Oocyte Atresia Characteristics and Effect on Reproductive Effort of Manila Clam *Tapes philippinarum* (Adams and Reeve, 1850)

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**OOCYTE ATRESIA CHARACTERISTICS AND EFFECT ON REPRODUCTIVE EFFORT OF MANILA CLAM **TAPES PHILIPPINARUM (ADAMS AND REEVE, 1850)

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**ABSTRACT** The histological characteristics of oocyte atresia were examined in the Manila clam *Tapes philippinarum* (Adams and Reeve, 1850), at Croisic Traict on the French Atlantic coast, over 4- and 5-month gametogenic periods (May to August 2015 and April to September 2016). Atresia was found at all stages of gametogenesis, as well as in residual oocytes, and was characterized by several characteristics: loss of the nucleus, nuclear degradation, altered cytoplasmic staining affinities, cytoplasmic retraction, and finally the loss of all cellular content. Histological observations indicated that *T. philippinarum* partially spawned repeatedly over the gametogenic period. Stereological counts showed that at least 15% of the oocyte volume was occupied by atresic oocytes (AO) at the onset of gametogenesis before any spawning activity; this increased to 30% in the middle of the gametogenic period (including both pre- and postspawning oocytes) and 80% at the end of the gametogenic period (postspawning atresia). Of all oocytes whose fate could be determined during active gametogenesis, nearly half were atresic. Similar observations were made for smaller sample sizes of clams from two other sites in nearby Bourgneuf Bay over a 26-mo period. Both AO and nonatresic oocytes were observed in the same gonadal acini, suggesting that the process was either not propagated or not synchronized. The considerable proportion of oocytes affected by atresia underscores the need for better recognition, documentation, and integration of this process into models of reproductive effort and fecundity in this species. In particular, condition indices based on tissue : shell weights should be interpreted as estimations of reproductive investment, not as indications of potential reproductive outcome.

**KEY WORDS:** oocyte atresia, *Tapes philippinarum*, gametogenesis, reproductive effort, stereology

**INTRODUCTION**


The present study documents the phenomenon of atresia in the reproductive cycle of *Tapes philippinarum*. Two data sets were used, a detailed 4- and 5-mo study during the gametogenic period and a longer time series (26 mo) with fewer individuals. In addition to the qualitative description of this process, we use quantitative histological techniques to estimate its impact on reproductive effort (*R*).

**MATERIALS AND METHODS**

**Species, Sites, and Sampling**

For reasons unclear to most workers, *Tapes philippinarum* has a relatively long list of competing generic names, of which *Ruditapes* is the most frequent in recent years. The reasons for selecting *Tapes* are outlined in Beninger and Boldina (2014).

The detailed histological study was carried out at a *Tapes philippinarum* and *Cerastoderma edule* culture operation, situated in an extensive mudflat aquaculture region on the French Atlantic coast. Twenty-three adult clams were haphazardly sampled every 2 wk (May to August 2015 and April to September 2016; 2.6–5 cm along the anteroposterior axis).

For the longitudinal time series, one fish and one unfished site were chosen on the French Atlantic coast: a recreational mudflat fishing site for *Tapes philippinarum* in the Gois passage, and an isolated, unfished site accessible only by boat; all three sampling sites were within 50 km of each other (Fig. 1). The sediment characteristics, water temperature, salinity, turbidity, and
tidal regimes of the two sites were very similar (Boldina & Beninger 2013, Beninger & Boldina 2014, Boldina et al. 2014). In the course of other, unrelated manipulations, nearly commercial-size clams ($\geq 3$ cm) were haphazardly sampled monthly at low tide from the unfished and fished sites (July 2010 to September 2012). The sampling took place at the incoming tide, often limiting the number of individuals sampled ($\leq 15$); in addition, several technical problems reduced the number of sampling dates. The number of females investigated is specified in Figures 5–7.

**Histological Techniques**

Sampled clams were separated from their shells and fixed on-site in ice-cold aqueous Bouin’s solution for at least 48 h. Three-to 4-mm-thick slices were removed along the dorsoventral axis of the foot, continuing up to the dorsal extremity of the visceral mass. Samples were then rinsed overnight under running tap water, dehydrated in an ascending ethanol-Roti-Histol series, and embedded in paraffin. Sections were cut at 7 μm and stained with a modified Masson’s trichrome protocol (Martoja & Martoja-Pierson 1967, Beninger et al. 2010) using trioxihematin (3 min), acid fuscine (2 min), orange G-phosphomolybdic acid (3 min), and fast green (1 min). Observations and analyses of the photomicrographs were performed using an Olympus Provis light microscope, and LUCIA GF 4.80 image capture and processing software. A total of 12 micrographs were archived for each female at the unexploited and fished sites, and nine micrographs for the farmed site, for later examination and stereological counts.

**Stereology**

Preliminary investigations showed that the *Tapes philippinarum* gonad satisfied the requirements for stereological analysis: constant gonad tissue anatomical localization, synchronous gametogenesis throughout the gonad, sufficiently homogeneous.
gonad tissue, and sufficiently large patches of gonad tissue were available to perform counts (Beninger & Boldina 2012a). For the purposes of this study, only the oocyte types were quantified using stereological counts: atresic oocytes (AO), immature healthy oocytes (IO), and mature healthy oocytes (MO) (Beninger 1987, Beninger et al. 2001, Valdizan 2011; Fig. 2A).

Generally, three counts were performed on each of three sections per individual, and the data were pooled to provide mean counts for each cell type for each individual, date, and site. Counts were performed on all females sampled over the 26-mo study period at the unexploited and fished sites (July 2010 to September 2012) and over the gametogenic period at the farmed site (May to August 2015 and April to September 2016). A 13 × 14 counting grid was used for the unexploited and fished site micrographs, whereas an 11 × 11 grid was used for the farmed site. Given the small number of females for some samples, the range was used as an indicator of dispersion about the mean (Beninger & Boldina 2012b).

RESULTS

Qualitative Characteristics of Atresia

Using the modified Masson’s trichrome staining protocol, healthy oocytes were readily identified with a pink cytoplasm and a rounded, well-defined nucleus; depending on the plane of section, a distinct nucleolus was also visible (Fig. 2). Healthy oocytes were either mature (mature healthy oocytes—regular rounded shape, separated from the acinal wall, Fig. 2B) or immature (immature healthy oocytes—pear shape, attached to the acinal wall with a peduncle, Fig. 2C).

Oocyte atresia in clams was characterized by the appearance of several characteristics, concomitantly or not. These characteristics were observed in both mature and immature oocytes; some affected the nucleus, others the cytoplasm.

Characteristics affecting the cytoplasm were as follows:

1. Cytoplasmic discoloration (Fig. 3A–C). In most cases, the atresic cytoplasm stained more intensely, becoming darker compared with that of healthy oocytes. More rarely, the cytoplasm turned from a uniform pink-red to green and purple.

2. Cytoplasmic retraction and detachment from the cell membrane (Fig. 3D–F, I). This was visible on histological slides as a clear space between the membrane and the cytoplasm, either on a small portion of the cell or involving most of the cell (Fig. 3D–F, I).

3. An irregular geometric shape, noticeably different from the spherical shape of healthy oocytes (Fig. 3G–I).

Characteristics affecting the nucleus were as follows:

1. Disappearance of the nucleoli (Fig. 3A–F, H–L). This characteristic is not sufficient on its own, because the section plane can pass above or below a nucleolus. In many cases, however, nucleoli are often histologically visible in actively synthesizing cells; thus their absence is a clue to atresia, especially when observed in a large number of oocytes, where it can indicate widespread atresia.

2. Homogeneous chromatin, seen as a very uniform nucleus color, with no chromatin clumping (Figs. 3J–L and 4).

3. Disappearance of the nucleus in medially sectioned cells (Fig. 3C, I).

The aforementioned characteristics may be more or less pronounced based on the chronological development of atresia. It is important to note that oocytes presenting different characteristics can be physically close to one another and also close to apparently healthy oocytes in the same acinus (Fig. 3D, H, J, K). Some parts of the clam gonad may be almost exclusively occupied

<table>
<thead>
<tr>
<th>Phase</th>
<th>Period</th>
<th>Elements observed</th>
<th>Other characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gametogenesis (Fig. 4A, B)</td>
<td>April to August</td>
<td>MO, IO, and AO (AMO and AIO)</td>
<td>Repeated declines in MO (Figs. 5–7): dribble spawner</td>
</tr>
<tr>
<td>Resorption (Fig. 4C)</td>
<td>September to November</td>
<td>Few oocytes, mostly identified as AO, AL and IAT</td>
<td></td>
</tr>
<tr>
<td>Resting phase (Fig. 4D)</td>
<td>December to March</td>
<td>AL, IAT, macrophage cells No gametes</td>
<td>Inter-individual variation in the degradation of residual acini</td>
</tr>
</tbody>
</table>

AMO = atresic mature oocytes; AIO = atresic immature oocytes; IAT = interacinal tissue; AL = acinal lumen.

Figure 4. Seasonal histological profiles of Tapes philippinarum gonads. (A) Beginning of gametogenesis (April): small IO attached to the acinal wall, forming from interacinal tissue (IAT); prespawning, atresic mature (AMO), and atresic immature oocytes (AIO) were also observed. (B) In July, MO and larger IO were observed. (C) During the resorption phase (September) only residual AIO and AMO were visible in the acinal lumen (AL). (D) In winter, no gametes were visible in the acini, and the acinal walls were invaded by numerous macrophage cells (M); the acinal walls themselves may disappear.
by AO, whereas the rest of the gonad may be much less affected.
In the overwhelming majority of observations, areas most severely
affected were located in the dorsalmost region of the visceral mass.

Seasonal Pattern of Atresia

Histological observations allowed the identification of three
major phases of the *Tapes philippinarum* oogenic cycle (Table 1).
Characteristics of atresia were observed in early and late vitello-
genic oocytes (atresic immature oocytes), and in mature oocytes
(atresic mature oocytes), beginning in April and throughout
gametogenesis and postspawning resorption (Fig. 4). In July
(Fig. 4B), oocytes were predominantly late vitellogenic or mature,
and characteristics of atresia were again noted in both stages.
During the resorption phase (September to November), most

Figure 5. Atresic, mature, and immature volume fractions over the 4- and 5-mo gametogenic periods at the farmed site. REST is the resting period when there were no oocytes; N is the number of females used for counts.

Figure 6. Atresic, mature, and immature volume fraction over the 26-mo sampling period at the unfished site. REST is the resting period; N is the number of females used for counts. No sampling was possible in spring 2012.
OOCYTE ATRESIA IN *TAPES PHILIPPINARUM*

**Quantification of Atresia**

To assess the importance of oocyte atresia throughout the reproductive cycle of *Tapes philippinarum*, the following oocyte volume fractions were calculated (Weibel et al. 1966):

1. **Atresic volume fraction (AVF)**: the number of grid points occupied by AO divided by the total number of grid points occupied by all oocyte types.

\[
\text{AVF} = \frac{\text{AO}}{\text{IO} + \text{AO} + \text{MO}} \times 100
\]

2. **Mature volume fraction (MVF)**: the number of grid points occupied by mature oocytes divided by the total number of grid points occupied by all oocyte types.

\[
\text{MVF} = \frac{\text{MO}}{\text{IO} + \text{AO} + \text{MO}} \times 100
\]

3. **Immature volume fraction (IVF)**: the number of grid points occupied by immature oocytes divided by the total number of grid points occupied by all oocyte types.

\[
\text{IVF} = \frac{\text{IO}}{\text{IO} + \text{AO} + \text{MO}} \times 100
\]

4. **Minimum atresic impact (MAI)**: the minimum impact of atresia on the oocyte population, expressed as

\[
\text{MAI} = \frac{\text{AVF}}{\text{AVF} + \text{MVF}} \times 100
\]

The MAI was based on three assumptions: (1) MVF (composed of healthy, mature oocytes) represented the oocyte volume fraction with a high probability of being spawned as healthy; (2) AVF represented the oocyte volume fraction with no probability of being spawned as healthy oocytes; the fates of MVF and AVF were therefore known; (3) The fate of IVF was unknown as it could either remain healthy or become atresic. This index, therefore, represents the minimum oocyte volume fraction known to be atresic, compared with the total oocyte volume fraction whose fate is known.

The evolution of MVF and IVF values over the sampling period at the farmed site reveals several gametogenic cycles and spawns (Fig. 5). The AVF was at least 15% before the first spawn, increasing to 25%–30% in subsequent spawns. At the end of the reproductive period (September 2016), all residual oocytes showed at least one characteristic of atresia, notably the disappearance of nucleoli (Fig. 4C), indicating generalized atresia in the gonad.

### Table 2.

<table>
<thead>
<tr>
<th>Sites</th>
<th>MAI</th>
<th>AVF</th>
<th>MVF</th>
<th>IVF</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmed</td>
<td>48.8 ± 5.7</td>
<td>33.7 ± 5.2</td>
<td>35.0 ± 4.4</td>
<td>31.4 ± 5.5</td>
<td>139</td>
</tr>
<tr>
<td>Unfished</td>
<td>44.3 ± 6.4</td>
<td>33.2 ± 6.4</td>
<td>41.2 ± 8.3</td>
<td>25.6 ± 10.5</td>
<td>49</td>
</tr>
<tr>
<td>Fished</td>
<td>41.9 ± 13.7</td>
<td>31.2 ± 11.2</td>
<td>37.5 ± 9.3</td>
<td>31.3 ± 10.6</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>32.7</td>
<td>37.9</td>
<td>29.4</td>
<td>232</td>
</tr>
</tbody>
</table>

Figure 7. Atresic, mature, and immature volume fraction during the 26-mo sampling period at the fished site. REST is the gametogenic resting period; N is the number of females used for counts. No sampling was possible in spring 2012.
oocytes were obviously destined for atresia. These results at the farmed sampling site in 2015 and 2016 extend and confirm the longer term observations from the fished and unfished sites (2010 to 2012; Figs. 6 and 7).

To clearly summarize these data, only those dates corresponding to MVF $\geq 20\%$ were chosen to represent the period of active gametogenesis (Table 2). The excluded periods were thus as follows:

1. The end of the gametogenic periods, when most oocytes were atresic and undergoing resorption.
2. The resting phase, when there was no gametogenesis.
3. The beginning of the gametogenic periods, when most oocytes were in early gamete stages and their fate is thus unknown.

Over the active gametogenic period at all sites, approximately 33% of gamete volume was occupied by AO and the MAI was 45% (Table 2). No marked differences in volume fractions were noted between sites.

**DISCUSSION**

**Histological Features of Atresia**

Histological observations of the present study establish the following indicators of atresia: absence of the nucleolus (an early indicator), as well as nuclear degradation (irregular nuclear envelope or chromatin degradation), cytoplasmic discoloration and retraction, and cellular distortion (puzzle shape). Although it is not possible to establish a firm chronological sequence at this point, certain atresic characteristics do present a temporal sequence, especially for the nucleus (Fig. 8).

The features described previously correspond to the categories of characteristics previously outlined in the Bivalvia (Beninger 2017). No discernable differences were noted between pre- and postspawning atresia, indicating a common process.

Generalization of atresia within acini has been reported for several bivalve species, being easily recognized by major distortions of oocyte shape (Dorange & Le Pennec 1989, Suárez et al. 2005, Beninger & Le Pennec 2006, Dutertre et al. 2009). Such generalization may also occur in *Tapes philippinarum* acini, but this appears to lack synchrony, and cellular distortions are much less severe. Instead, clarification of the nucleus is the revealing feature.

**Temporal Dynamics of Oocyte Atresia**

Based on all of the data from the different study sites and years, the temporal dynamics of oocyte atresia in *Tapes philippinarum* are summarized in Figure 9. Oocyte atresia steadily increased in the spring, before the first spawning. A precipitous decrease in all oocyte types characterized the first spawning; from this point onward, throughout the subsequent
gametogenic activity, both pre- and postspawning AO were present in the gonad simultaneously.

Of the three functional types of atresia proposed by Motavkine and Varaksine (1989), it is clear that the resorption phase of the reproductive cycle corresponds to residual atresia. The atresia observed before the resorption phase may be either physiological (i.e., a regulatory mechanism) or ecological (i.e., a response to unfavorable environmental conditions). Much further research will be necessary to refine this analysis.

**Impact of Atresia on $R_e$**

To the authors’ knowledge, this is the first study to quantify oocyte atresia and its effect on reproductive effort $R_e$. Previously, Morvan and Ansell (1988) calculated a percent of AO in *Tapes rhomboides*, using a complex estimation based on oocyte diameters and the Williams’ equation (Williams 1981); however, these authors only appear to have included mature AO in their estimations. The losses of 11% fecundity in spring and 3.3% in summer reported by these authors, therefore, represent considerable underestimations.

The stereological technique used in this study is based on the number of counting points occupied by particular cell types. This type of data does not allow precise oocyte numbers to be determined, but it can obviously be used as a proxy for such numbers, in addition to representing the amount of energy invested. If all oocytes are viable, the $R_e$ simply equals the total volume of oocytes in the gonad at time $t$. If some oocytes are not viable, the effective reproductive effort ($E_{Re}$) is the total oocyte volume fraction minus the volume fraction of all AO:

$$E_{Re} = R_e - AO$$

The results of the present study show that the minimum level of atresia in the *Tapes philippinarum* reproductive cycle was 15%, at the beginning of gametogenesis; this volume fraction climbed to approximately 33% during active gametogenesis, when both pre- and postspawning atresia are present (Figs. 9 and 10). Furthermore, the MAI obtained during active gametogenesis, from the three sites, was approximately 45%. Thus, nearly half of the oocyte volume fraction produced during active gametogenesis would be lost to atresia, reducing $R_e$ by approximately 45%. It should be remembered that this is the MAI, so the figure may well surpass 50%. As there was no evidence of AO resorption during active gametogenesis (no empty cells or macrophage invasion), it is assumed that these cells are lost at spawning;
arthria, therefore, seems to represent a net loss of energy for *T. philippinarum* females.

It is useful to place this result in the context of *R* estimates, approximated by indices such as the condition index (Lucas & Beninger 1985). To illustrate this point, such a condition index was calculated at the farmed site for 18 *Tapes philippinarum* individuals concomitantly sampled biweekly over 20 mo of one study period (March 2015 to December 2016, Fig. 11). The high values of this index during active gametogenesis are obviously misleading and should therefore be interpreted with caution because almost half of the gamete volume produced was artresic. The data of the present study show that although tissue:shell weight condition indices can be used to quantify reproductive investment, they cannot be used to indicate reproductive outcome.

From the preceding, it is clear that artresia can be a major cause of oocyte mortality in *Tapes philippinarum*; in itself, this result assists in the understanding of the high-level mortalities typically found in the early life stages of this and many other bivalve species, as well as allowing more realistic estimations of *R* and fecundity.

**ACKNOWLEDGMENTS**

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