

Article

Allometry for Eyes and Optic Lobes in Oval Squid (*Sepioteuthis lessoniana*) with Special Reference to Their Ontogenetic Asymmetry

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Abstract: Eyes develop in relation to body size and brain area for visual processing in some vertebrates. Meanwhile, it is well known that many animals exhibit left–right asymmetry in both morphology and behavior, namely, lateralization. However, it remains unclear whether the eyes and visual processing brain areas synchronously develop for their asymmetry. Oval squid (*Sepioteuthis lessoniana*) exhibits lateralization of optic lobe volume and left or right eye usage toward specific targets during their ontogeny. We address the question of how left–right asymmetry of the eyes and optic lobes exhibit an allometric pattern. To examine this question, we estimated the left and right volumes of eyes and optic lobes using microcomputed tomography. We found that, for the optic lobe volume, the right enlargement that appeared at ages 45 and 80 days then shifted to the left at age 120 days. In contrast, the volume of eyes did not show any left–right asymmetries from hatching to age 120 days. We also found that the volume of the eyes and optic lobes showed a slower increase than that of the whole-body size. Within these two visually related organs, the eyes grew faster than the optic lobes until age 120 days. These results are discussed in the context of the survival strategy of oval squid that form schools, two months post-hatching.

Keywords: brain; cephalopod; lateralization; micro computed tomography; vision



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1. Introduction

Vision is a sensory system that perceives information from the surrounding environment. In vertebrates, as the eyes become larger, finer visual acuity and/or greater light sensitivity are acquired [1]. In many vertebrates that depend on vision, the eyes develop allometrically to body size. Furthermore, larger animals tend to have relatively smaller eyes than those of smaller animals [2–4]. Differences in eye size across the species affect ecological and behavioral factors, such as activity patterns [3], habitats [5], and feeding strategies [6]. It is also known that the growth of eyes and body size is partially faster during ontogenetic development. For example, larval Siberian sturgeon, *Acipenser baerii* (Brandt, 1869), exhibits a positive allometry between eyes and body size, but it shifts to being closer to isometry in later stages, which suggests a change in visual function during ontogeny [7]. Eyes possibly have an allometric relationship with the brain areas that process visual information. This is suggested by the fact that, in birds, large eyes are affiliated with large brain areas for visual processing [3].

Many examples of brain lateralization exist, where the functions between the left and right brain hemispheres differ [8]. This allows animals to process different information simultaneously in each hemisphere, increase cognitive capacity, and quicken decision-making [8,9]. This lateralization is reflected in related specific behavioral lateralization. For example, the domestic chick, *Gallus gallus* (Linnaeus, 1758), uses the left eye, projecting

visual information in the right hemisphere, for prey recognition [10] and the right eye, processing visual information in the left hemisphere, for predator recognition [11]. Eye lateralization has been investigated in some animals and is linked to differences in visual abilities, such as visual acuity and sensitivity between the left and right eyes. For instance, the European starling, *Sturnus vulgaris* (Linnaeus, 1758), has more medium- and long-wavelength-sensitive single cones in the left eye than in the right eye, suggesting that the left eye can discriminate color more than the right eye [12]. In addition, lateralization can also be considered an ontogenetic process. In deep-sea squids (Histioteuthidae), the eyes and optic lobes (brain area processing visual information) continue to grow, with the left eye becoming twice as large as the right, from the hatching to the adult phase [13,14]. It is likely that the lateralized pattern in the sensory organs could influence those in the brain areas corresponding to their sensory function. However, information remains limited on whether these two morphological traits (eyes and their related brain area) synchronously develop owing to asymmetry.

Coleoid cephalopods (squid, cuttlefish, and octopus) exhibit many visually guided behaviors, such as predation, camouflage, and communication [15]. Cephalopods possess camera-type eyes similar to those of vertebrates [16] and the largest brain among invertebrates, which is equivalent to the vertebrate brain [17]. Cephalopod eyes, positioned laterally on the head [18], receive different visual information in their left and right fields, except for their binocular visual field (Figure 1) [19]. Optic lobes are the largest areas in the cephalopod brain and are located immediately behind the eyes (Figure 1) [20,21]. The optic lobes process visual information [20,21], regulate body color patterns [22,23], as well as motor responses [24]. Visual information received from the eyes is delivered to the ipsilateral optic lobes [20,21].

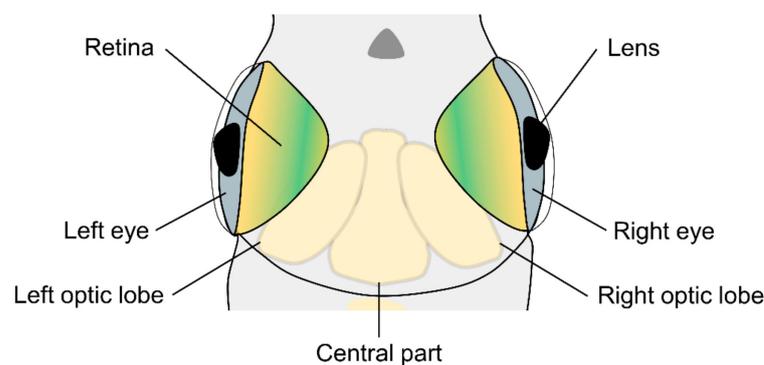


Figure 1. Diagram representation of the oval squid, *Sepioteuthis lessoniana*, head (dorsal view).

With regard to visual lateralization, some cephalopods use the left or right eye to identify specific targets, such as prey [25,26], predators [26,27], and conspecifics [26,28]. Their lateralization is also expressed for turning behavior in a T-maze [29,30] and for camouflage on shaded substrates [31]. Moreover, some cephalopods show lateralization of the brain volume, such as in the optic lobes [14,26,29] and the vertical lobe, which is the area processing learning and memory [29]. Furthermore, lateralization of cephalopods also appears to increase the concentration of monoamines in the optic lobes [29].

The vision of the oval squid, *Sepioteuthis lessoniana* (Férussac, 1831 in Lesson 1830–1831), has been studied anatomically and behaviorally. For instance, they form a school upon visually interacting with conspecifics [32–35] and the school corresponds with the ontogeny of their brain [36]. A retinal bump, a dent on the retina formed by being pressed on by the ipsilateral optic lobe, appears in the eyes of juveniles, but not of adults, which causes the intentional blurring of an image [37]. In combination with the retinal bumps and a vertical bobbing behavior (rhythmic slight movement of the body up and down) during hovering, different focal lengths (image blur) between the bump and other regions in the eyes are produced, allowing oval squids to estimate the size of objects and their distance, from either eye, in a low-contrast water environment [37]. Moreover, the left–right sides of the eye use

for visualizing specific objects changes during ontogeny in oval squids, which suggests adaptation of the visual strategy of this species [26]. With respect to the optic lobes of the oval squid, the right optic lobe develops faster than the left one after oval squids complete the process of forming a school; however, this bias does not appear during the pre-school forming phase, from hatching to 2 months of age [26]. Despite many studies on the vision of cephalopods, few studies have highlighted the asymmetric relationship between the eyes and optic lobes during ontogeny. Here, we addressed the question of how left–right asymmetry of the eyes and optic lobes exhibit an allometric pattern in oval squids.

2. Materials and Methods

2.1. Animal Rearing and Sample Collection

Egg cases of the oval squid *S. lessoniana* (identical to “Shiroika,” *Sepioteuthis* sp. 2) [38] were collected from the coastal water of Okinawa Island, Ryukyu Archipelago, Japan. Such collections do not require specific permissions in Japan. The egg cases were immediately transferred to a laboratory at the University of the Ryukyus, Nishihara Campus. Upon arrival, they were transferred to a circular tank (700 mm diameter, 120 L volume) with a closed seawater system connected to a temperature controller and an ultraviolet sterilizer (Multihydense[®], Aqua Inc., Shinagawa, Tokyo, Japan). Water temperature, salinity, and pH were maintained at 25.0–25.5 °C, 32–34 psu, and above 7.8, respectively. Fluorescent lights were fitted above the tank, and a light/dark cycle was set to 12/12 h with a timer. The tank was enriched by adding artificial corals and seaweeds to the bottom. Since hatching occurred within a range of several days, the day when the greatest number of hatchlings occurred was representatively defined as day 0. Hatchlings were fed live adult mysids, *Neomysis japonica* (Nakazawa, 1910), thrice a day, and juveniles were fed thrice a day with frozen anchovies, *Engraulis japonicus* (Temminck and Schlegel, 1846), and living guppy fries, *Poecilia reticulata* (Peters, 1859). Dead individuals, excrement, and any remaining prey were removed from the tank immediately after feeding was completed.

We sampled ten squids at each age: 0, 15, 30, 45, 80, and 100 days, and nine squids at age 120 days, which were euthanized by soaking in 10% ethanol in seawater. We then measured the dorsal mantle length (ML) and wet body weight (BW) of the specimens (Table 1). After measurements, we fixed the entire body of the squids at ages 0, 15, and 30 days and the head without the mantle and the arms of squids at ages 45, 80, 100, and 120 days in 10% formalin in seawater. These fixed specimens were preserved at 4 °C until further treatment was performed. Because we treated according to a previous study [39] whole eyes and the surrounding cartilage and muscle were connected, which prevented morphological changes to the eyeballs.

Table 1. Summary of oval squid (*Sepioteuthis lessoniana*) selected for micro-CT observations. Dorsal mantle length and wet body weight are shown as mean \pm standard deviation. Eyes volume and optic lobes volume shown in median (25–75th percentiles). * Volume of eyes was calculated for seven of nine squids at age 120 days (two squid eyes were deformed due to fixation and were excluded). Abbreviation: BW, wet body weight; ML, dorsal mantle length.

Age (Days) and Sample Size	ML (mm)	BW (g)	Eyes (mm ³)		Optic Lobes (mm ³)	
			Left	Right	Left	Right
0 (n = 10)	3.8 \pm 0.4	0.03 \pm 0.005	0.85 (0.6–1.3)	0.89 (0.6–1.2)	1.4 (0.9–1.5)	1.3 (0.9–1.5)
15 (n = 10)	10.2 \pm 1.3	0.2 \pm 0.06	8.0 (5.1–9.0)	8.1 (5.1–8.8)	5.4 (3.7–5.8)	5.2 (3.9–5.4)
30 (n = 10)	10.5 \pm 1.9	0.3 \pm 0.1	7.0 (6.0–8.5)	7.0 (5.9–8.4)	5.3 (5.1–5.7)	5.4 (5.1–5.6)
45 (n = 10)	17.6 \pm 2.2	1.1 \pm 0.3	25.5 (23.0–29.4)	25.3 (22.8–29.7)	14.7 (13.7–16.1)	14.8 (13.8–16.1)
80 (n = 10)	48.5 \pm 6.9	12.0 \pm 4.7	285.7 (267.7–444.9)	293.4 (266.1–447.7)	75.5 (70.8–102.1)	78.1 (72.9–106.0)
100 (n = 10)	69.1 \pm 10.1	27.9 \pm 11.2	537.3 (501.5–831.0)	522.2 (503.4–787.8)	112.0 (110.7–153.9)	112.3 (110.8–155.9)
120 (n = 9)	84.2 \pm 3.7	48.2 \pm 7.0	1088.9 (1041.0–1218.4)	1054.5 (933.5–1237.2)	176.6 (166.3–232.1) *	173.8 (164.1–230.9) *

2.2. Micro-CT Imaging

We performed the following treatments to observe the specimens with a micro-computed tomography (micro-CT) system, as previously described by Sakurai and Ikeda [26,39]. The specimens were rinsed with distilled water for 10 min. The specimens were then stained with 1% or 3% iodine potassium iodide in water (I₂KI; 0, 15, 30, and 45 days old, 1% for 1 day; 80 days old, 3% for 7 days; and 100 and 120 days old, 3% for 10 days). After staining, the specimens were rinsed with distilled water for 10 min. A micro-CT system (R_mCT2, RIGAKU, Tokyo, Japan) located at the Institute for Animal Experiments, Faculty of Medicine, University of the Ryukyus, was used for all imaging procedures. The X-ray tube voltage and tube current were set to 90 kV and 160 μ A, respectively. The field of view was set to 10 mm or 30 mm to scan the whole head of the squid (0, 15, 30, and 45 days old, 10 mm; 80, 100, and 120 days old, 30 mm). The voxel was set to $20 \times 20 \times 20 \mu\text{m}^3$ for a 10-mm field of view and $59 \times 59 \times 59 \mu\text{m}^3$ for a 30-mm field of view. The scanning time was set to 3 min to obtain high-resolution images. All images were exported as TIFF formats.

2.3. Data Analysis

The eyes and optic lobes of squids were identified according to previous studies on loliginid cephalopods [39,40]. We measured the volumes of the left and right eyes and optic lobes of squids (Figure 1) using Fiji (version 1.53h) [41]. The volume was calculated using the following formula:

$$t \times \Sigma a$$

where t is the thickness between the CT images and a is the area of the eyes or the optic lobes. We then calculated the volume ratio of the eyes and optic lobes (right volume/left volume) to assess these asymmetries, following Sakurai and Ikeda [26].

To test left–right asymmetry in the volume of the eyes and the optic lobes for each age of the squids, we used a one-sample Wilcoxon signed-rank test. To compare these left–right asymmetries between each age group, we used the Kruskal–Wallis test and the Steel–Dwass test for multiple comparison. For analysis of ontogenetic allometry, isometry (same relative growth rate between two variables) was defined as the slope of three for allometry of the volume vs. the ML, and slope of one for allometry of the volume vs. the BW, and of the eyes volume vs. optic lobes volume. These differences in the slope of isometry are matched with the physical dimension in this allometric scaling [42], which is based on the following relationship: volume (mm^3) = mass (g) = length³ (mm)³. The allometric equation is generally described using the following equation:

$$y = ax^b$$

where y and x are two variables, a is proportionality constant, and b is the exponent [42]. Both sides of this equation are log₁₀-transformed:

$$\log_{10} y = \log_{10} a + b \log_{10} x.$$

Considering the physical dimension, because \log_{10} volume = $3 \log_{10}$ ML and \log_{10} volume = \log_{10} BW in this study, these isometries defined the allometry with a slope of three or one, respectively. If the slope of the scaling is higher or lower than the slope of the isometry, scaling indicates hyperallometry (relative growth rate of one variable larger than the other) or hypoallometry (relative growth rate of one variable lower than the other), respectively. To test the correlation between body size and the volume of the left and right eyes or optic lobes, we used Pearson's product-moment correlation with a \log_{10} volume for the left–right eyes and optic lobes vs. \log_{10} ML or \log_{10} BW. The volume of the left–right eyes and optic lobes, ML, and BW were \log_{10} transformed to be able to use linear regression analyses. To compare the differences between ontogenetic scaling of the eyes and the optic lobes on the left and right sides, we used two-way analysis of covariance (ANCOVA) with measured areas (the eyes and the optic lobes) and the left–right sides as independent

variables, \log_{10} volume as dependent variables, and \log_{10} ML or \log_{10} BW as covariables. To compare the differences between ontogenetic scaling of the left and right eyes to that of the left and right optic lobes, we used one-way ANCOVA with the left–right sides as an independent variable, \log_{10} eye volume as a dependent variable, and \log_{10} optic lobe volume as a covariable. All statistical analyses were performed using R Studio version 2021.09.0 [43].

2.4. Animal Welfare

The oval squid were reared and the experimental treatments were conducted following the rules generally applied in European Union countries [44]. Experiments with live cephalopods are not regulated by the Japanese government or at the University of the Ryukyus, where all the experiments were conducted.

We fed living guppies to the squid for maintenance. As the University of the Ryukyus does not regulate the use of teleost fish for experiments, we used the guppies without ethical controls. However, we used them solely for the maintenance of the oval squids.

3. Results

3.1. Ontogenetic Asymmetry of the Left and Right Eyes and the Optic Lobes

The volumes of the optic lobes were calculated for all individuals at all ages, while the volume of the eyes was calculated for all individuals at ages 0, 15, 30, 45, 80, and 100 days, and for seven of nine individuals, at age 120 days (whose eyes were deformed due to fixation). No significant asymmetry in the volumes of the eyes was observed at any age (0 days old, $W = 32$, $p = 0.70$; 15 days old, $W = 23$, $p = 0.70$; 30 days old, $W = 26$, $p = 0.92$; 45 days old, $W = 30$, $p = 0.85$; 80 days old, $W = 28$, $p = 1$; 100 days old, $W = 22$, $p = 0.63$; 120 days old, $W = 10$, $p = 0.58$; Figure 2). In addition, the volume asymmetry of the eyes was not significantly different between age groups ($\chi^2 = 1.79$, $p = 0.94$; Figure 2).

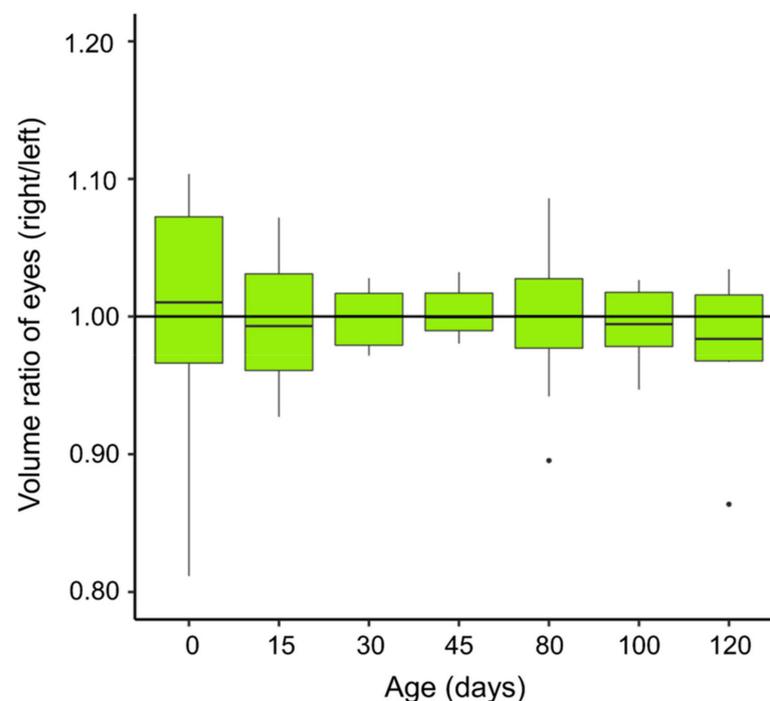


Figure 2. Ontogenetic change in volume ratio of left–right eyes (right volume/left volume) of the oval squid, *Sepioteuthis lessoniana*. Values > 1 show that the right optic lobe is larger whereas values < 1 show that the left optic lobe is larger. Box plots show median (internal line) and 25th and 75th percentiles (edge of box), whiskers show values within 1.5 times interquartile range, and dot shows an outlier.

In contrast to eye volume, lateralization of the optic lobes was observed. The volume of the right optic lobe was significantly larger than that of the left one at ages 45 and 80 days, and the volume of the left optic lobe was significantly larger than that of the right one at age 120 days (45 days old, $W = 47$, $p = 0.049$; 80 days old, $W = 55$, $p = 0.002$; 120 days old, $W = 1$, $p = 0.0079$; Figure 3). No such lateralization was observed at the ages 0, 15, 30, and 100 days (0 days old, $W = 24$, $p = 0.77$; 15 days old, $W = 23$, $p = 0.70$; 30 days old, $W = 38$, $p = 0.32$; 100 days old, $W = 39$, $p = 0.28$; Figure 3). The asymmetry significantly differed between age groups ($\chi^2 = 19.33$, $p = 0.0036$; Figure 3). The volume of the optic lobes at age 80 days was significantly lateralized more rightward than that at 30 and 100 days of age (Table 2 and Figure 3). Moreover, the volume of the optic lobes showed a significant opposite bias between ages 45 and 80 days for the larger right lobe and at 120 days for the larger left lobe (Table 2 and Figure 3). However, there were no significant differences between the other groups (Table 2 and Figure 3).

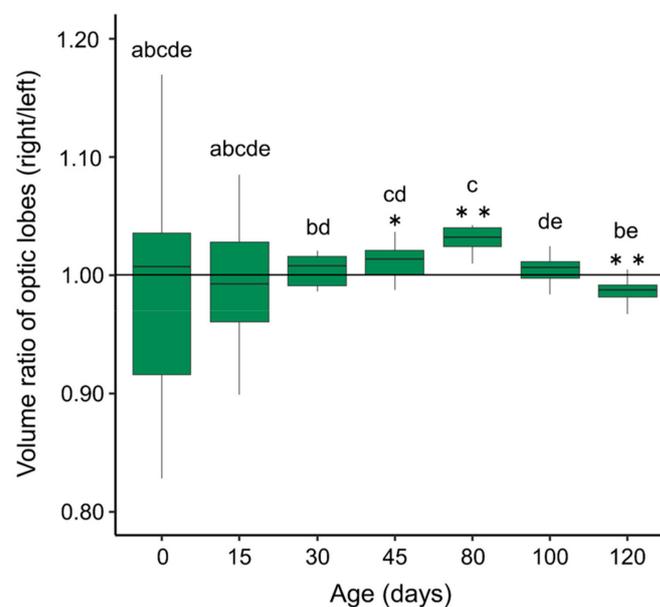


Figure 3. Ontogenetic change in volume ratio of left–right optic lobes (right volume/left volume) of oval squid, *Sepioteuthis lessoniana*. Values > 1 show that the right optic lobe is larger whereas values < 1 show that the left optic lobe is larger. Box plots show median (internal line) and 25th and 75th percentiles (edge of box), whiskers show values within 1.5 times interquartile range. * $p < 0.05$; ** $p < 0.01$; one-sample Wilcoxon signed-rank test. Letters represent results of a Steel–Dwass test and the different letters indicate a significant difference between two ages.

Table 2. Comparison between six ages for volume ratio of left–right optic lobes in oval squid (*Sepioteuthis lessoniana*). A Steel–Dwass test was used to test the difference between each age. The significant values are shown in bold.

Vs. Age. (Days)	Age (Days)											
	0		15		30		45		80		100	
	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
vs. 0	–	–	–	–	–	–	–	–	–	–	–	–
vs. 15	0.15	1.0	–	–	–	–	–	–	–	–	–	–
vs. 30	0	1.0	0.45	0.99	–	–	–	–	–	–	–	–
vs. 45	0.30	1.0	0.45	0.99	1.13	0.87	–	–	–	–	–	–
vs. 80	1.29	0.79	1.06	0.90	3.17	0.018	2.49	0.13	–	–	–	–
vs. 100	0.075	1.0	2.04	0.32	0.23	0.99	1.13	0.87	3.17	0.018	–	–
vs. 120	0.65	1.0	0.82	0.98	2.37	0.21	3.10	0.032	3.67	0.005	2.69	0.10

3.2. Ontogenetic Allometry among Body Size, Eyes, and Optic Lobes

There was hypoallometric scaling (slope < 3 (defined isometry was 3 for the ML), negative allometry) between the eye volume and ML (left, slope \pm SE = 2.38 ± 0.024 , intercept \pm SE = -1.53 ± 0.033 , $R^2 = 0.99$, $t = 99.2$, $p < 0.001$; right, slope \pm SE = 2.38 ± 0.025 , intercept \pm SE = -1.53 ± 0.035 , $R^2 = 0.99$, $t = 94.8$, $p < 0.001$; Figure 4A) and between the optic lobe volume and ML (left: slope \pm SE = 1.67 ± 0.016 , intercept \pm SE = -0.93 ± 0.022 , $R^2 = 0.99$, $t = 105.7$, $p < 0.001$; right: slope \pm SE = 1.67 ± 0.015 , intercept \pm SE = -0.93 ± 0.022 , $R^2 = 0.99$, $t = 108.1$, $p < 0.001$; Figure 4A). Two-way ANCOVA demonstrated that there was a significant effect of measured areas (eyes and optic lobes; $F_{1,267} = 224.2$, $p < 0.001$), but not for left–right sides ($F_{1,267} = 0.001$, $p = 0.97$) and these interaction effects ($F_{1,267} = 0.006$, $p = 0.94$).

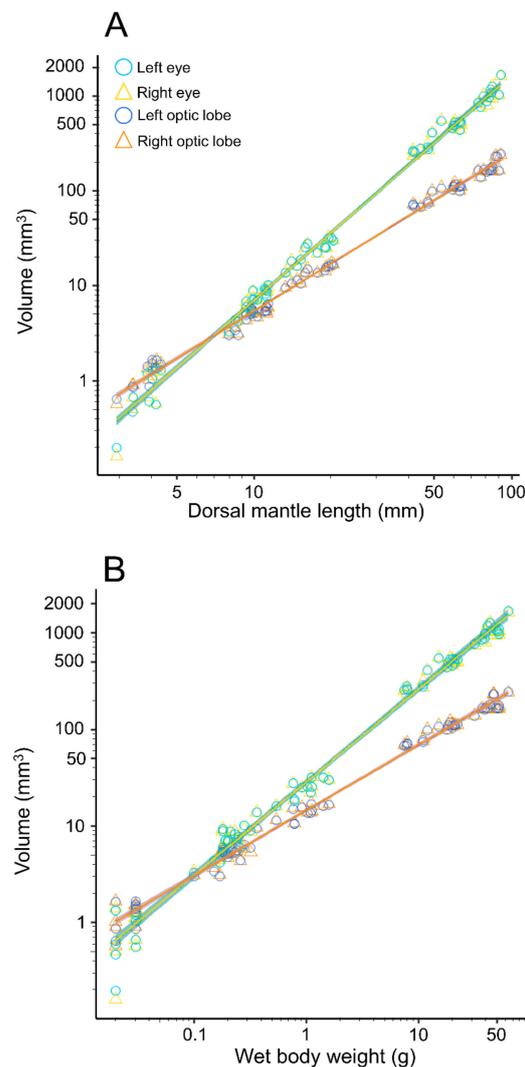


Figure 4. Allometry for left–right eyes, left–right optic lobes vs. dorsal mantle length (ML). (A) Left–right eyes, left–right optic lobes vs. wet body weight (BW), (B) of oval squid *Sepioteuthis lessoniana*. For correlation test, the ML, the BW, and the eyes volumes and optic lobes volumes were transformed into a common logarithm. For analysis of allometry, isometry (relative growth rate between two variables is same) was defined a slope of three for allometry of the volume vs. the ML and one for allometry of the volume vs. the BW so that the isometry would occur in this allometric scaling. Symbols show measured values in each individual. Regression lines with 95% confidence intervals represent Pearson product–moment correlation results, and colored by the left–right sides of eyes and optic lobes.

There was slight hypoallometric scaling (slope < 1 (defined isometry was 1 for BW), negative allometry) between the eye volume and BW (left, slope \pm SE = 0.97 ± 0.013 , intercept \pm SE = 1.46 ± 0.015 , $R^2 = 0.99$, $t = 74.6$, $p < 0.001$; right, slope \pm SE = 0.96 ± 0.014 , intercept \pm SE = 1.45 ± 0.015 , $R^2 = 0.99$, $t = 70.6$, $p < 0.001$; Figure 4B) and between the optic lobes volume and the BW (left, slope \pm SE = 0.68 ± 0.008 , intercept \pm SE = 1.16 ± 0.009 , $R^2 = 0.99$, $t = 86.5$, $p < 0.001$; right, slope \pm SE = 0.68 ± 0.008 , intercept \pm SE = 1.16 ± 0.009 , $R^2 = 0.99$, $t = 84.1$, $p < 0.001$; Figure 4B). Two-way ANCOVA demonstrated that there was a significant effect of measured areas (eyes and optic lobes; $F_{1,267} = 200.4$, $p < 0.001$), but not on the left–right sides ($F_{1,267} = 0.001$, $p = 0.94$) and these interaction effects ($F_{1,267} = 0.005$, $p = 0.94$).

Hyperallometric scaling (slope > 1 (defined isometry was 1 for the optic lobe volume), positive allometry) between the eyes and the optic lobe volume (left, slope \pm SE = 1.43 ± 0.012 , intercept \pm SE = -0.2 ± 0.017 , $R^2 = 0.99$, $t = 122.8$, $p < 0.001$; right, slope \pm SE = 1.42 ± 0.012 , intercept \pm SE = -0.2 ± 0.018 , $R^2 = 0.99$, $t = 117.8$, $p < 0.001$; Figure 5). One-way ANCOVA demonstrated that there was no significant effect of the left–right sides ($F_{1,131} = 0.084$, $p = 0.77$).

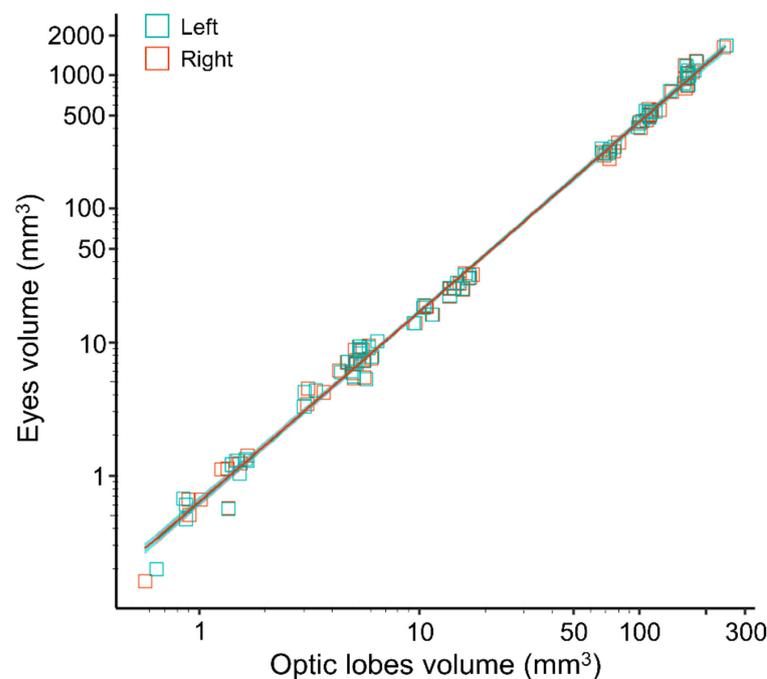


Figure 5. Allometry between left–right eyes and left–right optic lobes of oval squid, *Sepioteuthis lessoniana*. For correlation tests, the eyes volume and optic lobes volume were transformed to common logarithm. For analysis of allometry, isometry (relative growth rate between two variables is same) was defined a slope of one for allometry of the eyes volume vs. the optic lobes volume so that the isometry would occur in allometric scaling. Symbols show measured values in each individual. Regression lines with 95% confidence intervals represent Pearson product–moment correlation results, and colored by the left–right sides.

4. Discussion

4.1. Symmetry and Asymmetry of Left and Right Eyes and Optic Lobes during Ontogeny

We found that the volume of the left and right eyes in oval squid remained symmetrical up to 120 d post-hatching (Figure 2). Werner and Seifan [4] found that 32 of 33 species of gecko had left–right symmetrical eyes (an exception was the elegant gecko, *Stenodactylus sthenodactylus* (Lichtenstein, 1823), which has a larger right eye than left eye). However, the number and densities of retinal cells in some birds differ between the left and right eyes [12,45]. Moreover, the house-hunting ant, *Temnothorax albipennis* (Curtis, 1854), has a different number of ommatidia between left and right compound eyes, which influenced

the turning behavior in a branching nest maze, in which individuals with a greater number of ommatidia in the left eye turn towards the right, and vice versa for the other eye, in the maze [46]. Therefore, we should be cautious about such a possibility, as the left–right asymmetry of the eyes could appear at the cellular level in oval squid. Cells in the retina (i.e., photoreceptors and retinal ganglion cells) affect visual properties such as acuity and contrast sensitivity [47]. The lens modulation transfer function (an estimate of visual acuity) in the southern reef squid, *Sepioteuthis australis* (Quoy and Gaimard, 1832), is higher in the right eye than in the left eye at 100 days, post-hatching [48]. Although we did not find lateralization for the whole eye level (eye volume), possible cellular level lateralization of the eyes must be studied further for oval squid.

In contrast to the eye volume, we found asymmetry of the optic lobe volume, which shifted from right to left up to 120 days post-hatching in oval squid (Figure 3). Histioteuthidae (deep sea squid) have a left eye and optic lobe that is twice as large as that of the right eye, [14] and their asymmetries appear solely in adults, which means this asymmetry does not reverse during ontogeny [13]. Nevertheless, in some species, a dominant hemisphere examined by behavioral tests ontogenetically shifted to another hemisphere. For instance, domestic chicks shift the usage of the dominant hemisphere from the left to right during the early development phase after hatching, which coincides with their visual behaviors [49]. However, it is not known whether these dominant hemispheric shifts from the left to right coincide with asymmetrical hemispheric shifts of in volume. To the best of our knowledge, this study is the first to reveal a shift in size asymmetry of the brain hemisphere from the right to left in animals during ontogeny. The size asymmetry of the brain is found in some animals, such as in the habenular of the convict cichlid, *Amatitlania nigrofasciata* (Günther, 1867) [50], optic tectum of the yellow eye mullet, *Aldrichetta forsteri* (Valenciennes, 1836) [51], and the neocortex of rats [52]. In the convict cichlid, males and females with larger left habenular turn in the right and left directions in a T-maze, respectively [50]. This difference between sexes could be due to differences in motivation [50], where control of motivation is one of multiple functions in the habenula [53]. However, it is necessary to consider which function in the brain area relates to the specific behavioral lateralization if the brain area has multiple functions for the habenula.

Oval squid at the ages 45 days (ML \pm standard deviation (SD) = 17.6 \pm 2.2), 80 days (ML \pm SD = 48.5 \pm 6.9), and 120 days (ML \pm SD = 84.2 \pm 3.7) in this study (Table 1) correspond to those of juvenile (ML = 30–50 mm) and subadult (ML = 80–150 mm) in a previous study [54], in which morphological changes in the optic lobes of oval squid through ontogeny were investigated. The optic lobes of oval squid grow by increasing the size of the cell somata in the medulla of the optic lobes, rather than the cortex, from embryo to adult [54]. This suggests that the volume asymmetry in the optic lobes occurs in the medulla, consisting of a radial column zone, a region of visual information processing [21] and memory storage [20], tangential zone, visuomotor [24], and chromatophore control [22,23]. The optic lobes of pharaoh cuttlefish, *Sepia pharaonis* (Ehrenberg, 1831), could be more lateralized in the cortex and radial column zone than in the tangential zone [55]. Thus, the volume asymmetry of the optic lobes in oval squid could be related to the radial column zone rather than to the tangential zone. The cortex, also called the deep retina, possibly functions as a detector of object features, such as shape, extent direction, and brightness, which is similar to the function of the retina in vertebrates [20,21]. However, it is unclear how the radial column zone functions in visual information processing, but it could correspond to the cerebral cortex of vertebrates as both the radial column zone and the primary visual cortex (V1) are column structures [21]. Lateralization in the V1 of humans was found to be involved in processing the spatial frequency (expressing brightness changes in an object as frequency changes) of images, where the left and right V1 processes low- and high-spatial frequency content of images, respectively [56]. The difference in the spatial frequency represents the difference in the way the images, such as a visual scene, are seen. Images with high-spatial frequencies have enhanced edges and texture, whereas those with low-spatial frequencies enhance the rough global orientation

and proportions of the images [57]. Given that high-spatial frequencies are rapidly reduced over a distance in shallow water due to optical effects, such as scattering and veiling [58], it can be said that oval squids living in shallow water pay more attention to detailed features of close conspecifics at ages 45 and 80 days (larger right optic lobe) compared to at other ages. This could play a role in school formation by increasing the sensitivity to signals expressed from close conspecifics. In addition, this may serve a function in communication. For instance, male Australian giant cuttlefish, *Sepia apama* (Gray, 1849), display agonistic body patterns with high-spatial frequencies over a relatively short distance to other males during male–male competitions [59]. In addition, during the competition, the male of the Australian giant cuttlefish keeps the right eye (minority type) directed to rival males and has a higher fighting success than males with the left eye (majority type) directed at rival males [28]. This success suggests that the right-eye users are able to efficiently process signals with high-spatial frequencies, such as the edges and texture of rival males. Considering that visual information from the eye, in cephalopods, is sent to the ipsilateral optic lobes [20,21], it can be thought that the right optic lobe of oval squid at 45 and 80 days becomes larger to preferentially process signals with high-spatial frequencies from close conspecifics for school formation, which can be detected using the right eye. This is supported by a previous study where oval squid at 37–55 days old frequently used the right eye to watch conspecifics [26]. The left optic lobe becomes larger at 120 days post-hatching, when two months have passed since the onset of schooling behavior (i.e., 60 days post-hatching) in oval squid [34]. This might suggest that oval squid at an age of 120 days preferentially processes low-spatial frequencies. The low-spatial frequencies from the visual images can be perceived from far away, but not those at high frequencies [60]. It can be thought that the left optic lobe becomes larger to process information over far distances in shallow water, which can be detected by the left eye. Although five of nine individuals at age 71–90 days used the left eye for predators [26], it is unknown whether the left eye is used for predator at 120 days. The processing of the low-spatial frequencies of images increases survival opportunity because looking at a space over a long distance plays a role in the rapid detection of predators. The distance to a predator, trajectory, and swimming speed affect defensive behavior in cephalopods, such as shifts in body pattern, inking, and jetting [61,62]. Such spatiotemporal information of predators is important for school decision-making. For instance, oval squid display multiple schooling behaviors, such as forming a tight ball and belt [35], but it is unknown how these behaviors affect the spatiotemporal information of the predator. In summary, the shift in volume asymmetry appears in the optic lobes, but not in the eyes, which indicates that active neural processing for visual attention toward surroundings occur with a priority in particular phases of post-hatching.

Although visual information from the eyes of cephalopods is sent to the ipsilateral optic lobes [20,21], the information from the optic lobes can transfer to the contralateral optic lobes via the vertical and superior frontal lobes and the optic commissures [63–66]. In *Octopus vulgaris* (Cuvier, 1797), transfer via the vertical and superior frontal lobes is related to long-term memory [63], whereas transfer via optic commissures is related to short-term memory [66]. Such information transfers between the optic lobes may complicate the relationship between the optic lobes and behavioral lateralization, as suggested by Sakurai and Ikeda [26]. For example, one hemisphere excites and/or inhibits another hemisphere in humans [67]. Moreover, the transfer speed from the left to right hemispheres differs from that of the right to left hemispheres. For spatial stimuli, transfer speed from left to right hemispheres is faster than that from right to left hemispheres [68]. To understand the functional lateralization of the optic lobes in cephalopods, the effect of the connections between the optic lobes on visual lateralization should be investigated.

We previously found that the optic lobes of oval squid do not lateralize at 15–25 days old and 37–55 days old (when the squid is exhibiting pre-schooling behavior), whereas they become larger in the right lobe than in the left one at 71–90 days old (when squid are schooling) [26]. The right optic lobe was found to be larger than the left one at age 80 days,

which is consistent with a previous study [26]. However, this is inconsistent with Sakurai and Ikeda [26], because squid at 45 days exhibited rightward asymmetry for optic lobe volume in this study. This inconsistency could be explained by individual differences in the timing of the right-bias occurrence. In fact, the optic lobe volume of oval squid at 37–55 days tended to lateralize to the right, but this trend was not statistically significant [26]. Some genes could possibly drive the timing of the appearance of left or right optic lobe volume asymmetry, as in other animals. For example, honeybee, *Apis mellifera* (Linnaeus, 1758), expresses more protein-coding genes in the right hemisphere than the left hemisphere [69]. In addition, they express a higher number of genes related to nervous system development and signaling in the left hemisphere, and biological regulation compared to the right hemisphere [69]. A similar phenomenon can occur in the optic lobes of oval squid, which causes individual variations in the timing of the appearance of asymmetry. Further details on the mechanisms of asymmetry have been studied in other animals. For example, Notch signaling is a part of the genetic signaling cascades that regulate the development of epithalamic asymmetry in zebrafish, *Danio rerio* (Hamilton, 1822) [70]. It was revealed that Notch proteins are necessary for photoreceptor differentiation and retinal organization in the embryo of longfin inshore squid, *Doryteuthis pealeii* (Lesueur, 1821) [71]. Therefore, it is possible that Notch proteins also regulate the development of the left–right asymmetry in cephalopods. Gene expression in the left and right optic lobes and central part of the brain must be investigated in the future with respect to lateralization.

4.2. Allometry among Body Size, Eyes, and Optic Lobes during Ontogeny

We found that the ratio of volume of eyes and optic lobes to body size (expressed by ML and BW) in oval squid was relatively larger in hatchlings than in juveniles (Figure 4). Younger individuals of Siberian sturgeon, *Acipenser baeri*, possess relatively larger eyes than older ones, which suggests the importance of vision during the early life phase [7]. Furthermore, some brain areas in smaller individuals of the bluespotted stingray, *Neotrygon kuhlii* (Müller and Henle, 1841), are larger than that the larger ones [72]. In cephalopods, similar findings were also reported for the European common cuttlefish, *Sepia officinalis* (Linnaeus, 1758) [73], and the oval squid [36]. As cephalopods lack parental care at early stages, visual abilities at these stages are subject to a strong evolutionary pressure in which they catch prey and avoid predators by themselves [73]. However, in primates that benefit from parental care at early stages, eye and brain sizes are relatively large at the prenatal stage compared to older stages [74,75], similar to the oval squid, observed presently. These consistent results between primates and cephalopods might be caused by a high nutrition supply at the prenatal and embryonic stages. Primates and cephalopods, respectively, are supplied with high nutrition from mothers at the prenatal stage [76] and the inner yolk at the embryonic stage [77]. Thus, the relatively larger eyes and brain in younger individuals could be common developmental features across animals that depend on vision.

There was hypoallometric scaling for body size vs. the eye volume and for body size vs. the optic lobe volume, while eye volume rapidly increased more than the optic lobe volume (hyperallometry, slope > 1), although the former is slightly smaller than the latter in the very early phase of life (ML < 7 mm, BW < 0.1 g; 0–15 days old) (Figures 4 and 5). These facts tentatively suggest that a gradual process for visual perception, which can be used as neural circuits for visual information processing in the brain (i.e., optic lobes), has already developed in the early phase post-hatching, while eyes that acquire surrounding information are small but functional. This possibility could be explained by the visual environment that cephalopods experience during embryonic development. The European common cuttlefish, *S. officinalis*, can visually recognize prey and predators when they are in egg cases [78,79]. Moreover, if this cuttlefish embryo has learned predatory behavior, these hatchlings can visually use social information by observing the behavior of other hatchlings [80]. The optic lobes of oval squid begin to develop from an early embryonic stage (stages 15), when other brain areas have not initiated development [81]. Because oval squid hatchlings can hunt prey immediately after hatching [34], oval squid embryos have

already completed a particular visual information processing to detect prey using the optic lobes. This is also reflected in the higher proportion of cortex in the optic lobes of embryos compared with juvenile oval squid [54].

The relative volume of the eyes overtakes that of the optic lobes at 15 days and maintains this trend until 120 days (Figure 4). Although a similar ontogenetic shift from hatchlings to juveniles (ML 30 mm) has already been reported for oval squid [81], the timing of its shift (i.e., 15 days post hatching) was determined for the first time in this study. Such a size shift between eyes and brain area related to vision through ontogeny is unknown in other animals. In early post-hatching stages of six coleoid species (*Sepia officinalis*, *Rossia macrosoma* (delle Chiaje, 1829), *Sepietta obscura* (Naef, 1916), *Idiosepius notoides* (Berry, 1921), *Loligo vulgaris* (Lamarck, 1798), and *Octopus vulgaris*), the optic lobes are larger than the eyes [82]. In addition, three coleoid species (*I. notoides*: ML = 9 mm, *S. lessoniana*: ML = 19 mm, and *Liocranchia reinhardtii* (Steenstrup, 1856): ML = 20 mm) have larger optic lobes than eyes, whereas four species (*Abraliopsis falco* (Young, 1972): ML = 20 mm, *Pyroteuthis margaritifera* (Rüppell, 1844): ML = 19 mm, *Spirula spirula* (Linnaeus, 1758): ML = 42 mm, and *Bathyteuthis abyssicola* (Hoyle, 1885): ML = 16 mm) have larger eyes than optic lobes [83], although these seven species were not determined according to developmental stages. These results suggest that the timing of the shift reflects the variation in their morphological and ecological changes through ontogeny. The growth ratio of eye size in the Siberian sturgeon, *Acipenser baerii*, drastically changes from hyperallometry (slope > 1) from the prelarval stage to approximately isometry (slope \approx 1) at the larval stage with changes in foraging style and metamorphosis [7]. The body size of oval squid grows two-fold, and their fins cover half of their mantles to up to an age of 20 days [34,84]. At 20 days, oval squid are able to maintain a spatial position in a water column with their physical traits. Such development of swimming ability seems to be related to the size shift between the eyes and optic lobes. They can move freely in water once they acquire swimming ability and simultaneously encounter prey, predators, and conspecifics. These numerous visual inputs might accelerate the development of eyes, in which a high acuity and contrast sensitivity are facilitated, as in vertebrates [1]. This is possibly also supported by the fact that the European common cuttlefish, *S. officinalis*, has a greater acuity and discrimination ability if raised under higher light intensities (rich visual input) compared with those in lower ones (poor visual input) [85].

Although the volume of the optic lobes indicated left–right asymmetry in this study, the allometric scaling for optic lobes did not differ between the left and right parts (Figure 4). These results seem to be caused by a slight difference in the absolute volume of the left and right optic lobes and the asymmetrical shift up to 120 days, post-hatching. The European common cuttlefish, *S. officinalis*, with a slightly larger right optic lobe and right vertical lobe tend to turn left in a T-maze [29]. However, in oval squids, the larger right optic lobe is not associated with the direction and strength of the lateralized eye to look at prey, predators, and conspecifics [26]. This inconsistency indicates that species-specific differences exist for mechanisms causing visual-related lateralization among cephalopods. Because optic lobes have several functions, such as processing of visual information [21], storage of memory [20], control of body color pattern [22,23], and control of motor response [24], it is important to examine the asymmetry of visual behavior and optic lobes of oval squid and of other cephalopods from various aspects relating to vision.

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