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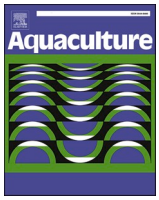

Refinement of a classification system for gonad development in the triploid oyster *Crassostrea gigas*

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ABSTRACT

Breakthroughs in polyploidy technologies have enabled the scaling-up of the commercial utilization of phenotypically-sterile triploid *Crassostrea gigas*. Compared with diploid oysters, gonad development in triploids is retarded, but not absent. Although histological methods of characterizing gonadal development in triploid *C. gigas* has been reported, the selection of sampling sites and densities might lead to bias when taking seasonal variations into consideration. Moreover, previously-employed classification systems were found to be incompatible with our long-term observations. In this study, by sampling Pacific oysters at different seasons, over several years and from different locations, we were able to establish a new classification system for gonadal development in triploid Pacific oyster based on histological analysis. Based on the types of germ cells, triploids were grouped into female (divided into female α and female β), male and hermaphrodites during the reproductive season. Surprisingly, female α was partially sterile, showing active gametogenesis and few abnormal germ cells, which were defined as β gonia. Unlike female α, triploid female β had the most severely retarded gonad along with abnormal morphological features in the gonia. We inferred that β gonia were correlated with female triploid sterility. Despite the presence of numerous gametes in triploid male gonads, the fecundity of triploid males was still severely reduced compared with that of diploids. In addition, maturation arrest of spermatogenesis in triploid males was detected histologically. Based on the types of germ cells and the presence of β gonia, hermaphrodites were further divided into hermaphrodite I and hermaphrodite II. An almost male-like triploid was redefined as a hermaphrodite triploid. The gonadal development of triploids from different sites was mostly similar. As shown via annual histological analysis, the increase in the percentage of female α and the decrease in the percentage of female β matched the seasonal development of the oyster gonad. Hence, we speculated that gonad development in female triploid oysters was delayed.

1. Introduction

Triploid aquatic species are widely used in aquaculture since their growth, survival rates and flesh quality are significantly enhanced compared to diploids (Rasmussen and Morrissey, 2007; Maxime, 2010). Optimization of polyploidy technologies has succeeded in producing triploids in many species, such as Atlantic salmon (*Salmo salar*) (Murray et al., 2018), rainbow trout (*Oncorhynchus mykiss*) (Weber et al., 2014), shrimp (Manan and Ikhwanuddin, 2021) and oysters (Nell, 2002). In addition, potential genetic contamination with gene-editing technology has driven researchers to find an alternative method for reproductively sterile aquatic animals. The polyploidy approach is a viable strategy to induce sterility.

In general, triploids have an extra set of chromosomes, which usually prevents meiotic sister chromatids from separating and makes it difficult to produce functional gametes (Zhang et al., 2021). Theoretically, triploids are sterile (Benfey, 1999). However, some triploid aquatic animals are not completely sterile. For example, triploid females of *Oncorhynchus masou* exhibited abnormal gonadal development without germ cells, whereas triploid males showed active gametogenesis (Gray et al., 1993; Tiwary et al., 2001). A similar phenomenon has been reported in several triploid fish (Krisfalusi and Cloud, 1996; Carrasco et al., 1998). In triploid shellfish, the potential to produce gametes and viable offsprings was limited, particularly in triploid oysters (Meng et al., 2012; Suquet et al., 2016; Matt and Allen Jr, 2021). The reproductive capabilities of triploid oysters vary between individuals.

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Relative to diploid oysters, the gonads in triploids were retarded but not absent in Pacific oysters (*C. gigas*) (Guo and Allen, 1994a; Beaumont and Fairbrother, 1991; Gong et al., 2004 ; Suquet et al., 2016). Jouaux et al. (2010) reported that 25% of triploid *C. gigas* could produce numerous gametes, and Hermabessiere et al. (2016) found that 46% of triploids produced numerous gametes. In *Crassostrea virginica*, 19% of triploids were observed with substantial numbers of gametes (Matt and Allen Jr, 2021). Moreover, fertile triploid oysters could be found in both sexes, and mating experiments using triploid parents could produce viable gametes (Guo and Allen, 1994a; Gong et al., 2004; Suquet et al., 2016).

Triploid oysters can be applied as an interesting model to elucidate the effect of triploidy on gametogenesis. Therefore, characterization of gametogenesis and a reasonable and scientific classification system developed for gonadal development in triploid oysters are necessary for further studies. Jouaux et al. (2010) previously established a classification method for gametogenesis in triploid Pacific oysters. Two types of gametogenic patterns were defined: α-patterns, which had a great number of gametes, and β-patterns, showing abnormal, disturbed gametogenesis. The appearance of the β-pattern was due to the presence of abnormal gonia. However, this classification system in triploid Pacific oysters seemed inapt for triploid *C. virginica* because gonad development in triploid *C. virginica* was quite different from that described by Jouaux et al. (2010) for triploid *C. gigas*. The fecundity described in triploid *C. gigas* was not observed in triploid *C. virginica* (Matt and Allen Jr, 2021). Based on the features of gonia and the presence of mature gametes, a classification system was developed to assess gonad development in triploid *C. virginica* (Matt and Allen Jr, 2021). Matt and Allen Jr (2021) hypothesized that the nature of gonia indicated sex, of which 'normal' gonia were associated with males, while irregular gonia were associated with females. In addition, they proposed that it was difficult to determine the stage of gametogenesis in triploids because of the lack of synchrony between follicle development and gamete production. Therefore, it is of great interest to note the stark difference in gonad development between the two *Crassostrea* oysters. In addition, different sampling sites and densities might cause bias when considering seasonal variation. Attention has been given to specific gonad development in triploid *C. gigas* over recent years. Jouaux et al. (2010) documented similar phenomena in which abnormal gonia and a small portion of triploid oysters contained numerous gametes. Nonetheless, the classification criteria proposed by Jouaux et al. (2010) did not fully explain our results based on long-term observations of triploid *C. gigas*. Some findings bear a marked resemblance to those described in triploid *C. virginica* (Matt and Allen Jr, 2021).

In this study, we aimed to characterize the gametogenesis of triploid Pacific oysters from different sites and compare them with diploids through fine histological analysis. The extent of gonad development in triploid Pacific oysters during an annual reproductive cycle was analyzed to obtain a better understanding of the fecundity of triploids. We developed a method to distinguish the sex of triploid *C. gigas* in early development using the nature of the type of germ cells and proposed a new classification system for gonad development in triploid Pacific oysters based on histological observations of a great number of samples. This study provided new insights into the gonad development of triploid oysters and valuable information to the aquaculture industry. Since triploid eggs have been successfully used to induce tetraploid oysters (Guo and Allen, 1994b), understanding gonad development in female triploids is of great significance for the induction of tetraploid oysters.

2. Materials and methods

2.1. Sample collection

All diploid and triploid Pacific oysters in this study were located in Shandong Province in China. The triploids were bred by crossing tetraploids and diploids. Two hundred triploids and 100 diploids were cultured in Rushan (Rs), Shandong (36.4 N, 121.3 E) and were sampled in June 2018. To assess the annual development cycle of gonads in triploid Pacific oysters, a total of 1000 one-year-old crossed triploids and 700 one-year-old diploids were cultivated in Qingdao (Qd), Shandong (36.2 N, 120.6 E), in 2018. The diploid and triploid oysters in Qingdao (Qd) were collected each month from April 2019 to March 2020. In April, 100 diploid and 100 triploid *C. gigas* were collected. From May to July, 100 diploids and 200 triploids were collected, while 40 diploids and 40 triploids were collected from other months. To compare gonad development of triploids in different locations in the same year, 300 crossed triploids and 100 diploids in Rongcheng (Rc), Shandong (37.1 N, 122.4 E) were collected in June 2019.

2.2. Ploidy verification

Before sampling, ploidy was evaluated in all triploid Pacific oysters. After dissection, approximately 1 mm^3 of gill tissue was dissected in 1 ml PBS (phosphate buffer saline) and diluted in 3 ml 100% ethanol. After a short centrifugation (300 *g*, 25 °C, 5 min) and removal of the supernatant, cell pellets were resuspended in 1 ml PBS. Thirty microliters of the solution containing propidium iodide was added. After a 20 min incubation, ploidy was analyzed using flow cytometry (CytoFlex Beckman Coulter, US).

2.3. Histology

Gonad tissue from each sample was fixed in Bouin's fluid for 24 h and then stored in 70% ethanol. Subsequently, sections were dehydrated, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin. The sections were observed and photographed using an Olympus BX53 microscope (Olympus, Japan).

2.4. Gonad development

Histological assessment covered each triploid and diploid. Stages of gametogenic development of diploids were assigned according to Franco et al. (2008) and Normand et al. (2008). Diploids were divided into six stages: stage 0: inactive stage; stage I: proliferative stage; stage II: growth stage; stage III: mature stage; IV, spawning stage; and V, reabsorption stage, based on the morphology of the follicles, follicle contents, and follicle coverage.

As gonad development in triploids deviated considerably from that in diploids, we did not adopt the standard of diploids to judge the stage of triploid gonad development. The observation of triploid gonads was mainly through the type of germ cells, the number and location of gametes, and the morphology of the follicles. Different gonads of triploids from the same time and location were compared and classified. To further analyze the differences in triploid gonad development, diploid and triploid gonads at the same time and location were compared.

3. Results

3.1. Classifying gonad development in triploids during the reproductive season

The gonad development of triploids was abnormal, which was quite different from that of diploids. One of the most striking differences was the abnormal gonadal cells in triploids, as shown in Figs. 1, 2, and 3 (arrowhead). Abnormal gonadal cells always presented with condensed and rod-shaped chromosomes that were acidophilic. This kind of gonadal cell was along the inner side of the gonadal tubule with a changeable number and was named β gonia according to Matt and Allen Jr (2021). We found that β gonia were present in most triploid gonads and throughout the course of gametogenesis. The presence of abnormal cells (β gonia) and the relative abundance of gametes and triploids were classified according to the types of germ cells. Because germ cells could be observed during the proliferative stage, the categories of triploids

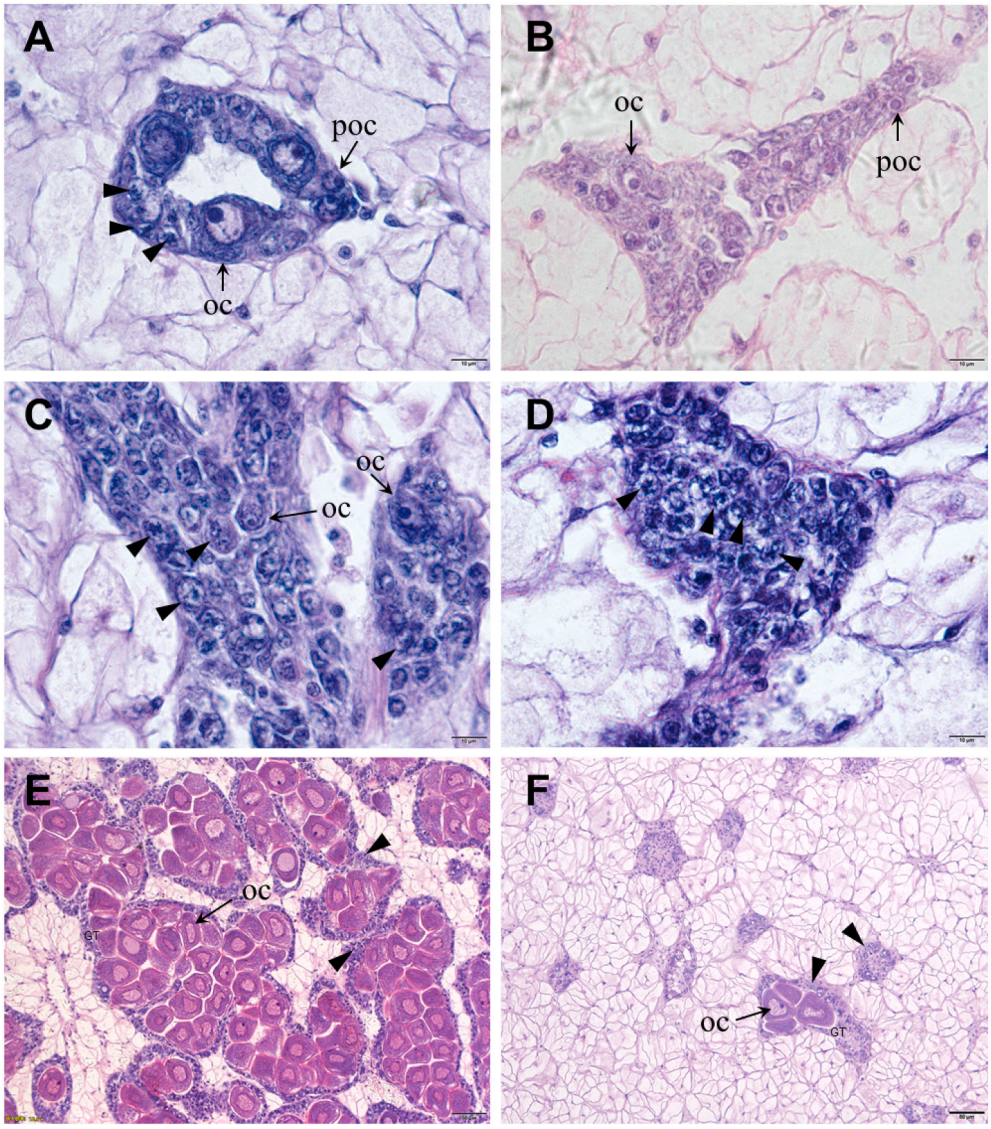

Fig. 1. Gonadal follicles of female diploid and triploid *C. gigas*. A, C&D. Female triploid at early stage; B. Female diploid at early stage; E. Female α triploid; F. Female β triploid. GT: gonadal tubule; poc: previtollogenic oocyte; oc: oocyte; β gonia (arrowhead). A–D, Scale bar = 10 μm; E–F, Scale bar = 50 μm.

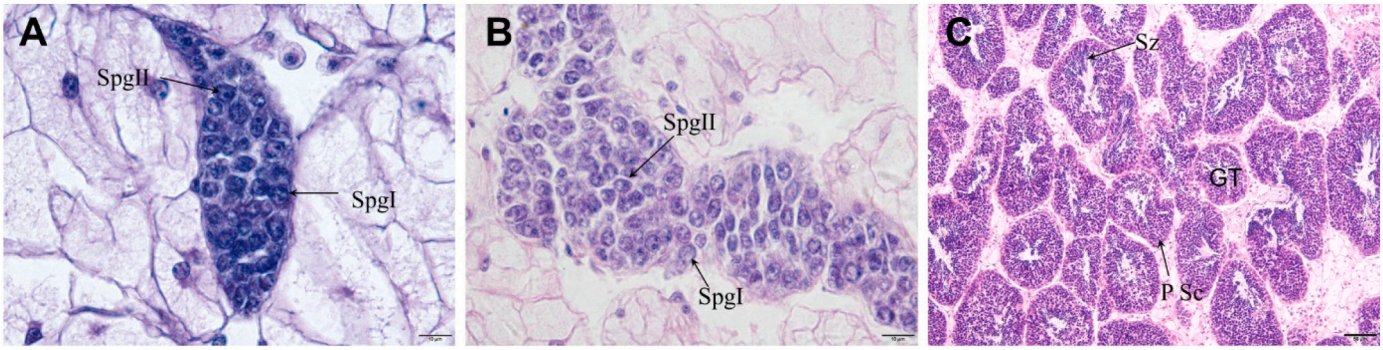

Fig. 2. Gonadal follicles of male diploid and triploid *C. gigas*. A. Male triploid at early stage; B. Male diploid at early stage; C. Male triploid. GT: gonadal tubule; SpgI: Type I spermatogonium; SpgII: type II spermatogonium; P Sc: primary spermatocyte. Sz: spermatozoa. β gonia (arrowhead). A–B, Scale bar = 10 μm; C, Scale bar = 50 μm.

started from the early development of triploid gonads.

3.1.1. Female

The sex of triploids can be distinguished in the early stage of gonad development. At the early development of triploids, previtellogenic

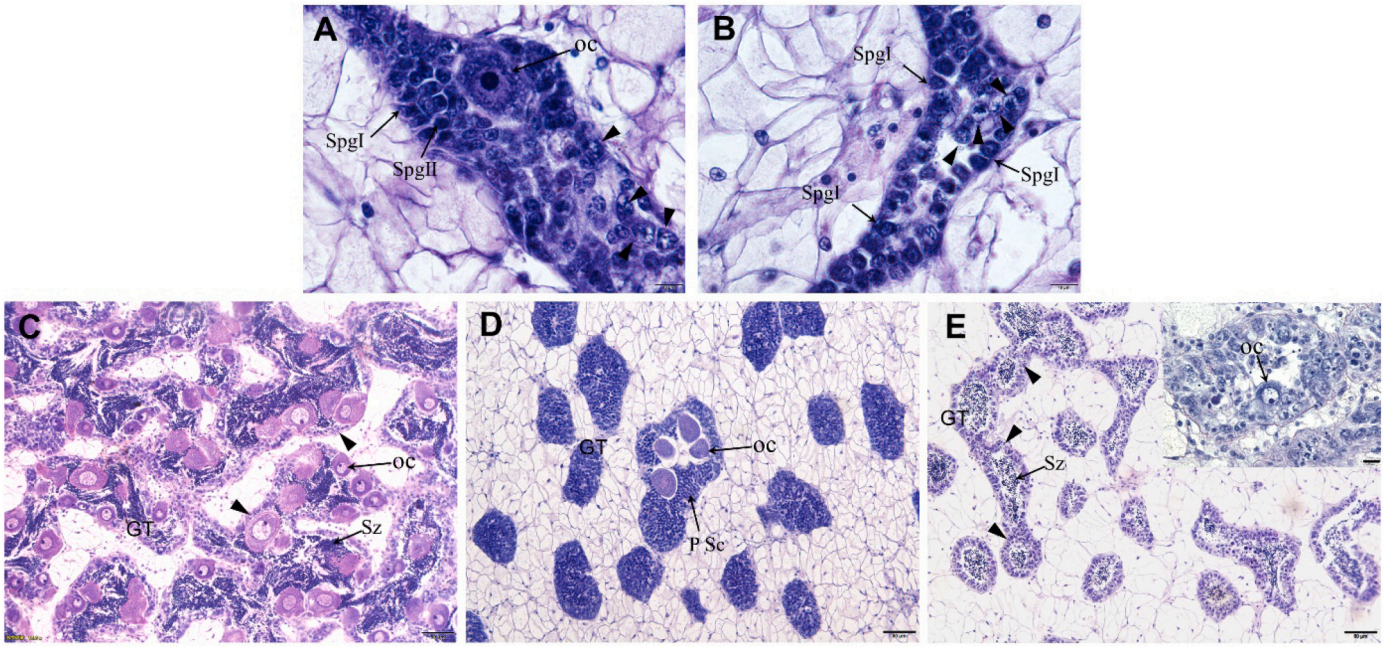

Fig. 3. Gonadal follicles of hermaphrodite triploid *C. gigas*. A& B. Hermaphrodite at early stage; C&F. Hermaphrodite I; E. Hermaphrodite II. GT: gonadal tubule; SpgI: Type I spermatogonium; SpgII: type II spermatogonium; P Sc: primary spermatocyte. Sz: spermatozoa; oc: oocyte; β gonia (arrowhead). A–B, Scale bar = 10 μm; C–E, Scale bar = 50 μm.

oocytes, oocytes and β gonia could be observed (Fig. 1A). There was an obvious nucleolus in the center of the previtellogenic oocyte, which accounted for a great proportion of the cells. The volume of the oocytes was larger than that of previtellogenic oocytes. In comparison, female diploids at early gametogenesis consisted of primary oocytes of homogeneous size (Fig. 1B), while the size of oocytes in female triploids was inhomogeneous (Fig. 1A). Sometimes β gonia occupied a large proportion of the follicle. As shown in Fig. 1C, two oocyte were detected, while the others were β gonia. Some follicles were even filled with β gonia (Fig. 1D).

With the development of gonads in female triploids, fecundity varies from individual to individual. The number of oocytes in triploid was varied. Some produced plenty of oocytes, while some produced few oocytes. To distinguish these two kinds of female triploids, female α and female β were used.

3.1.1.1. Female α. The female α triploid produced many oocytes (Fig. 1E). Follicles were filled with mature oocytes, and β gonia were distributed at the inner side of follicles. The gonads of female triploids were generally inhibited, and the number of oocytes in triploids was generally lower than that in diploids. However, a few female α were similar to diploids in the mature stage per histological sections, in which oocytes filled the gonad without connective tissue. In our study, only seven female triploids from 456 triploids in June were close to diploids.

3.1.1.2. Female β. Most triploid females were classified as female β, which had no or a few oocytes (Fig. 1F). The follicles of female β always consisted of β gonia.

3.1.2. Males

The follicles of male triploids contained closely arranged and orderly spermatogenic cells (Fig. 2). The gonads of male triploids (Fig. 2A) were similar to those of diploids (Fig. 2B) during early gametogenesis. However, with the development of gonad, the gonads of male triploids did not change substantially, and exhibited numerous primary spermatocytes, spermatids and few sperm in the follicles of male triploids, similar to the diploids of the same growth stage (Fig. 2C). Primary spermatocytes or spermatogonia were present along the follicle wall of male triploids, while a few spermatids were at the center of the follicle. No male triploid that produced as many spermatozoa as the mature diploids was observed. In addition, β gonia were absent in male triploids.

3.1.3. Hermaphrodites

Hermaphrodites could be identified according to gametogenesis cells and β gonia at early gametogenesis (Fig. 3). As shown in Fig. 3A, oocytes, primary spermatocytes and β gonia were observed at the same follicle. Primary spermatocytes and β gonia without oocytes were found in some hermaphrodites (Fig. 3B). Through histological observation, two types of hermaphrodites could be identified based on germ cells: hermaphrodite I (Fig. 3C) and hermaphrodite II (Fig. 3D). The major difference between the two types of hermaphrodites was the presence of β gonia.

3.1.3.1. Hermaphrodite I. Most triploid hermaphrodites were classified as hermaphrodite I, which contained spermatogenic cells, oocytes and β gonia. β gonia were present along the follicle wall of hermaphrodite I. Spermatogenic cells in hermaphrodite I were apparent, including primary spermatocytes, spermatids and sperm. In most cases, mature spermatids were more abundant than other spermatogenic cells in follicles. Many sperms detached from the germinal lining of the wall. The number of oocytes was variable. Sometimes it was difficult to observe oocytes in histological sections. As shown in Fig. 3E, the triploid contained spermatozoa and β gonia without oocytes, but when more sections of this sample were observed, the oocytes could be found (shown in the small picture). Thus, we also classified this type of triploid as hermaphroditic I.

3.1.3.2. Hermaphrodite II. Hermaphrodite II was rarely observed. Gonadal tubules of hermaphrodite II were filled with a number of spermatocytes surrounded by a few oocytes, and no β gonia were observed.

3.2. Categories of triploids in different locations

To assess gonad development in triploids from different locations,

triploids from Rushan (Rs), Rongcheng (Rc) and Qingdao (Qd) were classified according to the system standard mentioned above (Table. 1). The diploids, collected at the same time, were already in the mature stage (Supplement Fig. S1). The ratios of triploids assigned to five categories were similar at the three sites. The percentage of female β was the largest, and ranged from 44.44% to 52.20%. Hermaphrodite I ranged from 26.42–33.33%. Hermaphrodite II accounted for a very small proportion, with only six individuals found. The percentages of females α and males were similar, and were 5.56%–13.21% and 7.55%–12.59%, respectively.

3.3. Annual analysis of gonad development in triploids

Annual histological analysis of gonad development in 1000 triploid Pacific oysters began in April 2019 and lasted over a period of one year at Qd. The gonad development of triploid *C. gigas* could be divided into two phases: the gametogenesis phase from April to August and the reproductive inactivation phase from September 2019 to March 2020. Males and females were indistinguishable at the reproductive inactivation phase. Thus, only the triploid oysters of the gametogenesis phase were classified (Fig. 4); the gonadal development of triploids of the other phase were presented in Supplement Fig. S2-S4. From April to August 2019, the percentage of female α increased from 2.41% to 35.14%. From April to June 2019, the percentage of female β increased from 32.53% to 52.20% and then decreased to 16.22% in August. The percentage of males changed slightly in April (34.95%) and May (30.30%) and then decreased in July and August (22.22% and 24.32%) but reached its lowest value in June (7.55%). The ratio of hermaphrodite I decreased from April (30.12%) to July (13.89%). Hermaphrodite II made up a small, relatively unchanging percentage (0–4.05%).

In September, the diploids were in the reabsorption stage, while most triploids were in reabsorption except that some triploids spawned (Supplement Fig. S2). From October 2019 to February 2020, gonads of diploids and triploids were at resting (Supplement Fig. S3). In March 2020, the diploid and triploid gonads began to redevelop (Supplement Fig. S4).

3.4. Comparison of gonad development between diploid and triploid males

We compared diploid and triploid males in the same culture area from April to September 2019 (Fig. 5 and Fig. 6). The gonad tubules of diploid males in April contained a number of primary spermatocytes (Fig. 5A). Triploid males were similar to diploid males (Fig. 6A). In May, the diploid male gonads were filled with spermatogenic cells without connective tissue, and spermatocytes, spermatocytes, or spermatozoa appeared at the same time (Fig. 5B). For triploids, there was space between follicles, and spermatocytes and spermatocytes appeared (Fig. 6B). In June, diploids were in the mature stage, and the follicles were full of mature sperms. Follicles became loose, and sperms began spawning in July. During August, connective tissue appeared again, and sperms became less abundant. However, with the growth of gonads from June to August, the follicles in male triploids seemed to stop spermatogenesis, and the gonads looked similar, containing many spermatocytes and spermatocytes and few spermatozoa. In September, gonadal tubules of diploids were empty, while the composition of triploid follicles did not change.

Table 1
Percentage of individuals classified in each category of gonad development.

	Female α	Female β	Male	Hermaphrodite I	Hermaphrodite II
2018 Rs	10.37%	44.44%	12.59%	30.37%	2.22%
2019 Qd	13.21%	52.20%	7.55%	26.42%	0.63%
2019 Rc	5.56%	48.77%	11.11%	33.33%	1.23%

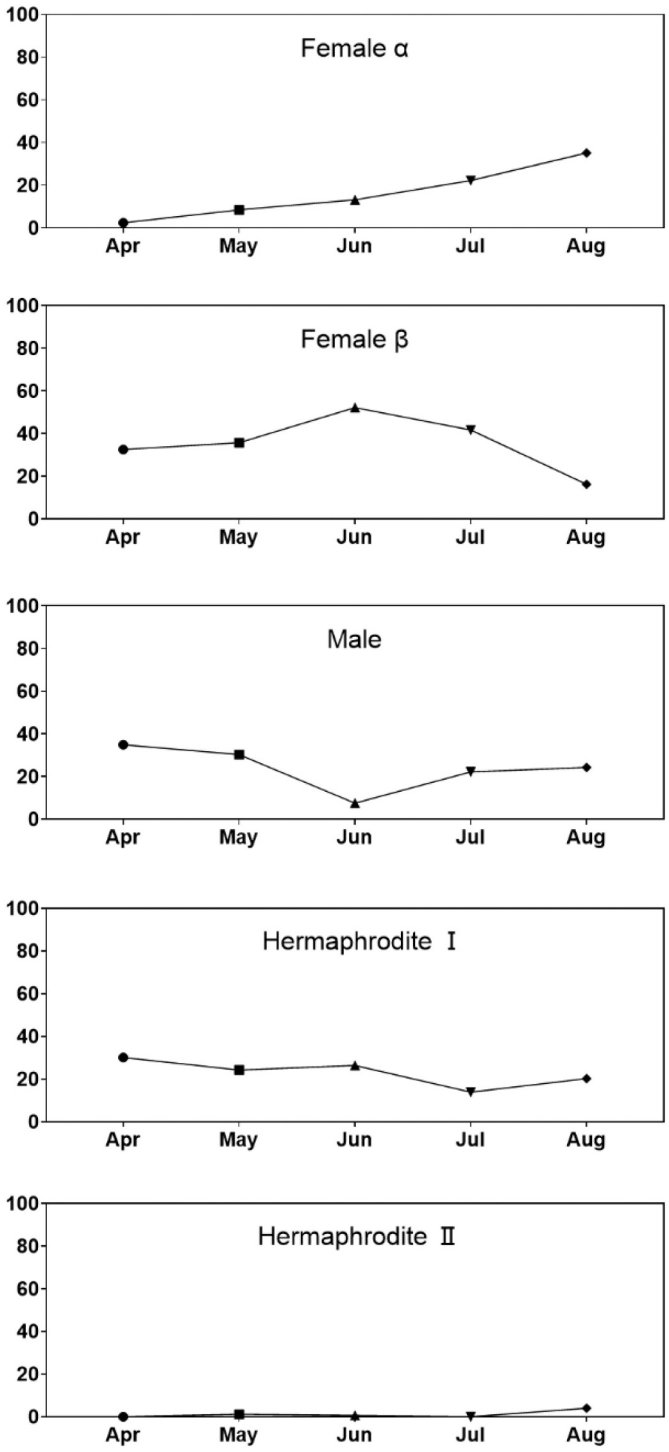

Fig. 4. Percentage of triploid *C. gigas* individuals of each category of gonad development sampled from Qingdao, Shandong.

4. Discussion

4.1. Characteristics of triploid gonad development

Our findings indicated that triploid oysters were characterized by abnormal gonad cells and β gonia. In keeping with previous reports, β gonia had rod-like condensed chromosomes that were basophilia

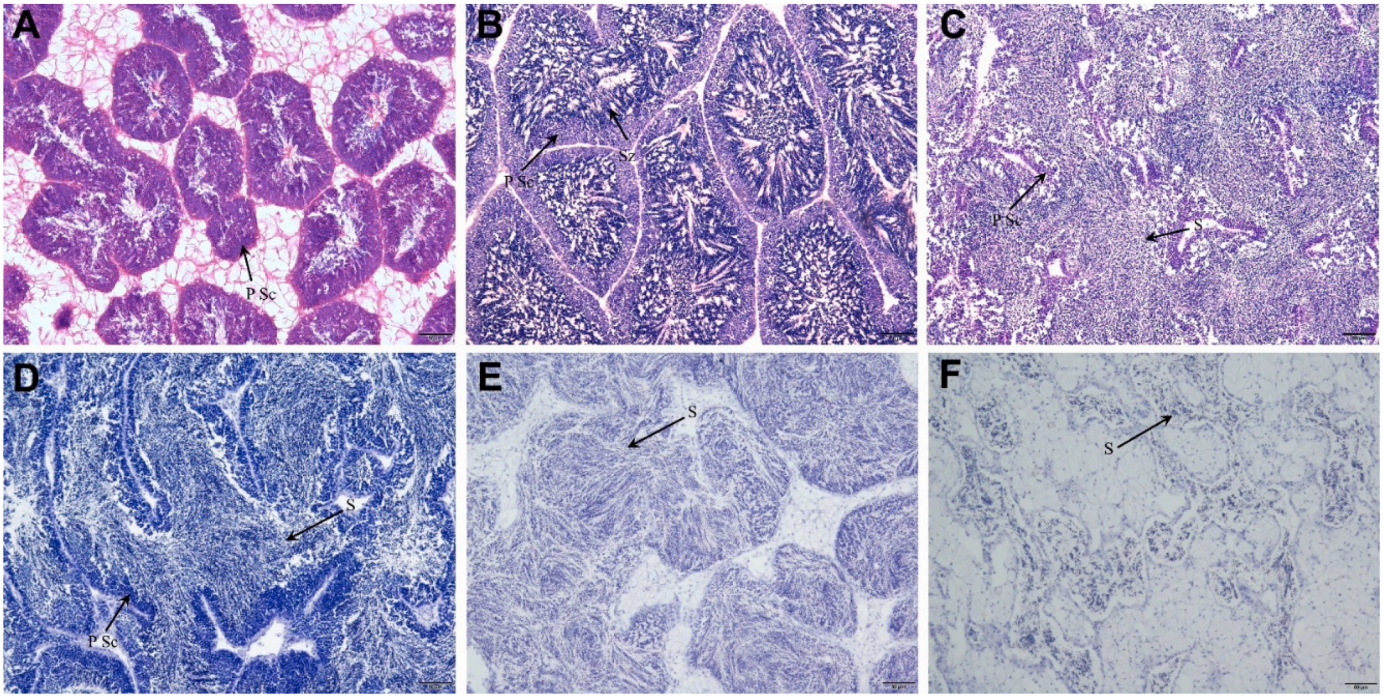

Fig. 5. Male diploids from April to September sampled from Qingdao, Shandong. A. April; B. May; C. June; D. July; E. August; F. September. Scale bar = 50 μm.

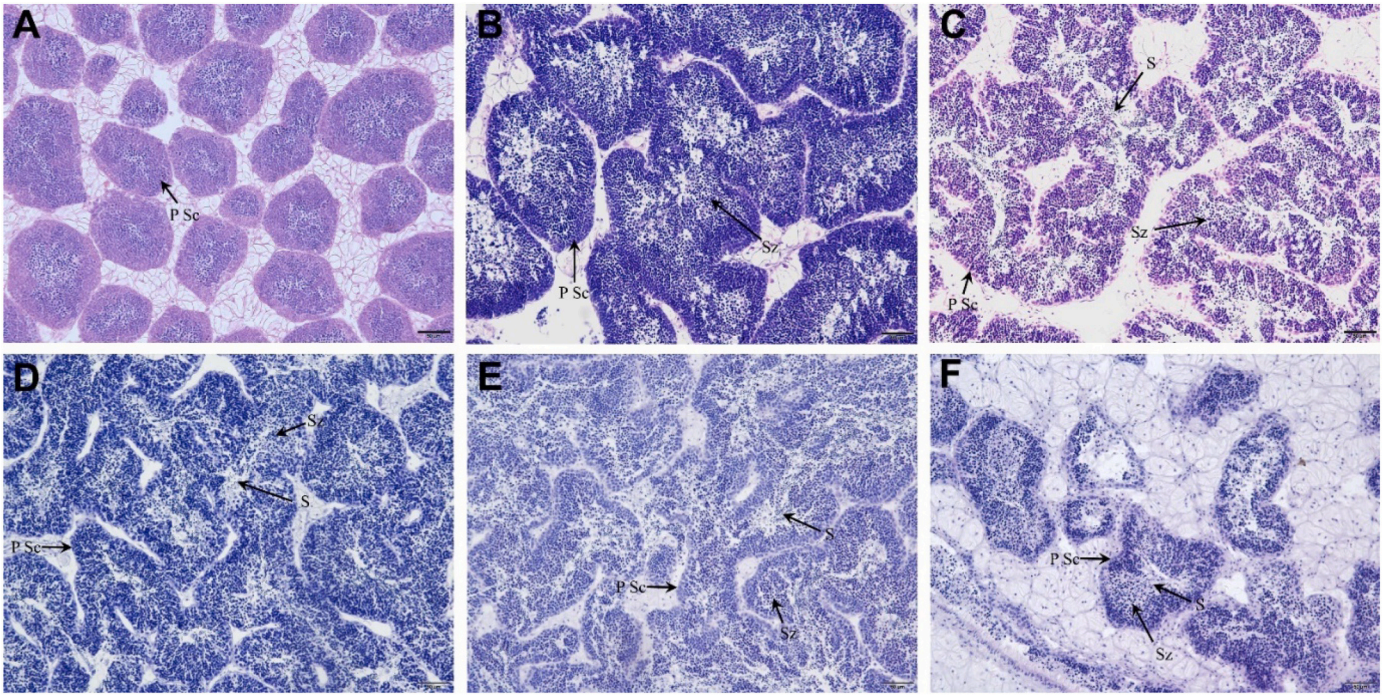

Fig. 6. Male triploids from April to September sampled from Qingdao, Shandong. A. April; B. May; C. June; D. July; E. August; F. September. Scale bar = 50 μm.

(Jouaux et al., 2010; Matt and Allen Jr, 2021). There were different explanations for the appearance of abnormal gonad cells. Jouaux et al. (2010) found abnormal cells present in sterile triploids that exhibited locking of gonial mitosis events in both males and females. However, Matt and Allen Jr (2021) observed abnormal gonad cells in all triploid *C. virginica* females and referred to them as abnormal oogonia. Consistent with the latter observation, the appearance of β gonia was associated with the oogenesis of triploid *C. gigas*.

β gonia were observed in the early stage of gonad development in triploid *C. gigas*, in accordance with the previous observation by Jouaux et al. (2010). Unlike diploid oysters, males and females had obvious phenotypic differences at stage I. Jouaux et al. (2010) considered that sex identification was never possible for triploid *C. gigas* at that stage due to the abnormal aspect of the gonial proliferating fold in the inner part of the gonadal tubule. Conversely, with fine microscopic observation, apparent oogonia and spermatogonia made it easy to distinguish the sex of triploids. Additionally, we identified β gonia from normal germ cells at the early stage of gametogenesis. In addition, β gonia were present in

triploids throughout the reproductive season, especially in triploids with oocytes. Therefore, β gonia served as an important criterion in our classification system for triploid Pacific oysters. In addition, the number of gametes varied greatly among triploid *C. gigas*. We used female α and female β to define triploid females with more oocytes and fewer oocytes, respectively.

The types of gonadal cells at the early stage largely indicated subsequent gonadal development. At the early stage of triploid gonad development, we observed different patterns of germline cells arrangement. Some gonads exhibited a compact and homogeneous germ cell arrangement in appearance, with deep alkaline staining, indicating an association with male fate. We also found that some male germ cells were interspersed with β gonia at early stage, indicating that these individuals would become Hermaphrodite I. Previtellogenic oocytes and oocytes were found in the early development of triploid female gonads. β gonia were present in all triploid females at the early growing stage, suggesting that β gonia were associated with triploid oogenesis. Based on the number of oocytes and β gonia, the females could be portioned into two types. The individuals with rare oocytes or previtellogenic oocytes but many β gonia in the gonads were female β, and the others were female α. The triploids with oocytes and spermatocytes would undoubtedly be hermaphrodites. The histologic types could be identified at early stage.

High variation in reproductive capacity exists among triploid oysters. The number of gametes in some triploids was comparable to that in normal diploid organisms, while others were sterile (Guo and Allen, 1994a; Eudeline et al., 2000; Normand et al., 2008; Jouaux et al., 2010). It was quite difficult to define the stage of gonad development in triploids. In our study, we found that the beginning and end of the reproductive period in triploids were similar to those observed in diploid gonads. Thus, to facilitate the study of triploid gonads, we suggested that judging the stage of triploid gonad development could refer to diploids raised at the same time and same area, which could be classified as stage 0, inactive stage; stage I: proliferative stage; stage II: growth stage; stage III: mature stage; IV, spawning stage; and V, reabsorption stage.

4.2. The sterility of triploid females

Sterility is common in triploid organisms. The meiosis process was obstructed in triploids because the extra haploid set of chromosomes made it difficult to develop functional gametes (Zhang et al., 2021). In practice, fecundity varies in triploids from different species. In *C. gigas*, oocyte maturation was arrested in the metaphase of the first meiosis (Li et al., 2000). Given the high error rate seen in meiotic products (Martin, 2008), the level of sterility in females was supposed to be lower. In fact, it was generally high in females, with 25% of triploids producing plenty of eggs (Jouaux et al., 2010). In our study, 5.56% ~ 13.21% of triploids produced numerous oocytes.

According to histological analysis, the percentage of female α increased gradually with gonad development, while the percentage of female β decreased in July and August, which matched the seasonal development of the oyster gonad. Therefore, we speculated that gonad development in female triploid oysters may be delayed. As previously reported, the diameter of mature eggs in triploid oysters was 11% larger than that of diploids (Jeung et al., 2016). The growth of triploid eggs requires more energy material than diploid eggs, which may result in a delay in gonad development in female triploids. Abnormal cells, β gonia, associated with undeveloped oocytes have already been mentioned above. We hypothesized that β gonia were directly related to female triploid sterility. However, studies on β gonia were quite limited.

4.3. The fecundity of triploid males

To reveal fecundity in triploid males of *C. gigas*, we specifically compared gonad development between diploid and triploid males. The results showed that normal diploids underwent the normal maturation of spermatogenesis during the reproductive season. However, when oysters were in the growth stage, the gonads of male triploids were similar to those of diploids only in April (Fig. 4A and Fig. 5A). After that, the gonad development of the triploid males seemed to be arrested, along with the maintenance of the dominance of spermatocytes and spermatids and the minority of sperms in gonad tubules. The same phenomenon was also reported by Allen and Downing (1990). Similarly, Xiang et al. (2006) claimed that even though sperms were observed in the vas deferens of triploid shrimp, ultrastructural observation indicated that they were not mature sperm but spermatids. In addition, comparative analysis of testis transcriptomes in cyprinid fish revealed that genes associated with sperm flagellar assembly and motility were downregulated in triploid fish, suggesting that the variational expression of these genes may be a critical factor for sperm formation and lead to sterility of triploid male fish (Xu et al., 2015; Li et al., 2019). Previous studies speculated that male triploid *C. gigas* could proceed with early meiosis normally and produce spermatocytes, but problems occurred in spermatocytes and spermatids in later meiosis (Lee, 1988; Allen and Downing, 1990; Barber and Mann, 1991; Guévélou et al., 2019). Thus, we speculated that unusual meiosis and obstacles of spermatozoa assembly may lead to the reduced fecundity of triploid males.

Additionally, the diploid and triploid oysters entered the resting stage simultaneously at the end of the reproductive season. We did not find any evidence that gonad development of male triploids was retarded. Although the gonads of triploid males contained numerous gametes, most of them might not be able to develop into mature sperm. The fecundity of male triploid *C. gigas* was still severely reduced, which was overestimated in the previous study (Jouaux et al., 2010). Moreover, the male-like triploid, which was regarded as β male triploid and a kind of sterile triploid by Jouaux et al. (2010), was confirmed to be hermaphrodite triploid in the present study.

Credit author statement

Qiong Yang: Writing - original draft. **Hong Yu**: Conceptualization, Writing - review & editing. **Qi Li**: Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Allen, S.K., Downing, S.L., 1990. Performance of triploid pacific oysters, *Crassostrea gigas*: gametogenesis. Can. J. Fish. Aquat. Sci. 47, 1213–1222.

Barber, B.J., Mann, R., 1991. Sterile triploid *Crassostrea virginica* (Gmelin, 1791) grow faster than diploids but are equally susceptible to *Perkinsus marinus*. J. Shellfish Res. 10, 445–450.

Beaumont, A.R., Fairbrother, J.E., 1991. Ploidy manipulation in molluscan shellfish: a review. J. Shellfish Res. 10, 1–18.

Benfey, T.J., 1999. The physiology and behaviour of triploid fishes. Rev. Fish. Sci. 7, 39–67.

Carrasco, L., Doroshov, S., Penman, D.J., Bromage, N., 1998. Long-term, quantitative analysis of gametogenesis in autotriploid rainbow trout, *Oncorhynchus mykiss*. Reproduction 113, 197–210.

Eudeline, B., Allen, S.K., Guo, X.M., 2000. Delayed meiosis and polar body release in eggs of triploid Pacific oysters, *Crassostrea gigas*, in relation to tetraploid production. J. Exp. Mar. Biol. Ecol. 248, 151–161.

Franco, A., Berthelin, C.H., Goux, D., Sourdaine, P., Mathieu, M., 2008. Fine structure of the early stages of spermatogenesis in the Pacific oyster, *Crassostrea gigas* (Mollusca, Bivalvia). Tissue Cell 40, 251–260.
Gong, N., Yang, H., Zhang, G., Landau, B.J., Guo, X., 2004. Chromosome inheritance in triploid Pacific oyster *Crassostrea gigas* Thunberg. Heredity 93, 408–415.
Gray, A.K., Evans, M.A., Thorgaard, G.H., 1993. Viability and development of diploid and triploid salmonid hybrids. Aquaculture 112, 125–142.
Guévélou, E., Carnegie, R.B., Small, J.M., 2019. Tracking triploid mortalities of eastern oysters *Crassostrea virginica* in the Virginia portion of the Chesapeake Bay. J. Shellfish Res. 38, 101.
Guo, X., Allen, S.K., 1994a. Reproductive potential and genetics of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). Biol. Bull. 187, 309–318.
Guo, X., Allen, S.K., 1994b. Viable tetraploids in the Pacific oyster (*Crassostrea gigas* Thunberg) produced by inhibiting polar body 1 in eggs. Mol. Mar. Biol. Biotechnol. 3, 42–50.
Hermabessiere, L., Fabioux, C., Lassudrie, M., Boullot, F., Long, M., Lambert, C., Hegaret, H., 2016. Influence of gametogenesis pattern and sex on paralytic shellfish toxin levels in triploid Pacific oyster *Crassostrea gigas* exposed to a natural bloom of *Alexandrium minutum*. Aquaculture 455, 118–124.
Jeung, H.D., Keshavmurthy, S., Lim, H.J., Kim, S.K., Choi, K.S., 2016. Quantification of reproductive effort of the triploid Pacific oyster, *Crassostrea gigas* raised in intertidal rack and bag oyster culture system off the west coast of Korea during spawning season. Aquaculture 464, 374–380.
Jouaux, A., Heude-Berthelin, C., Sourdaine, P., Mathieu, M., Kellner, K., 2010. Gametogenic stages in triploid oysters *Crassostrea gigas*: irregular locking of gonial proliferation and subsequent reproductive effort. J. Exp. Mar. Biol. Ecol. 395, 162–170.
Krisfalusi, M., Cloud, J.G., 1996. Effects of exogenous estradiol-17β on early growth and gonadal development of diploid and triploid female rainbow trout (*Oncorhyncus mykiss*). Dev. Genet. 19, 302–303.
Lee, M.M., 1988. Abnormal gametogenesis in triploid American oysters *Crassostrea virginica*. J. Shellfish Res. 7, 201–202.
Li, Q., Osada, M., Kashihara, M., Hirohashi, K., Kijima, A., 2000. Cytological observations on nuclear behavior in normal and gynogenetic eggs of the pacific oyster, *Crassostrea gigas*. Suisan Zoshoku 48, 193–198.
Li, W.H., Tan, H., Liu, J.M., Hu, J., Cui, J.L., Wang, S., Liu, S.J., 2019. Comparative analysis of testis transcriptomes associated with male infertility in triploid cyprinid fish. Reprod. Fertil. Dev. 31, 248–260.
Manan, H., Ikhwanuddin, M., 2021. Triploid induction in penaeid shrimps aquaculture: a review. Rev. Aquacult. 13, 619–631.
Martin, R.H., 2008. Meiotic errors in human oogenesis and spermatogenesis. Reprod. BioMed. Online 16, 523–531.
Matt, J.L., Allen Jr., S.K., 2021. A classification system for gonad development in triploid *Crassostrea virginica*. Aquaculture 532, 735994.
Maxime, V., 2010. The physiology of triploid fish: current knowledge and comparisons with diploid fish. Fish Fish. 9, 67–78.
Meng, Q.L., Bao, Z.M., Wang, Z.P., Wang, S., Hu, J.J., Hu, X.L., Huang, X.T., 2012. Growth and reproductive performance of triploid Yesso scallops (*Patinopecten yessoensis*) induced by hypotonic shock. J. Shellfish Res. 31, 1113–1122.
Murray, D.S., Kainz, M.J., Hebberecht, L., Sales, K.R., Hindar, K., Gage, M.J.G., 2018. Comparisons of reproductive function and fatty acid fillet quality between triploid and diploid farm Atlantic salmon (*Salmo salar*). R. Soc. Open Sci. 5.
Nell, J.A., 2002. Farmingtriploidoysters. Aquaculture 210, 69–88.
Normand, J., Le Pennec, M., Boudry, P., 2008. Comparative histological study of gametogenesis in diploid and triploid Pacific oysters (*Crassostrea gigas*) reared in an estuarine farming site in France during the 2003 heatwave. Aquaculture 282, 124–129.
Rasmussen, R.S., Morrissey, M.T., 2007. Biotechnology in aquaculture: transgenics and polyploidy. Compr. Rev. Food Sci. F 6, 2–16.
Suquet, M., Malo, F., Quere, C., Leduc, C., Le, G.J., Benabdelmouna, A., 2016. Gamete quality in triploid Pacific oyster (*Crassostrea gigas*). Aquaculture 451, 11–15.
Tiwary, B.K., Kirubagaran, R., Ray, A.K., 2001. Plasma levels of Gonadotropin-II and gonadal sex steroids in triploid catfish, *Heteropneustes fossilis* (Bloch). Fish Physiol. Biochem. 24, 9–14.
Weber, G.M., Hostuttler, M.A., Cleveland, B.M., Leeds, T.D., 2014. Growth performance comparison of intercross-triploid, induced triploid, and diploid rainbow trout. Aquaculture 433, 85–93.
Xiang, J.H., Li, F.H., Zhang, C.S., Zhang, X.J., Yu, K.J., Zhou, L.H., Wu, C.G., 2006. Evaluation of induced triploid shrimp *Penaeus (Fenneropenaeus) chinensis* cultured under laboratory conditions. Aquaculture 259, 108–115.
Xu, K., Wen, M., Duan, W., Ren, L., Hu, F.Z., Xiao, J., Liu, S.J., 2015. Comparative analysis of testis transcriptomes from triploid and fertile diploid cyprinid fish. Biol. Reprod. 92, 95.
Zhang, C., Li, Q., Zhu, L., He, W.C., Yang, C.H., Zhang, H., Liu, S.J., 2021. Abnormal meiosis in fertile and sterile triploid cyprinid fish. Sci. China Life Sci. https://doi.org/10.1007/s11427-020-1900-7.