CHAPTER III

Recruitment patterns of *Mytilopsis leucophaeata* in the harbour of Antwerp: implications for an ecologically and economically sound biofouling control

Chapter modified from:

VERWEEN A, VINCX M, MEES, J, DEGRAER S (2005)
Seasonal variability of *Mytilopsis leucophaeata* larvae in the harbour of Antwerp: implications for ecologically and economically sound biofouling control
Belgian Journal of Zoology 135 (1): 91-93.

Abstract

Mytilopsis leucophaeata (Conrad, 1831), the brackish water mussel, is a typical resistant estuarine species which invaded European waters in the nineteenth century, but only became subject of attention recently since it caused economic problems as an industrial fouler. In the harbour of Antwerp, the recruitment patterns of the species were studied during an intensive monitoring study in 2000-2004. Not only was *M. leucophaeata* monitored in its simulated natural environment, also a simulation of the heated conditions inside a cooling water system was used.

Larvae arrived at the end of May – early June and stayed in the water column for about five months. Threshold temperature for gamete maturation in *M. leucophaeata* may be 13 ± SE 1°C. Although the natural densities of larvae showed a high year-to-year variability, the period of larval occurrence was markedly similar. This strict timing of larval presence can be used as a tool to combat biofouling by *M. leucophaeata*; to prevent new biofouling, a targeted dosage of biocides during the period of larval presence would be as effective as a continuous dosage throughout the year. Although this study could not provide proof, the hypothesis can be raised that because of the high temperature inside the cooling system, adults present in the system can give rise to an unceasing source of new larvae to the natural environment.

Settlement data were subject to some monitoring and statistical restrictions. Nevertheless they could be used to formulate some interesting speculations. Settlement of individuals larger than 2 mm started 2.5 - 3.5 months after larval arrival, with peak densities in late summer. Settlement occurred almost throughout the whole year, although at much lower densities in winter. This emphasizes again the importance of using larval data to prevent new biofouling by *M. leucophaeata*; once the individuals are settling, seasonality becomes less clear and a pointed combat becomes impossible.

Keywords

Mytilopsis leucophaeata, biofouling control, ecology, pelagic larvae, settlement, Schelde

REMARK

Part of this chapter has been published as a short note in the Belgian Journal of Zoology (Verween et al., 2005). The short note comprises the biological monitoring of *M. leucophaeata* larvae in the harbour of Antwerp, and its implications on biofouling control. However, we have chosen to expand this research into a full chapter in order to complete the biological information of *M. leucophaeata*, deduced from an intense monitoring study. The added information is important, since it goes a step further than data given in Verween et al. (2005); (1) monitoring happened not only in simulated natural conditions, but also in a simulation of the cooling water system, with a constantly elevated temperature, and, (2) next to the larvae, juvenile settlement was also monitored during this study. The reason why this information was not added in the original publication was two-folded: (1) the aim of the publication was only to give a first report on the importance of *M. leucophaeata* as an invaded fouling species in Belgium, recently causing problems and (2) data on settlement of *M. leucophaeata* were difficult to quantify on a statistically satisfying way, making the information less suitable for publication.

Nevertheless, although subject to some monitoring and statistical restrictions, this extra information stays valuable and – with this critical remark in mind – can significantly contribute to the knowledge on the biology and possible fouling consequences of *M. leucophaeata*.

INTRODUCTION

Mytilopsis leucophaeata (Conrad, 1831), the brackish water mussel, is a mytiliform bivalve (Mollusca, Bivalvia, Veneroida, Dreissenidae), which produces strong byssus to attach to hard substrates. *Mytilopsis leucophaeata* is a typical estuarine species, and thus resistant to a wide range of oligo- to mesohaline conditions (Siddall, 1980). The species originates from the southern coast of the U.S. to Tampico, Mexico (Marelli and Gray, 1983).

In 1835, it was first detected in Europe, in the harbour of Antwerp (Nyst, 1835). After a period of apparent absence, *M. leucophaeata* is currently found along the coast of the North Sea from Germany into France and recently in Great Britain (Oliver et al., 1998). Ballast water discharges from ships were identified as a major vector in the transfer of nuisance aquatic species, such as *M. leucophaeata*, from one area of the world to another. The fact that the species was not detected in Belgian waters over more than 50 years does not necessarily indicate the absence of *M. leucophaeata* along the European coast. Because of the morphological resemblance with the closely related *Dreissena polymorpha*, the zebra mussel, species-confusion may have arisen. When *M. leucophaeata* became an economic problem in the nineties as an important industrial fouler, attention was brought back to this relatively unknown species.

Any surface exposed to untreated water provides an opportunity for the settlement and subsequent growth of organisms. Because of the high temperature and the constant supply of food and oxygen, cooling water systems are an ideal habitat for *M. leucophaeata*. Given these perfect conditions, settlement occurs readily and growth can be rapid until it causes fouling at the heat exchangers and the tubes in the conduits and finally leads to the failure of the operational systems. This phenomenon is known as biofouling (Jenner et al., 1998). Of all organisms causing fouling in cooling systems, mussels are known to cause the most serious problems (Rajagopal et al., 1996).

The freshwater zebra mussel *D. polymorpha* causes major fouling problems in freshwater lakes and great rivers in the U.S.. Hence, the biology and possible control methods of the species are well examined throughout the years. Brackish water species, on the other hand, are far more resistant to environmental changes, which makes them particularly robust fouling species. The most effective and cheap control measure is the use of chlorination. It was only when the legislation on biocide draining became stricter (VLAREM II, 4.2.4., VLAREM II, annex 2.3.1.), that the magnitude of the bio-fouling

problem by *M. leucophaeata* in the harbour of Antwerp became clear. In the near future, specific research on cooling water draining will be conducted and standard concentrations will be lowered. When the legislation on biocide draining in Belgium will get stricter, the use of merely chlorine will no longer be effective against biofouling. Other, (more expensive) methods have to be searched for to prevent fouling problems, caused by *M. leucophaeata*.

Adult mussels can shut their protective shell valves and stop byssus production to isolate their body from changes in the external environment (Khalanski and Bordet, 1981), such as biocide-passage. The planktonic larvae and plantigrades are the most vulnerable life stages, and thus susceptible to the biocides. Hence, knowledge on the cyclic presence of *M. leucophaeata* larvae provides a basis for an ecologically and economically proper use of these detrimental chemicals (Relini, 1984).

Although *M. leucophaeata* is known to cause major biofouling problems throughout Europe, there is a large discrepancy with the information on the species. We do for example not have information on the potential magnitude of fouling problems, caused by *M. leucophaeata*, as well on a temporal as on a spatial scale. Basic knowledge on the autecology of such biofouling species is a first step in comprehending its ecology, thus forming a baseline for a successful future biofouling treatment.

The aims of this intensive monitoring study were:

- To investigate the annual and seasonal variations in D-shaped larvae of *M. leucophaeata* in relation to temperature (°C) and salinity (PSU) in the cooling water system of BASF N.V. in the period 2000 – 2004. As such, the recruitment period(s) of *M. leucophaeata* were determined.
- To investigate the period and to qualify the success of settlement of *M. leucophaeata* in relation to temperature (°C) and salinity (PSU).
- To deduce from this information possible consequences for *M. leucophaeata* biofouling control.

MATERIAL AND METHODS

All field work was conducted at the industrial site of BASF, Antwerpen N.V. The industrial site is situated along the Schelde river, near the Dutch-Belgian border. There are two intake points at the site, at approximately one km distance from each other, accepting water from intermediate salinity, coming from the Schelde and the Rijn-Schelde channel (**Fig. 1**). The Schelde estuary extends from the mouth at Vlissingen (The Netherlands) until Gent (Belgium) over a distance of 160 km (Ysebaert et al., 1993), covering as such the whole salinity gradient from salt to freshwater. The Rijn-Schelde channel is a freshwater channel, connecting the Volkerak Lake with the international harbour of Antwerp, as such realising a tide-free connection between Rotterdam and Antwerp. The study area is limited to the oligohaline zone where *M. leucophaeata* is present and causing fouling problems.

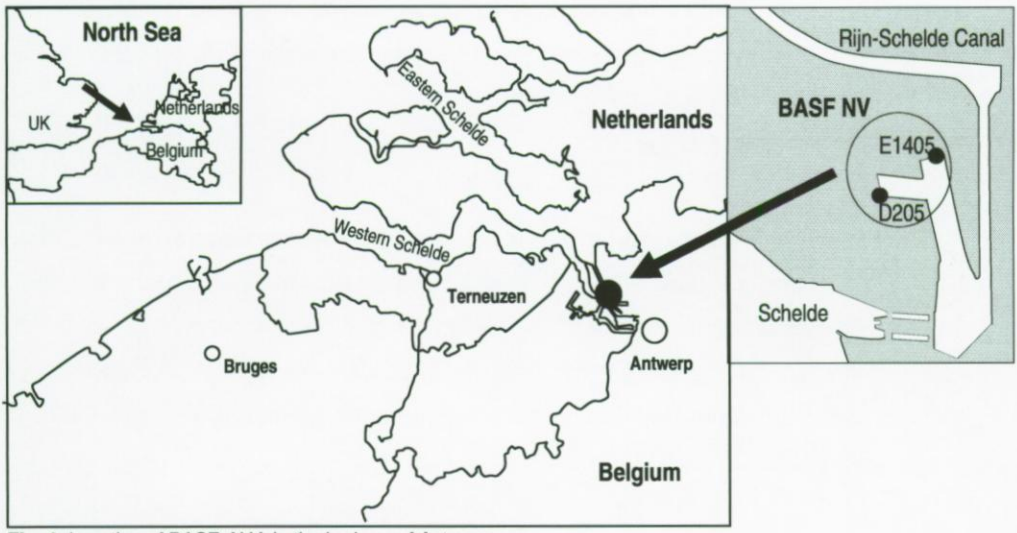

Fig. 1: Location of BASF, N.V. in the harbour of Antwerp.

At both intake points, D 205 and E 1405, a test-installation was build to allow biological monitoring of *M. leucophaeata*. Both points are situated along the dock, at a distance of approximately 1 km from each other. At D 205, part of the incoming water was artificially heated to create a constant temperature of approximately 20 °C, as to simulate the behaviour of *M. leucophaeata* larvae in the cooling water conduits. Another part of the incoming water was not heated, creating the opportunity to compare population dynamics of *M. leucophaeata* in the dock, being its natural environment, and the industrial installation. At E 1405, incoming water could not be heated due to practical limitations and data were merely used to obtain average information on the population dynamics of *M. leucophaeata* in the dock.

Both test-installations were kept free from biocide-dosage, as to allow biological continuance of the species. Each condition (heated and non-heated) consisted of three PVC-tanks (volume: 0.2 m^3), each with an average water flow of 1 m^3/h. Sampling of *M. leucophaeata* population dynamics occurred from February 2000 until December 2004, although the heated situation was not monitored before March 2001.

1. SAMPLING OF VELIGERS

Three replicate quantitative plankton samples were taken at each condition by sieving 50 l water over a 63 µm mesh sieve. From 4 February 2000 until 20 December 2000, *M. leucophaeata* veliger densities were monitored on a weekly basis. From March 2001 on densities were monitored weekly from spring until late autumn, but in wintertime, in absence of larvae, a biweekly monitoring interval was chosen. Environmental variables were monitored weekly all year long. Plankton samples were preserved in 70 % ethanol and veliger abundance was expressed as number larvae per cubic meter.

2. SAMPLING OF SETTLERS

Settlement of mussels preferentially occurs first on filamentous structures (i.e. primary settlement), while young mussels will eventually move to a hard substrate as final colonisation habitat (i.e. secondary settlement) (Bayne, 1964). To ensure the availability of filamentous as well as hard surfaces, three petticoat nets were attached crosswise on the water flow in the PVC-tank. Sampling of settlers happened by removing all individuals on the petticoat gauze and the PVC-walls in all PVC-tanks on a two weekly basis.

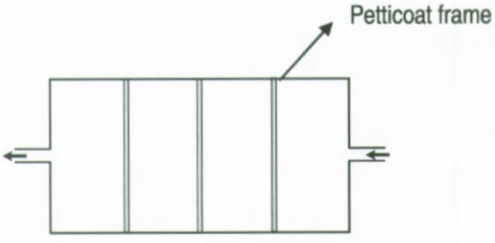

Fig. 2: PVC-tank with petticoat nets.

Quantifying settlement data proved to be very difficult (see discussion). However, data were solid enough to determine the period of presence of settled larvae, as such giving information on appearance

of this rather invulnerable life stage, large enough to create fouling problems in an industrial cooling water system. Therefore, the average number of individuals per tank was only interpreted as density classes: absent, low (1 - 30 individuals), high (31 - 60 individuals) and peak abundances (61 - 355 individuals).

RESULTS

1. LARVAL ABUNDANCE

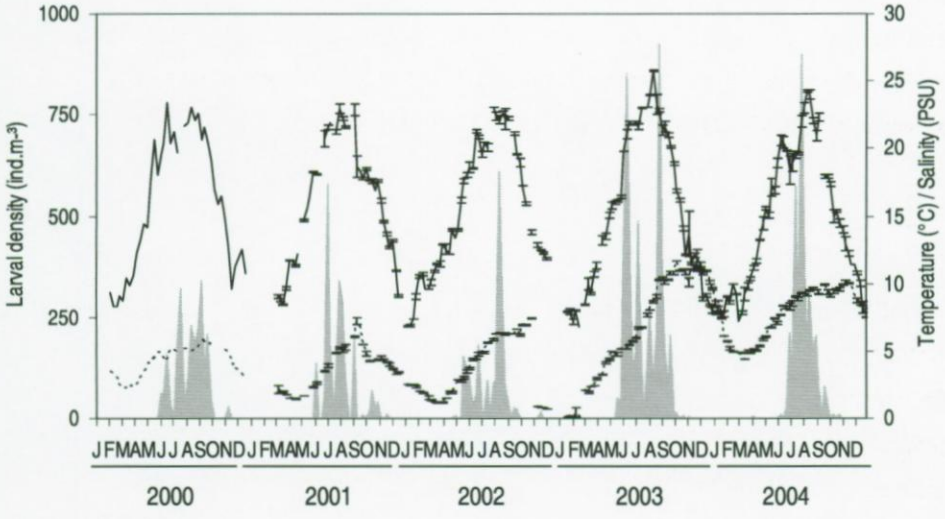

Fig. 3: Seasonal variation in larval arrival of *M. leucophaeata* in the simulated natural water system at BASF, Antwerpen N.V. (full line: temperature (°C), dashed line: salinity (PSU), grey shaded: larval density (ind./m³)).

Results indicated that in all years spawning began end of May – early June and lasted for about five months (**Fig. 3**). In 2000 larvae first appeared in the plankton at 3 June, in 2001 at 6 June, in 2002 at 21 May, in 2003 at 20 May and in 2004 at 8 June. Temperature at first detection ranged between 16.2 – 20.6 °C, salinity ranged from 2.6 to 7.7 PSU.

In all years, two or more distinct larval peaks could be observed. In 2000, 2002 and 2003 the highest peak occurred at the end of August – September (2000: 340 ind./m³ at 7/9; 2002: 613 ind./m³ at 20/8;

2003: 927 ind./m³ at 26/8) at an average temperature of 21.4 ± SE 0.4 °C and salinity ranging from 5.1 PSU in 2000 to 10.3 PSU in 2003. In 2001 and 2004, highest densities (2001: 580 ind./m³; 2004: 900 ind./m³) were recorded earlier, at respectively 3 and 27 July, when the water was 21.6 °C and 3.9 PSU in 2001 and 22.0 °C and 9.4 PSU in 2004. After this peak, two or more peaks were detected. The last peak occurred at 4 September in 2001 and 31 August in 2004 and coincided with the highest peaks in the other years.

In 2000, 2002, 2003 and 2004 larval densities declined after the highest peak and no veligers were found later then 19 November with average temperature 13 ± SE 0.4 °C. Again, salinities were highly variable, ranging from 0.8 PSU in 2002 to 10.1 PSU in 2003 and 2004. In 2001 last veliger densities were found on 20 November (13.7 °C, 4.2 PSU).

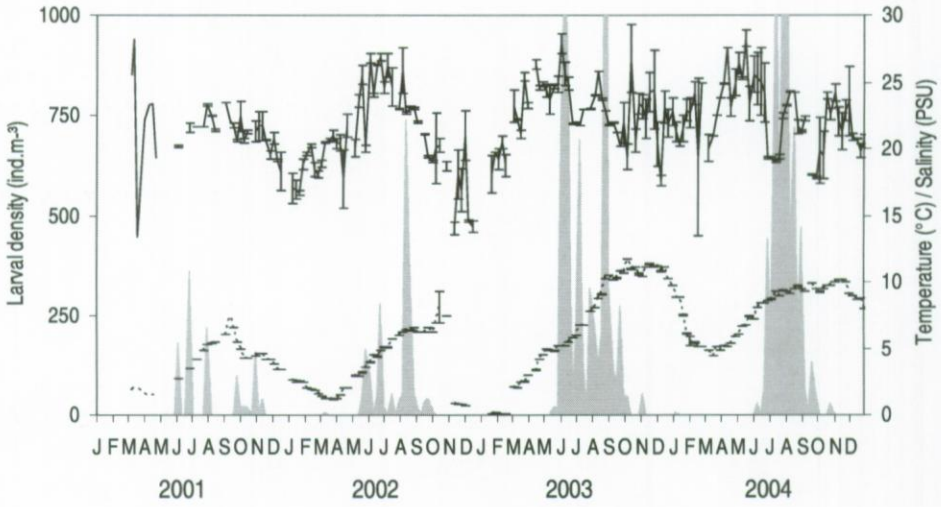

Fig. 4: Seasonal variation in larval arrival of *M. leucophaeata* in the simulated warm cooling water system at BASF, Antwerpen N.V. (full line: temperature (°C), dashed line: salinity (PSU), grey shaded: larval density (ind./m³)).

The artificially heated water had an average temperature of 21.9 ± SE 0.2 °C with a maximum of 28.2 °C and a minimum of 13.4 °C with similar salinity pattern as the environmental conditions (**Fig. 4**). Although temperature was always above the premising threshold temperature for gamete maturation for *M. leucophaeata*, this had no effect on the measured larval densities in the system.

Simulation conditions of the warm cooling water system did not have a significant effect (Wilcoxon Matched Pairs test: $p = 0.70$) on larval arrival and abundance (**Fig. 5**); abundance data were very much alike, with maximal weekly densities of 1810 larvae per m^3 in the simulation system and 1580 larvae per m^3 in the natural system.

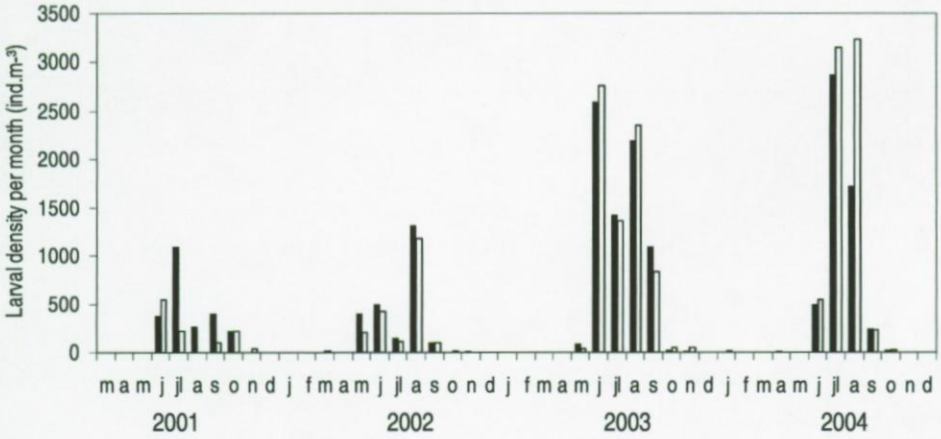

Fig. 5: Monthly comparison of larval abundance of *M. leucophaeata* in the simulated natural and heated conditions. (■ = natural environment; □ = heated system).

2. PRESENCE OF SECONDARY SETTLEMENT

Based on juvenile settlement, a clear distinction could be made between the years 2001-2002 and 2003-2004 with higher densities of settlers in the latter years (**Fig. 6**).

Although quantification in this study is subject to discussion, transformed data (i.e. density classes) could be used to extract some hypotheses. The start of the secondary settlement season was characterized by a major peak of settlers in late summer. In 2001, settlers arrived in low densities in the system at 4 September, in 2002 at 17 September, in 2003 at 5 August and in 2004 at 10 August. Temperature at first detection ranged between 21.2 - 24.4 °C at a salinity of 6.1 - 9.1 PSU. The duration of the settlement season was difficult to outline in 2001, but lasted about 12 months in the following years. In January - March 2003 a complete lack of settlers was observed while in the same period the following year, settlers were detected, although at low abundances.

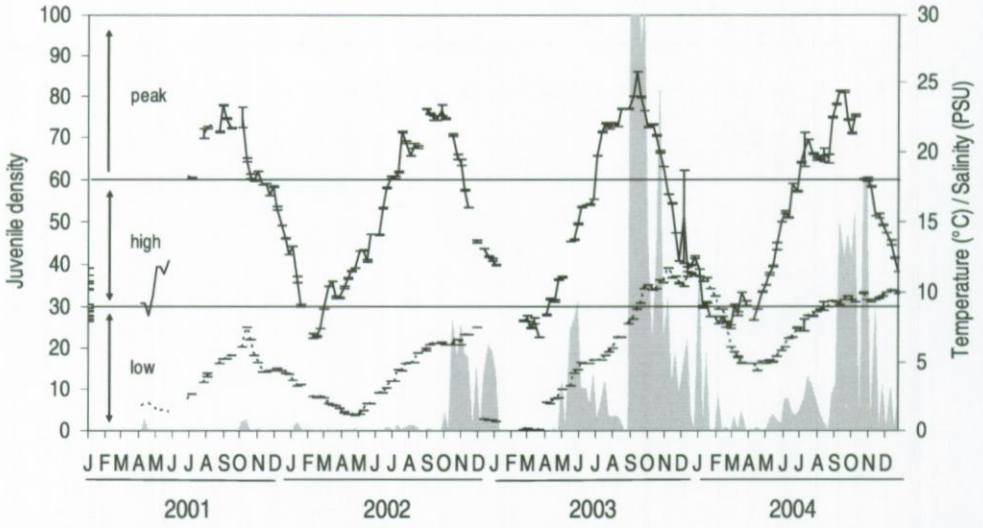

Fig. 6: Seasonal variation in presence of *M. leucophaeata* settlement in the natural water system at BASF, Antwerpen N.V. with indication of the density classes (full line: temperature (°C), dashed line: salinity (PSU), grey shaded: juvenile density).

Temperature in both periods was much alike, varying between 8 - 9.5 °C in 2003 and 7.6 - 10 °C in 2004 but salinity differed a lot with values of 0.1 - 2 PSU in 2003 and 4.9 - 8.8 PSU in 2004. The peak abundance of settlers was observed about 2.5 – 3.5 months after first detection of larval arrival with 93 days in between in 2001, 103 in 2002, 75 in 2003 and 81 in 2004.

In the heated condition the yearly start of the settlement season was also characterized by a visual peak in settlement although only at high densities, occurring at the same date as the peak abundances in the natural situation in 2002 - 2004, and one week earlier, on 11 September, in 2001 (**Fig. 7**). Remarkable is the fact that settlement was detected in spring 2003 and was absent in spring 2004, opposing the natural situation. Temperature in both periods was much alike, varying between 18.1 – 20.5 °C in 2003 and 19.4 – 22.5 °C in 2004 but salinity differed with values of 0.1 – 2 PSU in 2003 and 4.4 – 5.1 PSU in 2004.

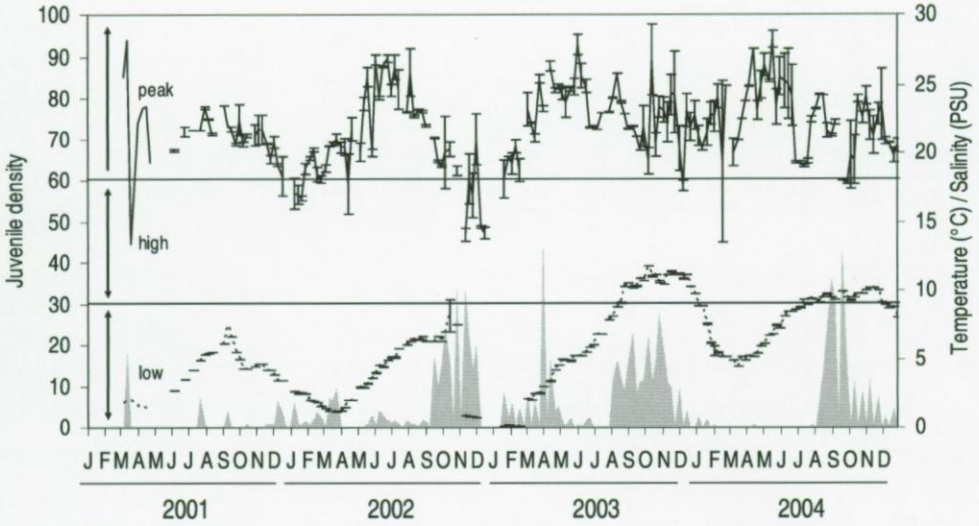

Fig. 7: Seasonal variation in presence of *M. leucophaeata* settlement in the simulated warm cooling water system at BASF, Antwerpen N.V. with indication of the density classes (full line: temperature (°C), dashed line: salinity (PSU), grey shaded: juvenile density).

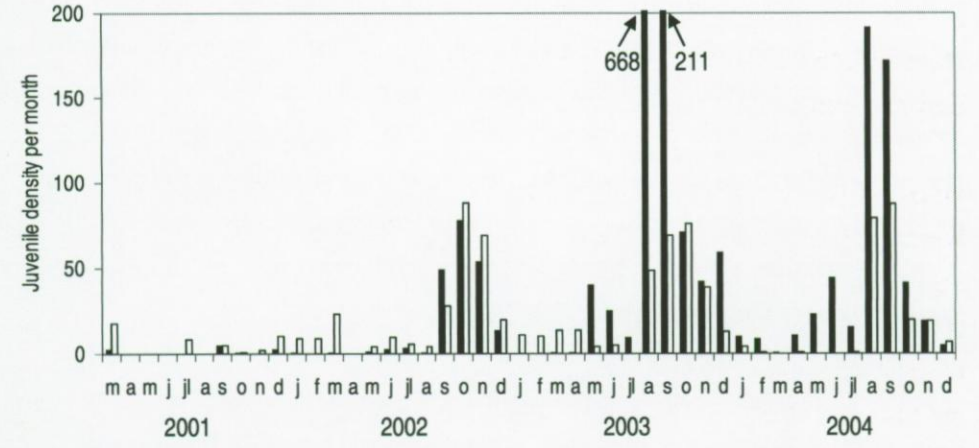

Fig. 8: Monthly comparison of abundance of *M. leucophaeata* settlers in the simulated natural and heated conditions. (■ = natural environment; □ = heated system).

Although the temperature in the cooling water system was much higher than in the natural water system, simulation conditions of the warm cooling water system did not have a significant effect (Wilcoxon Matched Pairs test: $p = 0.27$) on presence of settled individuals (**Fig. 8**); abundance data were very much alike, ranging from 0 to 45 individuals per tank in the simulation system and 0 to 60

individuals per tank in the natural system. Only the peak densities of autumn 2003 in the natural system (134 ± 63 individuals per tank) were absent in the heated system.

DISCUSSION

1. LARVAL ABUNDANCE

Dreissenidae - such as *M. leucophaeata* and *D. polymorpha* - are sequential spawners, and the duration of larval production in *D. polymorpha* can vary from 6 to 52 weeks (Sprung, 1993). The seasonal flexibility in larval production patterns indicates that adults carry ripe gametes for a very long time. After initial spawning, the exposure to ripe eggs and sperm in the water column often triggers gamete release by other ripe mussels, as such creating variability in recruitment, recognizable in the different larval peaks. Peak larval densities occur 1 – 2 weeks after spawning, corresponding to the normal time of development from veliger to veliconcha (Loosanoff and Davis, 1963).

Annual and geographic variation in temperature has been identified as the primary factor triggering reproduction of *D. polymorpha* where veligers typically appear in the water at temperatures above 12 °C. Also the mass spawning in *Mytilus edulis* is coordinated by reaction after a temperature shock (de Vooys, 1999). Severe winter temperatures, as monitored in January 2002 and 2003 (min. 6.8 °C), cause a marked decrease in basal metabolism, resulting in reduced depletion of energy reserves and consequently in an increase in gamete production when temperature increases (Pulfrich, 1997). If the following temperature rise in spring is more rapid than in other years, a synchronous and more intense spawning is obtained, as seen in summer 2003.

The intensity and duration of reproduction is believed to be controlled by an interaction of environmental factors (Kautsky, 1982), such as temperature, food availability and salinity. For *D. polymorpha*, 12 °C is the minimum temperature allowing gonad maturation and no veligers will appear in the water column at lower temperatures (Ram et al., 1996). Data show that for *M. leucophaeata*, this threshold temperature for gamete maturation may be 13 ± 1 °C, indicating that mussels in the cooling water system (± 20 °C) can be ripe at any moment of the year. Although this study does not provide proof, the hypothesis can be raised that adult mussels, present in the warm conduits of an industrial installation, can become an unceasing source of new larvae to the natural environment. The reason why the simulation conditions of

the warm cooling water system had no effect on larval arrival and abundance is the fact that it is the environment that triggers spawning of the natural adults. The natural conditions regulated the monitored larval pattern, independent of the receiving water system.

The natural densities of larvae showed a high year-to-year variability, with moderate values in 2000 – 2002 (yearly average densities 2169 ± 78 ind./m³) and high values in 2003 (yearly densities 5273 ind./m³) and 2004 (yearly densities 3557 ind./m³). Although major differences in densities between months and years were found, the period of larval occurrence was however markedly similar.

2. PRESENCE OF SECONDARY SETTLERS

De Blok and Geelen (1958) stated that mussel larvae attach preferentially to filamentous structures, such as algae (i.e. primary settlement). After this temporarily surface of attachment, they pass on to their final place of settlement, a hard substrate (i.e. secondary settlement). In experimental setups, petticoat gauze has proved to be a suitable substrate for the attachment of pediveliger larvae for metamorphosis (de Vooys, 1999) but, during the study two considerations arose. (1) Although pediveliger larvae prefer filamentous structures such as petticoat gauze for settlement in artificial circumstances, this monitoring study showed that if also natural filamentous algae were present, *M. leucophaeata* larvae preferred these for attachment. *Cordylophora caspia* (Pallas, 1771), a colonial hydroid, was found in enormous densities in the test-installation during autumn 2002 and 2003. Although samples of this hydroid were covered with juvenile *M. leucophaeata*, at the same time almost none were detected on the petticoat gauze. (2) *Mytilopsis leucophaeata* is able of movement from one place to another, even in its pediveliger stage (Rajagopal, pers comm). Therefore, distinguishing between primary and secondary settlement of *M. leucophaeata* became impossible and the settlement considered in this study was defined as secondary settlement.

Secondary settlement of *M. leucophaeata* was first detected in late summer, when a peak of small individuals of about 2 – 3 mm was found. This process started about 2.5 – 3.5 months after larval arrival. In most areas, peak mussel settlement period of *M. edulis* occurred during summer, 1 – 2 months after spawning, with sometimes a second peak in autumn – winter (Seed, 1969a). The delay in *M. leucophaeata* settlement can easily be explained by the lack of primary settlement data for *M. leucophaeata*; plantigrades of *M. edulis* become competent to settle at a shell length of 250 µm, a length undetectable without microscope, with four weeks seeming to be the average time spent by the majority of plantigrades on filamentous algae (Bayne, 1965). Growth of postlarvae from metamorphosis

to a shell length of 2 mm then takes one to two months (Seed, 1969a; Sprung, 1984), reaching the length, detected in this study.

Settlement of *M. leucophaeata* occurred almost throughout the whole year, although at much lower densities during wintertime. This is a general pattern in mussel species. Many populations of *M. edulis* also show at least a low level of settlement throughout the year (Snodden and Roberts, 1997), with different peaks of settlement in late summer and the following spring. These latter can be explained by the fact that early settlers grow more slowly during unfavourable conditions. A reduced temperature together with reduced food availability during winter leads to a cessation of growth and feeding, up to 6 months (Lane et al., 1985), keeping length of these settlers below detectable range. So, small settlement peaks during spring may still be originating from the larval bloom from the past summer.

Study of settlement in the heated system did not reveal a significantly different pattern from the natural circumstances. On the contrary, the pattern of opposing presence of settlement in heated and natural conditions during spring 2003 – 2004 indicated that *M. leucophaeata* settlers have no preference what so ever for the heated system. We would expect a rather high level of settlement of *M. leucophaeata* throughout the whole year since water temperature does not drop in wintertime. However, two patterns have to be taken into account. (1) Although water temperature is kept artificially high, food abundance and quality do follow the seasonal pattern of the natural environment. Therefore, growth can be delayed in the heated system in winter, although Bayne (1965) stated that this cessation in growth is triggered by reduced temperature rather than reduced food availability. (2) Considering the small surface of the PVC-tanks and the rather high velocity of the passing cooling water, *M. leucophaeata* larvae have a short residence time in the tanks, possibly too short for *M. leucophaeata* to experience the higher temperature conditions and decide to settle.

No conclusions could be made on the amount of settlement or the relation between larval abundance and true recruitment of *M. leucophaeata* since primary settlement was not included in this study. However, it has been estimated that pelagic mussel larvae have a high mortality of more than 99% (Sprung, 1984). Not only are they highly vulnerable to external influences, their transformation to a benthic phase is also the most sensitive one in their life cycle. Larval survival and development is markedly affected by fluctuations in food abundance, quality and predator abundance and is dependent upon species-specific temperature ranges (Pulfrich, 1997). These biotic and abiotic parameters affect various larval species differently and resultant annual variations in larval percentages surviving to metamorphosis can differ completely between species and locations.

CONCLUSIONS: IMPLICATIONS FOR ECOLOGICALLY AND ECONOMICALLY SOUND BIOFOULING CONTROL

The strict timing of larval presence of *M. leucophaeata* is a first indication that knowledge of the bivalve's life cycle can be an important tool in the combat against biofouling. To prevent new biofouling, a pointed dosage of biocides during the period of larval presence would be as effective as a continuous dosage throughout the year. A targeted dosage will decrease the amount of biocides needed, allowing to (1) meet the VLAREM II criteria on the use of biocides and (2) explore the use of ecologically less harmful, but more expensive biocides.

The almost all year round presence of secondary settlers emphasizes the importance of combating *M. leucophaeata* biofouling while they are still in their larval phase. Once the individuals are settling, seasonality becomes less clear, eliminating a successful targeted combat. Research also shows that settlement data are difficult to obtain, while planktonic samples are simple and reliable, emphasizing the importance and ease of working with larval data.

Studies of the heated system typical for an industrial cooling water system prove that biofouling by *M. leucophaeata* is not expected to be significantly enhanced. Most probably, since salinity, food quality and quantity will be the same as in the natural system and temperature is constantly above spawning level, the hypothesis can be raised, that the continuous possibility of spawning by adult mussels in the cooling water system can become an unceasing source of new larvae into the natural environment. The conditions in this natural environment however can make survival of these larvae practically impossible in unfavourable periods, but no research has been conducted on this topic.

However, whereas plankton samples remain a useful means of confirming spawning events of the local adult populations, all mentioned factors of variance can be used to conclude that making predictions of recruitment success to adult mussel stocks from larval densities is quasi unreliable (Pulfrich, 1997). Therefore, monitoring studies of planktonic larvae of *M. leucophaeata* are an ideal tool to determine the limited period in which new biofouling by the species can be combated, but they are insufficient to predict the extent of future biofouling problems of *M. leucophaeata*.