Comparison of Behavior, Histology and *ImpL2* Gene Expression of *Octopus sinensis* Under Starvation and Senescence Conditions

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Offshore cephalopods usually experience a short and extreme senescence process at the end of their lives. Unique behaviors such as brooding and fasting occur during this period. The cause of senescent cephalopod death is still unknown. Excessive energy consumption under prolonged starvation is one possible reason for death. A comparison analysis between normal starvation and senescence may provide valuable information to deepen our understanding of the senescence process of cephalopods. In this study, a comparison of behavior, histology and expression of a candidate senescence-specific gene (Imaginal morphogenesis protein-Late 2, ImpL2) of female Octopus sinensis under normal starvation and senescence was conducted. The females in the starvation group had similar behaviors, histology and ImpL2 expression level to the control group. The females in the senescence group exhibited typical behaviors and symptoms of senescent octopus, including fasting and unhealed lesions on the skin. Moreover, swimming and grasping ability was weakened in senescent individuals. The muscle tissue in the mantle and arm of senescent females showed a histolytic appearance. The secretory cells and their microvilli in the digestive gland gradually degraded and disappeared during senescence. The expression level of ImpL2 gene significantly changes in multiple organs of senescent females. The results showed that O. sinensis underwent different physiological and molecular processes during starvation and senescence. Starvation alone could not lead to senescent symptoms in the octopus. Tissue degeneration in the mantle, arm and digestive gland would increase the death risk of the octopus.

Keywords: octopus, senescence, starvation, behavior, histology, *ImpL2*

INTRODUCTION

The majority of shallow-water cephalopod species experience a short and extreme senescence process at the end of their lives. The senescence of female octopuses usually begins with spawning, and they tend to their clutches of eggs and blow water to clean and oxygenate the eggs (Gabe, 1975; Cosgrove, 1993). During this brooding period, females rarely leave their clutches and abstain from

feeding (Mangold, 1987). Females usually die when their eggs hatch (Anderson et al., 2002). Symptoms such as retraction of skin around the eyes, undirected or uncoordinated activity and unhealed lesions on the skin also occur in senescent females (Anderson et al., 2002; Wang and Ragsdale, 2018; Roumbedakis and Guerra, 2019). The male octopuses do not go through the brooding process, but the aforementioned senescent conditions will gradually appear from sexual maturity, and they die approximately the same time as the females (Hanlon, 1983; Mangold, 1983; Van Heukelem, 1983).

The cause of senescent cephalopod death is still unclear. A significant loss of body weight often occurs in senescent females. In the wild, female giant Pacific octopus (*Enteroctopus dofleini*) lost 50% to 71% of their body weight during the brooding period (Cosgrove, 1993). In captivity, female common octopus (*Octopus vulgaris*) lost 50% of their body weight (O'Dor and Wells, 1987); big blue octopus (*Octopus cyanea*), 36% (Van Heukelem, 1976); and *Octopus mimus*, 25% (Cortez et al., 1995). Therefore, excessive energy consumption under prolonged starvation may lead to the death of cephalopods. However, female octopuses under normal starvation and senescence have different behaviors. Compare with the drastic behavioral changes found in senescent female octopuses, starving females remain alert and active even after 38 days of fasting with substantial body weight loss (Wells and Wells, 1975). This implies that the octopus may undergo completely different physiological processes during starvation and senescence. However, little is known about this physiological difference. Most studies have focused on either the starvation or senescence process (Castro et al., 1992; Jackson and Mladenov, 1994; Zamora and Olivares, 2004; Roumbedakis et al., 2018), but the comparison analysis of these two conditions is limited.

At present, the molecular mechanism of cephalopod senescence is largely unknown. The optic gland has been found to play an important role in prompting senescence (Wodinsky, 1977). The transcriptional level of the Imaginal morphogenesis protein-Late 2 gene (*ImpL2*) is significantly up-regulated in the optic gland of the California two-spot octopus (*Octopus bimaculoides*) during senescence, making it a candidate senescence-specific gene in cephalopods (Wang and Ragsdale, 2018). However, the role of *ImpL2* in cephalopod senescence is still unclear.

To gain insight into the mechanisms underlying cephalopod senescence, a comparative analysis based on behavior, histology and gene expression (*ImpL2*) between starvation and senescence in the East Asian common octopus (*Octopus sinensis*) was conducted in this study.

MATERIALS AND METHODS

Octopus Collection and Management

A total of 146 *O. sinensis* (70 females and 76 males, body weight 300 g~600 g) were caught from the coastal waters of Lianjiang (Fujian Province, China) in June 2020. The octopuses were used for larvae rearing purposes, and they provided a sampling pool. Only the individuals that met the experimental requirements were included in this study. They were separated into 3 tanks

(6 m×6 m×1 m; dark yellow wall and white bottom). Each tank placed approximately 50 individuals with a 1:1 male/female sex ratio. Each tank placed 40 artificial dens at the bottom. The clams (*Ruditapes philippinarum*) and Zhikong scallops (*Chlamys farreri*) were provided twice a day, and the daily feed amount was approximately 15% of the octopus body weight. The same amount of live swimming crabs (*Charybdis japonica*) were offered every two days. The daily water exchange rates of the tanks were 200%. Water parameters were as follows: temperature, 19.5~23.1°C; salinity, 28~32 ppt; pH, 7.8~8.2; illumination intensity, 600~1000 Lux; and dissolved oxygen above 5 mg/L. A 14 h light:10 h dark photoperiod was maintained during the experiments. The octopuses were cultivated for two weeks, and mating frequently occurred during this period.

Experimental Design and Sample Collection

Females were chosen to conduct the tests in this study, because it was easier to identify their senescence stage. They were separated into fiberglass buckets (diameter-120 cm, height-70 cm), and each bucket contained one individual. The water parameters during the tests were as follows: temperature, 19.2~23.5°C; salinity, 28~32 ppt; pH, 7.8~8.2; and dissolved oxygen above 5 mg/L. The daily water exchange rate was 200%. The octopuses were divided into control, starvation and senescence groups. The control group included females that had not laid eggs with a feed supply. The starvation group included females that had not laid eggs without feed supply. The senescence group included females that had laid eggs. The octopuses in the control and starvation groups were chosen randomly from the tanks. They were kept undisturbed for one day to habituate to the bucket environment. Then the live clams (approximately 10% of the body weight) were provided for three days to test the condition of each octopus. The individuals that fed actively were kept to conduct subsequent experiments. The individuals in the control group were fed clams for another 3 days, and the samples were collected on the third day. No live prey items were provided in the starvation group, and the samples were collected on the 15th and 30th days after starvation. The octopuses in the senescence group were the females that just spawned in a day. The artificial dens were checked at 9 am every morning. Once the female was found to lay eggs, both the female and the artificial den were transported from the tank to the experimental bucket. The clams were offered every morning and cleaned on the next morning. The feeding amount of the senescent individuals was investigated by counting the empty shells of clams in the morning. The samples of the senescence group were collected on the 1st, 15th, 30th and 45th days after spawning. Three replicate samples were taken at each time point. A total of 21 females were used to conduct real-time quantitative PCR (qPCR) and histological analysis. The remaining individuals were used for larvae rearing. A diagram of the experimental flow can be found in **Figure S1**.

Octopuses were submerged in 20 g/L magnesium chloride seawater solution for at least 20 min to achieve deep anesthesia, which was certified by loss of reaction to all stimuli (Zhu et al., 2020). Once anesthetized, the individual was placed in a clean

tray, and the body weight (BW) was recorded. The optic gland was first collected for qPCR test. Then the individual was immediately euthanized by destroying the brain to confirmation of death as method of human killing (Fiorito et al., 2015). The digestive gland weight (DGW) and ovary weight (OW) of each individual were recorded. The digestive gland index (DGI) was calculated using $DGI = \frac{DGW}{BW\text{-}DGW}$, and the calculation of gonadosomatic index (GSI) was carried out using $GSI = \frac{OW}{BW\text{-}OW}$ (Castro et al., 1992; Sieiro et al., 2014). The mantle muscle, arm muscle, digestive gland, ovary, kidney, systemic heart and gill were collected for qPCR analysis and histological observation. The mantle samples were the muscle tissue near the funnel. The arm samples for qPCR were the muscle tissue corresponding to the 4th-6th row of suckers distal from the mouth on the second arm on the right side. The arm samples for histological analysis were the muscle tissue corresponding to the 7th-8th row of suckers on the same arm. The histological samples were fixed in Davidson fixative solution, and the qPCR samples were frozen in liquid nitrogen and stored at −80°C until RNA extraction. All of the animal experiments in this study were approved by the Animal Care and Use Committee of the Chinese Academy of Fishery Sciences (IACUC-2020-03).

Behavioral Observation During Starvation and Senescence

The females in the starvation and senescence treatments were also used for behavioral observation. They were observed three times daily (9 am-10 am, 2 pm-3 pm, 7 pm-8 pm). An underwater camera (Hero 5 Black, GoPro, USA) was used to record the behaviors. To record the predation behaviors of the females in the starvation group, they were fed empty shells of clams. The individuals in the senescence group rarely left their dens. To record their appearance and behaviors, several females were driven out of their home dens.

Histological Analysis During Starvation and Senescence

The samples were fixed in Davidson fixative solution for 48 hours. The fixed samples were dehydrated in a sequence of 75%, 85%, 95% and 100% ethanol solutions, and 95% and 100% ethanol dehydration was conducted twice. Each dehydration process lasted 20 min. The dehydrated samples were then cleared and impregnated with paraffin wax. The paraffin-embedded sections were cut into 6 μm sections using a microtome (RM2235, Leica, Germany), and sections were mounted onto glass slides. Then, the samples were dried at 45°C for 4 hours, followed by hematoxylin eosin staining. The stained samples were examined under a light microscope (MD4000 B, Leica, Germany).

RNA Extraction, Reverse Transcription, and Real-Time Quantitative PCR

Total RNA was extracted using an RNA extraction kit (DP419, TIANGEN, China). The quality and quantity of total RNA were detected by UV spectrophotometry (NanoDrop 2000, Thermo Scientific, USA) and 1.2% agarose gel electrophoresis. First strand cDNA was synthesized using the Evo M-MLV Mix Kit with gDNA Clean for qPCR (AG11728, Accurate Biology, China) according to the manufacturer's instructions. The primers for *ImpL*2 were designed based on our genomic data of *O. sinensis* (Li et al., 2020), and the elongation factor 1-α (EF) gene was used as the internal reference (Sirakov et al., 2009; García-Fernández et al., 2016). The primer sequences (5'-3') were as follows: *ImpL*2-F: TTCTAACGGAATGGGAGC, *ImpL*2-R: TCGGCAACTTAACTGGACAGC; EF-F: ACGAAGGCTGGGAAATTGA, EF-R: TGGTCTCTCCGTTGGTCTCT (Sirakov et al., 2009). The *ImpL*2 expression level of the optic gland in the control, starvation and senescence groups was detected using real-time quantitative PCR. Moreover, the *ImpL*2 expression levels of different tissues in the control and senescence groups (30 days after spawning) were also assessed. The qPCR was conducted using ChamQ SYBR Color qPCR Master Mix (Q411-02, Vazyme, China) and ABI StepOne Plus Sequence Detection System (Applied Biosystems, Foster City, CA, USA). A total of 20 μL of the PCR reaction volume contained 10 μL of 2×ChamQ SYBR Color qPCR Master Mix (Low ROX Premixed), 0.4 μL of 50×ROX Reference Dye I, 0.4 μL of forward and reverse primers (10 μM each), 1 μL of diluted cDNA templates, and 7.8 μL of sterile distilled water. The thermal cycling program was as follows: 95°C for 30 s, 40 cycles of 95°C for 10 s and 60°C for 30 s. A melting curve analysis was performed following the cycling stage to confirm the presence of a single PCR product. Each sample was detected in triplicate. The expression level of the EF gene remained stable across different samples. Relative gene expression levels were normalized to EF and quantified as the fold change relative to the expression levels in the control group according to the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Statistical Analysis

Statistical analysis was carried out with SPSS Statistics 21 (IBM, USA). Data were first tested for normality (Shapiro-Wilk test) and variance homogeneity (Levene's test). The *ImpL*2 expression level in the optic gland was analyzed with one-way analysis of variance (ANOVA). Multiple comparison was carried out using LSD test. The Kruskal-Wallis and Dunnett's T3 tests were conducted for DGI, GSI and feeding amount data. The *ImpL*2 expression level in different tissues was analyzed using independent t test. All data are presented as the mean ± standard deviation (SD). Statistical significance was considered a *p* value of< 0.05.

RESULTS

Behavioral Observation of *O. sinensis* in Different Groups

Control group: The females in the control group were active predators and spent time outside of their home dens (**Figure 1A**). They were able to change body color according to environmental variations. The females hunted clams through a swift pounce, and
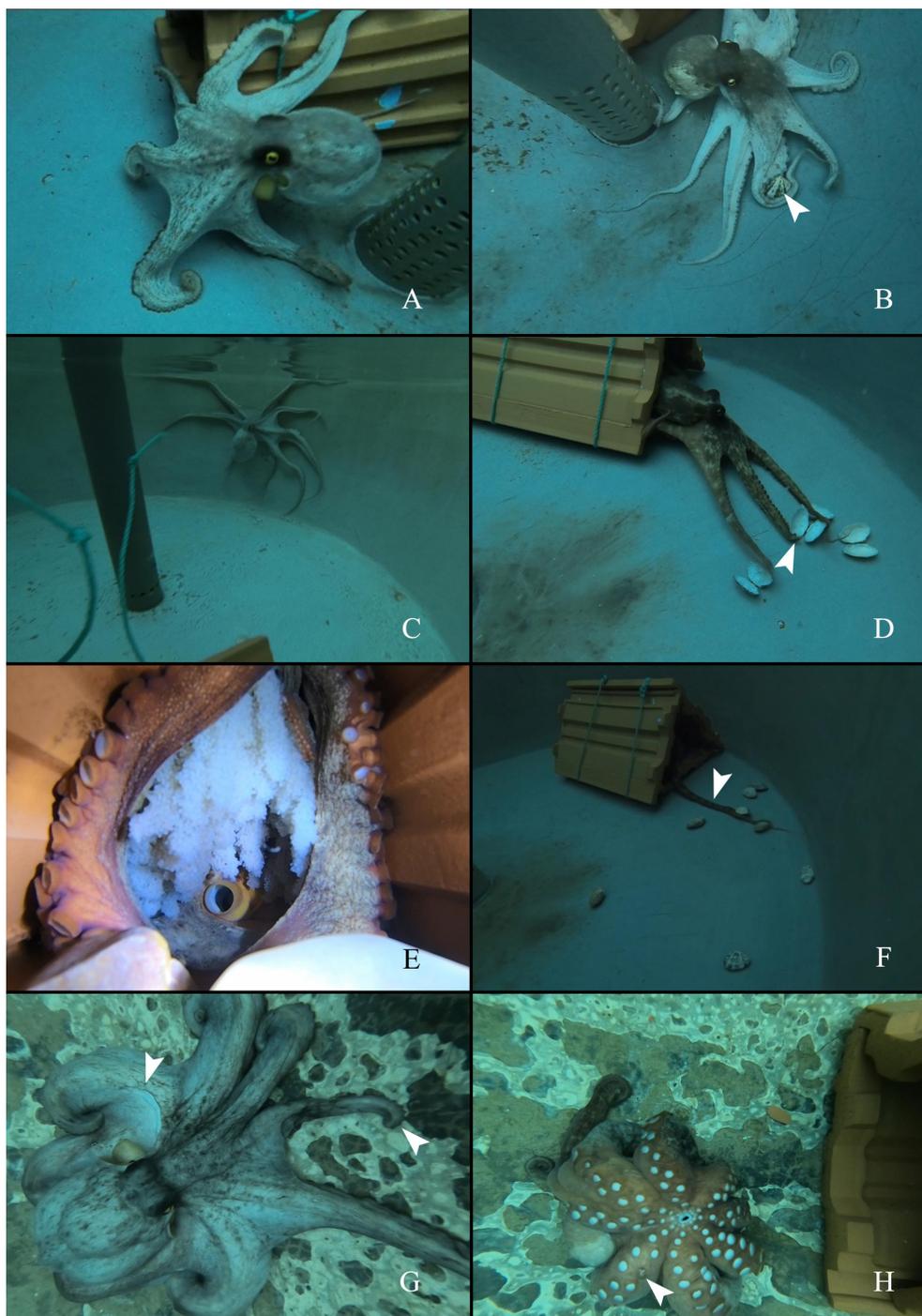
FIGURE 1 | The appearance and behaviors of *O.sinensis* in control, starvation and senescence groups. **(A)** *O.sinensis* in control group spends time outside of its home den. **(B)** *O.sinensis* in control group attacks the clams (several clams are restrained under the interbranchial web). White arrow head indicates the clam. **(C)** *O.sinensis* in starvation group is active outside of its home den. **(D)** *O.sinensis* in starvation group attacks the empty shells of clams. White arrow head indicates the missing arm tip. **(E)** A brooding female. **(F)** *O.sinensis* in senescence group stays inside their home den, uses arm to grasp clams. White arrow head indicates the arm stretches out the den. **(G)** The appearance of a senescent female. White arrow heads indicate a missing patch of skin and a missing arm tip. **(H)** A senescent female cannot turn over (one day before death). White arrow head indicates a missing sucker.

the prey were captured under the interbrachial web (**Figure 1B**) (Movie-Control group feeding). The clams were consumed fast due to the good appetite of the females.

Starvation group: The females in the starvation group began laying eggs on the 35th day, and the last spawning was found on the 39th day. Thus, behavioral observation was carried out for 39 days. They had similar behaviors to those in the control group and spent quite a long time outside of their dens (**Figure 1C**). The females kept attacking the empty shells during the 39-day starvation experiment (**Figure 1D**) (Movie-Starvation group feeding). Some individuals had missing arm tip (**Figure 1D**).

Senescence group: In the senescence group, the food intake decreased gradually from 7-8 clams per day to 1-2 clams per day, and eventually the females stopped feeding (**Supplementary Figure S2**). There were feeding amount variations between different individuals (**Supplementary Figure S3**). The females had brooding activity, and they rarely left their home dens (**Figure 1E**). Instead of capturing prey by pouncing, the females used one arm to grab the clam back to the den (**Figure 1F**) (Movie-Senescence group feeding). One female was found to leave its clutch to obtain the prey (Movie-Senescent individual goes out). During this short period, she did not pounce on the clams; instead, she grabbed and carried the prey back to its den. Moreover, the grasping ability of the arms became weaker, and the clams could not be held by the arms very well. Unhealed wounds and missing arm tips were found in the senescent females (**Figure 1G**) (Movie-Appearance and behaviors before death). The females seemed to lose swimming ability before death, and they could only crawl on the bottom of the tank (**Figure 1H**) (Movie- Appearance and behaviors before death).

Histological Analysis of Different Tissues During Starvation and Senescence

In the present study, only mantle muscle, arm muscle, digestive gland and ovary showed histological variations during senescence. The kidney, systemic heart, and gill exhibited similar histological structures in the control, starvation and senescence groups. The mantle muscle of the females in the control and starvation groups had similar structures, and bundles of circular muscle fibers were separated by thin layers of radial muscle fibers. The muscle tissue on 15, 30 and 45 DAS (days after spawning) showed a histolytic appearance, the muscle fibers were broken and dissolved, the radial muscle fibers became incompact, and the gaps between the fibers were enlarged (**Figures 2A–C**; **Supplementary Figure S4**). A similar histological change pattern was observed in the arm muscle. Both the control and starvation groups possessed arms with a typical array of longitudinal muscle and trabeculae fibers. However, rather than since 15 DAS, the breakdown and disorganization of muscle fibers had occurred since 30 DAS. The histological structure on 15 DAS was the same as that of the control group (**Figures 2D–F**; **Supplementary Figure S5**). The digestive gland in the control and starvation groups exhibited a similar histological structure, with characteristic secretory cells and microvilli. The microvilli at the top of secretory cells began to disappear on 15 DAS. In the senescence group, the secretory

cells were found to shrank and disappear on 30 DAS, while most of the secretory cells disappeared on 45 DAS (**Figures 2G–I**; **Supplementary Figure S6**). The digestive gland index of the starvation, 30 DAS and 45 DAS groups was significantly lower than that of the control group (**Supplementary Figure S7**). A large number of mature follicles were observed in the ovaries of the control and starvation groups. The epithelium of follicles infolded to form elongated crests. Most of the follicles degraded and disappeared on 15, 30 and 45 DAS (**Figures 2J–L**, **Supplementary Figure S8**). The gonadosomatic index of the 30 DAF (days after fasting) and 1 DAS groups was significantly higher than that of the control group, while the index of the 15, 30 and 45 DAS groups was significantly lower than that of the control group (**Supplementary Figure S9**).

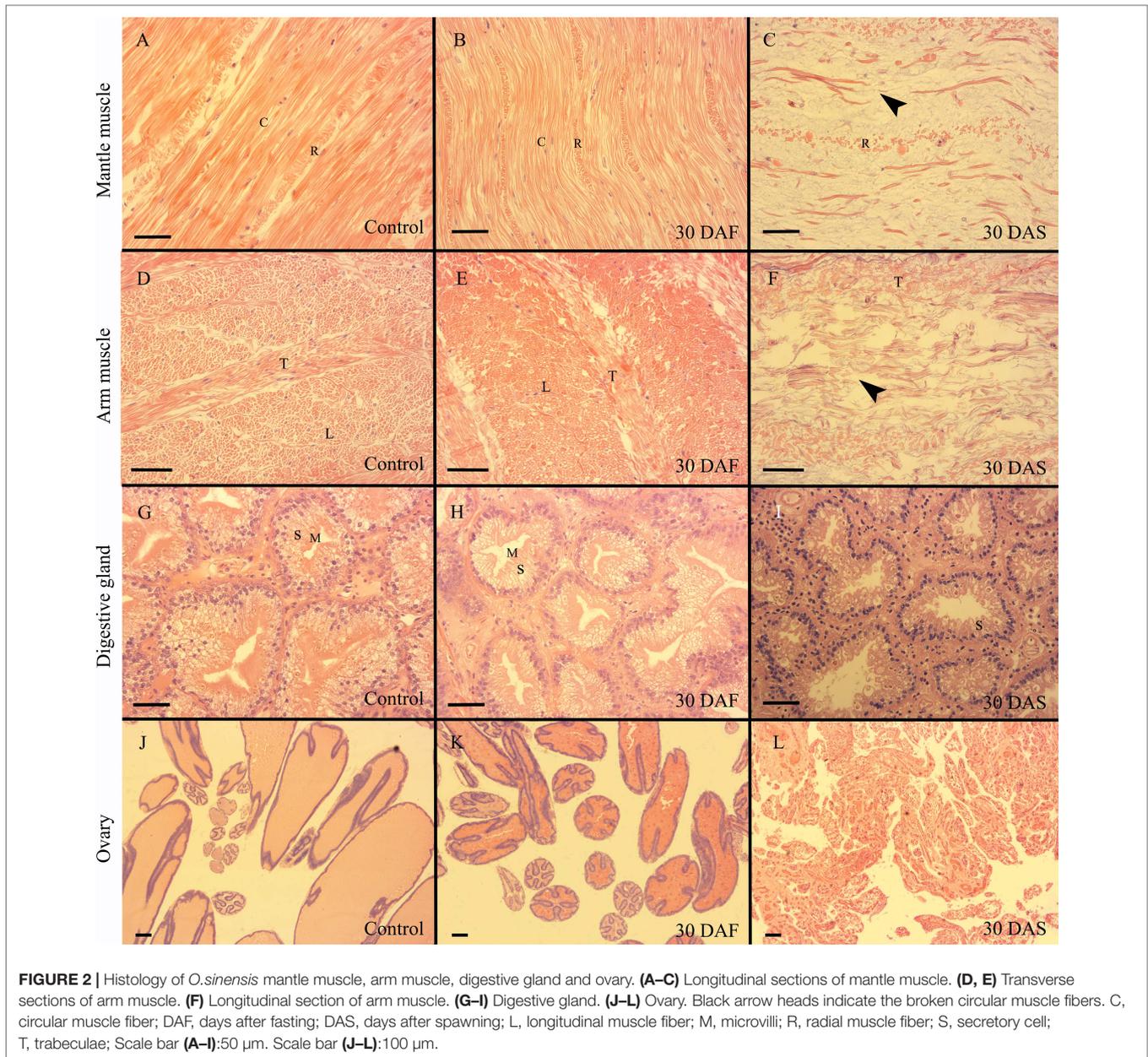## Expression of *ImpL2* During Starvation and Senescence

The *ImpL2* expression level in the optic gland of starvation and control groups was significantly lower than that in the senescence group, and the peak expression level was found on 30 DAS (**Figure 3**). The *ImpL2* expression level of optic gland, arm muscle, mantle muscle and ovary on 30 DAS was significantly higher than that of the control group (**Figure 4**). The largest difference was found in the optic gland, followed by the ovary and arm muscle (**Figure 4**). The down-regulation of *ImpL2* expression was found in kidney and gill in the senescence group (**Figure 4**). While the *ImpL2* expression level in digestive gland and systemic heart was similar between the control group and senescence group (**Figure S4**).

DISCUSSION

Behaviors of *O. sinensis* During Starvation and Senescence

The females in the control and starvation groups had similar behaviors. In general, octopuses can easily learn to recognize empty shells and stop capturing them. Interestingly, the individuals in the starvation group kept attacking the empty shells during the 39-day starvation experiment. Thus, it may be a better strategy for starving individuals to capture all potential prey rather than spending time identifying them. Overall, the octopus did not show obvious behavioral and appearance changes after nearly 40 days of starvation and remained alert and active, similar to the findings in *O. vulgaris* (Wells and Wells, 1975).

Compared to the control and starvation groups, the females in the senescence group exhibited completely different behaviors. First, the females rarely left their home dens after spawning. They had brooding activity before egg hatching, which is typical in octopus (Gabe, 1975; Cosgrove, 1993; Anderson et al., 2002; Hanlon and Messenger, 2018; Wang and Ragsdale, 2018). The individuals continued to stay in their home dens after eggs hatched; even if they were driven out, they would soon crawl back to their dens. However, *O. bimaculoides* has been shown to spend most of the time outside of the dens before death (Wang

FIGURE 2 | Histology of *O.sinensis* mantle muscle, arm muscle, digestive gland and ovary. **(A–C)** Longitudinal sections of mantle muscle. **(D, E)** Transverse sections of arm muscle. **(F)** Longitudinal section of arm muscle. **(G–I)** Digestive gland. **(J–L)** Ovary. Black arrow heads indicate the broken circular muscle fibers. C, circular muscle fiber; DAF, days after fasting; DAS, days after spawning; L, longitudinal muscle fiber; M, microvilli; R, radial muscle fiber; S, secretory cell; T, trabeculae; Scale bar **(A–I)**:50 μm. Scale bar **(J–L)**:100 μm.
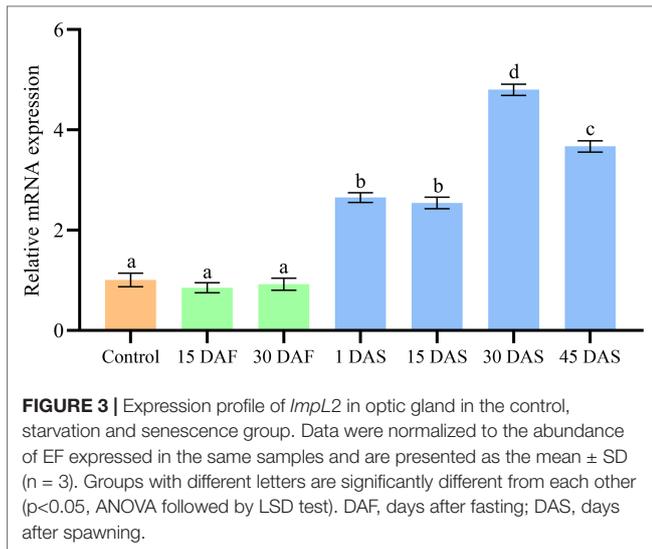
and Ragsdale, 2018). This indicated that senescent behaviors are variable in different species. Both *O. sinensis* and *O. bimaculoides* lost most of their motor ability after hatching. In the wild, slow-moving octopuses are easy targets for predators when they leave their dens. Thus, *O. bimaculoides* is likely to be captured by predators before the senescence process kills it.

Second, senescent females lost their appetite and abstained from feeding, which is also a typical symptom in octopus (Van Heukelem, 1977; Wodinsky, 1977; Cortez et al., 1995; Anderson et al., 2002). The females in the senescence group also exhibited different predatory behaviors; rather than pursuing prey, the individuals stayed in their home dens and used one arm to grab the clams. The predation of *O. bimaculoides* before spawning

occurs through a visually directed jet-propelled "pounce", while brooding females grab crabs as they move past the den (Wang and Ragsdale, 2018). The senescent octopus changes from an active predator to a lurker that can only wait for prey.

Third, the females in the senescence group showed weakened motor ability. Individuals rarely swam before death; they could only crawl on the bottom of the tank. The pupils of senescent *O. bimaculoides* have been found to gradually lose their horizontal orientation with respect to gravity, and the females spend time sitting on the bottom of the tank (Wang and Ragsdale, 2018). Moreover, the arms were less flexible in grabbing clams than the control group. This would further decrease their predatory ability to obtain prey.

FIGURE 3 | Expression profile of *ImpL2* in optic gland in the control, starvation and senescence group. Data were normalized to the abundance of EF expressed in the same samples and are presented as the mean ± SD (n = 3). Groups with different letters are significantly different from each other (p<0.05, ANOVA followed by LSD test). DAF, days after fasting; DAS, days after spawning.

FIGURE 4 | Expression profile of *ImpL2* in different tissues in control group and senescence group (30 DAS). Data were normalized to the abundance of EF expressed in the same samples and are presented as the mean ± SD (n = 3). *p<0.05, independent t test. DAF, days after fasting; DAS, days after spawning.

Fourth, the females in the senescence group had unhealed wounds, missing arm tips and suckers. Similar symptoms have also been found in senescent *O. bimaculoides* (Wang and Ragsdale, 2018). In addition, arm regeneration may not occur in injured senescent European Cuttlefish (*Sepia officinalis*) (Féral, 1988). Resistance against parasite infection may be impaired in senescent *O. vulgaris* (Pascual et al., 2010). Thus, the senescence process of cephalopods is likely accompanied by a reduction in immunity.

Histological Variations During Senescence

The muscle tissue in the mantle and arm of *O. sinensis* had a high degree of muscle histolysis during senescence. In the post-spawning stage of deep-sea squid *Moroteuthis ingens*, the thick, elastic mantle becomes thin, inelastic and gelatinous, and the muscle tissue has a very disorganized structure and histolytic appearance, consisting predominantly of scattered collagen fibers with considerable gaps between (Jackson and Mladenov, 1994). In *O. mimus*, the arm muscle fibers of senescent individuals present atrophy and histolysis, with a great enlargement of the gaps between the fibers (Zamora and Olivares, 2004). The breakdown of muscle structure inevitably leads to a decrease in motor ability. The grabbing and swimming ability was reduced in *O. sinensis*, which would lower their chance to capture prey and escape from predators, eventually increasing the risk of death. There was no such muscle tissue wasting in the starvation group, indicating that starvation would not lead to muscle dissolution, and there must be other reasons for muscle histolysis in the senescent octopus.

The digestive gland index (wet weight) gradually decreased in the starvation and senescence groups, indicating that the digestive gland is one of the main energy reserve organs during these two processes. A similar phenomenon could be found in other cephalopods (Castro et al., 1992; Zamora and Olivares, 2004). The digestive gland structure of *O. sinensis* in the starvation group remained intact, while the secretory cells and microvilli in the senescence group continually degraded and disappeared. In the post-spawning stage,
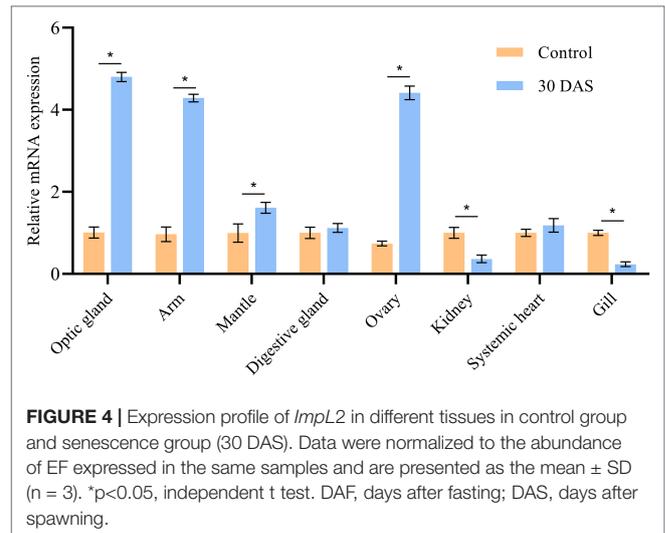
the secretory cells of the digestive gland in *O. mimus* lose their apical portion and are reduced to cubic cells (Zamora and Olivares, 2004). The degeneration of the digestive gland will destroy the digestion and absorption function, which would lead to death by starvation.

Expression Pattern of *ImpL2* During Senescence

In the present study, most of the tissues showed *ImpL2* expression variations during senescence, suggesting that *ImpL2* plays important role in senescent octopus. The *ImpL2* expression level of optic gland, arm muscle, mantle muscle and ovary in the senescence group was higher than that in the control group. IMPL2 is a homolog of insulin-like growth factor binding protein (IGFBP) that serves as a suppressor of the insulin/IGF signaling pathway in *Drosophila* (Honegger et al., 2008; Kwon et al., 2015; Roed et al., 2018). The up-regulated expression of *ImpL2* in *Drosophila* has been shown to induce muscle tissue wasting (Figueroa-Clarevega and Bilder, 2015; Kwon et al., 2015), which is similar to the muscle histolysis found in senescent octopus. The function of *ImpL2* in octopus is still unknown, but the similar histological changes imply a conserved function between octopus and *Drosophila*. As an important endocrine organ, the *ImpL2* expression level in the optic gland increased significantly during senescence. This result is consistent with the findings in *O. bimaculoides* (Wang and Ragsdale, 2018). There was no *ImpL2* expression difference between the starvation and control groups, indicating different insulin/IGF signaling regulation between normal and senescent octopus. Down-regulation of *ImpL2* expression was found in kidney and gill during senescence, indicating different functions of *ImpL2* played in these tissues. Only limited studies have been conducted on *ImpL2*, its function in different tissues during senescence needs further investigation in the future.

CONCLUSION

Based on behavioral, histological and gene expression analyses, it was found that *O. sinensis* underwent different physiological and

molecular processes during starvation and senescence. Prolonged starvation alone could not lead to senescent symptoms in the octopus. Histological evidence corresponding to some senescent symptoms was found in the present study. The reduced motor ability of the senescent octopus was related to muscle tissue wasting in the mantle and arm, and the degeneration of secretory cells in the digestive gland weakened digestive function. The expression level of *ImpL2* significantly changes in multiple tissues during senescence, implying its function in promoting senescence. We plan to focus on the muscle wasting of senescent octopus in the future. The regulation mechanism of *ImpL2* on Insulin/IGF pathway and the function of *ImpL2*-Insulin/IGF signaling pathway on muscle wasting will be investigated through molecular biology techniques such as recombinant protein stimulation, RNA interference and pathway inhibitor/activator treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This study was reviewed and approved by Animal Care and Use Committee of the Chinese Academy of Fishery Sciences.

AUTHOR CONTRIBUTIONS

LB conducted the sample collection, data curation and draft writing. FL performed the histological analysis. QC conducted the writing-review and editing. CL and XL performed the molecular work. JT conducted the statistical analysis. SZ performed the data visualization. ML, YS and RX were involved in the sample collection. SC contributed to the concept and design of the study. 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.2022.874777/full#supplementary-material

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