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Molecular and Morphological Identification and Seasonal Distribution of Eggs of Four *Decapterus* Fish Species in the Northern South China Sea: A Key to Conservation of Spawning Ground

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Although species of *Decapterus* form important pelagic fisheries in the northern South China Sea, information on their spawning grounds is limited because identification of fish eggs based on their morphology is difficult. We identify eggs of four *Decapterus* species (*D. macrosoma, D. maruadsi, D. macarellus*, and *D. tabl*) with DNA barcodes from the fishery resources surveys in spring and autumn 2018 in Xisha islands, and spring and later summer–autumn 2019 along the continental shelf of the northern South China Sea, and describe egg morphology. Of 1405 fish eggs with obtained cytochrome c oxidase subunit I (COI) sequences, 81 were successfully attributed to four *Decapterus* species; eggs of each are spherical, have a smooth chorion and narrow perivitelline space, and can be partly differentiated by diameter, melanophore drops on the oil globule, and the notum of the embryo. Seasonal distributions of eggs reveal spawning grounds, with that of *D. maruadsi* located mainly off the Pearl River estuary in spring; eggs of *D. maruadsi* rarely co-occur with those of *D. macrosoma*. Spawning grounds of *D. macrosoma* are probably further south, where water temperature and salinity are higher. Spawning periods of these four *Decapterus* species overlap slightly. Spawning habitat of *D. maruadsi* has been lost, and the spawning season of *D. macrosoma* has extended. Identification of *Decapterus* eggs using DNA barcodes can assist with the identification of eggs using traditional morphological approaches. Spatial and temporal information on the distributions of *Decapterus* eggs can be used for improved conservation of spawning grounds and fisheries management in the northern South China Sea.

Keywords: fish eggs, DNA barcode, morphology, *Decapterus*, spawning ground, northern South China Sea

INTRODUCTION

Pelagic fish play important roles in marine ecosystems, occupy a large proportion of commercially important global fisheries, and are considered increasingly important as a source of protein (Lainez del Pozo, 2013; Food and Agricultural Organization [FAO], 2019). However, being r-strategists, their stocks can vary considerably within and between years and decades, related to changes in climate and oceanography, and physical–biological process at regional and global scales (Checkley et al., 2009; Kawakami et al., 2010; Watson et al., 2018; Ma et al., 2019). Understanding early life stage recruitment is therefore important in fisheries management (Chambers and Trippel, 1997; Fuiman, 2002; Checkley et al., 2009).

Scads in the genus *Decapterus* are commercially important pelagic fish. Of these, the small Japanese scad *D. maruadsi* is commercially important in China and, from 1998 to 2006, was the first number catch in the northern South China Sea (SCS). However, the average catch of this species decreased by 28%, relegating it to the third number catch in 2007–2018, with catch varying considerably between years (China Agriculture Press), 1997–2019). While *D. maruadsi* is caught mainly by pair trawlers, and single bottom trawl and light purse seine nets, two other species (in predominantly subtropical–tropical waters), Shortfin scad *D. macrosoma* and Mackerel scad *D. macarellus*, are targeted by light purse seine and light falling nets (Yang et al., 2009; Li et al., 2018). A fourth species, Roughear scad *D. tabl*, found on the edge of the continental shelf from 200 to 360 m, is uncommon (Froese and Pauly, 2010).

The northern SCS continental shelf is an important spawning and nursery ground for various commercial fishes, such as jack mackerel, sardines, and Japanese scad (Zhang et al., 1985). Recent progress in understanding the biology and ecology of early life history stages and recruitment processes of these pelagic species has been made (Xie and Watanabe, 2007; Kasai et al., 2008; Nishiyama et al., 2014; Sciascia et al., 2018). However, due to difficulties identifying fish eggs and larvae, information on the early life stages of these species, especially their spawning grounds based on ichthyoplankton surveys in the SCS, is limited (Zhang et al., 1985; Zhou et al., 2011; Li et al., 2014; Chen et al., 2018).

Accurate identification of fish eggs based on morphology can be difficult and time-consuming, especially when dealing with samples containing eggs of many species at various stages of development. This is further aggravated by fish adapting to oceanic conditions, changing their reproduction and egg morphology, such as incubation time, egg diameter, pigmentation, and spawning season duration (Neira et al., 2015). Fish egg identification is further complicated by many characters used to distinguish taxa being evident only during later developmental stages, mainly after the sarcomere period, leaving eggs of even unrelated species sometimes morphologically indistinguishable (Shao et al., 2002; Ikeda et al., 2014).

DNA barcoding using molecular markers [e.g., cytochrome c oxidase subunit I (COI)] enables accurate identification of fish eggs and larvae, regardless of developmental stage or morphology (Valdez-Moreno et al., 2010; Frantine-Silva et al., 2015; Hubert et al., 2015). Considerable sequence data for fish taxa are deposited in the BOLD (Barcode of Life Data System[1]) database; by early 2020, more than 19,000 fish species based on over 260,000 specimens were reported (Ratnasingham and Hebert, 2007). These data enable discrimination of adults and larvae of many fish species.

We use DNA barcodes to discriminate eggs of *D. maruadsi*, *D. macarellus*, *D. macrosoma*, and *D. tabl* from the northern SCS. We aim to (i) differentiate the eggs of these *Decapterus* species using DNA barcodes and describe their morphology, (ii) determine the main spawning grounds and reproductive periods of *Decapterus* species in the northern SCS, and (iii) appraise the value of information on fish reproduction and recruitment in sustainable exploitation and management.

MATERIALS AND METHODS

Sample Collection

Fish eggs were collected during the austral spring (April–May) and later summer–autumn in the northern SCS and Xisha islands (**Figure 1**). In all, 85 stations were surveyed, including 84 stations in the Guangdong coastal sea area (19.12–23.15°N, 110.7–117.21°E) in April and September–October 2019 and 1 station off the Xisha islands (16.98°N, 112.28°E) in May and September 2018. Eggs were collected with a 2.7-m-long, 80-cm-diameter zooplankton net, with a 0.505-mm mesh and a cod-end container mesh of 400 μm. Nets were fitted with a General Oceanic flowmeter for estimating filtered water volume. In horizontal trawls, nets were dragged 10–15 min at 1.5–2.2 knots. Samples from each station were fixed in ∼75% ethanol/seawater solution.

Sample Photographs

All eggs from each station were sorted beneath a stereomicroscope in a laboratory; up to 15 eggs were randomly selected (if more than 15 eggs at a station, or all eggs if less) and photographed. Alcohol-preserved eggs were rehydrated in hydrogen peroxide for ∼8 min for cleaning and to better measure egg diameter (to the nearest 0.001 mm). Eggs were photographed using a Zeiss microscope (Axioplan 2 imaging E) and numbered, and their DNA was subsequently extracted (**Figure 2**).

DNA Extraction and PCR Amplification

Each numbered egg was firstly put in a cleaned centrifuge tube, dried in the air and volatilized alcohol, and then quickly punctured by a fine needle within 50 s. Total genomic DNA was then extracted using an Axygen Genomic DNA Miniprep Kit (Axygen, Shanghai, China). COI sequences (∼648 bp) were amplified using the universal primers FishF1 and FishR1 (Ward et al., 2005). The polymerase chain reaction (PCR) contained 20 μl of Tsingke TM Master Mix, 1 μl of each primer (10 pmol), 1–10 μl of template DNA and 8–17 μl of ddH_2O (total template DNA + ddH_2O = 18 μl), to make a total volume of 40 μl. PCR conditions were 94°C for

[1]http://www.barcodinglife.org and http://www.boldsystems.org

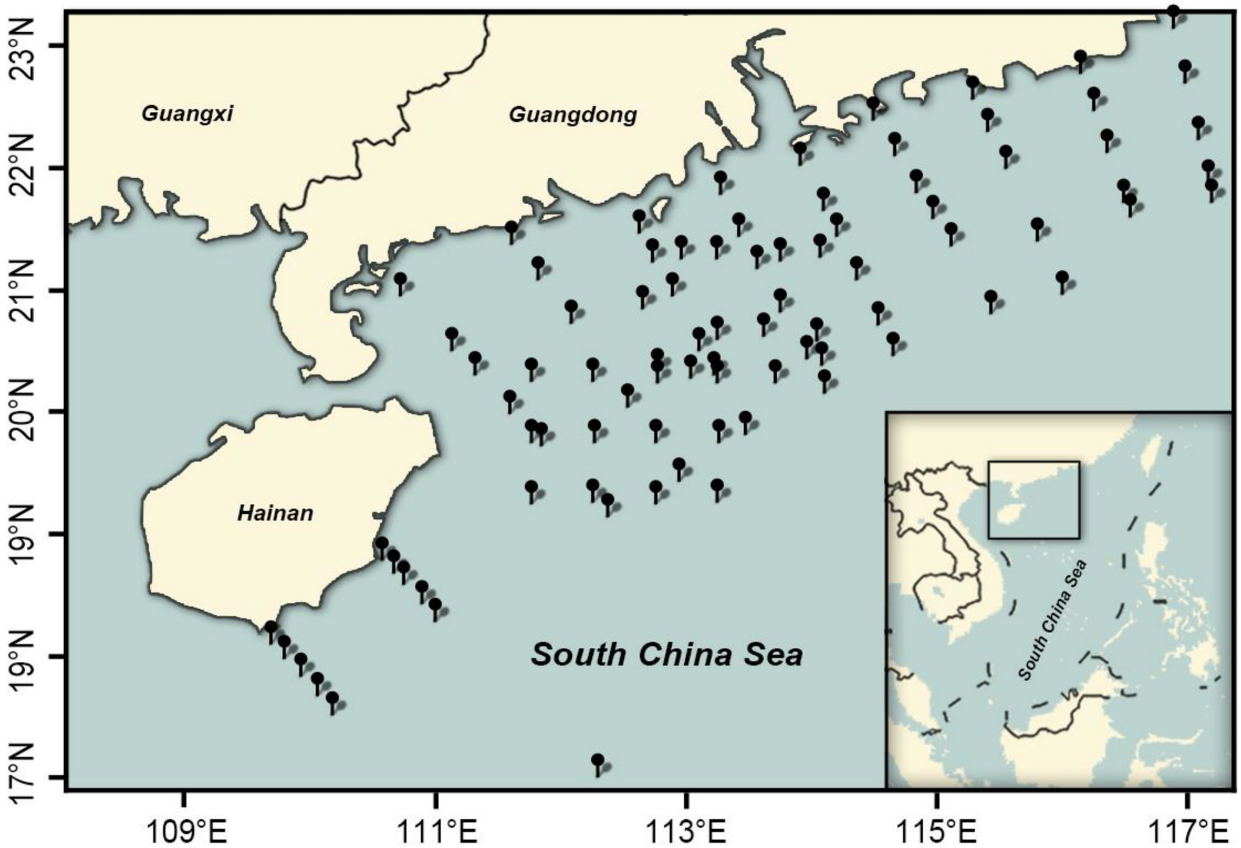

FIGURE 1 | Sampling location in the northern South China Sea.

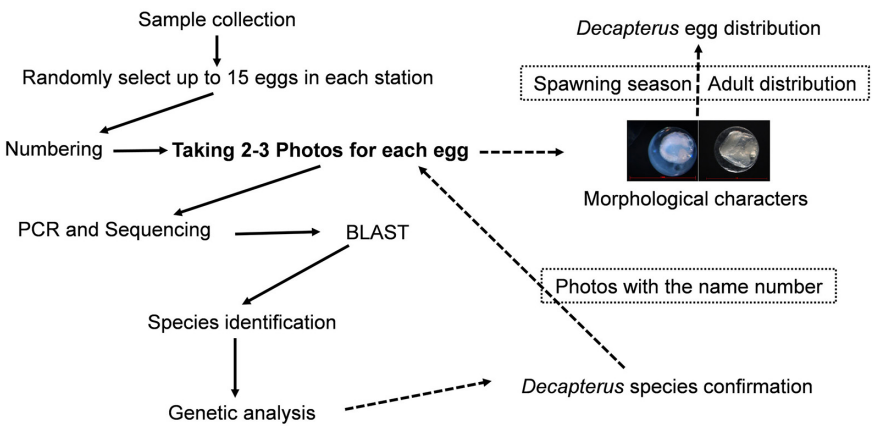

FIGURE 2 | Research workflow.

3 min, 35 cycles at 94°C for 30 s, 51°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min. Amplification products of PCR reactions were purified using 1% low-melting agarose by electrophoresis, and sequenced bidirectionally on an ABI 3730 XL DNA system following manufacturer protocols. High-quality DNA sequences were edited with MEGA v6.0 (Tamura et al., 2013).

Sequence Analysis

Egg sequences were initially differentiated by Blast search in BOLD (the Barcode of life Data System, BOLD)[2]. Sequences exceeding 98% similarity and 2% divergence threshold with the nearest neighbor were tagged with species name following

[2]http://www.boldsystems.org/

the criteria of Hubert et al. (2015). The DNA barcode library under the project "South China Sea Fishes" in BOLD comprised six locally and proximally occurring *Decapterus* species; it was used in combination with BOLD to confirm egg species when aforementioned criteria were not met, using *Selar crumenophthalmus* as an outgroup in tree-based delimitation (Jaafar et al., 2012; Chang et al., 2017; Hou et al., 2018). Available *Decapterus* COI sequences were downloaded from Genbank[3] (8 February 2020), combined with our *Decapterus* egg sequences and submitted to statistical parsimony networks for haplotype network construction (Templeton et al., 1992) using the default 95% connection limit in TCS 1.2 (Clement et al., 2000).

Egg Morphology

Images of identified *Decapterus* eggs were used to describe their morphology following Shao et al. (2001) and Ikeda et al. (2014).

Egg Distribution

Catch rates of *Decapterus* eggs for each station are presented as numbers of eggs per 100 m^3 of filtered water (flowmeter data). Depth (D), surface sea temperature (SST), and salinity (S) were measured and analyzed under the guidance of "Specification of Oceanographic Investigation" (GB12763-2007). The distribution and egg catch rates at stations with *Decapterus* spp. were plotted using Golden Software Surfer (version 15.0, Golden Software Inc., Golden, CO, United States).

RESULTS

Fish Egg Identification by DNA Barcode

A total of 81 *Decapterus* eggs were screened from 1,405 successfully amplified samples (referable to 120 fish species). All sequences of *Decapterus* eggs were of high quality, with no evidence of insertions/deletions, heterozygous sites, or stop codons; each has been uploaded to NCBI (Genbank accession numbers: MT609955–MT610035). The 81 sequences were 619 nucleotides in length after alignment and trimming of noisy sites; a BOLD Blast search identified four candidate species belonging to four species of genus *Decapterus* (based on 98% similarity and 2% of genetic divergence among species); all egg sequences reached a match of similarity 98–100%, with matching results for the nearest neighbor ranging similarity 90.78–100%. Only four sequences fitted the criterion and were identified as *D. tabl* or *D. macarellus*; the remaining 77 sequences were 98–100%, similar to both the best match and nearest neighbor, with the genetic divergence from the nearest neighbor less than 2% (indicating that 95% of the samples could not be unambiguously assigned to species directly using Blast in Bold; Supporting information S1). Thus, haplotype analysis and genetic distance were conducted for the downloaded COI sequences of genus *Decapterus* from the Bold system and Genbank, to detect if there is haplotype sharing with low genetic divergence among them (**Supplementary Table S2**). Five groups, i.e., *D. macrosoma* and *D. maruadsi*, *D. kurroides,* and *D. maruadsi*, etc., were detected to likely share the COI haplotype but with high genetic divergence (>2%), which indicated that they are not really sharing haplotypes but with wrong identification or mismatch species name among the sequences (**Figure 3** and **Supplementary Table S3**). The tree-based method was then used to delimit the fish eggs with local DNA barcode library, which revealed six species clusters in genus *Decapterus* supported by bootstrap values of 100%, attributing 61 sequences to *D. macrosoma*, 16 sequences to *D. maruadsi*, 2 sequences to *D. macarellus*, and 2 sequences to *D. tabl* (**Figure 4**).

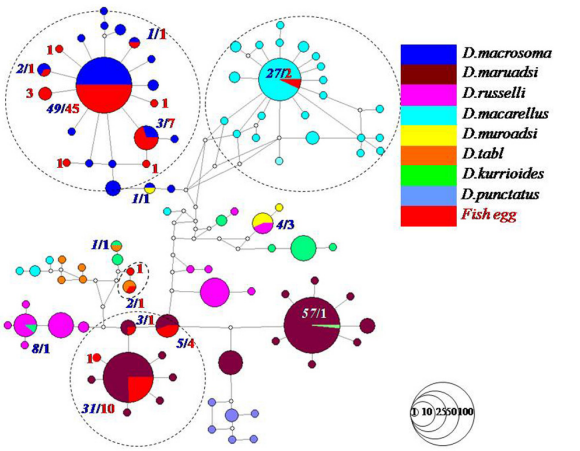

FIGURE 3 | *Decapterus* spp. egg haplotype network analysis combined with downloaded sequences from NCBI. Red numbers in bold and italic indicated fish egg sequences, and blue and white numbers in bold and italic indicated downloaded fish sequences. Circles represent haplotypes, and each circle is proportional to the number of occurrences of the corresponding haplotype. The dotted circles contained the eggs and the downloaded sequences of four *Decapterus* fish species.

Decapterus Egg Morphology

Eggs of all four *Decapterus* species are spherical, with a smooth chorion and diameters ranging 0.60–0.86 mm; the smallest is *D. maruadsi* (n = 16; mean ± SE = 0.75 ± 0.06), and the largest is *D. macarellus* (n = 2; mean ± SE = 0.81 ± 0.01) (**Figure 5**). All eggs have a narrow perivitelline space, with that of *D. maruadsi* widest after the myotome stage (**Figure 6**). Yolk segmentation was not observed in alcohol-preserved eggs.

Oil globules occurred in the rear position of each species and were numerous in *D. maruadsi* and *D. tabl*. Some *D. maruadsi* eggs had a single large oil globule (0.16 mm) and three to five smaller oil globules (0.030–0.039 mm), with their number dependent on developmental stage. Eggs of *D. tabl* had one large oil globule (0.18 mm) and three small oil globules (0.065–0.118 mm). Oil globules in *D. macrosoma* are orange colored, while in the other three species, they are transparent. Melanophore drops occurred on the large oil globule of both *D. maruadsi* and particularly *D. macrosoma*; no melanophore drops were found in *D. macarellus* or *D. tabl*. The myotome stage was pigmented in each species. Melanophore drops were widely scattered on the embryo notum from the cephalosome to the tail sarcomere, more densely in *D. macrosoma* (**Table 1** and **Figure 6**).

[3]https://www.ncbi.nlm.nih.gov/genbank/

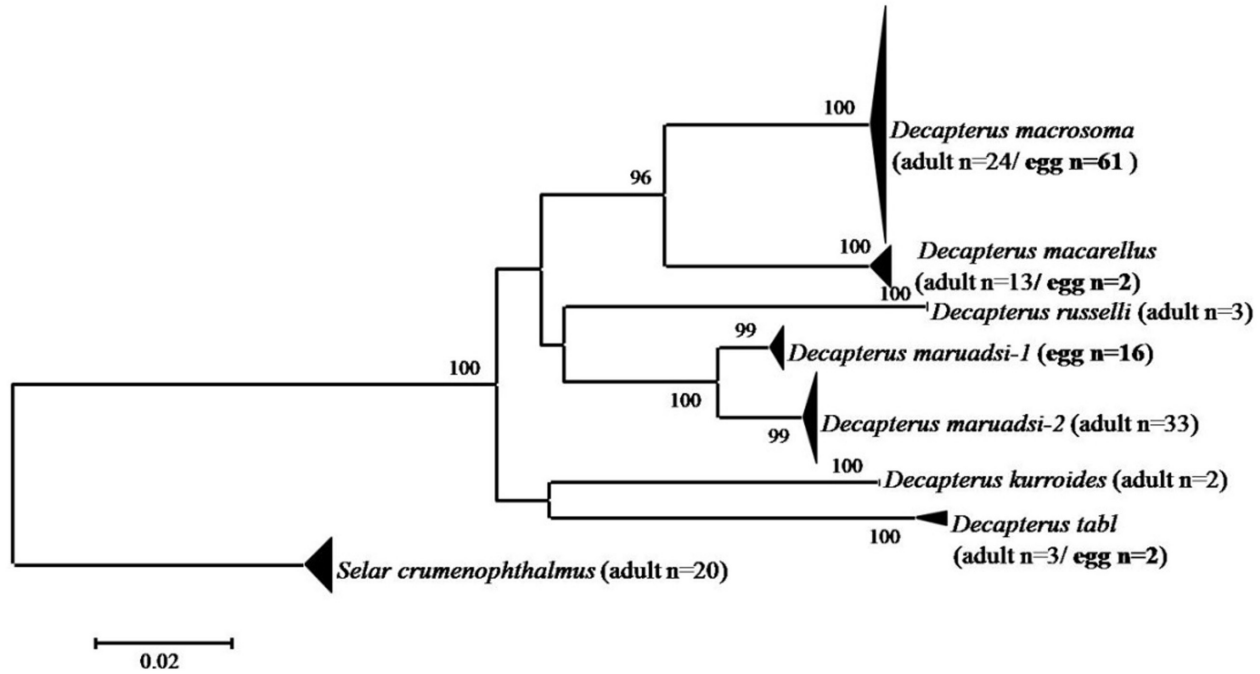

FIGURE 4 | Neighbor-joining tree of fish egg sequences combined with local DNA barcode library of *Decapterus* species. The numbers at the nodes are bootstrap values based on 1,000 replications.

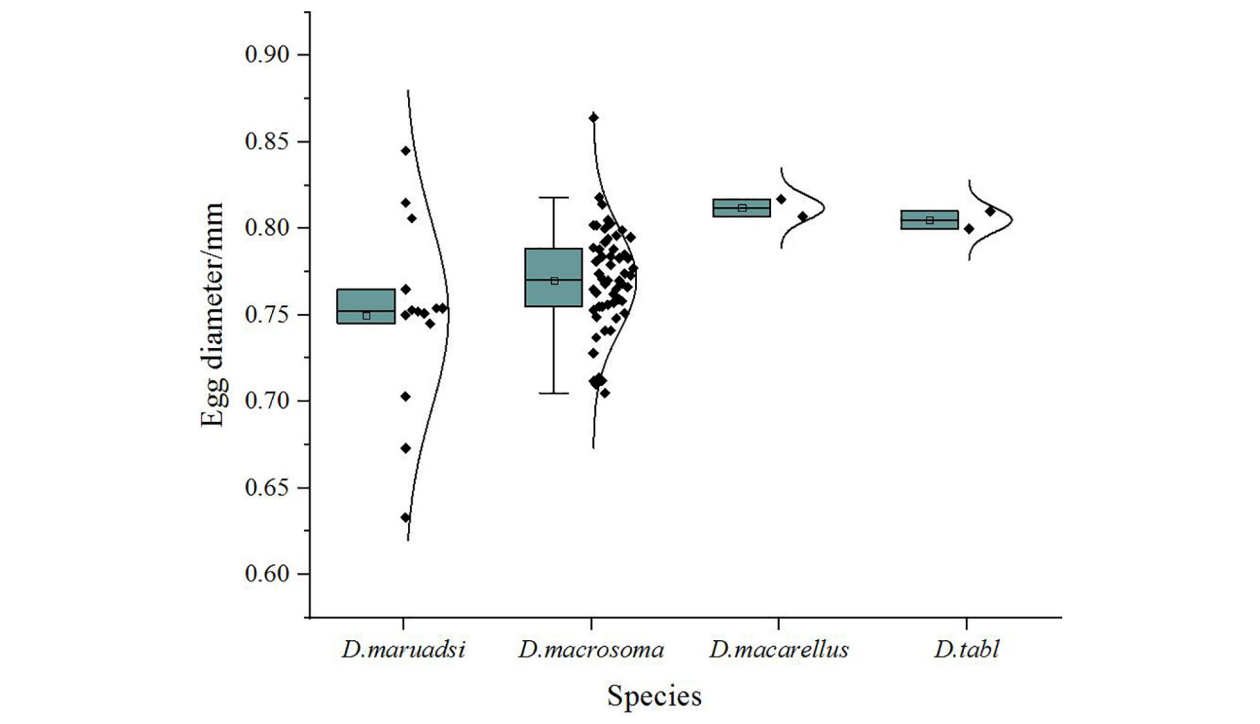

FIGURE 5 | Egg diameter (mm) in *Decapterus* species, northern South China Sea. Whisker plots depict median, mean, 1st and 3rd quartiles, and ranges (minima, maxima).

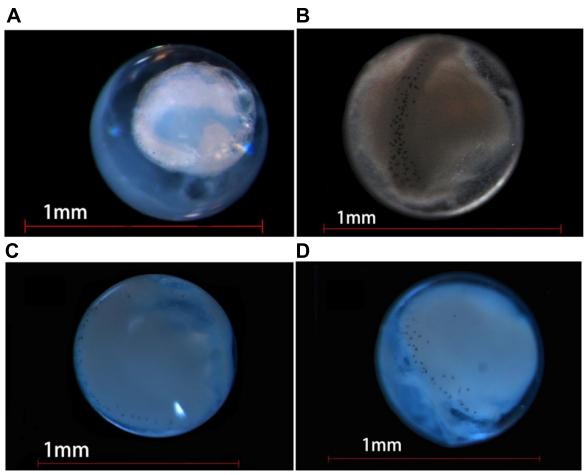

FIGURE 6 | Photographs of eggs of four *Decapterus* species delimited by COI. **(A)** *D. maruadsi*, **(B)** *D. macrosoma*, **(C)** *D. macarellus*, and **(D)** *D. tabl*.

Seasonal Distribution of *Decapterus* Eggs

Eggs were collected at 85 stations, with those of *Decapterus* at 9 stations in both spring and autumn. Eggs of *D. maruadsi* occurred at four stations concentrated off the Pearl River Estuary in spring, at 46.7–83.6 m, SST 25.4–27.2°C, and salinity 32.24–34.61 (**Table 2** and **Figure 7A**). Eggs of *D. maruadsi* were not found in autumn. Eggs of *D. macrosoma* occurred at four stations from 55.5 to 108.0 m in spring, at SST 25.08–28.42°C and salinity 32.24–34.48. In autumn, eggs of *D. macrosoma* occurred at nine stations from 45.3 to 118.2 m from the Pearl River Estuary to south of Hainan Island (**Figure 7B**) at SST 27.60–29.42°C and salinity 33.86–34.42. Eggs of *D. macarellus* occurred at one station in spring of 2018, and eggs of *D. tabl* occurred at one station in spring of 2018 and one station in spring of 2019 (**Table 2** and **Figure 7A**).

DISCUSSION

Molecular Identification by DNA Barcode

The lack of morphological characters in descriptions of ontogenetic stages of fish eggs renders their identification by light microscopy difficult, especially for early stages (Kawakami et al., 2010; Nishiyama et al., 2014). DNA, however, facilitates their identification. We apply DNA barcodes (COI sequences) to identify eggs of *Decapterus* species and demonstrate the importance of this technique for egg identification, particularly in spawning ground surveys (Lewis et al., 2016; Leyva-Cruz et al., 2016; Ahern et al., 2018).

Disadvantages of using COI sequences for DNA barcodes include sequence alignment, where sequences match multiple species in BOLD: 16 sequences were highly similar (≥99%) to *D. maruadsi* and *D. russelli*, and 61 sequences were highly similar (≥99%) to *D. macrosoma* and *D. maruadsi* (S1). Identification can be ambiguous, possibly because of

TABLE 1 | textitDecapterus egg morphology (identified by DNA barcode).

Species	Number for measurement	Egg shape	Diameter (mm)	Chorion		Perivitelline space	Yolk segmentation	Oil globule			Pigmentation in myomere
				Color	Surface			Number	Color	Diameter (mm)	Melanophore
D. maruadsi	14	Spherical	0.63–0.85	Transparent	Smooth	Narrow	Non-visible	1 + (3–5)	Transparent	0.16	Punctiform drops on notum
D. macrosoma	56	Spherical	0.74–0.86	Transparent	Smooth	Narrow	Non-visible	1	Orange	0.17–0.23	Punctiform drops on notum
D. macarellus	2	Spherical	0.6	Transparent	Smooth	Narrow	Non-visible	1	Transparent	0.16	Punctiform drops on notum
D. tabl	2	Spherical	0.80–0.81	Transparent	Smooth	Narrow	Non-visible	1 + (3)	Transparent	0.18	Punctiform drops on notum

TABLE 2 | *Decapterus* egg distributions in the northern South China Sea.

Species	Year	Season	Density ind./100 m^3	Depth (m)	SST (°C)	Salinity (PSU)	Location
D. maruadsi	2019	Spring	6.35–26.91	46.7–83.6	25.4–27.2	32.24–34.61	20.60–21.33°N 113.4–114.1°E
D. macrosoma	2019	Spring	1.49–23.34	55.5–108	25.08–28.42	32.24–34.48	18.77–21.33°N 110.56–113.82°E
		Later summer–autumn	0.37–62.07	45.3–118.2	27.60–29.42	33.86–34.42	18.68–20.53°N 110.56–114.66°E
D. macarellus	2018	Spring	153.1	57.6	29.1–29.4	34.17–34.22	16.96°N 112.22°E
D. tabl	2018	Spring	76.55	57.6	29.1–29.4	34.17–34.22	16.96°N 112.22°E
	2019	Spring	28.42	120.7	27.71	34.56	18.42°N 110.87°E

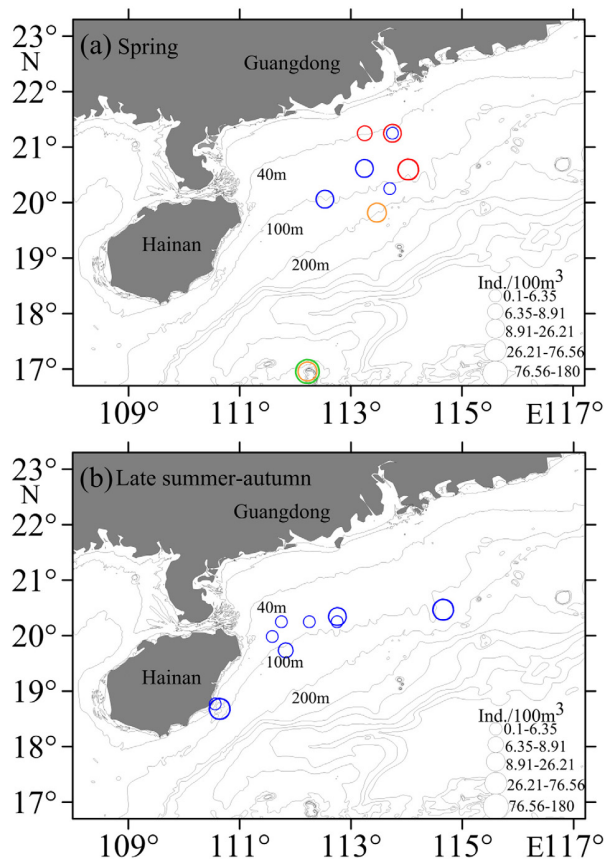

FIGURE 7 | Horizontal distributions of *Decapterus* eggs in the northern South China Sea. **(a)** Spring; **(b)** Late summer-autumn. Symbols represent catch rates per 100 m^3. Red circles indicate *D. maruadsi*, blue circles indicate *D. macrosoma*, green circles indicate *D. macarellus*, and orange circles indicate *D. tabl*.

hybridization, recent divergence, inadequate taxonomy, or incorrect identification (Meyer and Paulay, 2005; Ivanova et al., 2007). Hybridization is a problem for DNA barcode delimitation for matrilineal inheritance. The two carangids *Caranxignobilis* and *C. sexfasciatus* were artificially crossed with *C. melampygus*, with *C. melampygus* demonstrated to be the maternal parent of the hybrid (using 16S and COI sequences) (Murakami et al., 2007). Murosaba was even considered to be a natural hybrid between *Scomber australasicus* and *D. muroadsi*, but mitochondrial and nuclear markers demonstrated this to be untrue (Yanagimoto and Mahito, 2015). No further evidence proves that hybridization and introgression naturally occur in the Carangidae, especially in Genus *Decapterus*.

Some closely related species have recently diverged and may be closely genetically related or/and even share identical haplotypes in isolated gene pools, e.g., in tunas (Arnaud et al., 1999; Vinas and Tudela, 2009; Chang et al., 2017; Hou et al., 2018). However, the haplotype sharing may not have been found in Genus *Decapterus* (**Figure 3** and **Supplementary Table S3**). Due to poor taxonomy, two species may share the same DNA barcode if nominal species come from the same gene pool, and were in fact conspecific (Dahruddin et al., 2017). Homonyms, synonyms, and misidentifications within the reference library could also introduce cases of apparent haplotype sharing or deep intraspecific divergence (Chakraborty et al., 2006; Tzeng et al., 2007; Chen et al., 2020). For *Decapterus* species, misidentification may be the main reason for the haplotype sharing, according to the different retrieval systems, categories, books, or multiple independent identifications in different oceans (Sun and Chen, 2013). This could be proven by haplotype and genetic divergence analysis in our study (**Figure 3**).

Morphology and Identification

Photographs can be used to describe egg morphology for species identification and would be a valuable approach in ecological studies combining DNA barcode analysis and morphology (Kawakami et al., 2010; Harada et al., 2015). Eggs of the four *Decapterus* species were similar in their spherical shape, smooth chorion, narrow perivitelline space after the myotome stage, and in having melanophores distributed in myotome—features that may inadequately separate taxa. Nishiyama et al. (2014) suggested that egg diameter and yolk segmentation may be diagnostic characteristics for differentiation of *Trachurus japonicas* and *D. maruadsi* eggs. The average diameter of *D. maruadsi* eggs was smaller than for the three other *Decapterus* species, but ranges in diameter overlap (**Figure 5**); accordingly, egg diameter can be used to differentiate fish eggs to specific taxa but not in species level. We report the egg diameter of *D. maruadsi* as ranging from 0.63 to 0.85 mm, similar to dimensions reported from China (0.67–0.85 mm, Zhang et al., 1985) and Japan (0.67–0.80 mm, Ikeda et al., 2014). However, our *D. macrosma* (0.74–0.86 mm) and *D. macarellus* (0.81–0.82 mm) egg diameters differ from previous measurements reported for these two species (0.6–0.64 mm and 0.6 mm, respectively) by Zhang et al. (1985).

We summarize information on eggs of nine fish species with similar morphology, including the eggs of *Parapristipoma*

trilineatum and *Selar crumenopthalmus* that occurred simultaneously in the field surveys (**Supplementary Table S4**; Mito, 1966). Yolk segmentation can be observed in formalin-preserved eggs of *T. japonicus*, *D. maruadsi*, *P. trilineatum*, *S. crumenopthalmus*, and *S. boops*, but not for the alcohol-preserved eggs—species whose eggs have similar egg diameter and oil globule diameter. Matuda (1969) and Ikeda et al. (2014) emphasized the importance of spawning seasons for fish egg identification; however, the spawning seasons of these nine species overlap in the northern SCS, so this cannot be used to differentiate eggs either (Matuda, 1969). We even found that the eggs of *D. macrosma* identified by molecular method co-occurred in the survey of April and August–October, much longer than reported by Zhang et al. (1985). Thus, using morphological characteristics, i.e., egg diameter, oil globule, yolk segmentation, melanophore drops, etc., together with spawning season information can partly differentiate the fish eggs to some special taxa, but cannot differentiate them to species level. This further highlights the importance and benefits of using a DNA barcode method for fish egg species identification and spawning ground surveys in the subtropical tropical waters in the SCS.

Spawning Ground Seasonal Distribution

We report seasonal distribution patterns of *Decapterus* eggs in the northern SCS. Eggs of *D. maruadsi* occurred mainly off the Pearl River Estuary in spring, over 40–100 m, identifying this area as a key spawning ground for this species; eggs were not, however, found in autumn. Eggs of *D. macrosoma* occurred during both spring and autumn, mainly over 45–118 m (**Table 2**). Eggs of *D. macrosoma* co-occurred with those of *D. maruadsi* at one station only in spring, probably indicating a greater spawning area further south where water temperature and salinity were higher (**Table 2**). Compared with surveys in the 1960s and 1970s, no eggs of *D. maruadsi* occurred west of the Pearl River estuary or Hailing Islands, indicating that the spawning sea area may have decreased in these two areas (South China Sea Fisheries Research Institute [SCSFRI], 1966, 1979). As one of the last remaining core fishing grounds in the SCS, human activities such as overfishing, aquaculture, and coastal reclamation have already contributed to the collapse of many fish resources and damaged key habitat (Li and Huang, 2008; Darwall and Freyhof, 2016; Amorim et al., 2017; Hamilton et al., 2017). Furthermore, the preferred temperature range of *D. maruadsi* (<28°C) indicates that a shift in its spawning habitat may be a response to climate change (Kienast et al., 2001; Liu et al., 2008; Wernberg et al., 2016; Cai et al., 2017; Asch and Erisman, 2018; Asch et al., 2019; Sandø et al., 2020). For *D. macrosma,* Zhang et al. (1985) reported its spawning season to continue from March to July, but we identified 56 eggs by molecular method from eight stations from August to October, indicating that the spawning period of this species has extended.

Fishery Conservation and Management

We demonstrate the value of DNA barcoding for identifying fish eggs and for this technique to assist with identification of fish eggs in more traditional morphological studies. Spawning ground protection and moratoria on fishing activities in the SCS should take into consideration possible losses of *D. maruadsi* spawning habitat and the extension of the spawning season of *D. macrosma*. The SCS is a complex environment, with high fish diversity and inconspicuous variation in water temperature, so fish egg morphology, spawning season, spawning ground distribution, and hydrological conditions in this region require further study.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI (accession: MT609955–M610035).

ETHICS STATEMENT

The animal study was reviewed and approved by the Department of Ocean and Fishery, Guangdong Province.

AUTHOR CONTRIBUTIONS

GH and HZ analyzed the data and completed the first draft. GH and ZC performed the filed survey. ZC and HZ provided the guidance on data analysis and structure. GH, JW, JZ, and WH conducted the experiments. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.590564/full#supplementary-material

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