* eggs in the
301 western North Pacific can be identified using the conventional egg identification keys;
302 however, species identification can only be accurate by incorporating the effect of water
303 temperature on egg size. Although water temperature is an important factor for fish
304 ecology, it has rarely been considered for the species identification of marine fish eggs.
305 Our results revealed that the relationship between water temperature and egg size
306 provides useful information for the species identification of planktonic fish egg.

307 There are two possible reasons for the negative correlation between the egg size
308 of mackerel and water temperature. First, temperature affects egg size in individual
309 females and age groups (Yoneda et al., 2022), as has been found in several fish species

(Chambers & Waiwood, 1996), due to the physiological response of female fish to temperature; the temperature during vitellogenesis influences the size of eggs produced by female fish (Laptikhovsky, 2006; Yoneda et al., 2014). Secondly, there appears to be a difference in the timing of spawning onset in relation to body size, age, and/or spawning experience of fish (Watanabe & Yatsu, 2006; Watanabe, 2010). Large, old, and repeat spawners start spawning earlier than young, small, and first-time spawners. Because first-time chub mackerel spawners produce smaller eggs than repeat spawners even at high temperatures (Yoneda et al., 2022), the egg size of the spawning population may decrease in warmer seasons in association with increased spawning by first-time females later (warmer) in the season. Although the spawning strategy of blue mackerel is currently unclear, it may be reasonable to consider the effect of water temperature on egg size to distinguish between the eggs of these two mackerel species.

The new discrimination criterion used in this study was more practical than the conventional criterion. According to the stock assessment report, the biomass and catch of blue mackerel continuously decreased from 2010 to 2021 (Yukami et al., 2022a). The TEP estimated using the new criterion (hereafter referred to as the new TEP) showed a continuous decreasing trend between 2010 and 2021, similar to the stock assessment report (Figure 6). However, the TEP estimated using the conventional criterion (hereafter referred to as the conventional TEP) increased instantaneously in 2018. The rapid increase in the conventional TEP may have been caused by species misidentification of eggs (Kanamori et al., 2021). Because the TEP of blue mackerel was considerably lower than that of chub mackerel, the impact of species misidentification of eggs on TEP estimation would be greater for blue mackerel. The

333 new criterion used in this study allowed the quantitative consideration of egg size
334 variability in relation to water temperature and the estimation of reasonable TEPs.
335 Therefore, we recommend the use of this new criterion to estimate the TEPs of chub and
336 blue mackerel in the western North Pacific. However, future studies are required to
337 clarify when, where, and how SSTs affect the egg size of these two mackerel species,
338 particularly in 2018.

339 Chub mackerel is widely distributed in the North Pacific, including the Kuroshio
340 Current region (this study) and California coast, whereas blue mackerel is mainly
341 distributed from the Australian coast to New Zealand and off the Pacific coast of
342 Mexico (Collette & Nauen, 1983; Nakabo & Doiuchi, 2013). Although the main
343 distribution areas of the two mackerel species are far apart, there are some overlaps.
344 Although the available data on egg size and SST in other areas is limited for both
345 species, the egg size of chub mackerel is 1.06−1.14 mm and the SST is 14.0−21.0 °C in
346 the eastern North Pacific (Kramer, 1960), while that of blue mackerel is 1.05−1.3 mm
347 and the SST is 15.8−22.8 °C in southeastern Australia (Neira & Keane, 2008). These
348 data are comparable with those obtained in the Kuroshio Current region: egg size of
349 0.875−1.15 mm and 1.05−1.35 mm and SST of 9.9−25.5 °C and 10.9−25.3 °C for chub
350 mackerel and blue mackerel, respectively (Figure 5; Table 4). This finding suggests that
351 the new discrimination criteria developed in this study can also be applied to other
352 habitats. The Atlantic mackerel (*S. scombrus*) and Atlantic chub mackerel (*S. colias*) are
353 distributed in the North Atlantic Ocean and have similar egg morphologies. The egg
354 diameter of the Atlantic mackerel is 1.0−1.38 mm (Rodríguez et al., 2017; Russell,
355 1976), while that of the Atlantic chub mackerel is 1.04−1.14 mm (Rodríguez et al.,

2017). In the eastern North Atlantic, a species that is easily confused with *S. scombrus* is the European hake *Merluccius merluccius* (Hofmann et al., 2017; Russell, 1976). The methods used in this study may contribute to improving the accuracy of the identification of other *Scomber* species.

This study had some limitations. First, a small sample size of blue mackerel was used for the DNA analysis because of its low stock biomass. Therefore, obtaining samples at high temperatures is particularly important. Second, spatial and temporal variations in egg size were unclear in this study. By addressing spatiotemporal variations in egg size in future studies, our understanding of the association between egg size, population status (e.g., age and size structures of maternal individuals), and environmental factors may be improved. Third, it was unclear at which depths mackerel eggs were distributed in the collection depth range (i.e., 0–150 m) in the present study. Gaining more knowledge on the vertical distribution of adult fish and their eggs (e.g., Yasuda et al., 2023) would improve our understanding of the relationship between temperature and egg size.

In conclusion, the new species identification approach that incorporates the effects of temperature on the egg size of chub and blue mackerel was effective at distinguishing between the eggs of these two mackerel species. Moreover, the TEP estimated using the new species identification key was more accurate than that calculated using the conventional identification key. This approach not only contributes to the study of the spawning ecology of marine fishes by understanding egg size variability but also to the sustainable use of fish resources through the reasonable

estimation of TEPs. We also summarize the currently available information on fish eggs and suggest that the proposed approach could potentially be applied to other fish taxa.

AUTHOR CONTRIBUTIONS

MW, TY, MN, AT and MY designed the study. SN, MN, and MW collected the DNA samples and conducted the analysis. MW, JK, and TY analyzed the data. MW and TY wrote the original manuscript. All authors contributed to manuscript editing. All the authors have read and agreed to the published version of this manuscript.

ACKNOWLEDGEMENTS

The wide-area and long-term data used in this study were obtained from spawning surveys conducted by the fisheries research institutes in Aomori, Iwate, Miyagi, Fukushima, Ibaraki, Chiba, Tokyo, Kanagawa, Shizuoka, Aichi, Mie, Wakayama, Tokushima, Kochi, Ehime, Oita, and Miyazaki Prefectures, and by the Fisheries Resources Institute (formerly the National Research Institute of Fisheries Science), Japan Fisheries Research and Education Agency (FRA). We would like to express our deepest gratitude to the staff at the research institutes and the senior researchers at the FRA who conducted the survey. The spawning surveys were conducted as a part of the Promotion Program for the Marine Fisheries Stock Assessment and Evaluation for Japanese Waters (Fisheries Agency). This study was also supported by Challenging Research (Pioneering) (KAKENHI No. 20K20455) from the Japan Society for the Promotion of Science.

DATA AVAILABILITY STATEMENT

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

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TABLES

Table 1. Sample sizes of *Scomber* spp. eggs collected in the western North Pacific from 2006 to 2021. The total number of stations (i.e., tows or stations) are listed for all the sampling stations (all) and the positive stations where the eggs were captured (positive). In addition, the mean sea surface temperature (SST) and standard deviation (SD) at the station where the eggs were collected are listed.

Year	Station		SST	
	All	Positive	Mean (°C)	SD
2006	6,052	289 (4.8 %)	19.6	2.1
2007	5,749	243 (4.2 %)	19.8	1.9
2008	6,173	124 (2.0 %)	19.5	1.8
2009	6,328	178 (2.8 %)	19.8	1.7
2010	6,230	284 (4.6 %)	20.0	1.8
2011	6,066	271 (4.5 %)	18.2	2.1
2012	6,000	278 (4.6 %)	19.2	1.7
2013	6,222	247 (4.0 %)	19.2	1.7
2014	6,043	294 (4.9 %)	18.4	1.7
2015	6,431	270 (4.2 %)	18.7	2.0
2016	7,372	292 (4.0 %)	19.3	2.0
2017	7,199	391 (5.4 %)	18.1	1.7
2018	7,391	513 (6.9 %)	18.9	1.4
2019	8,335	454 (5.4 %)	19.4	1.8
2020	7,801	435 (5.6 %)	18.7	1.3
2021	8,268	349 (4.2 %)	18.5	1.0

Table 2. Bayesian information criterion (BIC) of the Gaussian mixture models applied to the size composition analysis of mackerel eggs for each sea surface temperature (SST) range. The values in bold font indicates the highest BIC model. ΔBIC indicates the difference between each model. It is note that the best model in the "mclust" package is the one with the highest BIC among the fitted models (Scrucca et al., 2023).

SST range (°C)	n	One-group model			Two-group model			
		log-likelihood	df	BIC	log-likelihood	df	BIC	ΔBIC
15 >	218	349	2	**687**	-	-	-	-
15 ≤	731	1031	2	2049	1275	5	**2518**	469
16 ≤	2772	4040	2	8065	4757	5	**9474**	1409
17 ≤	5861	8945	2	17872	9734	5	**19424**	1552
18 ≤	9680	14140	2	28262	15519	5	**30992**	2730
19 ≤	8797	11516	2	23015	12972	5	**25899**	2884
20 ≤	4791	5722	2	11427	6781	5	**13520**	2093
21 ≤	2366	2654	2	5292	3163	5	**6287**	995
22 ≤	1320	1927	2	3840	2040	5	**4044**	205
23 ≤	273	392	2	773	434	5	**841**	67
24 ≤	227	249	2	487	280	5	**533**	46
25 ≤	268	321	2	631	370	5	**711**	80

620 Table 3. Mean egg diameter, variance, and mixing probability for the small and large groups
621 estimated by Gaussian mixture model, mean egg diameter between the two groups, and mean
622 sea surface temperature (SST) for each SST range.
623

SST range (°C)	Mean SST (°C)	Egg diameter						
		Small group			Large group			Mean of the two groups (mm)
		Mean (mm)	Variance	Mixing probability	Mean (mm)	Variance	Mixing probability	
15 >	13.8	-	-	-	-	-	-	-
15 ≤	15.5	1.060	0.001	0.947	1.275	0.001	0.053	1.167
16 ≤	16.5	1.053	0.001	0.931	1.244	0.001	0.069	1.148
17 ≤	17.5	1.044	0.002	0.968	1.236	0.002	0.032	1.140
18 ≤	18.5	1.040	0.002	0.953	1.222	0.002	0.047	1.131
19 ≤	19.4	1.031	0.002	0.915	1.209	0.002	0.085	1.120
20 ≤	20.4	1.018	0.002	0.891	1.210	0.002	0.109	1.114
21 ≤	21.4	1.008	0.002	0.864	1.198	0.003	0.136	1.103
22 ≤	22.3	0.997	0.002	0.971	1.184	0.004	0.029	1.091
23 ≤	23.3	0.974	0.002	0.925	1.134	0.002	0.075	1.054
24 ≤	24.5	0.966	0.001	0.490	1.107	0.002	0.510	1.037
25 ≤	25.3	0.960	0.001	0.260	1.104	0.002	0.740	1.032

624
625

626 Table 4. Mean egg diameter of the small and large groups estimated using the sea surface
627 temperature (SST) function and annual mean SST for the two groups.

Year	Small group						Large group					
	n	Egg diameter (mm)			SST (°C)		n	Egg diameter (mm)			SST (°C)	
		Min.	Mean	Max.	Mean	SD		Min.	Mean	Max.	Mean	SD
2006	965	0.875	1.009	1.125	19.6	2.06	22	1.100	1.180	1.275	20.2	1.62
2007	1256	0.900	1.019	1.125	19.9	1.99	228	1.075	1.212	1.325	19.4	1.35
2008	434	0.900	1.026	1.125	19.7	1.61	157	1.100	1.232	1.325	19.1	2.10
2009	514	0.900	1.011	1.150	19.7	1.79	88	1.100	1.230	1.325	20.1	1.26
2010	1137	0.900	1.007	1.150	20.1	1.89	170	1.075	1.190	1.300	19.7	1.47
2011	1186	0.900	1.042	1.150	17.9	2.11	412	1.075	1.218	1.325	19.0	1.68
2012	2264	0.875	1.025	1.125	19.6	1.71	180	1.100	1.203	1.300	19.8	1.49
2013	2167	0.900	1.022	1.200	19.2	1.72	422	1.075	1.196	1.325	19.3	1.38
2014	1380	0.900	1.025	1.150	18.2	1.67	305	1.100	1.223	1.350	19.1	1.43
2015	1680	0.900	1.031	1.125	18.7	1.45	104	1.100	1.217	1.325	19.0	1.60
2016	1540	0.900	1.028	1.150	18.9	1.57	244	1.050	1.167	1.300	22.3	2.08
2017	3765	0.900	1.040	1.150	18.1	1.65	95	1.075	1.198	1.325	19.2	1.64
2018	5139	0.875	1.041	1.150	18.9	1.36	186	1.100	1.176	1.325	19.1	1.05
2019	5960	0.900	1.037	1.150	19.2	1.43	365	1.050	1.134	1.325	22.9	2.83
2020	3061	0.900	1.029	1.125	18.6	1.14	227	1.050	1.186	1.300	19.5	0.89
2021	1602	0.900	1.039	1.125	18.6	1.19	49	1.125	1.180	1.300	19.40	2.10

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630

631 Table 5. Date of sample collection in 2020 and 2021, egg diameter of each species of DNA-identified mackerel eggs [sample size (n), mean, and
632 standard deviation (SD)], and sea surface temperature (SST, mean, and SD).
633

Date	*Scomber japonicus*					*Scomber australasicus*				
	Egg diameter (mm)			SST (°C)		Egg diameter (mm)			SST (°C)	
	n	Mean	SD	Mean	SD	n	Mean	SD	Mean	SD
2020/2/24−2020/3/6	92	1.051	0.034	17.8	0.66	2	1.24	0.09	18.5	0.21
2021/2/24−2021/2/24	5	1.015	0.038	19.6	0.53	0	-	-	-	-

634

FIGURE LEGENDS

Figure 1. Egg survey points from 2006 to 2021. Gray represents the survey points where no mackerel eggs were obtained; blue represents the survey points where mackerel eggs were obtained. This figure includes the survey points for all years shown in Table 1.

Figure 2. Annual number of eggs measured (a), histogram of egg diameter (b), and histogram of sea surface temperature in the sampling station (c) of the mackerel eggs examined in this study.

Figure 3. Egg diameter distribution of the mackerel species by sea surface temperature (SST) range from 2006 to 2021 and the establishment of two density distributions estimated using the Gaussian mixture model. The histograms represent the number of eggs per diameter. The red and blue lines are the estimated curves for the small and large groups, respectively. The dotted line represents the mean of each group, and the black dotted line represents the mean of the two groups.

Figure 4. Relationship between the mean sea surface temperature (SST) and egg diameter estimated using the Gaussian mixture model. Different colors indicate different groups (red: small group, blue: large group, and black: the intermediate between the two groups). Lines represent the mean values of the linear regressions for the small group ($y = 1.23424 - 0.01080x$, $n = 11$, R^2 adjusted = 0.9579, $t = -20.91$, $p < 0.001$), large group ($y = 1.53390 - 0.01668x$, $n = 11$, R^2 adjusted = 0.9776, $t = -11.48$, $p < 0.001$), and the intermediate between two groups ($y = 1.38407 - 0.01374x$, $n = 11$, R^2 adjusted = 0.9290, $t = -15.12$, $p < 0.001$). The gray areas indicate 95% confidence intervals for each linear regression.

Figure 5. Relationship between the egg diameter of mackerels and the sea surface temperature in the survey points. The regression line is the boundary for classifying the two groups developed using the Gaussian mixture model (see text, and the same of intermediate line between two groups from Figure 4). The black points indicate mackerel eggs (n = 37,304) whose species are unidentified. The red and blue points represent the chub (n = 97) and blue (n = 2) mackerel eggs identified through DNA analysis.

Figure 6. Comparison of the annual total egg production (TEP) estimated from the different criteria for the small (a) and large (b) groups. The broken and solid lines show the

TEP estimated using the new criterion developed in this study and that using the conventional criterion, respectively.

Figure 7. Morphological characters of planktonic eggs in the coastal areas of Japan (modified from Ikeda et al., 2014). For each type with the same morphological characteristics, the number of species and traits (egg shape, chorion characteristics, perivitelline space, yolk characteristics, embryo characteristics, and number of oil globules), egg diameter, and oil globule diameter are shown. The egg diameters of *Scomber* spp. indicated by asterisk are the results of the present study. X is shown in the appropriate trait columns and, in some cases, the following traits are indicated by alphabet letters. Perivitelline space characteristics: thin film inside (a) (Kuroda et al., 1982). Oil globules: distinct coloration [pink (b), golden (c), colorless, or yellow (d)], present in large numbers (e, more than 50). Chorion characteristics: hexagonal sculpturing (f), small spines scattered in part or over entire surface (g), degenerating filaments (h), chorion with a double structure (i), distinct coloration (j), protrusions like verrucae (k), triple-bladed projections (l, more than 200 on hemispherical surface), triple-bladed projections (m, less than 100 on hemispherical surface), thick shell (n), gelatinous or triangular protrusions over entire surface (o), pustulate inner surface (p), and smooth (q) (Ikeda et al., 2014). See Table S1 for the species included in each type.

Figure S1. Cumulative distribution function (a) and quantile-quantile plots (b) showing the estimated egg diameter from the two-group model.