RESEARCH NOTE

Seasonal variation in gametogenesis and spawning of *Mytilopsis leucophaeata*, an invasive bivalve in Europe

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Mytilopsis leucophaeata (Conrad, 1831) is a brackish-water species with a typically high resistance to environmental changes (Verween, Vincx & Degraer, in press). The species invaded European waters in the nineteenth century (Nyst, 1835), but it was only when it became a severe fouling species in the 1990s (Rajagopal, Van der Velde & Jenner, 1995; Verween *et al.*, 2005), clogging European industrial cooling-water systems, that it attracted the attention of the industrial and scientific communities.

Knowledge of the species' life history is almost completely lacking, both within its original habitat and its newly invaded environment (Verween *et al.*, in press). Populations of the closely related *Dreissena polymorpha*, the zebra mussel, have an annual gametogenic cycle with one or more spawning events during summer and autumn and a high degree of gametogenic synchronization (Borcherding, 1991). Temperature is a key environmental factor governing the timing of both gametogenesis and spawning in dreissenid mussels (Borcherding, 1991; Fong *et al.*, 1995; Ram, Fong & Garton, 1996; Nichols, 1996; Claxton & Mackie, 1998) and food availability has also been suggested as an important regulator (Borcherding 1991; Nichols, 1996; Ram *et al.*, 1996). Based on data on the presence of larval *M. leucophaeata* in the water column (Verween *et al.*, 2005), we hypothesized that *M. leucophaeata* has only one spawning period, and does not display the bimodal pattern often found in *D. polymorpha*. In addition, we investigated the correlation between gametogenesis and environmental variables such as temperature, salinity and food availability.

Perhaps the most useful and reliable information concerning seasonal trends in gametogenesis is obtained from histological preparations of the gonads (Seed & Suchanek, 1992). Although laborious (probably the major reason for its limited use), this method can give detailed information about the entire reproductive cycle, including the actual time of spawning. The scale of gametogenic development for both male and female mussels was determined using an original arbitrary classification system, described by Seed (1969) for *M. edulis*. This system has been successfully applied to *D. polymorpha* (Borcherding, 1991) and was compared with *M. leucophaeata* to distinguish more easily between the classification stages. This classification method is widely used to identify broad trends of the sexual cycle but, as in any system of arbitrary classification, intermediate stages inevitably occur, resulting in some subjectivity. To make the classification as objective as possible, Seed (1969) used multiple criteria in the assessment of each stage (Table 1). The classification stages include the resting or spent condition (0), the gamete development period (Developing I–V) and the spawning period (Spawning IV–I).

The mean gonad index (MGI), defining the breeding condition of any sample, was determined monthly by multiplying the number of individuals in each stage by its numerical score (0–V, deduced from the arbitrary rating of the stage) and by dividing the sum of these products by the total number of individuals in the sample. The resulting value ranges between 0, when all the individuals are spent or resting, to V, when all individuals are sexually mature. An increase in the MGI indicates a period of development in gonadal tissue, while a decrease in the MGI indicates a period of active spawning (Seed, 1975).

All data originated from an industrial site in the harbour of Antwerp, along the Schelde River in Belgium (51°21.37′N; 4°17.30′E). The study area is situated in the oligohaline zone where *M. leucophaeata* causes fouling problems. Mussels were collected monthly from January to December 2006. The gonads were removed carefully from the surrounding tissues, fixed in Bouin's fluid and embedded in paraffin (60°C) and sections cut at 5–10 μm were stained with toluidin blue (Pearce, 1985). An average of 25 individuals was processed every month. The slides were analysed using a Leica DMLB microscope at ×200 and ×400 magnification. Mussel length averaged 13.58 mm ± SE 0.11. Monthly differences in gametogenic stages of *M. leucophaeata* were tested by analysis of variance (ANOVA). Univariate Pearson correlation matrices were used to determine correlations between environmental variables and the MGI.

Gametogenesis started in January with a slowly increasing trend in late winter, accelerating through spring and early summer until the highest MGI was reached in July–August (Fig. 1), which we interpreted as the main spawning period for *M. leucophaeata*. Data on larval abundances of *M. leucophaeata* (Verween *et al.*, 2005) endorse this pattern, with larvae appearing in the water column from June onward, with maximal densities occurring in August. The development from an egg to a D-shaped larva, large enough to be monitored in this study, takes *c.* 3–4 weeks (Mackie & Schloesser, 1996). In early autumn (September–October), the MGI rapidly decreased towards the winter minimum. The analysis of the gonadal conditions confirmed this pattern of gametogenesis and provided further detail. The redevelopment of the spent or resting gonads started in January, with an average of 52.0% of the individuals under developing conditions, but still 32.0% resting or spent. In the following months, the percentage of resting gonads decreased to 0% in July and August. From June onwards, the development percentages decreased, and many individuals (69.6%) began to spawn. The percentage of spawning individuals reached a maximum in September, but values exceeded 50% until December. Only a small part of the individuals were already spent or resting in September (4.2%). From October onwards, a high percentage of individuals was spent (average 26.2% ± SE 6.2), with few gonads being in development (average 8.3 % ± SE 2.5). The most significant feature of the annual spawning season of *M. leucophaeata* was its duration; the period over which more than 50% of the individuals were spawning extended over 6 months. Thus although only a single spawning period was detected, it lasted for a very long time from June to September. This echoes the pattern observed by Bamber & Taylor (2002)

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Table 1. Microscopic classification system used for gonads of *Mytilopsis leucophaeata*, after Seed (1969) and Buchanan (2001).

General description	
Resting or spent 0	No trace of sexuality; stores of fat and glycogen are accumulated in the connective tissue, frequently obscuring genital canals.
Developing I	Onset of gametogenesis; islands of gametogenesis (follicles) appearing in matrix of dense connective tissue; no ova or spermatozoa are present in this stage.
Developing II	Ripe gametes appear in centre of follicles, although mainly occupied by early stages of gametogenesis.
Developing III	Follicle size increases; follicles half filled with ripe gametes, half with early stages.
Developing IV	Almost maximal proliferation of follicles; general reduction of early stages of gametogenesis and increase of ripe gametes.
Developing V	Fully ripe gonad; ova compacted into polygonal configuration and male gonads distended with ripe sperm arranged in compact laminae.
Spawning IV	Active discharge of gametes in progress, but follicles still relatively full; reduction of follicle pressure induces rounding of ova and loss of laminar appearance in males
Spawning III	Follicles approximately half full with mature gametes but with relatively few early development stages; in females, eggs are rounded rather than polygonal.
Spawning II	Follicles are considerably less than half full with mature gametes.
Spawning I	Follicles collapsing; only residual gametes remain, sometimes undergoing cytolysis; centre of follicles can be filled with yellow-brown matrix of cytolysis.
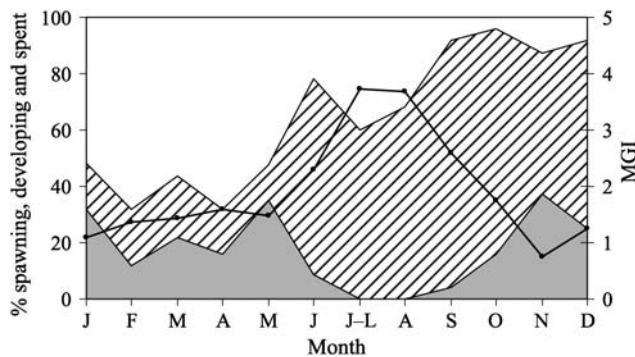
Figure 1. Breeding cycle of *Mytilopsis leucophaeata*, as defined by the average MGI over a 1 year period, with total percentages in the spawning, developing and spent conditions $\pm$ SE (filled circles, MGI; white area, developing; striped, spawning; grey, spent).

who also indicated a spawning period for *M. leucophaeata* from June to September in the Noordzeekanaal. Literature information concerning gametogenesis in *M. leucophaeata* is limited, leaving the question of dependency of breeding cycle on location open for speculation.

In many mussel species, gametogenesis starts in winter, with maturity reaching a peak in late winter, which is followed by a first spawning event during spring–summer. During the warm water conditions of summer, and given adequate nutrition, gonads may mature again for a second late summer–autumn

spawning (Buchanan, 2001). The pattern of one prolonged spawning season in *M. leucophaeata*, confirmed by Bamber & Taylor (2002), thus deviates from the general patterns found in other mussels (Sprung, 1987; Borcherding, 1991). However, the process of spawning can occasionally be completely non-synchronous in *D. polymorpha*, occurring throughout the year with even minor changes in environmental conditions leading to substantial differences in the timing of larvae and ripe gametes (Nichols, 1996).

This study does not identify different spawning periods among individuals. Laboratory studies revealed that *D. polymorpha* is in principle able to spawn four times per season (Walz, 1978), although in nature an annual gameto-genic cycle with a high level of synchronization between the members of a population was found (Borcherding, 1991). This pattern might also be the case here, with the different spawn-ing periods within an individual being obscured by the mass spawning of the whole population.

The rise in MGI clearly coincided with a rise in temperature (Fig. 2; Pearson $r = 0.82$; $P < 0.01$). The highest MGI (MGI = 3.72) coincided with maximal temperatures. This pattern sup-ports the theory that temperature is the main regulator of game-togenesis in mussels (Sprung, 1983; Seed & Suchanek, 1992). Gametogenic index increased in early summer with increasing water temperature. Following spawning, gametogenesis com-menced again after water temperature began to decrease in early autumn. Hence, the general activation of the gonads seems to be activated by declining temperatures, just as in *D. polymorpha* (Garton & Haag, 1993).

Lubet (1959) hypothesized that food abundance was the primary controlling factor for gonad growth in *M. edulis*, based on the fact that gonadal development was immediately resumed at the phytoplankton outbreak in the spring, although tempera-ture was still low at that time. In this study, the MGI also
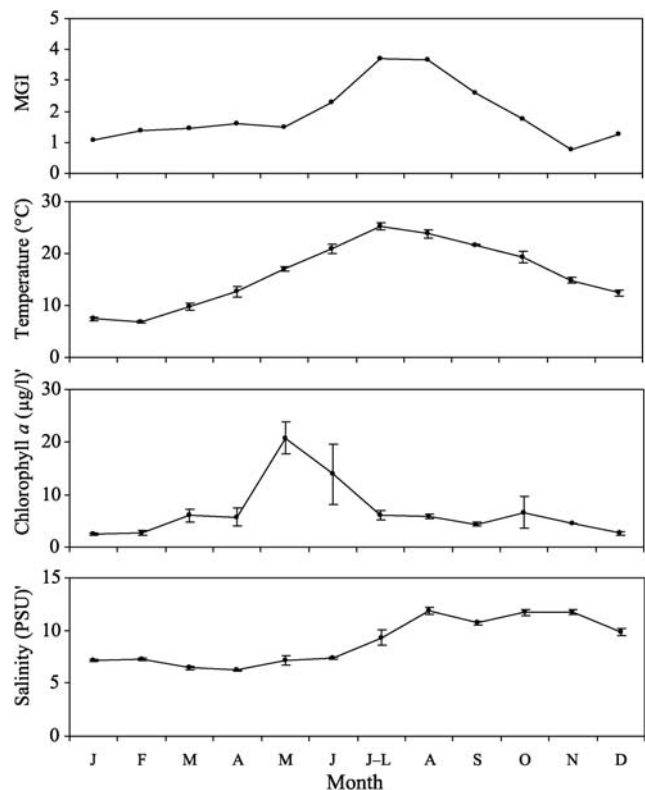
Figure 2. Seasonal changes in MGI in *Mytilopsis leucophaeata* and environmental factors ($\pm$ SE) during 2006.

increased rapidly during the spring phytoplankton bloom (maximum of 20.8 µg/l $\pm$ 3.0 in May) after a slow rise in winter. The maximal MGI was reached only after this bloom. Food quantity and quality, expressed as the chlorophyll *a* concentration, proved to be a significant determinant in *M. leucophaeata* gametogenesis, taking into account a time delay of 2 months ($r = 0.76$; $P = 0.01$). This makes chlorophyll *a* also a significant factor in governing gametogenetic index and spawning. The time delay can be explained by the need for food by the mussels; the phytoplankton is ingested and it is only when enough food is available for the daily metabolism of the organism that the surplus is used for gametogenesis. Numerous authors have observed a link between food availability and gametogenesis (Borcherding, 1991; Ram *et al.*, 1996; Claxton & Mackie, 1998; Buchanan, 2001), and others have identified temperature as well as nutrition as the most important factors controlling the reproductive cycle in mussels, causing temporal variation in reproductive events between some populations (Sastry, 1979; Sprung, 1983; Hawkins, Salkeld & Bayne, 1985; Seed & Suchanek, 1992). It can be suggested that both factors, the presence of a temperature threshold and the food concentration, may be more or less simultaneously responsible for the onset of spawning, although not of equal importance.

For salinity, neither a clear pattern nor a correlation with MGI ($r = 0.31$; $P = 0.33$) was found. This absence of correlation can be explained by the euryhaline nature of *M. leucophaeata*; its adaptation to a wide range of salinities reduces the effect of salinity on vital processes in its life history. However, the lack of information on the effect of salinity on gametogenesis in other mussel species could also indicate the insignificance of this variable in the reproductive cycle, independent of the salinity tolerance of the species.

In contrast to *D. polymorpha*, which can expand very rapidly as soon as a new freshwater basin is colonized, *M. leucophaeata* is a rather slow natural colonizer with low dispersal capacities, and is restricted to brackish water bodies. The presence of a prolonged spawning period in the life cycle of *M. leucophaeata*, however, is advantageous for successful invasion. Species which spawn more or less continuously, like *M. leucophaeata*, might be expected to have a more stable adult population than seasonally breeding species such as *D. polymorpha*, since damage caused by unsuccessful recruitment is likely to be reduced (Seed & Brown, 1977). Although the high physiological plasticity of zebra mussels in response to local conditions is not immediately confirmed in this study of *M. leucophaeata*, densities are likewise high with maxima of 15,000–28,000 individuals per m^2, depending on the location (Darr & Zettler, 2000; Laine, Mattila & Lehikoinen, 2006). So although *M. leucophaeata* is a slower colonizer than *D. polymorpha* (Verweel *et al.*, in press), it is definitely a seriously invasive species.

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