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Article

Observed Pattern of Diel Variation in Specific Gravity of Pacific Mackerel Eggs and Larvae

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Abstract : Although Pacific mackerel (*Scomber japonicus*) is an important commercial species in Korea, its recruitment mechanism remains largely unknown. Diel vertical positioning of larvae in the water column, which is affected by their specific gravity and the surrounding water density, may help to provide an understanding on recruitment success through predator avoidance and prey availability. The specific gravity measurement on Pacific mackerel eggs and larvae would seem to be essential information necessary to learn about the transport process from spawning to nursery grounds, and consequently the recruitment success. Eggs were artificially fertilized, and larvae were fed with rotifer when their mouths opened 3–4 days after hatching. We conducted the experiment using a density gradient water column to measure the ontogenetic changes in specific gravity from fertilization to 10 days after hatching. Egg specific gravity was stable during most of the embryonic period, but a sudden increase to 1.0249 g cm^{-3} happened just before hatching. However, the specific gravity of newly hatched larvae was much lighter (1.0195 g cm^{-3}), and specific gravity tended to increase continuously after hatching. Comparison of specific gravity with seawater density reveals that eggs and newly hatched larvae can float in the surface layer of the ocean. For the later period of the experiment, the specific gravity showed a cyclic diel pattern: the highest in the evening while the lowest at dawn. The fullness of larval stomach may be responsible for the observed differences in specific gravity, because stomach fullness was lower (40–60%) at midnight, and higher (80–85%) in evening. The diel pattern of specific gravity might provide clues regarding how larvae match the diel vertical migration of prey organisms.

Key words : Pacific mackerel, fish eggs and larvae, specific gravity, density-gradient water column, diel vertical movement

1. Introduction

In many mid and high latitude marine ecosystems, fish show the seasonal migration pattern through their life histories among feeding, spawning, and nursery grounds (Harden-Jones 1968; Houde 2008). Fishes move into the spawning area for the spawning, and the fish eggs and larvae emerged in spawning ground should be transported to nursery ground for better survival in coastal areas (Norcross and Shaw 1984). The advection of plankton including fish eggs and larvae was influenced by their vertical positioning in the water column, because the horizontal current strength generally varies with depth. Seawater properties such as temperature and salinity were

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vertically different in ocean, so that it is also true that the duration of embryonic period which depends upon the surrounding seawater temperature could be influenced by the vertical positioning of fish eggs (Kendall and Kim 1989). Due to the different vertical distribution pattern of fish eggs and larvae each year, therefore, the successful transport of fish eggs and larvae into the nursery ground would be different, and consequently resulted in various year class strengths of marine fishes (Bailey 1981; Kendall and Kim 1989; Sundby 1991). Since they enter the nursery ground by the existing current, they transform to juvenile stage by metamorphosis, and their mortality generally would be much reduced (Parrish et al. 1981). Therefore, knowledge of vertical egg and larval distribution is critical to improving sampling methods, modelling dispersal pathway from spawning to nursery grounds as well as understanding recruitment success (Sundby 1991; Adlandsvik et al. 2001; Boyra et al. 2003).

The main factor for determining vertical positioning of fish eggs is the density difference between organisms and surrounding seawater. The interaction between them is very dynamic. Due to the changes in temperature and salinity properties of water mass from time to time, the vertical locations of eggs and larvae is adjusted depending upon the density difference (Sundby 1997; Sundby and Kristiansen 2015; Coombs et al. 2004; Goarant et al. 2007; Petereit et al. 2009). The content of body fluid inside the egg, the presence of oil droplets in the yolk, and the thickness of chorion would be the significant contributors for determining specific gravity of eggs. In addition, the continuous internal chemical reaction during the embryonic period causes the changes in composition of egg materials, and impacts on specific gravity (Thorsen and Fyhn 1996; Laurel et al. 2010). The yolk decreases of yolk-sac larva, and development of internal organs including ossification would result in the increase of larval specific gravity.

The Pacific mackerel *Scomber japonicus* is distributed in temperate and subtropical areas throughout the northwestern Pacific Ocean. The Pacific mackerel is one of the most dominant commercial species in the East China Sea, the East Sea, and the Yellow Sea. The geographic distribution of monthly catches in Korean waters suggests that the Tsushima Current stock spawns and overwinters around Jeju Island (Yukami et al. 2009), migrates northward to the Yellow Sea and the East Sea during spring through summer, and returns to the southern wintering ground during winter (Hwang et al. 2001). The spawning season of Pacific mackerel is earlier in the northeastern East China Sea from late March to May (Shiraishi et al. 2009; Yukami et al. 2009), and is typically occurring in the south coastal area of Korean Peninsula from May to June (Hwang and Lee 2005). The Pacific mackerel eggs spawned and fertilized are buoyant pelagic, and they are transported by the Tsushima Warm Current toward the southern coastal areas of the Korean Peninsula or the East Sea as ichthyoplankton (Jung et al. 2013).

Our knowledge on the ecological characteristics of the vertical distribution, feeding habits, transportation route in Korean waters, and location of nursery ground on the real ocean during early life history of the mackerel was very limited. A few measurements on the specific gravity of Pacific mackerel eggs were reported recently (Jung et al. 2013), it was known that the specific gravity of mackerel eggs was lighter than the density of sea surface water at the spawning ground around the Jeju Island, Korea. The measurement of the larval specific gravity was not reported elsewhere due to the nature of active and fast swimming capability. High mobility of mackerel larvae requires more ossified body structure, so that we speculated that larva might have a heavier specific gravity than the egg, and larval specific gravity would increase as they get old. This research is extended from a previous study conducted by Jung et al. (2013). We hypothesized that (1) larvae has a heavier specific gravity than eggs and (2) larval specific gravity would increase as they grow. To test our hypotheses, we have measured the specific gravity of eggs and larvae using a density gradient water column. The result of laboratory experiments should be the initial data for horizontal advection model projection on the mackerel egg and early larval stage (passive movement period) and on future Pacific mackerel stock in the Korean waters.

2. Materials and Methods

We conducted experiments for the specific gravity measurement on Pacific mackerel eggs and larvae in two consecutive years. Pacific mackerel eggs were provided by the Fisheries Institute of the Gyeongsangnam-do, Tongyeong-si, Korea, in May of 2014 and 2015. About 200 adults of Pacific mackerel were reared in the inland seawater tank (3 m diameter × 2 m depth) with water temperature of 17–19°C. Adult fish spawned eggs in rearing tank after the injection of hormone at mid-night, and eggs were naturally fertilized in the rearing tank. Fertilized eggs were collected by filtering net at outflow in rearing tank after 2–3 hours later, and brought to

Pukyong National University for the specific gravity measurement. The eggs were reared in small aquaria (ichthyoplankton rearing tanks, diameter and height are 60 cm, respectively) where the UV-filtered seawater was supplied. The water temperature of rearing tank was maintained in 19–20°C and salinity of 34 PSU. The eggs were hatched in rearing tank within 2–3 days and larvae were kept for about two weeks until the end of experiment. The larvae were fed with rotifer 3 times a day in 2014, and 4 times a day in 2015, and specific gravity was measured one hour after feeding. Based on the astronomical chart of sunrise and sunset (https://www.kasi.re.kr, the Korea Astronomy and Space Science Institute), light condition was controlled in considering day and night duration in the southern coast of the Korean Peninsula: about 14.5 hours light and 9.5 hours darkness conditions within 24 hours.

We measured the specific gravity of fish eggs and larvae using the density gradient water column (DGC, Martin Instrument Co. Ltd., UK) which prototype was originally introduced by Coombs (1981) (Fig. 1). Water temperature of 70 cm height DGC was maintained in 20°C during the experiment, and sea salt (RedSea Co., USA) was added in making density gradient water. For the experiment, each column was filled by continuously graded seawater solution ranging from 1.0127 g cm^{-3} at the surface of the column to 1.0260 g cm^{-3} on the bottom for egg experiment, while 1.0098 g cm^{-3} at the surface to 1.0680 g cm^{-3} on the bottom for larval experiment. To estimate the specific gravity of individual egg and larva, 5–8 glass floats of known densities were introduced into the DGC. After 2–3 hours (minimum 2 hours) the glass floats had positioned at the neutral buoyancy in the column and could be used as reference levels of specific gravity. The center position of the glass floats was read with a precision of 1.0 mm. The specific gravities of eggs and larvae located between glass floats in DGC were interpolated at every observation by linear regression based on the known specific gravity and height information of glass float. The density of glass floats was originally reported at 23°C, and a density correction at 20°C was obtained according to the equation based on thermal expansion:

$$\text{calibrated density (g cm}^{-3}) = (\text{float density}) + (\text{temperature difference}) \times (\text{float density}) \times 0.000028$$

Once the eggs were dropped into the DGC, they were not removed from DGC to avoid the destruction of water column stability. The position of eggs in the DGC was read 30 min after the introduction into the column. The egg specific gravity was continuously measured from fertilization to hatching with the interval of 2 hours. The experiment was performed until all eggs were hatched. And the specific gravity of the hatched larvae was measured in the density gradient column during 10 or 14 hours after hatching at every two hours, as same as eggs, cause of we want to know difference between egg specific gravity and just hatched larval specific gravity. This egg and just hatching larval specific gravity measurement is different part from larval specific gravity measurement. The specific gravity of Pacific mackerel eggs and larvae were measured over time until all eggs hatched or eggs/

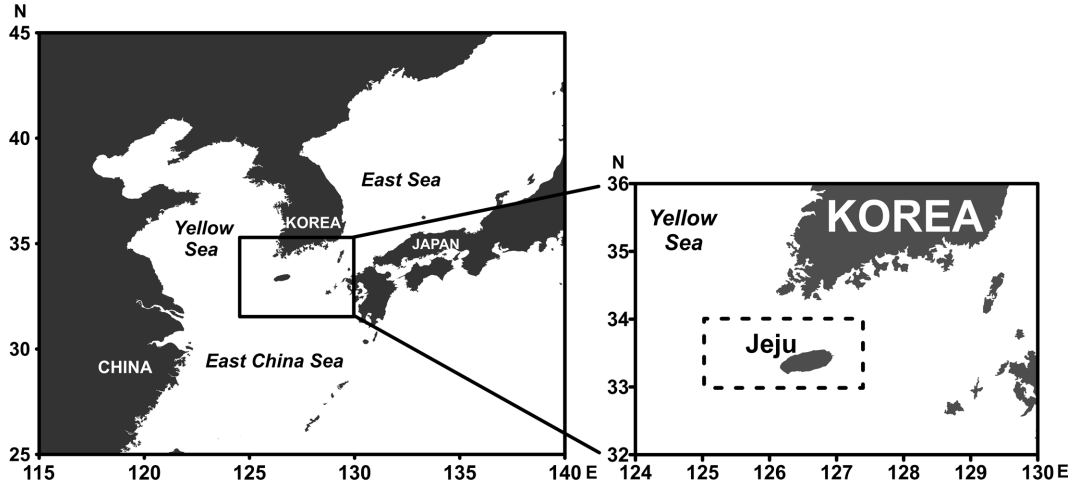

Fig. 1. The areas of interest in this study. Pacific mackerel (*Scomber japonicus*) eggs used were spawned in the southern coast of the Korea, and the seawater density was calculated around the Jeju Island (125.0–127.5°E, 33.0–34.0°N)

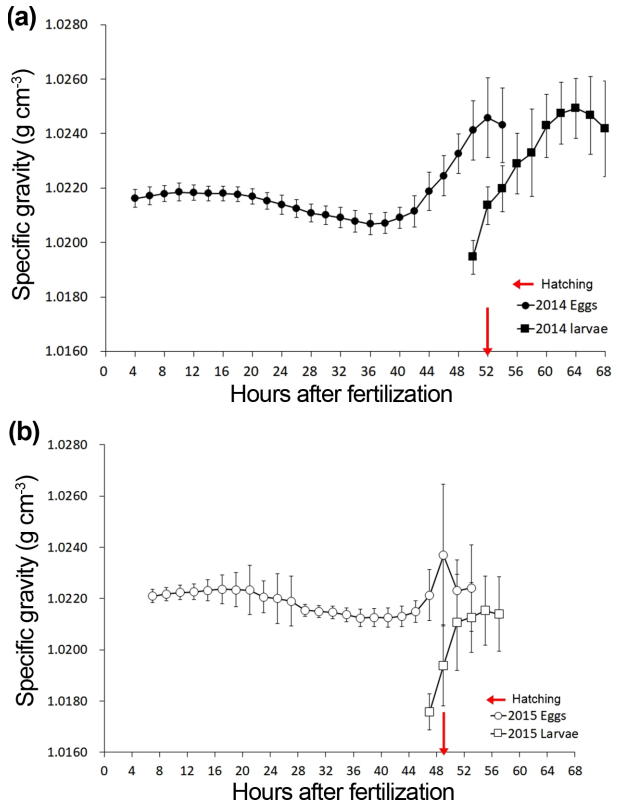

Fig. 2. Changes in specific gravity of Pacific mackerel (*Scomber japonicus*) eggs and larvae. (a) 2014 and (b) 2015. Vertical bars indicate the standard deviation at each observation

larvae died (Fig. 2).

Water temperature of the DGC were maintained 20°C in which the embryonic period was a roughly 50 hours. Due to the swimming capability of larvae, on the other hand, larval specific gravity was measured instantaneously. As larvae grew in larval tank, various sizes of larvae were used for such point measurement of specific gravity. Larvae were dropped into the DGC several times a day. Time intervals for observation were 3 times and 4 times a day in 2014 and 2015, respectively. The observation was carried out every 8 hours (02:00, 10:00, and 18:00) in 2014, while every 6 hours (00:00, 06:00, 12:00, and 18:00) in 2015. At each observation, 15 to 20 individuals were selected from the rearing tank. From the 2nd day after hatching, larvae were anesthetized for 20 minutes with 0.005% of MS222 prior to the observation. From 3rd day after hatching, the larvae were fed with rotifer in the rearing tank one hour before the observation. After 4th day after hatching, the DGC was made of 0.005% MS222 solution, because anesthetized larvae were awakened often during sinking, and swam to disturb the stability of water column. During the anesthetizing, the photos of larvae experimented were taken under a microscope and measured its total and notochord lengths in 0.1 mm precision at each observation.

To explain the change in specific gravity during larval period, we considered two possibilities to cause such changes: the larval sizes and the fullness of larval stomach. The size of the individual larva as well as the amount of food in each stomach experimented was measured separately in day and night, and compared and tested for the difference using statistical tool. The total length (TL) of all larvae experimented was measured under a microscope in precision of 0.1 mm. For the index of larval stomach fullness, we used the photos for judging the stomach fullness. Simply we calculated the ratio of the number of larvae with full stomach with rotifer to the total number of larvae examined at each observation. For the statistical test on the specific gravity difference between day and night observations, we used t-test in EXEL (two-sample t-test) with the measurements on specific gravity and total length (mm) observed at 00:00 and 18:00 during 3rd–9th day of experiment in 2014 and 2015. The stomach fullness index was calculated by the following formula below,

$$\text{Stomach fullness index } (\%) = (N_f / N) \times 100$$

where N_f is the number of larvae that the stomach is full with food, and N is the total number of larvae used in this experiment. The results of the 2014 study could not be calculated because the photographs could not be read properly.

Based on the indication of high catch around the Jeju Island (125.0–127.5°E, 33.0–34.0°N, Fig. 1), we assume that the spawning of Pacific mackerel in Korean waters is active in areas off the Jeju Island during May (Hiyama et al. 2002). The actual seawater density in spawning areas off the Jeju coast is compared with the specific gravity of Pacific mackerel eggs and larvae to elucidate where and how they locate in water column. The Korea Oceanographic Data Center (KODC) provides the serial oceanographic observation data (http://kodc.nifs.go.kr) that have been collected by the National Institute of Fisheries Science (NFRDI 2005) bi-monthly (February, April, June, August, October, and December) since 1960s. We used the seawater temperature and salinity information collected in upper 100 m of the sea surface layer in April and June 2014 and 2015, and seawater properties in May was determined by taking the average of April and June in 2014 and 2015. Seawater density were calculated by R

package 'marelac' (version 2.1.5). F-test and Independent Two-sample t-test used data analysis provided by Excel (Microsoft home and student 2016, Ver. 1708).

3. Results

Embryonic period of fertilized eggs was a little bit longer than two days. Hatching started from about 50 hours after fertilization, and completed at 56 hours during our experiment. The mean egg specific gravity during the early-mid developmental stage (until 44 hours after fertilization) ranged from 1.0207–1.0219 g cm^{-3}, and the variance of egg specific gravity at each measurement was comparatively small (0.0002–0.0006). However, a sudden increase from about 1.0207 to 1.0249 g cm^{-3} in mean specific gravity happened in the late stage between 44 and 54 hours, and maintained similar mean values again showing the high variance (0.0007–0.0019 g cm^{-3}) until just before hatching (Fig. 2a). In May 2014, the spawning area off the southern coast of the Korean Peninsula had a mean seawater density of 1.0241 g cm^{-3} in the surface layer, as shown in Table 1. Comparison of the specific gravity of eggs with seawater density of the spawning ground deduced that Pacific mackerel eggs spawned in subsurface (above 10 m depth) of the spawning ground generally rose toward the surface in early-mid developmental stages, and then might adjust vertical positioning within the surface layer until hatching.

In 2014 experiment, the average specific gravity of the newly hatched larvae (1.0190, 1.0199 g cm^{-3}, n = 2) was much lighter than the average specific gravity of the eggs (1.0246 ± 0.0015 g cm^{-3}, n = 109, 52 hours after fertilization) (Fig. 2a). Also, the mean larval specific gravity was lower than the density of surface sea water (1.0241 g cm^{-3}, 0 m depth in May 2014; Table 1). At 14 hours after hatching (i.e., 64 hours after fertilization), the specific gravity of larvae increased sharply to 1.0249 ± 0.0011 g cm^{-3} (n = 13). In 2015, the first measurement of egg specific gravity was 1.0221 ± 0.00027 g cm^{-3} (n = 83) at 7 hours after fertilization. The changing pattern of specific gravity of eggs was also like those observed in 2014. Egg specific gravity in the middle stage was slightly heavier (1.0224 ± 0.00056 g cm^{-3}, n = 81, at 17 hours after fertilization) than the early stage, and decreased continuously to 1.0212 ± 0.00035 g cm^{-3} (n = 49) for 20 hours (Fig. 2a). The specific gravity of eggs ranged from 1.0212 to 1.0213 g cm^{-3} from 37 to 43 hours after fertilization, but it increased rapidly to 1.0237 ± 0.00276 g cm^{-3} (n = 41) at 49 hours after fertilization. In the density gradient column, on the other hand, the first hatched larva appeared at 47 hours after fertilization showing the specific gravity of 1.0176 ± 0.0007 g cm^{-3} (n = 4). Considering the specific gravity (1.0221 ± 0.0010 g cm^{-3}, n = 51) of eggs observed at 47 hours after fertilization, newly hatched larvae were much lighter than eggs. The specific gravity of larvae was gradually increased for four hours, and stabilized thereafter showing 1.0214 ± 0.0014 g cm^{-3} (n = 30) at 10 hours after the first hatching in 2015, while the specific gravity of early larval stage was stabilized at 14 hours after hatching (Fig. 2b).

The mean and standard deviation of larval length at the first hatching was 3.36 ± 0.28 mm TL (n = 12, few hours after first hatched larvae observed) in 2014, and the first day specific gravity measurement was conducted for the larvae of 3.77 ± 0.23 mm TL (n = 6). Larvae continuously developed, and reached 4.61 ± 0.22 mm TL at the 10^{th} day after hatching, which is equivalent to the growth rate of 0.114 mm day^{-1}. There was a positive relationship between daily mean larval specific gravity and the mean larval

Table 1. The mean seawater density (g cm^{-3}) calculated based on seawater temperature and salinity around the Jeju Island (125.0–127.5°E, 33.0–34.0°N) in 2014 and 2015 at 0, 10, 20, 30, 50, 75, and 100 m depth. Seawater density in May was determined by taking the average of April and June

Depth	2014 seawater density (g cm^{-3})			2015 seawater density (g cm^{-3})		
	April	May	June	April	May	June
0	1.0254	1.0241	1.0228	1.0249	1.0237	1.0225
10	1.0259	1.0247	1.0234	1.0254	1.0243	1.0232
20	1.0264	1.0255	1.0246	1.0259	1.0252	1.0244
30	1.0269	1.0265	1.0261	1.0265	1.0261	1.0257
50	1.0278	1.0277	1.0276	1.0276	1.0274	1.0273
75	1.0290	1.0289	1.0288	1.0288	1.0287	1.0286
100	1.0299	1.0299	1.0299	1.0299	1.0299	1.0299

Table 2. Summary of the daily mean larval specific gravity (g cm^{-3}) and length (mm TL) in 2014 and 2015, respectively

	2014				2015			
	Specific gravity (g cm^{-3})		Length (mm, TL)		Specific gravity (g cm^{-3})		Length (mm, TL)	
	n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD
Few hours after hatching	13	1.0233 ± 0.0006	12	3.36 ± 0.28	13	1.0228 ± 0.0013	10	3.04 ± 0.09
Day 1	39	1.0245 ± 0.0009	32	3.77 ± 0.23	93	1.0238 ± 0.0008	64	3.35 ± 0.23
Day 2	46	1.0251 ± 0.0011	29	3.89 ± 0.13	86	1.0226 ± 0.0013	100	3.66 ± 0.11
Day 3	71	1.0274 ± 0.0013	68	4.05 ± 0.17	88	1.0209 ± 0.0014	114	3.72 ± 0.10
Day 4	87	1.0272 ± 0.0018	38	3.98 ± 0.17	88	1.0246 ± 0.0030	102	3.81 ± 0.10
Day 5	88	1.0287 ± 0.0017	47	4.00 ± 0.14	92	1.0286 ± 0.0024	109	3.98 ± 0.16
Day 6	64	1.0288 ± 0.0017	33	4.08 ± 0.18	62	1.0317 ± 0.0033	98	4.19 ± 0.18
Day 7	66	1.0296 ± 0.0020	50	4.21 ± 0.16	66	1.0361 ± 0.0040	80	4.49 ± 0.22
Day 8	45	1.0301 ± 0.0021	41	4.37 ± 0.23	83	1.0407 ± 0.0040	86	4.75 ± 0.25
Day 9	29	1.0300 ± 0.0022	25	4.52 ± 0.31	67	1.0420 ± 0.0015	75	4.93 ± 0.34
Day 10	8	1.0318 ± 0.0018	9	4.61 ± 0.22	36	1.0428 ± 0.0111	36	4.97 ± 0.34

length (Table 2). In 2014, the larval specific gravity at 3.77 mm (the first day) was about 1.0245 g cm^{-3}, and it was steadily increased to 1.0318 g cm^{-3} when larval size was 4.61 mm (at 10th day, increasing rate of specific gravity: 0.00678 g cm^{-3} per mm). In another word, the specific gravity of the Pacific mackerel larvae increased 0.0008 per each day during the experiment period in 2014 (Table 2). In 2015, the daily mean and standard deviation of larval length was 3.35 ± 0.23 mm TL (n = 93) at the first observation (1st day after hatching), and larval length was continuously increased and the mean and standard deviation of larval length was 4.97 ± 0.34 mm TL at the 10th day after hatching, which is equivalent to the growth rate of 0.176 mm day^{-1}. The daily mean larval specific gravity was also increased with increasing larval length, but increasing rate of larval specific gravity in 2015 was larger than that in 2014. In 2015, the specific gravity of the newly hatched larvae was relatively light (1.0238 ± 0.0008 g cm^{-3}, n = 93). From the first day to the third day after hatching, larval specific gravity decreased (1.0209 ± 0.0014 g cm^{-3}, n = 88) with 3.72 ± 0.10 mm TL larval length, and then continuously increased (Fig. 2b, Table 2). The larval specific gravity at 3.35 mm (the first day) was about 1.0238 ± 0.0008 g cm^{-3} (n = 93), and it was reached to 1.0428 ± 0.0111 g cm^{-3} (n = 36) when larval size was 4.97 ± 0.34 mm TL (at 10th day, increasing rate of specific gravity: 0.01034 g cm^{-3} per mm). In another word, the specific gravity of the Pacific mackerel larvae increased 0.00182 g cm^{-3} per each day during the experiment period in 2015 (Table 2).

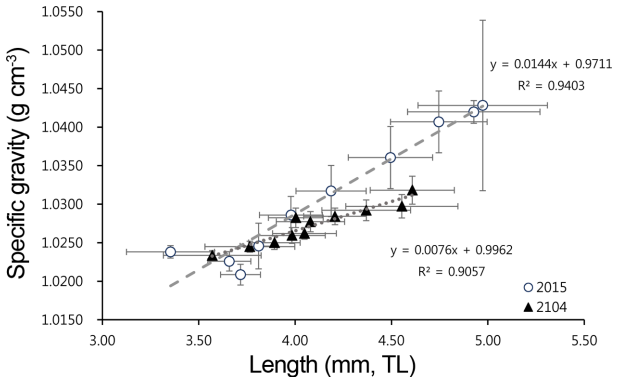

Fig. 3. Relationship between the mean specific gravity and mean total length (mm) of the Pacific mackerel (*Scomber japonicus*) larvae in 2014 and 2015. Triangles and circles indicate the mean specific gravity and mean length of the larvae with one standard deviation in 2014 and 2015, respectively

Fig. 3 showed that there was a significant, positive relationship between larval length and larval specific gravity. In 2015, the larval growth rate and specific gravity increase rate were higher than in 2014. This is interpreted because of the difference in the number of times the food can be eaten, which will be discussed in the discussion. In both 2014 and 2015, as the size of the larvae grows, the increasing rate of specific gravity is different (Fig. 3). When comparing the length growth of the larvae, growth in 2015 was significantly faster than that of 2014 (p = 0.1634, t = 1.022, t-test, one tail) and specific gravity increase in 2015 was significantly higher

than of 2014 (p = 0.4909, t = 0.023, t-test, one tail). Both the average length and the specific gravity of the daily increase with time showed a large value in the observation in 2015. At the same time, the specific gravity of the larvae observed in 2015 was larger when compared with the similar size (mean TL, mm). Fig. 3 shows two scatter graphs and two trend lines using the mean specific gravity corresponding to the average size of the larvae measured in the two years. The slope of the trend line with a specific gravity increase according to the length of 2015 is 0.0144, which is larger than the slope of 0.0076 in 2014. The slopes of these two trend lines were significantly different (p = 0.508, t = −0.02, see "Comparing the slopes for two independent samples", http://www.real-statistics.com).

Specific gravity of Pacific mackerel larvae showed a cyclic pattern within a day. Especially for 4- to 9-day old larvae in 2014, the pattern of the diel change in specific gravity was clearly observed. The specific gravity was the highest in the evening (18:00) while was the lowest at night (02:00) (Fig. 4a). In 2015, the increasing trend of specific gravity with cyclic pattern appeared also clearly from 4th day to the end of experiment. Larval specific gravity was the highest during the daytime (12:00 or 18:00) while was the lowest at the midnight (00:00) (Fig. 4b). In both 2014 and 2015, we found that there were significant differences in larval specific gravity between the daytime and the night time (Table 2). Two analyzes were conducted to determine the cause of the heavy and night-time phenomenon during the day, which is the same in 2014 and 2015. The first was to see if there was a difference between the average size of the larvae during the night and the day, and the second was to use the number of larvae in the digestive system that were full of food during the night and day. However, in both 2014 and 2015, no significant difference in larval length between day and night was found (Table 3). This result may indicate that other factors such as stomach fullness could affect changes in larval specific gravity between day and night. Additionally, in 2015, we found differences in the ratio of larval stomach fullness between day and night in relation to changes in larval specific gravity between day and night (Fig. 4b). Because larvae were fed with rotifer from 3th day after hatching in 2014 and 2015, larvae that have large amount of prey organisms in their stomach seems to have relatively higher specific gravity, and vice versa. In 2015, the proportion of stomach fullness was 74–83% at 18:00, while that of night was 67–25% at 00:00 (Fig. 4b). Also, this tendency associated with stomach fullness observed during 4th and 9th day (Fig. 4b).

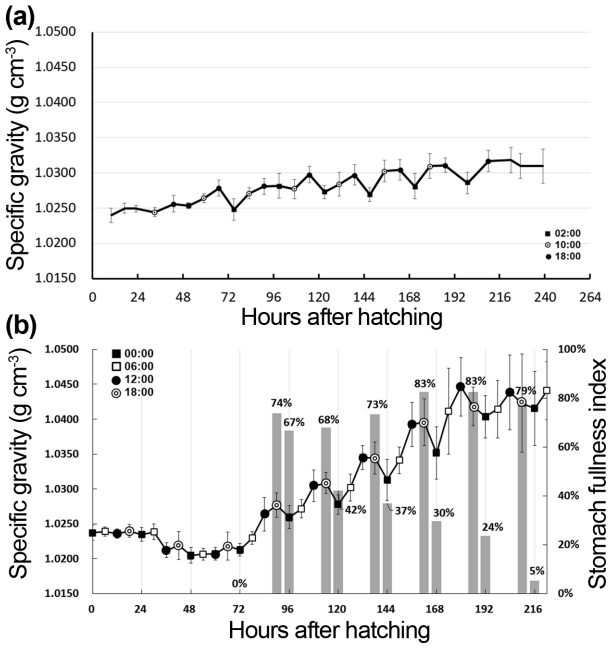

Fig. 4. Diel changing pattern of larval specific gravity of Pacific mackerel (*Scomber japonicus*). (a) 2014 and (b) 2015. Diel difference in Stomach fullness index was shown in (b)

Table 3. Independent two-sample t-test on larval specific gravity and total length difference for night and day time observations in a day. (a) 2014 and (b) 2015

(a) 2014

Day (old)	Specific gravity (g cm^{-3}) 00:00–18:00		Total length (mm) 00:00–18:00	
	t	*p*	T	*p*
4	−10.424	0.000	−2.814	0.010
5	−4.277	0.000	−0.848	0.405
6	−5.090	0.000	1.237	0.229
7	−8.607	0.000	−1.755	0.090
8	−5.359	0.000	−0.849	0.407

(b) 2015

Day (old)	Specific gravity (g cm^{-3}) 00:00–12:00		Total length (mm) 00:00–12:00	
	t	*p*	T	*p*
4	−8.731	0.000	−0.384	0.703
5	−7.602	0.000	−3.909	0.000
6	−11.171	0.000	−0.857	0.396
7	−7.184	0.000	−1.015	0.318
8	−7.177	0.000	−3.303	0.002
9	−2.388	0.024	−1.855	0.070

4. Discussion

In this study, the egg specific gravity throughout developmental stages gradually decreased until full development of the main organs, however, the egg specific gravity suddenly increased just before hatching (Fig. 2). Our results about the pattern of changes in egg specific gravity over time are consistent with Jung et al. (2013). The similar pattern of changes in the egg specific gravity throughout developmental stages was also observed in various marine fish including Atlantic mackerel (*Scomber scombrus*; Coombs et al. 2001), Atlantic cod (*Gadus morhua*; Nissling and Vallin 1996; Anderson and Young 1995; Jung et al. 2012), Red sea bream (*Pagrus major*; Kitajima et al. 1993), European anchovy (*Engraulis encrasicolus*; Ospina-Alvarez et al. 2012). This result may suggest that eggs rise in the water column during the early developmental stages and then sink in the water column just before hatching.

The newly hatched larvae or larvae during the early stages are relatively passive until they completely develop morphological characteristics for swimming, therefore, changes in the specific gravity during the early larval stage could directly alter their vertical distribution in the water column (Fig. 2). The specific gravity of larvae just after hatching was lighter than egg specific gravity (Fig. 2), then, larval specific gravity rapidly increased over time (about 10 days observation after hatching, Table 2). This result may suggest that the newly hatched larvae of Pacific mackerel in the ocean abruptly rise to the shallower depth in the water column due to drastic changes in the specific gravity from eggs to larvae just after hatching and then rapidly sink down due to increases in their specific gravity as they grow. It is possible that the specific gravity of the newly hatched larvae that are removed heavy chorion is lighter than that in eggs (Kjesbu et al. 1992). This similar change in specific gravity from eggs to larvae is also shown in other marine fish (Nissling et al. 1994; Miller and Kendall 2009). Buoyancy in marine fish larvae seems to be maintained mainly through the low specific gravity of the fluids of the yolk sac and subdermal space (Miller and Kendall 2009). As marine fish larvae grow, it is likely that larval specific gravity increase due to increased skeletal ossification and developed functional organs (Miller and Kendall 2009).

Our experiments in both 2014 and 2015 showed no differences in larval specific gravity between day and night for the newly hatched larvae (Fig. 4). However, from four days after hatching, there were variations in larval specific gravity between day and night (Fig. 4). The similar pattern of diel changes in the specific gravity were found in different marine fish larvae in both laboratory and field studies (Takashi et al. 2006; Kitajima et al. 1993; Shoji et al. 1999; Adlandsvik et al. 2001), though there was little evidence of diel migration for either mackerel larvae or horse mackerel larvae (Coombs et al. 2001). For example, Kitajima et al. (1993) reported the similar pattern of diel changes in the specific gravity of Red sea bream (*Pagrus major*) during their early larval period based on their laboratory study. Also, Takashi et al. (2006) showed that Pacific Bluefin tuna (*Thunnus orientalis*) larvae have a diel change in their specific gravity; larval specific gravity is high during the daytime while is low during the night time based on their laboratory study. Moreover, based on field sampling study, Shoji et al. (1999) showed diel vertical movements in larvae of Japanese Spanish mackerel (*Scomberomorus niphonius*) after yolk-sac absorption; larvae were found in the relatively shallower depth during night time while larvae were observed in the deeper water column during the daytime. Thus, it is possible that the observed changes in larval specific gravity of Pacific mackerel between day and night occur in the ocean, resulting in changes in their vertical distribution in the water column between day and night.

These previous studies also discussed factors such as inflation (or deflation) of swim-bladder and stomach fullness that could alter larval specific gravity and vertical distribution between day and night (Kitajima et al. 1993; Shoji et al. 1999; Takashi et al. 2006). Takashi et al. (2006) suggested that swim-bladder could affect the specific gravity of Pacific bluefin tuna larvae; swim-bladder volume was small during the daytime while was greater during the night time. Shoji et al. (1999) showed swim-bladder effect with feeding incidence on diel vertical movement of Japanese Spanish mackerel larvae; volumes of swim-bladder were greater during the night time than that during the day time, and feeding incidence increased during the daytime (high stomach fullness) while decreased during the night time (less stomach fullness). In our study, it seems that differences in the specific gravity of Pacific mackerel larvae between day and night are highly and positively related to the ratio of stomach fullness of larvae (Fig. 4b). In addition, we did not observe any Pacific mackerel larvae that completely developed swim-bladder or had inflation (or deflation) of swim-bladder. Although development of swim-bladder of marine pelagic fish larvae starts from the early larval stage, but it does no

function until larvae grow to certain size or age (e.g., Atlantic herring larvae; Miller and Kendall 2009). Therefore, we suggest that the degree of stomach fullness on Pacific mackerel larvae during the early life stages could play an important role to alter their specific gravity between day and night, resulting in changes in larval vertical distribution in the water column between day and night. Other previous studies also showed that feeding or starvation during larval stages could affect changes in larval specific gravity; starving larvae are typically lighter than fed larvae of equal age (Blaxter and Ehrlich 1974; Neilson et al. 1986). For example, Neilson et al. (1986) reported that the specific gravity of poorly fed larval cod (*Gadus morhua* L.) may decrease due to the low water and higher protein content of tissues. Also, Blaxter and Ehrlich (1974) suggested that specific gravity of larvae likely tend to decrease with starvation.

We observed differences in larval growth rate between 2014 and 2015; the mean body length of larvae in 2015 was larger than that in 2014. Also, we found differences in larval specific gravity at the same age between the two examine years; larval specific gravity in 2014 was lighter than that in 2015. In this study, larvae were fed three times a day in 2014 while were fed four times per day in 2015. Therefore, these differences in larval growth rate between 2014 and 2015 could be resulted from differences in the number of feedings per day between the two years. This result suggests that larval growth rate during the early life stages could be strongly affected by prey availability and feeding success in the ocean, causing changes in larval specific gravity at the same developmental stage (Fig. 3). In terms of the increases of the number of feeding and growth rate, larval specific gravity in 2015 could be heavier than that in 2014 at the same age.

Knowledge of the specific gravity of marine fish eggs and larvae is critical to better understanding their vertical distribution and horizontal transport in the ocean (Sundby 1991; Adlandsvik et al. 2001; Boyra et al. 2003; Myksvoll et al. 2013; Sundby and Kristiansen 2015). Vertical distributions of fish eggs and larvae in the ocean could be changed due to both abiotic (e.g., turbulent mixing) and biotic (e.g., specific gravity) factors (Kendall and Kim 1989; Sundby 1991; Sundby and Kristiansen 2015). It is likely that Pacific mackerel larvae just after hatching abruptly move upward in the water column and then larvae sink in the relatively deep water as they grow. Furthermore, Pacific mackerel larvae have diel vertical movements between day and night due to changes in larval specific gravity in relation to prey availability, feeding success, though depth-discrete field sampling data are not available in Korean waters that could support our results about their diel vertical movements. Changes in larval specific gravity within 24-hours could explain how mackerel larvae occupy the surface during night, and inhabit deeper layers during the day. Larvae could easily sink to the deeper water with high specific gravity in daytime, which reduces predation pressures in ocean surface layer. Collectively, our results provide baseline data for future modeling work of dispersal pathways from spawning to nursery grounds for this species. Moreover, our study provides the new information about egg and larval specific gravity in this species that would be useful for studying the relationship between vertical distribution and prey-predator interactions such as avoidance of predators and availability of prey organisms in future.

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