Oocyte Atresia and Its Effect on Reproductive Effort of the Common Cockle Cerastoderma edule (Linneaus, 1758)

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OOCYTE ATRESIA AND ITS EFFECT ON REPRODUCTIVE EFFORT OF THE COMMON COCKLE *CERASTODERMA EDULE* (LINNEAUS, 1758)

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ABSTRACT In an effort to elucidate the causes of early life stage mortality, the histological characteristics of oocyte atresia were examined biweekly in the European common cockle *Cerastoderma edule* (Linnaeus, 1758) over an 11-mo-period (January–November 2018), at a farmed site on the French Atlantic coast. Gametogenesis was continuous at the population level, with no apparent interindividual synchronicity. Atresia was observed throughout the year, at all stages of oogenesis, characterized by loss of the nucleolus, nuclear and chromatin degradation, and angular cell shape. Both atresic and nonatresic oocytes were observed in the same gonad acini, suggesting that the process was either not propagated or not synchronized. Stereological counts showed that atresic oocytes occupied an annual mean of 30% (range 12%–47%) of the oocyte volume. Estimation of the minimum atresic impact showed that more than 50% of the oocytes, whose fate can be determined from histological sections, were or would become atresic, reducing the fecundity accordingly. Together with previously reported results in other bivalve species, this underscores the need for better recognition, documentation, and integration of this process into models of fecundity, reproductive effort, population dynamics, and production.

KEY WORDS: oocyte atresia, common cockle, *Cerastoderma edule*, gametogenesis, reproductive effort, stereology

INTRODUCTION

The common cockle *Cerastoderma edule* (Linnaeus, 1758) is found on the Northeast Atlantic coast, from Mauritania to Norway. It is an economically important species in The Netherlands, United Kingdom, and France, with a peak fishery yield of more than 100,000 metric tons in the late 1980s; annual cockle-farming production has averaged 3–5,000 metric tons since the mid-1980s (http://www.fao.org/fishery/species/3535/en). The recreational fishery is also highly developed, particularly in France, with added dimensions of tourism and heritage (Boldina 2013, Beninger 2018).

A firm understanding of reproductive biology is obviously essential to the management of any exploited population. Although some data exist concerning the reproduction of *Cerastoderma edule* (Boyden 1971, Kingston 1974, Yankson 1986, Navarro et al. 1989, Guillou et al. 1990, Iglesias & Navarro 1991, Guillou & Tartu 1992, Martínez-Castro & Vásquez 2012, Pronker et al. 2015), little is known concerning the actual fecundity of this species. In particular, the proportion of viable oocytes has not yet been determined in any population. Recent studies of oocyte atresia (the degeneration of oocytes within the gonad) have revealed that this is a widespread phenomenon among bivalves, and in the one species in which its effect has been quantified, up to half of the oocytes may be nonviable (Beninger 2017, Chérel & Beninger 2017). Although atresia shares common general characteristics in all species studied to date (Beninger 2017), histological identification is often hampered by a lack of clear, detailed, species-specific criteria.

The present study documents the histological characteristics of oocyte atresia in cultured *Cerastoderma edule* and quantifies its importance with respect to total oocyte production.

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MATERIALS AND METHODS

Species, Site, and Sampling

The study was carried out at a clam—cockle farm on the French Atlantic coast (Fig. 1). Adult cockles (30 individuals, >2 cm) were haphazardly sampled biweekly from January to November 2018.

Histological and Stereological Techniques

Sampled cockles were shucked and fixed in Bouin's solution, embedded, and sectioned at 7 μm as per Chérel and Beninger (2017). Slides were first stained with Alcian blue to reveal the oocyte sheath (Beninger & Chérel 2019) (acetic acid 3% 1 min, dry 3 min, and Alcian blue 30 min), followed by a modified Masson's trichrome protocol (Beninger et al. 2010, Chérel & Beninger 2017) (trioxyhematein 2 min, acid fuschin 2 min, orange G–phosphomolybdic acid 4 min, and fast green 4 min). Observations were performed using an Olympus Provis light microscope and Olympus cellSens Standard software.

Stereological techniques are usually carried out to quantify relative gamete volume fractions for documentation of the reproductive cycle (Newell & Bayne 1980, Lowe et al. 1982, Newell et al. 1982, Lowe & Pipe 1986, MacDonald & Thompson 1986, Beninger 1987, Lee 1988, Morvan & Ansell 1988, Royet 1991, Pazos et al. 1996, Chérel & Beninger 2017); in that case, interindividual anatomical constancy is a prerequisite. An extensive preliminary histological study of the cockle showed that there is no interindividual constancy in the gonad location, with acini being found variably from the ventral extremity of the foot to the entire periphery of the digestive gland, and even between the outermost digestive tubules. The present study did not seek to quantitatively document the reproductive cycle, but rather the proportions of various oocyte types within the gonad acini, wherever they were found. To this end, nine micrographs were taken for each female in regions containing abundant acini.

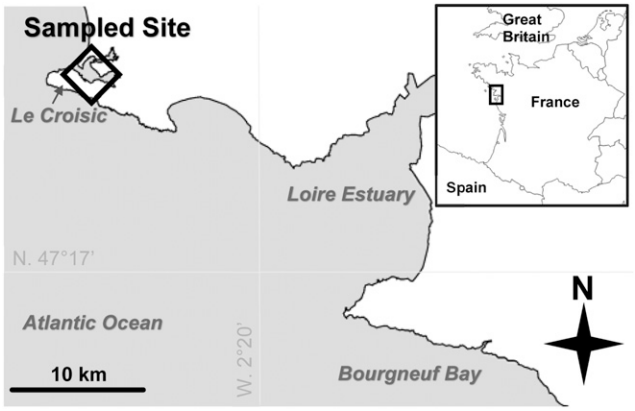

Figure 1. Location of the study site.

Stereology was carried out on gonad acini using an 11 × 11 grid. Counts were performed on five features of interest: atresic oocytes (AO), immature healthy oocytes (IO), mature healthy oocytes (MO), oocyte sheath (S), and intra-acinal lumen (Fig. 2). The following oocyte volume fractions were calculated (Chérel & Beninger 2017):

$$\text{Atresic Oocyte Volume Fraction}: \quad \text{AVF} = \frac{\text{AO}}{\text{AO}+\text{IO}+\text{MO}} * 100$$

$$\text{Mature Oocyte Volume Fraction}: \quad \text{MVF} = \frac{\text{MO}}{\text{AO}+\text{IO}+\text{MO}} * 100$$

$$\text{Immature Oocyte Volume Fraction}: \quad \text{IVF} = \frac{\text{IO}}{\text{AO}+\text{IO}+\text{MO}} * 100$$

On histological examination of the 30 individuals sampled, actively gametogenic females represented less than 15 individuals throughout the study (Fig. 6). Given that the resulting number of females on several samplings was prohibitively low for meaningful calculation of conventional measures of dispersion (Beninger et al. 2012), means and data ranges were calculated, expressing the latter as ½ (maximum–minimum) in figure graphs.

Minimum atresic impact (MAI) (Chérel & Beninger 2017) was expressed as:

$$\text{MAI} = \frac{\text{AVF}}{\text{AVF}+\text{MVF}} * 100.$$

RESULTS

Qualitative Characteristics of Atresia

Healthy mature or immature oocytes (IO) presented a rounded shape, with a pink cytoplasm and a well-defined cell membrane when stained with the modified Masson's trichrome protocol. The spherical nucleus contained a slightly condensed chromatin; in most sections, a well-defined nuclear envelope and nucleolus were visible (Fig. 3). The oocyte sheath stained strongly with Alcian blue, indicating abundant acid mucopolysaccharides; it was much more voluminous around healthy, vitellogenic oocytes, than young, IO, and much more variable around AO (Figs. 3–5).

Oocyte atresia was characterized by several histological features, either concomitantly or not. These features were observed in both mature oocytes and IO; some affected the nucleus, whereas others affected the cell shape and membrane.

1. An irregular, angular cell shape, noticeably different from the rounded, spherical shape of healthy oocytes (Figs. 4B, C and 5C, D).
2. Cell membrane rupture (Fig. 4D).
3. Dispersed yet strongly stained chromatin, seen as a uniform or finely mottled nucleus color (Figs. 4A, C, D and 5A–C).
4. Irregular nuclear envelope (Fig. 5B, C), a decrease in the nucleus size (Fig. 4D), or even a vestigial state (Fig. 5D).

The aforementioned characteristics were more or less pronounced, based on the chronological development of atresia. Interestingly, oocytes presenting advanced atresic characteristics were often observed adjacent to apparently healthy oocytes in the same acinus (Figs. 2 and 4A).

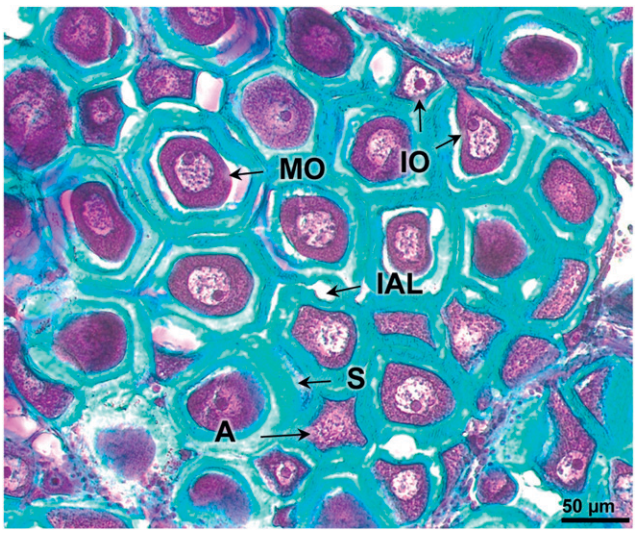

Figure 2. General view of the female gonad of *Cerastoderma edule* stained with Alcian blue and modified Masson's trichrome, showing the five histological features used for stereological counts: AO, mature oocytes (MO), IO, oocyte sheath (S), and intra-acinal lumen.

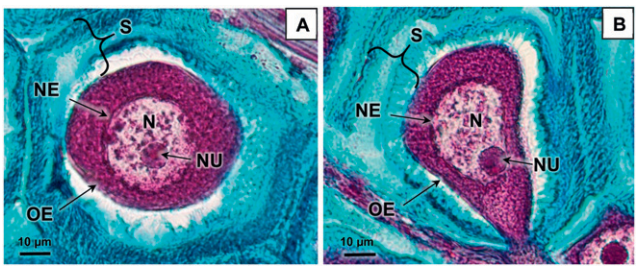

Figure 3. Healthy mature (A) and immature, pedunculated oocyte (B) of *Cerastoderma edule*. Note the smooth oocyte envelope (OE) and large oocyte sheath (S). The nucleus (N), containing a slightly condensed chromatin, is surrounded by a well-defined envelope comprising a double membrane (NE). Depending on the plane of the section, a nucleolus (NU) is often visible.

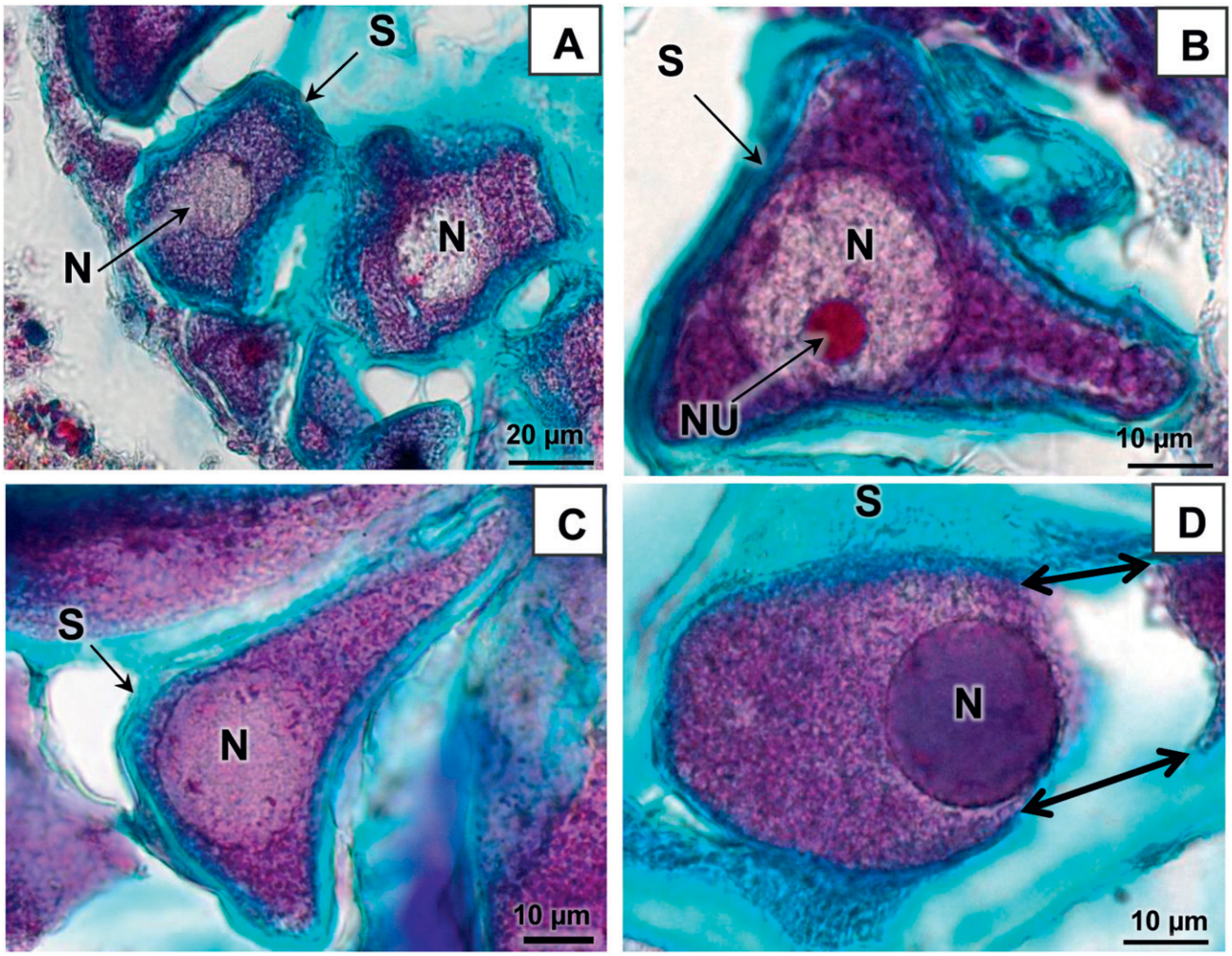

Figure 4. Characteristics of atresia in IO of *Cerastoderma edule*: altered cell shape and chromatin structure (A–C), thin oocyte sheath (B, C), nuclear shrinkage, and oocyte envelope rupture (double arrows, D). Note the slightly degraded chromatin structure in B, indicating an intermediate condition between the oocytes in Figure 3 and those in Figure 4A, C, and D. N, nucleus; NU, nucleolus; S, sheath.

Periods of Gametogenesis and Atresia

The sustained and relatively high proportion of IO (IVF) indicated a continuous gametogenic activity at the *Cerastoderma edule* population level (Fig. 6), although some individuals did show evidence of a resting period (no visible acini). Gametogenesis was not entirely synchronous; individuals with mature volume fraction (MVF) >20% (active gametogenesis) were present throughout the year, with a slight predominance at the end of winter and the end of summer. Characteristics of atresia were observed at all stages of oogenesis, throughout the year (minimum atresic volume fraction (AVF): 12.63% for October 29, maximum AVF: 46.85% for February 22; Fig. 6).

Quantification of Atresia

The AVF, MVF, immature volume fraction (IFV), and MAI means were calculated both for all individuals and for individuals showing active gametogenesis (MVF >20%) (Table 1).

The differences in values between all individuals studied and those in active gametogenesis are less pronounced for AVF than for the other indices. During active gametogenesis, MAI was almost 53%. The higher value of MAI calculated for all individuals (57.42%), despite the lower value of AVF (27.85%), was because of the higher value of the IVF. In both cases, the MAI showed that more than half of the oocyte volume fraction whose fate could be determined was lost to atresia.

DISCUSSION

Oogenetic and Atresia Dynamics

The presence of gametes throughout the year, particularly the presence of IO, confirms that the common cockle is a dribble spawner at this site, as has been observed elsewhere (Kingston 1974, Yankson 1986, Guillou et al. 1990, Guillou & Tartu 1992). In addition to significant interindividual heterogeneity in oogenesis, individuals showed resting states at different times of the year, with a peak during summer. It is, therefore, not possible to delimit a spawning period, and, therefore, not possible to determine whether the observed atresia occurred pre- or postspawning. These conclusions were supported by similar AVF, MVF, and IVF values obtained from an opportunistic study carried out on a reduced number of individuals over a 26-mo period at a nearby fishing site

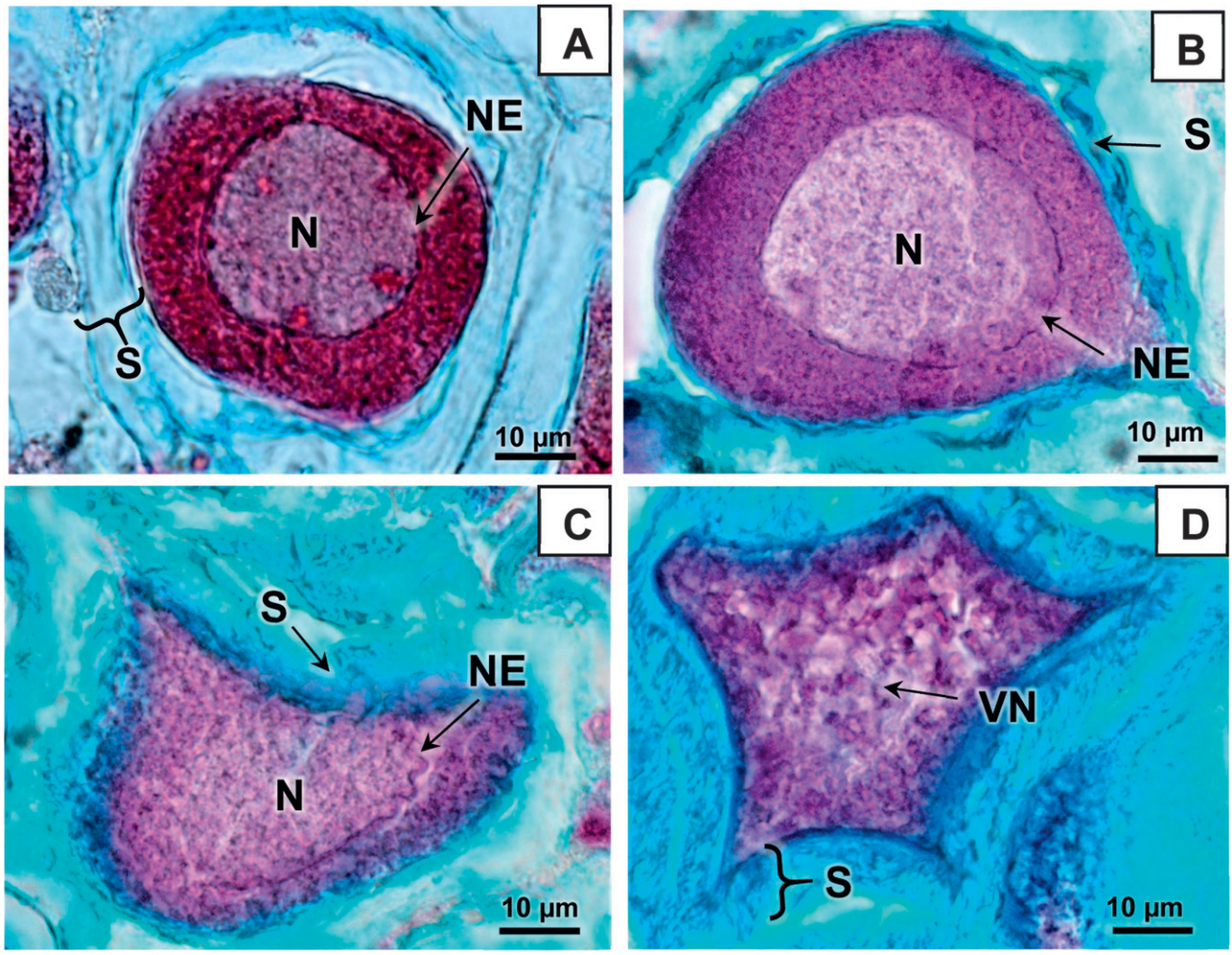

Figure 5. Characteristics of atresia in mature oocytes of *Cerastoderma edule*: altered chromatin structure (A–C) or even (D) vestigial nucleus (VN). Thin oocyte sheath (B–D), irregular nuclear envelope (B, C), and angular shape (C, D). N, nucleus; NU, nucleolus; S, sheath; NE, nuclear envelope.

(Daphné Chérel and Inna Boldina, Université de Nantes, unpublished data). Taken together, these observations indicate that approximately half of the *Cerastoderma edule* oocyte production was lost to atresia; similar results were also obtained for the sympatric venerid clam *Tapes philippinarum* (Chérel & Beninger 2017).

Histological Features of Atresia

The asynchronous oocyte atresia of *Cerastoderma edule* corresponds to the physiological type of atresia described by Motavkine and Varaskine (1989) and previously reported in the Manila clam *Tapes philippinarum* (Veneridae) (Chérel & Beninger 2017). This contrasts with the synchronous type of atresia, presumed to be "ecological" (i.e., triggered by ecological factors), in which all oocytes in a given acinus become atresic quasi-simultaneously, as observed in the Mytilidae (Suárez et al. 2005, Beesley et al. 2008, Suárez Alonso et al. 2010, Smolarz et al. 2017, García-Corona et al. 2018, Koagouw & Ciocan 2018, Rouabhi et al. 2019), Pinnidae (Camacho-Mondragón et al. 2012, 2015), Pectinidae (Paulet et al. 1992, Cantillanez et al. 2005, Beninger & Le Pennec 2006, Beninger 2017), Pteriidae (Saucedo et al. 2001), and Ostreidae (Steele & Mulcahy 1999, Dutertre et al. 2009, Vaschenko et al. 2013).

Although differing somewhat in presentation, the histological features of oocyte atresia in *Cerastoderma edule* correspond to the categories of characteristics previously outlined in the Manila clam *Tapes philippinarum* (Adams & Reeve, 1850) (Chérel & Beninger 2017) and bivalves in general (Beninger 2017). Although an altered, irregular oocyte shape is a common histological feature of all types of atresia (Beninger 2017), this characteristic presents differently in the aforementioned atresia types. In the physiological type of atresia, the oocytes become increasingly angular, whereas in the ecological type, they assume complex "puzzle-piece" shapes, as in *Mytilus* sp.(Suárez et al. 2005, Beesley et al. 2008, Suárez Alonso et al. 2010), *Argopecten* sp. and *Pecten* sp. (Paulet et al. 1992, Cantillanez et al. 2005, Beninger & Le Pennec 2006), and *Placopecten magellanicus* (Beninger 2017). In addition, the oocyte envelope becomes less distinct, and the boundaries of neighboring cells become difficult to distinguish.

Although in some species, such as *Tapes philippinarum* (Chérel & Beninger 2017) and *Crassostrea gigas* (Dutertre et al. 2009, Beninger 2017), oocyte atresia is accompanied by cytoplasmic shrinkage away from the plasma membrane, this was not observed in the *Cerastoderma edule* of the present study.

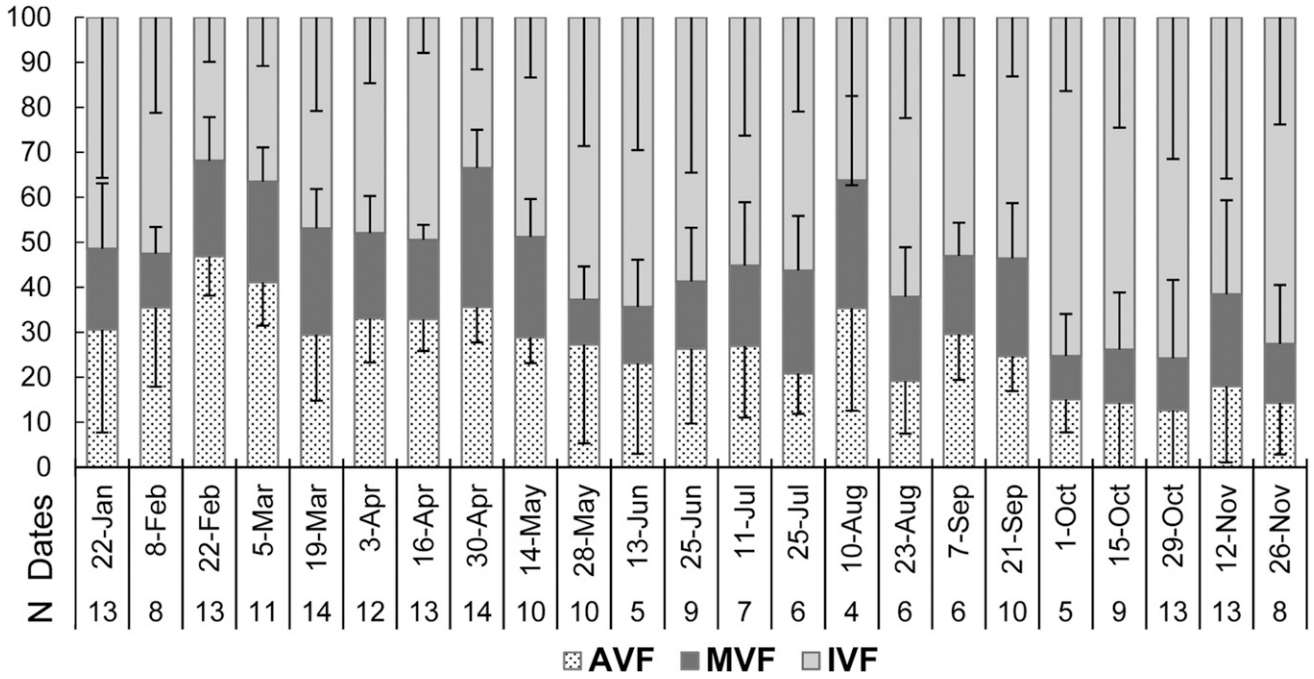

Figure 6. AVF, healthy MVF, and healthy IVF of oocytes. Error bars represent half the range of values. *N* is the number of females used for counts.

The nuclear characteristics of oocyte atresia observed in histological sections of the common cockle are very similar to those observed in most bivalves (Beninger 2017), notably chromatin degradation, with an endpoint homogeneous nuclear staining. Similar observations have been made for *Tapes philippinarum* (Chérel & Beninger 2017), *Pecten maximus* (Beninger 2017), *Atrina maura* (Camacho-Mondragón et al. 2015), *Mytilus galloprovincialis* (Suárez et al. 2005, Suárez Alonso et al. 2010), and *Crassostrea angulata* (Vaschenko et al. 2013). In other species, for example, *Pinctada mazatlanica* (Saucedo et al. 2001) and *Placopecten magellanicus* (Beninger 2017), chromatin degradation occurs in the form of clumping. In most cases, as in *Cerastoderma edule*, the nucleoli disappear, as may the nucleus itself in the end stages of atresia.

Impact of Atresia on Fecundity and Reproductive Effort

The present study shows that throughout gametogenesis in *Cerastoderma edule*, approximately one-third of the oocyte volume was occupied by AO, representing a 33% instantaneous oocyte mortality. When expressed as the total proportion of oocytes whose fate is known (AO/healthy mature oocytes + AO or MAI), this source of oocyte mortality represents, on average, over 50%. In other words, what is normally considered "fecundity" is overestimated by at least 30% to over 50%. To date, such mortality can only be detected using histology, a woefully under-used tool in marine ecology.

The absence of macrophage invasion in or around the gonad acini in any of the slides examined indicates that the AO are not resorbed by *Cerastoderma edule*. Further studies are necessary to determine whether this represents an energy loss for this species or whether the AO serve some other function in reproduction.

After the previously published work on the Manila clam *Tapes philippinarum* (Chérel & Beninger 2017), the present study represents only the second attempt to quantify the impact of oocyte atresia on bivalve reproduction. In both species, it is clear that atresia can be a major cause of oocyte mortality. These results elucidate one of the major causes of the high levels of early life stage mortality in coastal bivalves, a universal phenomenon in both wild and cultured species. Should similar studies confirm the 30%–50% oocyte mortality level due to atresia, more realistic bivalve fecundity estimations will be possible. This source of oocyte mortality should be included in revised models of bivalve reproductive effort, population dynamics, and production.

ACKNOWLEDGMENT

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TABLE 1.

Mean AVF, MVF, IVF, and MAI (%) for all individuals investigated (*N* total = 219) and for individuals in active gametogenesis (*N* MVF >20% = 98).

	AVF	MVF	IVF	MAI
N total	27.85 ± 1.71	18.65 ± 1.29	53.50 ± 2.60	57.42 ± 2.40
N MVF >20% (active gametogenesis)	31.94 ± 1.80	27.36 ± 1.04	40.70 ± 2.08	52.98 ± 1.87

±95% confidence interval.

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