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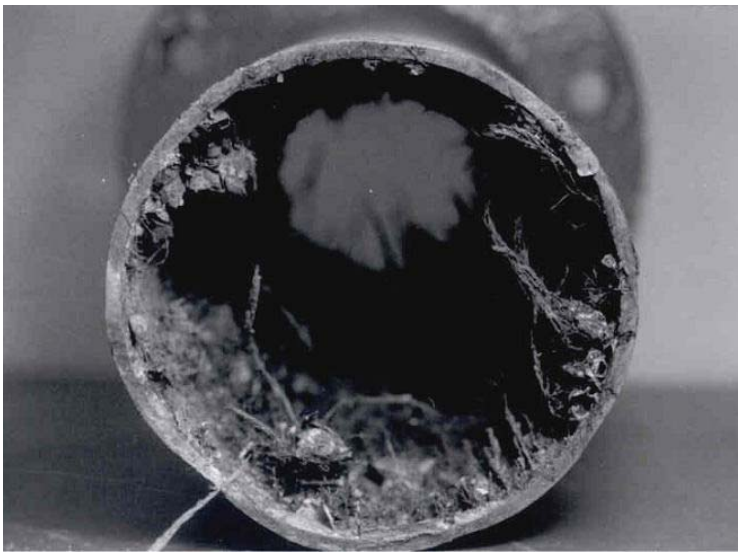
BIOLOGICAL KNOWLEDGE AS A TOOL FOR AN ECOLOGICALLY SOUND BIOFOULING CONTROL:

A CASE STUDY OF THE INVASIVE BIVALVE *MYTILOPSIS LEUCOPHAEATA* IN EUROPE

Biologische kennis als een instrument voor een ecologisch verantwoorde biofouling beheersing:

Een case study van de invasieve mossel *Mytilopsis leucophaeata* in Europa



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SUMMARY

SAMENVATTING

SUMMARY

Mytilopsis leucophaeata (Conrad, 1831), the brackish water mussel, is causing biofouling problems in the cooling water systems of several North-West European industrial sites (Rajagopal et al., 1994; Verween et al., 2005). Technical installations such as cooling water systems mainly use water from nearby seas or rivers to cool down electrical processes in the industrial plant. Larvae of *M. leucophaeata* are pumped up together with the cooling water and are dispersed in the cooling system. The larvae arrive in a beneficial environment: they enter a system with a constant water flow, which assures a continuous supply of food and oxygen, yet in absence of predators, which are kept out by means of the 1 mm sieving system at the water entrance. Given these perfect environmental conditions, settlement occurs quickly and growth can be rapid. This growth will eventually interfere with the industrial processes, finally leading to their failure. This process is defined as biofouling (Jenner et al., 1998).

Worldwide, biofouling problems yearly pose an enormous economic cost, especially in cooling water systems, leading to the development of control measures. The most effective and inexpensive mussel control measure in industrial stations is the use of chlorination as a biocide. Although the use of biocides stays well below the criteria defined by (inter)national legislation, the search for other, more specific and environment-friendly control measures is ongoing. Therefore, the ecology of *M. leucophaeata*, present in the harbour of Antwerp, is used as a tool in search for better, ecologically sound solutions against its biofouling. This general objective was divided in two aspects: (1) the provision of an advice for an efficient and rational use of biocides to control biofouling by *M. leucophaeata*, in order to further confine the damage of these chemicals to the surrounding environment and to the cooling water system itself and (2) if possible from a technological point of view, converting from biocides to more biological solutions in the battle against biofouling by *M. leucophaeata.*

Adult mussels can close their protective shell and stop byssus production to temporary isolate their body from the external environment, that may contain biocides (Khalanski and Bordet, 1981). In contrary, the planktonic larvae and plantigrades are considered more vulnerable life stages, and thus most probably susceptible to biocides. Hence, biological knowledge of the combatable species is an indispensable basis for an ecologically and economically proper use of these detrimental chemicals (Relini, 1984). Therefore, chapter II to V deal with the autecology (e.g. population dynamics aspects) of *M. leucophaeata* while chapter V and VI treat the development of specific control methods.

BIOLOGICAL KNOWLEDGE…

The currently available literature on *M. leucophaeata* is summarized in **Chapter II**. *Mytilopsis leucophaeata* is a mytiliform bivalve (Mollusca, Bivalvia, Veneroida, Dreissenidae) resistant to a wide range of oligo- to mesohaline conditions. Although the genus *Mytilopsis* originally existed in Europe more than 60 million years ago, it disappeared after its expansion to Central America. The species currently originates from the southern coast of the US to Tampico, Mexico and has re-invaded European brackish waters in the early 19th century. It was only when *M. leucophaeata* became a biofouling problem in the 1990s that attention was drawn to this relatively unknown species. *Mytilopsis leucophaeata* is a rather slow natural colonizer needing human-mediated vectors for its dispersal, but once established the species has all the advantages to become a severe fouling species. Expansion along European brackish waters is still taking place and speeding up, by means of e.g. ballast water and hull fouling of ships, with very recent discoveries in the Black Sea, the mouth of the Guadalquivir in Spain and the Baltic Sea in Finland. In the following chapters, lack of data on the autecology of *M. leucophaeata*, necessary for the development of future control measures, was gathered.

The recruitment patterns of *M. leucophaeata* in the harbour of Antwerp were investigated in **Chapter III**. Although the natural densities of larvae showed a high year-to-year variability, the period of larval occurrence was markedly similar with larvae arriving at the end of May – early June for a period of about five months. Threshold temperature for gamete maturation in *M. leucophaeata* appeared to be 13 ± SE 1°C. Although this study could not provide proof, the hypothesis was raised that because of the high temperature inside the cooling system, adults present in the system can give rise to a continuous source of new larvae to the natural environment. Secondary settlement occurred almost throughout the whole year, although at much lower densities in winter.

The study in **Chapter IV** investigated the reproductive cycle of *M. leucophaeata*. Gametogenesis slowly started late winter (January) and accelerated in spring during the spring plankton bloom until a main spawning period was reached in summer (August). A single uninterrupted spawning period was detected during the second half of the year in which more than 50% of all individuals were in an early or late spawning state. As was the case in other mussel species, temperature was defined as being the most important regulator affecting gametogenesis of *M. leucophaeata*. Additionally, food concentration proved to be a crucial environmental factor governing the timing of gametogenesis and spawning.

Mytilopsis leucophaeata followed an oscillatory growth pattern with a single summer growing period per year (**Chapter V**). *Mytilopsis leucophaeata* showed to be a slowly growing, small mussel species with high longevity. Growth decreased during wintertime, but never ceased completely. *Mytilopsis leucophaeata* has an average growth rate of less then 3 to 6 mm/year. Based on a combination of growth of different cohorts, hypothetical growth of an average individual mussel could be modeled over a 5 year period, resulting in a maximum length >19 mm with a growth constant of 0.41. Temperature was found to be the main environmental factor affecting growth.

… AS A TOOL FOR AN ECOLOGICALLY SOUND BIOFOULING CONTROL

A thorough study of the autecology of *M. leucophaeata* already yielded valuable information for the development of control measures against its biofouling. This 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 targeted dosage of biocides during the period of larval presence would be as effective as a continuous dosage throughout the year. Another advantage is that adults tend to be weaker after spawning when they have little energy reserves (Bayne et al, 1976), so it is obvious that effective control measures are best adopted during spawning periods (Rajagopal et al., 2005a). 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 settled, seasonality becomes less clear, eliminating a successful targeted combat. However, this strategy was considered a mere first step in the right direction: treatment still works reactively, i.e. larvae must be monitored before action can be undertaken, resulting in a laborious monitoring strategy to determine the period of biocide dosage.

By means of the development of a predictive model in **Chapter VI,** the larval presence of *M. leucophaeata* could be predicted, excluding the need for the laborious larval monitoring. To incorporate this modeling technique in industrial monitoring technologies, it was preferable that the complexity of the model was minimal, without reducing its predictive capacity. The developed temperature-time model makes it possible to predict larval presence in the water column just by monitoring water temperature. This is an advantage in industrial use as a simple automatic monitoring system is sufficient in determining the period of biocide dosage need.

In search for a more ecologically sound biofouling control for *M. leucophaeata* we tried to go one step further in **Chapter VII**. Since a mussel's life cycle consists of a vulnerable, larval and an invulnerable,

adult stage, we searched for a lethal combination of the prevailing environmental variables that made it technically possible to combat *M. leucophaeata* larvae without the use of detrimental chemicals. For *M. leucophaeata* however, this shift from the vulnerable, larval phase to the highly resistant, benthic phase needs to be nuanced emphasizing that only the first, very young embryos are vulnerable: a gradient in resistance might be expected from the early life stage to the benthic stage. Even the very first life stages, i.e. 4h old embryos, however are already remarkably resistant to abrupt changes in temperature and salinity indicating that a fully natural solution against *M. leucophaeata* biofouling is at the moment still not feasible.

This study provided a comprehensive overview of the biology of the invasive brackish water mussel *M. leucophaeata* in the harbour of Antwerp, being the first study of the species' autecology in European waters. It also emphasizes the important role of biological knowledge in developing efficient control measures against biofouling of *M. leucophaeata*: a more specific, ecologically sound control measure could be designed. This research program is the first predictive study related to biofouling problems, where knowledge on the biological processes initiating biofouling is used to prevent new problems.

SAMENVATTING

Mytilopsis leucophaeata (Conrad, 1831), de brakwatermossel, veroorzaakt biofouling problemen in de koelwatersystemen van verschillende industriële sites in Noordwest Europa (Rajagopal et al., 1994; Verween et al., 2005). Water uit nabij gelegen zeeën of rivieren wordt vaak gebruikt om de elektrische processen in het industriële bedrijf af te koelen. Larven van *M. leucophaeata* worden samen met het koelwater opgepompt en verspreid in het koelsysteem waar perfecte omgevingsomstandigheden voor broedval en groei heersen. Ten eerste garandeert een constante waterstroming een continue toevoer van voedsel en zuurstof en daarenboven zijn predatoren er afwezig, aangezien deze worden tegen gehouden door het 1 mm zeefsysteem aan de ingang. De groei van *M. leucophaeata* zal na verloop van tijd interfereren met de industriële processen en uiteindelijk leiden tot een storing. Dit proces wordt gedefinieerd als biofouling (Jenner et al., 1998).

Wereldwijd leiden biofouling problemen jaarlijks tot enorme kosten - voornamelijk in koelwatersystemen – en dringen controlemaatregelen zich op. De meest efficiënte en goedkope controle tegen mossel biofouling in industriële systemen is het gebruik van chlorering als biocide (IPPC, 2000). Hoewel het gebruik van biociden niet in strijd is met de (inter)nationale wetgeving, is het onderzoek naar andere, meer specifieke en milieuvriendelijke controlemaatregelen volop bezig. De doelstelling van deze studie was het ontrafelen van de ecologie van *M. leucophaeata* in de haven van Antwerpen. Dit kon gebruikt worden als een instrument in de zoektocht naar betere, ecologisch verantwoorde oplossingen tegen zijn biofouling. Deze algemene doelstelling werd opgesplitst in twee aspecten: (1) het voorzien van een advies voor een efficiënt en rationeel biocidegebruik om biofouling door *M. leucophaeata* te controleren, om zo de schade van deze chemicaliën aan de omgeving en het koelwatersysteem verder te beperken en (2) indien milieutechnisch mogelijk, over te schakelen van biociden naar eerder biologische oplossingen in de strijd tegen biofouling door *M. leucophaeata*.

Een literatuurstudie toonde aan dat de gevoeligheid van mosselen voor externe veranderingen varieert gedurende hun levenscyclus. Adulte mosselen kunnen hun beschermende schelp sluiten en byssusproductie stoppen om hun lichaam tijdelijk af te sluiten van hun omgeving (Khalanski and Bordet, 1981), terwijl de planktonische larven en plantigraden als meer kwetsbaar beschouwd worden (Ackerman et al., 1994). Dit bewijst dat biologische kennis van de te bestrijden soort een onmisbare basis is voor een ecologisch en economisch gepast gebruik van biociden (Relini, 1984). De specifieke biologische processen van *M. leucophaeata* worden behandeld in hoofdstukken II tot V, terwijl

hoofdstukken VI en VII de ontwikkeling van specifieke, milieuvriendelijkere controlemethoden bespreken.

BIOLOGISCHE KENNIS…

De beschikbare literatuur over *M. leucophaeata* is samengevat in **Hoofdstuk II**. *Mytilopsis leucophaeata* is een mosselsoort (Mollusca, Bivalvia, Veneroida, Dreissenidae), resistent aan een brede waaier van oligo- tot mesohaliene condities. Alhoewel het genus *Mytilopsis* meer dan 60 miljoen jaar geleden in Europa voorkwam, verdween het na zijn expansie naar Centraal Amerika. De soort werd in het begin van de 19e eeuw opnieuw geïntroduceerd in Europese brakwaters vanuit de zuidkust van de VS tot Tampico (Mexico). Het was echter pas toen *M. leucophaeata* een biofouling probleem werd in de jaren 90 dat de aandacht op deze relatief onbekende soort werd gevestigd.

Mytilopsis leucophaeata is een eerder trage natuurlijke kolonisator, die menselijke factoren zoals bv. scheepvaart nodig heeft voor zijn verspreiding. Eenmaal gevestigd echter heeft de soort alle kenmerken om een ernstige foulingsoort te worden. Expansie langs Europese brakwaters vindt nog steeds plaats en is aan het versnellen door bv. ballastwater in en fouling op de romp van schepen, met recente ontdekkingen van de soort in de Zwarte Zee, de monding van de Guadalquivir in Spanje en de Baltische Zee in Finland.

In de volgende hoofdstukken worden de hiaten in de autecologie van *M. leucophaeata*, nodig voor de ontwikkeling van toekomstige controle maatregelen, opgevuld. De rekruteringspatronen van *M. leucophaeata* in de haven van Antwerpen werden onderzocht in **Hoofdstuk III**. Hoewel de natuurlijke densiteiten van larven een hoge jaar-tot-jaar variabiliteit vertoonden, was de periode van larvale aanwezigheid duidelijk gelijkaardig, met larven die in het systeem aankomen vanaf eind mei – begin juni. De larven werden gedurende ongeveer vijf maanden gedetecteerd in de waterkolom. De drempeltemperatuur voor gameetrijping was 13 ± SE 1°C. De hypothese werd geopperd dat door de hoge temperatuur in het koelsysteem - steeds boven 13 °C - de aanwezige adulten een continue bron van nieuwe larven kunnen leveren aan de natuurlijke omgeving. Geen direct bewijs werd hier echter voor gevonden. Secundaire broedval vond ongeveer het ganse jaar plaats, al waren de densiteiten in de winter veel lager.

De studie in **Hoofdstuk IV** onderzocht de voortplantingscyclus van *M. leucophaeata*. Gametogenese startte traag in de late winter (januari) en versnelde in de lente gedurende de planktonbloei tot een

paaiperiode werd bereikt in de zomer (augustus). Eén enkele ononderbroken paaiperiode werd gedetecteerd gedurende de tweede helft van het jaar waarin meer dan 50% van de individuen zich in een vroege of late staat van paaien bevonden. Zoals ook bij andere mosselsoorten, werd temperatuur gedefinieerd als de belangrijkste regulator in het gametogeneseproces van *M. leucophaeata*. Ook voedselconcentratie bleek een cruciale omgevingsfactor in het bepalen van de timing van gametogenese en paaien.

Mytilopsis leucophaeata volgde een oscillerende groei met één enkele groeiperiode per jaar in de zomer (**Hoofdstuk V**). Groei verminderde gedurende de winter maar stopte nooit volledig. *Mytilopsis leucophaeata* bleek een traag groeiende, kleine mosselsoort met een lange levensduur. De gemiddelde groeisnelheid bedroeg minder dan 3 tot 6 mm per jaar, naargelang de grootte van de mossel. Gebaseerd op een combinatie van groei van verschillende cohorten kon de hypothetische groei van een gemiddelde individuele mossel gemodelleerd worden over een periode van vijf jaar, resulterend in een maximale lengte van > 19 mm met een groeiconstante van 0.41. Temperatuur bleek de belangrijkste beïnvloedende omgevingsfactor te zijn in het groeiproces.

… ALS EEN INSTRUMENT VOOR EEN ECOLOGISCH VERANTWOORDE BIOFOULING BEHEERSING

Een grondige studie van de autecology van *M. leucophaeata* leverde waardevolle informatie voor de ontwikkeling van controlemaatregelen tegen zijn biofouling. De strikte timing van de larvale aanwezigheid van *M. leucophaeata* is een eerste indicatie dat kennis van de levenscyclus van de tweekleppige een belangrijk instrument kan zijn in de strijd tegen biofouling. Om nieuwe biofouling te voorkomen zou een gerichte dosering van biociden tijdens de periode van larvale aanwezigheid even effectief zijn als een continue dosering gedurende het volledige jaar. Een ander voordeel is dat adulten zwakker blijken te zijn na het paaien, wanneer ze weinig energiereserves hebben (Bayne et al, 1976). Het is dus duidelijk dat effectieve controlemaatregelen best toegepast worden gedurende de paaiperiodes (Rajagopal et al., 2005a). De aanwezigheid van secundaire broedval gedurende praktisch het ganse jaar benadrukt het belang van het bestrijden van *M. leucophaeata* biofouling in zijn larvale fase; eens de individuen zich settelen wordt de seizoenaliteit minder duidelijk, waardoor een succesvolle gerichte behandeling onmogelijk wordt. Deze strategie werd echter pas als een eerste stap in de juiste richting gezien: de behandeling werkt nog steeds reactief, d.w.z. dat larven eerst gemonitord moeten worden voor actie kan worden ondernomen, resulterend in een arbeidsintensieve monitoringsstrategie die nodig blijft om de periode van biocide dosering te bepalen.

Door de ontwikkeling van een voorspellend model in **Hoofdstuk VI** kon de larvale aanwezigheid van *M. leucophaeata* voorspeld worden, waardoor de nood aan de arbeidsintensieve larvale monitoring overbodig wordt. Om deze modelleringtechniek te incorporeren in industriële technologieën was het aan te raden om de complexiteit van het model te minimaliseren zonder zijn voorspellende capaciteit te reduceren. Het ontwikkelde temperatuur-tijdsmodel maakt het mogelijk om larvale aanwezigheid in de waterkolom te voorspellen door enkel de watertemperatuur op te volgen. Dit is een voordeel in industrieel gebruik; een eenvoudig automatisch meetsysteem is voldoende om de periode van biocidegebruik te bepalen.

In **Hoofdstuk VII** probeerden we nog een stap verder te gaan in de zoektocht naar een meer ecologisch verantwoorde biofouling controle voor *M. leucophaeata*. Aangezien de levenscyclus van een mossel bestaat uit een kwetsbare, larvale en een onkwetsbare, adulte fase, zochten we een letale combinatie van de heersende omgevingsvariabelen die het milieutechnisch mogelijk kon maken om *M. leucophaeata* larven te bestrijden zonder het gebruik van schadelijke chemicaliën. De shift van de kwetsbare, larvale fase naar de resistente adulte fase voor *M. leucophaeata* moest echter genuanceerd moest worden. Enkel de eerste, zeer jonge embryo's bleken kwetsbaar en een gradiënt in resistentie kan verwacht worden van de vroege levensstadia tot de adulte, benthische stadia. Zelfs de allereerste levensstadia - 4 uur oude embryo's - waren al opvallend resistent tegen abrupte veranderingen in temperatuur en saliniteit, wat erop wijst dat een volledig natuurlijke oplossing voor *M. leucophaeata* biofouling op dit moment nog niet realiseerbaar is.

Deze studie geeft een uitvoerig overzicht over de biologie van de invasieve brakwatermossel *M. leucophaeata* in de haven van Antwerpen. Het is de eerste studie van de autecology van de soort in Europese brakwaters en benadrukt ook de belangrijke rol van biologische kennis in de ontwikkeling van efficiënte controlemaatregelen tegen de biofouling van *M. leucophaeata*. Een efficiëntere, ecologisch verantwoorde oplossing waarbij het gebruik van biociden drastisch verminderd kan worden wordt voorgesteld. Dit onderzoeksprogramma bevat de eerste voorspellende studie in verband met biofoulingproblematiek, waarbij kennis over de initiërende biologische processen van biofouling werden gebruikt om nieuwe problemen te voorkomen.

CHAPTER I

General introduction, aims and thesis outline

INTRODUCTION

BIOFOULING = the malfunctioning of a technical installation because of interactions between natural, biological processes (e.g. settlement and growth) and the operation of the installation itself (Jenner et al., 1998).

Technical installations such as cooling water systems use water to cool down electrical processes in the industrial plant. The enormous amounts of water, needed in cooling water systems, make the use of treated (tap) water economically not feasible. Therefore a lot of companies have to rely on the use of untreated water from nearby seas or rivers. However, together with the cooling water numerous organisms get pumped up and are dispersed in the system. Artificial substrates that are present in the system provide a new habitat for settlement and growth of these invasive species. Thus, biofouling is a special case of colonization of hard surfaces by living organisms in the water (Bott, 1988).

1. THE PROCESS OF BIOFOULING

Any surface exposed to natural waters provides an opportunity for the settlement and subsequent growth of organisms; together with the cooling water, numerous organisms get pumped up and become dispersed in the system. The cooling water system provides an ideal habitat for a lot of these species, since the majority are sessile suspension feeders: they enter a system with a constant water flow which assures a continuous flow of food and oxygen, but without predators, since these are kept out by means of the screening system. Giving these perfect conditions, settlement will occur quickly and growth can be rapid. This growth will eventually interfere with the industrial processes, finally leading to their failure.

The peculiarity of this biofouling process is its two-stage character (Little and Wagner, 1997). Micro-organisms (bacteria, unicellular fungi, algae, protists) are the first to colonize immersed surfaces; this succession stage is referred to as microfouling. In a next stage, propagules of macro-organisms, spores of macroalgae and larvae of invertebrates settle on the hard surfaces, a process referred to as macrofouling. Succession of communities of micro- and macrofoulers develop with the participation of phenomenologically similar colonization processes (biofouling) but cannot be reduced only to them (Valiela, 1984; 1995). The different processes of biofouling of any natural and artificial substrate by any organism are: (1) transport, (2) settlement of larvae, (3) attachment, e.g. the maintenance of the settled

micro-organisms and larvae, (4) development and (5) growth (Railkin, 2004). These elementary processes replace each other sequentially during surface colonization by micro- and macrofoulers.

1.1. Microfouling

When a surface is submersed in natural water, adsorption of organic and inorganic substances and ions starts immediately; the surface gets coated with a film of proteins, polypeptides, polysaccharides and lipids. Saturation of the surface with these molecules and the development of a dynamic balance with the environment is established within hours (Baier, 1984; Wahl, 1989, 1997).

Microfouling communities are frequently referred to as biofilms (e.g. Bryers and Characklis, 1982; Hamilton, 1987) because of the fact that bacteria and diatoms are dominant in these communities (Avelin, 1997). Development of microfouling communities proceeds as a biological succession in which the preceding stage of succession is conductive to the onset of the following one (Connell and Slatyer, 1977). The primary process of bacterial colonization includes (Characklis, 1984): (1) transport of organic molecules and bacteria towards a submerged surface, (2) adsorption of organic molecules, as a result of which the surface becomes conditioned and more favorable for attachment of bacteria, (3) attachment of bacteria to the conditioned surface, (4) metabolism of attached microorganisms, as a result of which they adhere to the surface faster, (5) growth of bacteria and (6) partial detachment of the bacterial film.

Within hours, bacteria will have consolidated their presence and after fourteen days the bacterial film will have reached its maximal thickness, rarely exceeding a few hundred micrometers (Jenner et al., 1998).

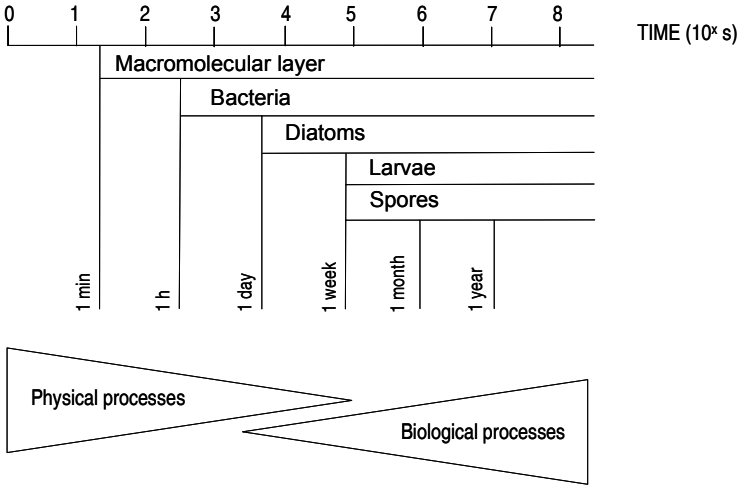

Fig. 1: Chronology of hard substrate colonization (modified from Wahl, 1989).

Bacterial colonization is of a mixed physical and biological nature, but when the settling of diatoms starts, the biological factors begin to prevail over the physical ones (Wahl, 1989) (**Fig. 1**). The presence of bacteria stimulates the development of diatoms (Gorbenko, 1977), thus facilitating the next phase of the succession. The secretion of sticky polysaccharides and the production of chemical elements by these primary residents can contribute to the corrosion of the surface. After this autotrophic phase, heterotrophic succession occurs with the settlement of protozoa and larvae of macroscopic species. If these larvae survive to grow on towards maturity, this will lead to macrofouling of the affected surface.

Microfouling does not only precede macrofouling chronologically, in many cases it also induces and stimulates macrofouling (Railkin, 2004), suggesting that macrofouling is an independent stage of autogenic succession.

1.2. Macrofouling

The microbial film is developed in a time span of several days to two or three weeks, but the macrofouling process develops much more slowly. At a first stage of macrofouling succession, the surface is colonized by fast-growing organisms, often colonial foulers like Hydrozoa, Bryozoa and Serpulidae (Polychaeta). This process takes between 2-3 weeks and 1-2 years.

The second stage is characterized by slow-growing organisms, e.g. mussels and sponges. This stage is often interrupted by physical factors and therefore lasts only for several years. The climax state, characterized by mussel dominance (**Fig. 2**), is achieved within a couple of years, which is much earlier than in terrestrial ecosystems (Connell and Slatyer, 1977).

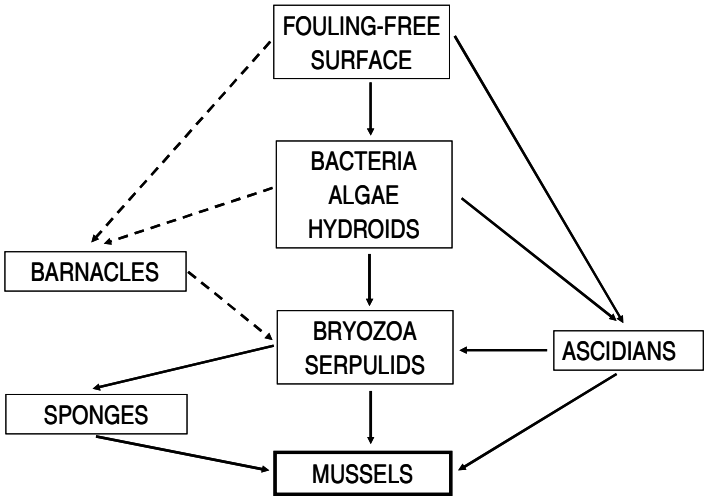

Fig. 2: Classical scheme of succession of a biofouling community. Dashed lines: collateral ways of succession, bold lines: climax communities (from Scheer, 1945).

However, under conditions of frequent disturbance, climax communities can be damaged or destroyed, throwing the succession process back to earlier stages, evolving again to the presence of higher fouling-organisms. This variation in final community structure gives some authors reason to doubt the existence of a climax community in macrofouling (Sutherland, 1974, 1984; Sutherland and Karlson, 1977). A dominance of bivalves, e.g. mussels and oysters, is only one of the possible final stages in an unstable habitat, such as coastal areas, shallow waters and estuaries. In more stable conditions, e.g. at greater depths, bivalves are generally replaced by algae, shaping the final community (Oshurkov, 1992; Connell and Slatyer, 1977).

The course of fouling development depends on the conditions under which biofouling takes place: (1) the geographical region and the location of the water, (2) the degree of physical stability of the environment, (3) the species assembly of the natural communities, (4) the season in which the substrate is submerged, (5) the duration of its submersion in the water and (6) the distance from the bottom (Railkin, 2004).

Jenner et al. (1998) observed that when larvae are ready to colonize a substrate, they do not necessarily need a biofilm for settlement: larvae of mussels and barnacles are capable of settling rapidly on surfaces free of microfouling. This strongly implies that elimination of microfouling is not a perfect tool in avoiding macrofouling.

2. COOLING WATER CIRCUITS

Water gets pumped up from the environment through a water intake system (**Fig. 3**). Since the water is needed as a cooling product, it is mostly withdrawn at relative depth. The tunnel which converts the water to the operational site normally consists of concrete and has a diameter of 2-3 meters. Trash racks, consisting of vertical sections with 10 cm wide openings, prevent drifting material from entering the system. A series of parallel culverts then lead to screen bays, with mesh size ranging between 1 to 10 mm, where fine materials and small animals are removed. However, a great diversity of fouling species, whose adults live attached to solid surfaces, disperse by means of planktonic larvae, which are much smaller than 1 mm.

Once the water is sieved, it enters the cooling system itself, consisting of a parallel series of heat exchanges. There are two types of heat exchangers - tube and plate heat exchangers - both serving to cool the industrial processes by heating the surrounding water. In the tube heat exchanger, the water passes through the medium to be cooled by a bundle of narrow parallel tubes, creating as such a laminar flow between both media. In a plate heat exchanger the water and the medium to be cooled pass each other while divided by a parallel series of closely adjoined plates, giving a much more complex turbulent flow increasing the efficiency of the heat transfer. Unfortunately, it is this specific set-up which increases the likelihood of fouling and the actual repair costs.

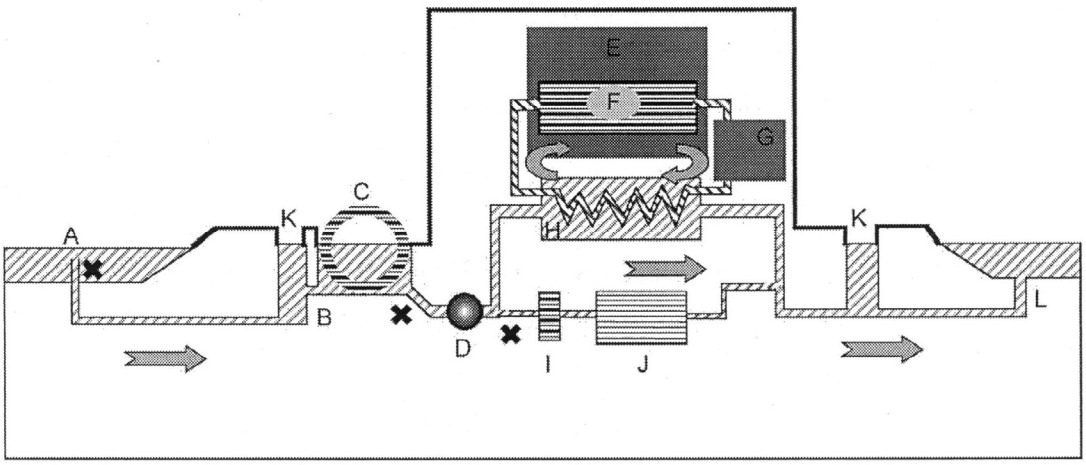

Fig. 3: Basic elements of a cooling water system of a direct cooled thermal power station. Arrows show direction of water flow in both open and closed circuits. A: offshore cooling water intake; B: coarse bar screens; C: 10 – 1 mm mesh size rotating drum screens; D: main cooling water pump; E: reactor; F: steam generators; G: turbines and electricity generators; H: steam condensers; I: 2 mm mesh size pressure strainers; J: auxiliary and emergency system oil coolers; K: intake and outfall surge shafts; L: offshore outfall; X: biocidal injection points (modified from Taylor, 2006).

There are two types of cooling systems: once-through and recirculating systems (**Fig. 4**). In an open recirculating system, the water comes in a recirculating cycle, transferring it between the power station's heat exchangers and a cooling tower. In the cooling tower, water temperature is lowered by evaporation. The cooled water is collected in a basin, from where it is pumped back to the heat exchangers. The heat loss has to be made up by adding natural water: the so-called 'make-up'. However, most cooling systems need such enormous amounts of water that they utilize a once-through system, where the water gets pumped up, passes through the heat exchangers and is discharged straight back to the environment. This last type limits the use of anti-fouling agents, leading to an increased biofouling risk in the installation. Also the environmental risk is greater in once-through

systems; leakages of process fluids are more problematic here, since the water is directly discharged again in the environment, while in a recirculating system contaminants are temporarily retained.

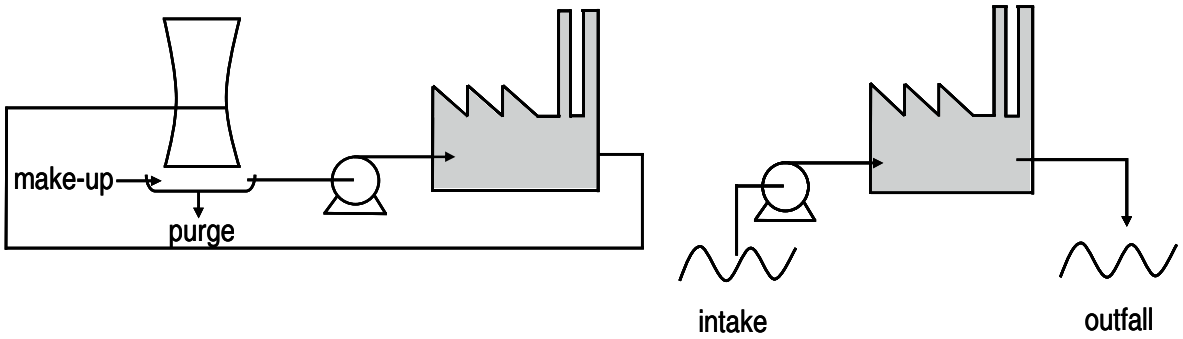

Fig. 4: Open recirculating system Once-through cooling water system .

In closed recirculating systems there is only a minimal loss of water, since there is no direct contact with the atmosphere; process heat is transferred into cooling water in one heat exchanger and in a second heat exchanger the cooling water is cooled by air or water. The cooled water is then returned to the heat exchanger that cools the industrial process. Compared to once-through and open recirculating systems, closed cooling water systems are ideal for fouling control, since little or no contamination of the water from outside takes place. In practice, however, this type of cooling water system is rather rare because of the high initial investment costs.

3. TYPES OF FOULING PROBLEMS

The main fouling types occurring in industrial cooling water systems are: corrosion, scaling, clogging and biofouling of the heat exchangers, conduits or cooling tower. The fouling potential is directly associated with the water source, which may contain particulate matter, debris, dissolved solids, micro- and macro-organisms.

3.1. Corrosion

Corrosion is an electrochemical process occurring on metal surfaces, where atoms are exposed to an electron acceptor, e.g. O_2, with a higher affinity than the potential donor. The result is a metal oxide or other salt, having little structural ability, which causes damage to the material. The presence of a biofilm on the metal surface often improves conditions for corrosion, as such accelerating the process (Bazanth, 1979). Mainly sulfate reducing bacteria are known to cause this kind of microbially induced corrosion.

Corrosion can only be prevented by keeping the metal surface of the heat exchangers clean, by the application of corrosion inhibitors or by using high integrity metals such as titanium. The process of corrosion can occur in both once-through and recirculating systems.

3.2. Scaling

Evaporation loss from the cooling water of the system concentrates inorganic and organic materials. If the concentration of salts in the water film exceeds their solubility limits, precipitation of particularly calcium carbonate and calcium phosphate occurs, resulting in a crust on the cooling water side of heat exchangers, referred to as scaling. Scaling reduces the performance of the heat exchanger and can also cause package problems in the cooling tower (Jenner et al., 1998) by increasing its weight, resulting in release of the package material.

Scale formation can be controlled by adjusting the pH-value of the incoming water, which can be achieved by dosing acid and by the use of scaling inhibitors (Bonné et al., 2000). This process can play a role in both once-through and open recirculating systems.

3.3. Biofouling and clogging

Together with the cooling water, a wide range of organisms is entrained into the cooling water system. These organisms can readily colonize the available substrates such as concrete, metal, wood and plastic surfaces in the heat exchangers, cooling water conduits and cooling tower.

Growth conditions in the cooling water system are ideal for sessile organisms: the steady water flow assures an abundance of nutrients and oxygen, while access for predators is limited. As a consequence of this undesired biological growth, (1) restriction in water flow, (2) blockage of the heat exchangers, (3) increased rate of corrosion and (4) loss of heat transfer may occur. All these have negative environmental and economical consequences.

4. BIOFOULING CONTROL

Several types of antifouling measures have been used in cooling water conduits. However, their developmental status is very different; some techniques are used widespread, while others (1) have proven low efficiency, (2) are only applicable in smaller systems or (3) are still in an experimental phase. An overview of most used physical and chemical antifouling techniques are given in **Table I** (Jenner et al., 1998). Hereafter, only the main chemical techniques that are applied at the moment will be discussed, i.e. chlorination, heat treatment and surface coatings, since it was the use of these products that gave rise to the research questions of this PhD. An important remark is that the success rate of all these techniques is very species-specific; populations of different or even identical species from different geographical locations can have higher or lower threshold levels for survival (Graham et al., 1975, Rajagopal et al., 2005b).

4.1. Chlorination

The antifouling procedure most favored by operators near north-western European coasts and estuaries is continuous low-level or intermittent chlorination with hypochlorite (Jenner et al., 1998; BREF, 2000; Rajagopal, 2003). Chlorination is also considered as Best Available Technique (BAT) in industrial cooling water systems within the cooling requirements of the industrial process (IPPC, 2000). Continuous chlorination involves adding a continuous low dose of oxidants to the cooling water, sufficient to restrict settlement and growth of fouling organisms through chronic toxicity but without an acute impact on the cooling circuits themselves (Taylor, 2006). However, because of the pH of brackish water or seawater, a large part of the used oxidants has only limited efficiency in controlling the organisms and a large amount needs to be dosed to reach the needed mortal toxicity level in the water. In intermittent chlorination, chlorine is dosed periodically at higher doses, killing organisms depending on the dose and contact time (Rajagopal et al., 2002a, b). Important criteria involved in choosing between continuous or intermittent chlorination are costs and environmental discharge specifications (Mattice and Zittel, 1976).

Concerns have risen about the likely release of chlorination byproducts (CBPs) in the effluent stream, such as organohalogens, chlorobromoform and phenols, and their possible damage to the aquatic life in the natural environment. However, it needs to be emphasized that the presence of organohalogenated compounds in surface waters is not solely due to the chlorination of cooling systems, also particular mention should be made of agriculture and natural production (IPPC, 2000). Scientific literature on this

topic only provides a limited understanding in the chemical dynamics of the effluent stream, leaving room for speculation (Taylor, 2006). An important disadvantage of chlorination is also the species-dependent toxicity level; some species, even from the same phylum, are far more resistant to chlorination than others. The non-specific toxicity of hypochlorite, on the other hand, makes it impossible to kill the combatable species without affecting other, non-fouling species. Chlorination efficiency is also temperature-dependent; if the water temperature is low (< 15°C), the time required for effective chlorination will be prolonged (Jenner and Janssen-Mommen, 1993).

4.2. Heat treatment

Heat treatment is accomplished by recirculating a part or the whole of the water discharge through the intake pipes, in order to raise the temperature of the water to the required lethal levels. This maximum temperature is maintained in the circuit for a short time, killing all macrofouling biota by the heat shock. Although heat treatment is a highly efficient way of eliminating macrofouling, it leads to a loss in production because of insufficient cooling of the installation (Harrington et al., 1997). Therefore, heat treatment is used only occasionally, depending on the breeding season of important fouling organisms at a given location (Rajagopal et al., 2003).

A disadvantage is that this treatment is restricted to few power plants; a special design is required for the cooling water system at an early stage of plant building. Adaptations afterwards are often expensive and technically difficult (Jenner and Janssen-Mommen, 1993).

4.3. Surface coatings

Rather than adding a toxic substance to the water, it would be far more valuable to treat the surfaces on which biofouling develops, thus preventing the fouling problem from happening. Paints or coatings can be applied at different locations in the cooling water system, but these provide the same problem as heat treatment; in a complex cooling circuit, it is impossible to treat the whole system with a coating. In new cooling water systems however, this technique can be applied on all the tubes and conduits.

In the past, the use of toxic paints was commonly used to control mussel fouling (Jenner et al., 1998). Foul-release or easy-release coatings are some of the major alternatives to heavy-metal based antifouling coatings. The mechanisms of these types of coatings are generally assumed to be related to physical surface properties, which interfere with the adhesion of marine organisms (Clare, 1998). These different surface properties can prevent adhesion or may reduce the adhesion strength of

the fouling organisms (Callow and Fletcher, 1994; Swain and Schultz, 1996), facilitating easy removal from the surface (Brady and Singer, 2000). Fouling organisms thus can settle and grow on the surfaces of these coatings, but adhere poorly and can be removed by light brushing, water sprays or by hydrodynamic self-cleaning (Afsar et al., 2003).

Table I: Overview of the most common physical and chemical antifouling techniques in European power stations (adapted from Jenner et al., 1998).

Processes and treatments	Methods		Targets	Use in European power stations
Water filtration	Gross filtration (1–10 cm)	Grids at water intake, mostly with trash racks	Removes drifting debris at water intakes	Everywhere
	Fine filtration (1–10 mm)	Rotating, band or drum screen bays at water intake, debris sieves to protect heat exchangers, removable grids with screens	Removes drifting debris at water intakes; Stops biological debris	Everywhere
Mechanical cleaning	Manual	Dry or underwater cleaning, often high pressure of pipes, basins, screens and heat exchangers	Removes settled macrofouling; Eliminates slime on condensers or plate heat exchangers	Everywhere
	Automatic	High-pressure cleaning of rotating screen bays	Removes drifting debris from the screens	Everywhere
		Continuous cleaning of condenser tubes by sponge balls	Removes bacterial slime (and some scale)	Widely
		Self-cleaning debris filters	Removes biological debris produced by macrofouling	Widely
		Cleaning plate heat exchangers by vacuum suction processes	Removes biological debris and mineral deposits	Sometimes
Other physical methods	High water velocity	Increasing water velocity above critical values	Avoids settlement of macrofoulers	Everywhere
	Heat treatment	Increasing water temperature above lethal levels	Removes settlement of macrofoulers	Some marine and freshwater stations
Chemical treatments	Low toxicity surface coatings	Applied on parts of the circuit with lower water velocity	Avoids settlement of macrofoulers	Used increasingly
	Chlorination	Continuous low-level treatment (generally < 1 mg/l)	Restricts macrofouling and bacterial slime; Eliminates Bryozoa	Widely
		Intermittent treatment at higher doses (8-40 mg/l for 4-6 h)	Restricts macrofouling; Eliminates blue algae and Bryozoa	Sometimes

5. LEGAL FRAMEWORK ON BIOCIDE CONTROL

5.1. Europe

The general trend at the moment is towards a tougher legislation to better protect public health and the environment from the overuse and misuse of biocides and pesticides. The European Union is making efforts to harmonize the rules, setting common standards for the various Member States and making sure that a product that is banned in one country cannot be freely bought in another. This harmonization process on biocides began recently because of the major differences between EU countries.

In 1998, an EU Directive (Directive 98/8/EC) was adopted on the placing of biocidal products on the market. This Directive covers the authorization and marketing of active substances and biocides, advertisement, use, classification, sale, monitoring, etc.. The scope of this Directive is very wide, covering 23 different product types. These include disinfectants used in different areas, chemicals used for preservation of products and materials, non-agricultural pesticides and anti-fouling products. The specific applications of this piece of legislation are being put into place quite slowly, both at a European level and in the Member States. The Member States first gathered information on the active substances and products authorized in the various countries and then started examining the dossiers.

In 2000, the Water Framework Directive (Directive 2000/60/EC) was formulated, being the most substantial piece of water legislation ever produced by the European Commission. The Directive will provide the major driver for achieving sustainable management of water in all the Member States for many years to come. It requires that all natural inland and coastal water bodies within defined river basin districts must reach at least good status by 2015, and defines how this should be achieved through the establishment of environmental objectives and ecological targets for surface waters. For strongly fluctuating and artificial water bodies, a good ecological status and potential has to be defined, taking into account economical functions such as navigation, as being the case for the harbour of Antwerp. The final result should be a healthy water environment achieved by taking due account of environmental, economic and social considerations. This Directive will clearly have future impacts on biocide restriction. A proposal for a Directive on environmental quality standards in the field of water policy (being an amendment of Addendum X of the Water Framework Directive), with a list containing environmental quality standards for priority chemical substances and certain other

pollutants, has only just been dispersed and although it needs to be implemented by 2007, it will probably be delayed because of international disagreements (S. Lammens, VMM, pers. comm.).

In 1996, the EU Directive on integrated pollution prevention and control (IPPC) (Directive 96/61/EC) was adopted, demanding that all annex-I companies (companies considered to be a nuisance according to VLAREM I) would act according to permit conditions based on Best Available Techniques (BAT) at least by 2007. In essence, the IPPC Directive is about minimizing pollution from various industrial sources throughout the European Union. In 2000, the IPPC published the Reference Document on the application of Best Available Techniques to Industrial Cooling Systems, considering the environmental performance of the cooling system in the context of the overall environmental performance of an industrial process. In this document, optimization of the application of oxidizing biocides in once-through systems is based on timing and frequency of biocide dosing. It is considered BAT to reduce the input of biocides by targeted dosing in combination with monitoring of the behavior of macrofouling species (e.g. valve movement of mussels) and using the residence time of the cooling water in the system. For cooling water systems where different cooling streams are mixed in the outlet, pulse-alternating chlorination is BAT (IPPC, 2000).

5.2. Belgium

No biocide may be placed on the market without prior authorisation from the Federal Ministry of Environment, with the assent of the High Council for Hygiene. According to Belgian legislation, "biocides" are defined as "active substances and preparations containing one or more active substances that are presented in the form in which they are delivered to the user, intended to destroy, deter or control harmful organisms by chemical or biological means" (see Royal Decree of 22/05/2003 Article 1 (1)). The specific legislation concerning power stations and the use of biocides is part of the Flemish government.

Over the past few years, the Belgian authorities, in tandem with the European Union, have been working to tighten up the regulations for placing these products on the market. To this end, the Risk Control Service is working to make a better assessment of the active substances contained in biocidal products. The federal government is also increasingly seeking to promote the proper use of these products through information campaigns and awareness-raising programs aimed at consumers, farmers and industry. The EU Directive was implemented in Belgium by means of the Royal Decree of 22 May 2003. The first dossiers on active substances were examined in 2004 and

at the end of the year there were some 450 biocides, based on approximately 100 active substances, on the Belgian market.

5.3. Flanders

In Flemish legislation, cooling water is defined as "water used in the industry for cooling down but which has not been in contact with the products that had to be cooled, neither with other polluting products" (VLAREM I, Art. 1.11).

For all surface waters, a basic quality norm for absolute chloride below 200 mg/l is maintained since July 1st 1995. Free chlorine has a maximum tolerable level of 0.004 mg/l (VLAREM II, Annex 2.3.1.).

The criteria concerning chemical substances in cooling water discharges into surface waters are clear; without specific permit the cooling water may not contain chemicals belonging to the groups defined in VLAREM I, Addendum 5.3.2., neither other chemicals in concentrations damaging - directly or indirectly - for human health, flora and fauna (VLAREM II, Art. 4.2.4.1). If a quantity of more than 100 m^3 per hour is discharged, a control-installation has to be present to control the quality and quantity of the discharged cooling water and to permit easy sampling of the discharged water (VLAREM II, Art. 4.2.5.1.2).

6. MACROFOULING ORGANISMS IN EUROPEAN WATERS

Most brackish water fouling species also have substitutes in fresh and seawater. An overview of the main fouling species in these European waters is given in **Table II**. An important remark is the alien nature of many fouling species. Harbours are excellent arrival and operating bases for alien species. Much of the harbours are directly surrounded by industrial activities, which may use the natural water in one of their processes, e.g. as cooling water, as such being the first human activities hampered by these invasive species.

6.1. Barnacles

Although often present in large densities in cooling water systems, barnacles are less of a problem than mussels. In theory, barnacles can settle and develop on any surface exposed to water, but in practice they prefer the rough concrete walls of the cooling conduits above the delicate structures of the

heat exchangers (Barnes, 1971). Since they are rather small (maximum 25 – 30 mm high), settled on enormous concrete structures, their impact on water flow is negligible.

Most barnacles are stenohaline, with only *Elminius modestus* (Darwin) found in a broader salinity range in estuaries and ports. *Balanus improvisus* (Darwin), the only brackish water barnacle, has a wide distribution throughout Europe and East America (Furman and Yule, 1991).

Barnacles are hermaphrodite, with internal fertilization. Eggs are laid inside the external plates and hatch into free-swimming larvae, easily dispersed inside the cooling water system. After several moults the larva moves onto a hard substrate, fixing itself by head and antennae.

6.2. Hydroids

Cordylophora caspia (Pallas), a colonial hydroid, is the main hydroid species causing fouling problems (Folino, 2000; Smith et al., 2002). It inhabits brackish and freshwater and colonizes all sorts of hard substrates. Colonies consist of polyps specialized for feeding (gastrozooids) or reproduction (gonozooids) and grow rapidly via asexual budding, most prolific during spring and summer months (Roos, 1979; Jormalainen et al., 1994).

Cordylophora caspia colonizes intake pipes and chambers in Europe and North America (Jenner and Janssen-Mommen, 1993; Khalanski, 1997). Although rather easily removed by the use of biocides, *C. caspia* poses another important problem in fresh waters; in an interaction between the hydroid and *Dreissena polymorpha* (Pallas), the filamentous structure of *C. caspia* enhances zebra mussel settlement by increasing the surface area available for settlement (Folino-Rorem et al., 2004).

6.3. Tube worms

One of the most important tube worms causing fouling is *Ficopomatus enigmaticus* (Fauvel), an Australian reef-building tubeworm that has been introduced to many estuaries in Europe and America (Cohen et al., 1995). It is a euryhaline species, living in salinities between 1 – 35 PSU (Hartmann-Schroeder, 1967). Settlement takes place on most substrates, but only when water flow is rather high, since the species is unable to withstand deposition of mud. The larvae settle and a tube length of 30 mm can be reached in two weeks (Ten Hove and Van den Hurk, 1993).

Although tube worms are easily killed by biocides, their calciferous tubes stay attached and the old encrustations will serve as substrates for new ones or for the attachment of mussels and hydroids.

6.4. Mussels

Of all organisms causing fouling problems in cooling systems, mussels are known to cause the most serious problems (Rajagopal et al., 1996). Uncontrolled growth of mussels in the precondensor regions of the cooling water system can disrupt normal operation of a power plant, irrespective of its geographical location (Claudi and Mackie, 1994). Three common fouling species can be distinguished, depending on their habitat: (1) the Blue Mussel, *Mytilus edulis* L., is a temperate marine species with a widespread distribution in both the northern and southern hemisphere, (2) the Zebra Mussel, *Dreissena polymorpha* (Pallas), is a temperate to subtropical freshwater species and possibly the most famous fouler, with an extremely rapid distribution throughout the Great Lakes in North-America and (3) the Dark False Mussel or Brackish Water Mussel, *Mytilopsis leucophaeata* (Conrad), a brackish water mussel with an extended tolerance to biocides.

The reason why these three species are efficient foulers has to be searched for in their life cycle (**Fig. 5**). After external fertilization, free-swimming larvae develop in the water column. Their planktonic nature is a perfect feature for rapid dispersal along different water bodies; larvae get spread by the water currents, until they settle. Since these stages are very small (< 500 µm), almost no physical man-made barriers exist. The planktonic stages are characterized by a weak shell, having little features to protect themselves from the outside world. However, after settling, the adult mussels develop a hard, protective shell. In a first stage, settlement happens on filamentous structures, but later in the year, these primary settlement sites are traded for permanent sites, which are usually hard substrates. Unless settlement is quickly identified, it is possible for this 'spat' to grow unnoticed to a size where they may interfere with the functioning of some parts of the cooling water installation. Adult mussels can shut their valves and stop byssus production to isolate their bodies from changes in the external environment (Khalanski and Bordet, 1981), such as biocide-passage. As a result, once established inside a cooling water system, they are very difficult to remove.

The biology of the target species of this PhD-research, *M. leucophaeata,* will be more thoroughly discussed in Chapter 2.

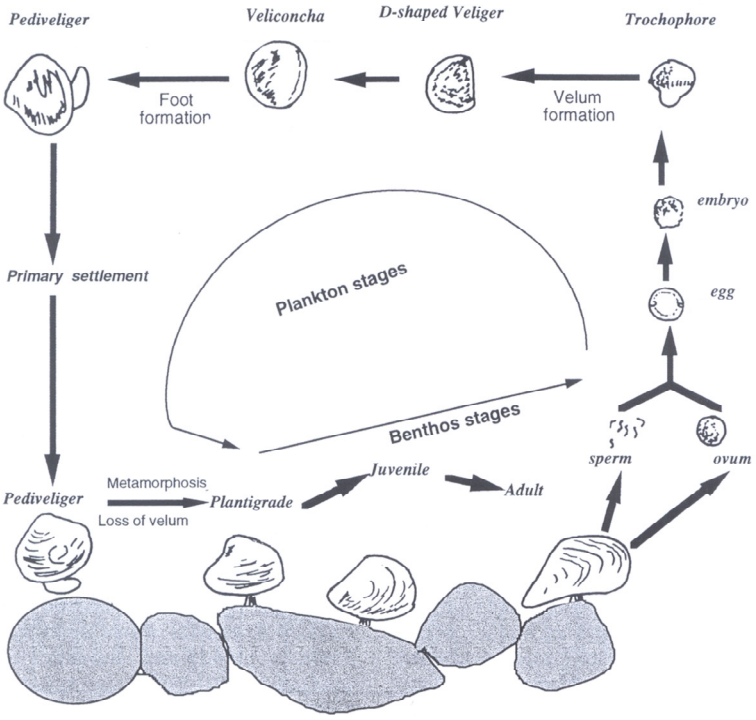

Fig. 5: Schematic lifecycle of mussels (from Ackerman et al., 1994).

Table II: Main biofouling species in European waters. * = alien species in European waters

Organism	Brackish water	Seawater	Freshwater
Barnacles	*Balanus improvisus* (Darwin)	*Balanus crenatus* (Bruguière) *Chthalamus stellatus* (Poli) *Semibalanus balanoides* (Linneaus) *Elminius modestus** (Darwin)	
Hydroids	*Cordylophora caspia* (Pallas)		
Tube worms	*Ficopomatus enigmaticus** (Fauvel)	*Ficopomatus enigmaticus** (Fauvel) *Pomatocerus triqueter* (Linneaus) *Serpula vermicularis* (Linneaus)	
Mussels	*Mytilopsis leucophaeata* * (Conrad)	*Mytilus edulis* L. *Mytilus galloprovincialis* (Lamarck) *Modiolus modiolus* (Linneaus) *Modiolus barbatus* (Linneaus)	*Dreissena polymorpha* (Pallas) *Dreissena bugensis* (Andrusov)
Oysters	*Crassostrea gigas* *(Thunberg)	*Crassostrea gigas* *(Thunberg) *Ostrea edulis* (Linneaus)	
Clams	*Rangia cuneata* *(Sowerby)		*Corbicula fluminea* (Muller)

7. STUDY SITE

All field work was conducted at the site of BASF, Antwerpen N.V. (Belgium), along the Schelde river. The industrial site is situated at the right bank in the harbour of Antwerp, near the Dutch border (**Figs. 6 - 7**), and receives water of intermediate salinity (1 – 12 PSU) coming from the Westerschelde river and from the Rijn-Schelde channel.

Fig. 6: Aerial overview of the plant at BASF, Antwerpen N.V. from south-east to north-west direction.

BASF, Antwerpen N.V. is the biggest chemical production center in Belgium and a daughter concern of the BASF-group, which belongs to the top five of chemical world concerns, with sites in Europe, North- and South-America, Central and Southeast-Asia. The production at Antwerp mainly consists of mineral fertilizers, complex and simple synthetics and their pre-products, synthetic fibers, basic chemicals and refinement products. Cooling water gets pumped up from canal dock B3 (**Fig. 8**), which is connected to the Schelde river by two large sluices, the Zandvliet and the Berendrecht Sluice, and which follows a once-through system. The dock is part of the large port of Antwerp, located at the Belgian-Dutch border and is approximately 1.5 km long and 0.35 km wide. Water is pumped into the cooling water system at two intake points, at a distance of one kilometer from each other.

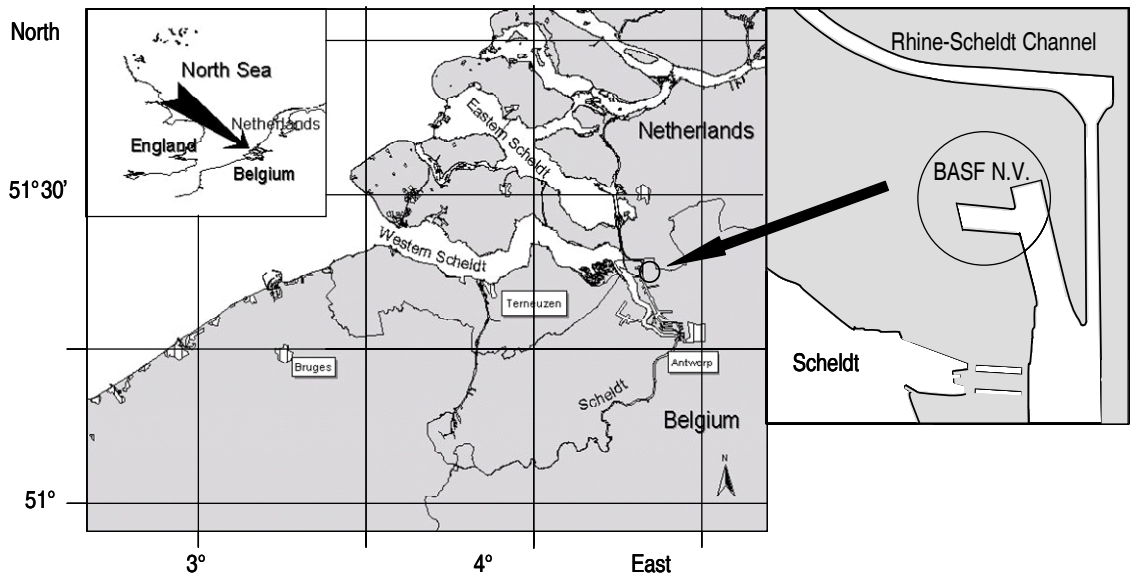

Fig. 7: Map of Westerschelde river with study site at BASF, Antwerpen N.V.

The topside of the northern inlet (I2) is located 3 m under the water surface; the inlet in the western part (I1) has topside at 3.65 m under the water surface. There are two major release points with one outlet at the southern area of dock B3 and one located in the Rijn-Schelde channel, both at relatively large distance from the inlets. The southern outlet (O1) with a width of 13.50 m is located from the water surface until 1.80 m under the water surface; the northern outlet (O2) has a width of 15.94 m and is located at a height of 0.85 until 3.05 m under the surface. The southern outlet is situated at B3 where the total water depth is 15.25 m, the northern outlet discharges in the relative shallow Schelde-Rijn channel with a depth of 5 m. During summer, the cooling water conduits of the site take in up to 80000 m^3 of 1mm-filtered, but untreated river water per hour and in wintertime, an average incoming flow of 30000 m^3 of water per hour occurs.

Most severe biofouling problems occur in the first part of the intake pipes and in the cooling towers. Especially places with reduced water flow and the heat exchangers are extremely vulnerable. However, *M. leucophaeata* is found throughout the entire cooling water installation of BASF, Antwerpen N.V. (Ooms, pers comm.).

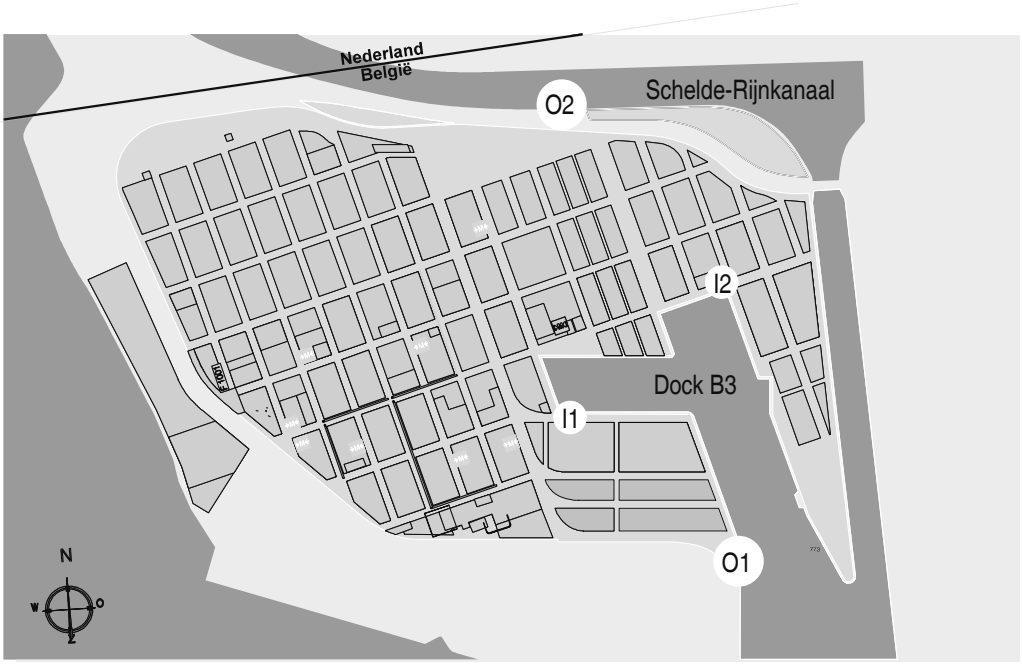

Figure 8: Inlet and outlet locations of the cooling water discharge. I1 = inlet 1, west part of dock B3; I2 = inlet 2, north of dock B3; O1 = outlet 1, south of dock B3; O2 = outlet 2, in Rijn-Schelde channel.

AIMS

The cooling water systems of several North-West European industrial plants are confronted with the problem of biofouling by the brackish water mussel *Mytilopsis leucophaeata*. Larvae entered the system together with the extracted water, where they could attach onto substrates, such as the heat exchangers and the tubes in the conduits. Until now, the general problem of biofouling is taken on by the use of biocide-dosage, mostly by chlorination. Although the use of biocides stays well below the criteria defined by (inter)national legislation, the search for other, more specific and environment-friendly control measures is ongoing. A case study on the biology of the invasive bivalve *M. leucophaeata* was used as a tool for its ecologically sound biofouling control.

This PhD thesis aimed at (1) the provision of an advice for an efficient and rational use of currently used biocides to control biofouling by *M. leucophaeata*, in order to confine the damage of these toxic chemicals to the surrounding environment and to the cooling water system itself and (2) investigating - on a lab scale - the possibility of converting from biocides to more biological solutions in the battle against biofouling by *M. leucophaeata.* However, in order to develop an ecologically and economically proper use of antifouling chemicals, biological knowledge of the combatable species is an indispensable tool (Hillman, 1977; Relini, 1984), with the life cycle of mussels being the perfect proof. More and more industries and technologies start with the implementation of biological research in their control programs in order to obtain a more efficient use of biocides in search for the most adequate battle against biofouling (Wianco and McKenna, 2002; Peterson and Suddard, 2002; Ng, 2004). As Khalanski (1997) mentioned, when mussel-fouling is a problem, monitoring is absolutely indispensable.

Therefore, the ecology of the brackish water mussel *Mytilopsis leucophaeata* in the harbour of Antwerp was used as a tool in search for better, ecologically sound solutions against its biofouling. In the first part of the thesis, basic biological processes of *M. leucophaeata* were studied to get a better insight in the ecological behaviour of this rather unknown species. These aims were extended by comparing the biology of *M. leucophaeata* with that of the closely related freshwater zebra mussel *Dreissena polymorpha*. As such, biofouling and invasion capacities of *M. leucophaeata* could be compared with this worldwide fouler in order to determine the level of possible future problems with the species.

In the second part of the thesis, this biological knowledge was used to search for alternative solutions against *M. leucophaeata* biofouling. The motivation of including biological knowledge in the search for a more optimal biofouling control of *M. leucophaeata* was two folded:

(1) Economically

By monitoring of the incoming cooling water, the presence of the vulnerable mussel larvae in the water column could be monitored. The use of biocides could then be reduced to this life stage in order to avoid new biofouling by *M. leucophaeata*. Smaller amounts of biocides would be needed but would still be as effective, leading to a financial saving.

(2) Ecologically

Chlorination is worldwide the most commonly used biocide and considered as Best Available Technology in cooling water systems (IPPC, 2000). The pH of the brackish water (6-8) however makes the greatest part of the used NaOCl little effective and leads to the formation of damaging chlorination by-products (CBPs) (Jenner, 1985). The reduction of biocide usage, by means of biological monitoring, could finally lead to a reduction of the amount of harmful chemicals in the water. The financial saving on the other hand could trigger the search for and development of potentially less harmful but more expensive chemicals in order to obtain a solution as effective as chlorination. The possible switch to more biological solutions, although still on small-scale laboratory experiments, can eventually make the use of biocides redundant.

THESIS OUTLINE

The thesis is divided in two main parts. In the first part, the biology of *Mytilopsis leucophaeata* is thoroughly studied (Chapter II, III, IV and V) while in the second part, this information is used in search for a tool for an ecologically sound biofouling control by *M. leucophaeata* (Chapter VI and VII). The general discussion, conclusions and future outlook are presented in Chapter VIII.

Several parts of this thesis have already been published in the international literature and the remaining data have been submitted for publication. Therefore, the outline and output of the chapters is exactly like the published papers, unless otherwise mentioned (Chapter III). Each chapter is as such intended to be an autonomous part, which can be read separately from the other chapters. Inevitably, there will be overlap between the introductions, the sections on the study site and material and methods of the different chapters of the thesis. **CITED LITERATURE** however is generalized and listed at the end of the thesis. The accepted manuscript concerning the first European record of the invasive brackish water clam *Rangia cuneata* (G.B. Sowerby I, 1831) (Mollusca: Bivalvia) is given in **APPENDIX I**. The author's publication list is given in **APPENDIX II**.

In **CHAPTER II**, the currently available literature on *Mytilopsis leucophaeata* was summarized. The systematic classification, evolution, ecology and biogeographical expansion of *M. leucophaeata* in European waters was discussed and invasion and biofouling capacities of the species was extracted from this. Because of the biological resemblance with the zebra mussel, *Dreissena polymorpha*, being the most well-known biofouler worldwide in industrial installations, and the fact that both species have overlapping habitat tolerances, both species were compared whenever possible. The chapter has been accepted as VERWEEN A, VINCX M, DEGRAER S (IN PRESS) *Mytilopsis leucophaeata*: the brackish water equivalent of *Dreissena polymorpha*? In: Van der Velde, G, Rajagopal, S, Bij de Vaate, A (eds) Zebra Mussels in Europe. Backhuys Publishers, The Netherlands.

In **CHAPTER III** the recruitment patterns of *Mytilopsis leucophaeata* in the harbour of Antwerp were investigated. This research aimed to investigate and describe annual and seasonal variations in D-shaped larvae and settlement of *M. leucophaeata* in relation to the abiotic environment. As such, a first extension of biological information concerning the species was accomplished and implications for ecologically and economically sound biofouling control were deduced. Part of this chapter has been

published as 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.

CHAPTER IV described detailed the strong seasonal development of the reproductive cycle of *Mytilopsis leucophaeata,* studied by means of histological preparations of the gonads. In addition, we investigated the influence of environmental variables such as temperature, salinity and food availability on the process of gametogenesis to determine their effect on timing and progress of the gametogenic cycle. This chapter has been submitted as: VERWEEN A, VINCX M, DEGRAER S (SUBMITTED) Seasonal variation in gametogenesis and spawning of *Mytilopsis leucophaeata*, an invasive bivalve in Europe. *Journal of Molluscan Studies*.

In **CHAPTER V**, growth of *Mytilopsis leucophaeata* was investigated in different size classes and the effects of prevailing abiotic variables (temperature, salinity, oxygen and chlorophyll a content) and mussel length on this process was evaluated. Average hypothetical shell growth of *M. leucophaeata* in the harbour of Antwerp was modeled by von Bertalanffy growth curves and the species' longevity was estimated. This chapter has been published as: VERWEEN A, VINCX M, DEGRAER S (2006) Growth patterns of *Mytilopsis leucophaeata*, an invasive biofouling bivalve in Europe. *Biofouling* 22 (4): 221 – 231.

The aim of **CHAPTER VI** was to develop a statistical model to predict larval response of *Mytilopsis leucophaeata* larvae to environmental conditions in estuarine ecosystems. Multiple logistic regression, taking into account temporal autocorrelation, was applied on a large dataset allowing us to predict the probability of occurrence of *M. leucophaeata* larvae at BASF Antwerpen N.V. as a response to the environmental variables. As such, a tool was searched for, resulting in an efficient and rational use of biocides to control biofouling by *M. leucophaeata.* The chapter has been accepted for publication as: VERWEEN, A, HENDRICKX, F, VINCX, M AND DEGRAER, S (IN PRESS) Larval presence prediction through logistic regression: An early warning system against *Mytilopsis leucophaeata* biofouling. *Biofouling.*

CHAPTER VII aimed at determining the limits of survival of *Mytilopsis leucophaeata* larvae, in order to define a possible range where the species can possibly induce fouling problems in the future and, if possible from a technological point of view, to develop as such a more biologically sound method against biofouling by *M. leucophaeata.* The vulnerability of different larval life stages of *M. leucophaeata*

to temperature and salinity were studied during standardized acute 48-h experimental tests and the survival limits of the most vulnerable larval life stage were determined at different temperature–salinity combinations. This chapter has been submitted for publication as: VERWEEN, A, VINCX, M AND DEGRAER, S (SUBMITTED) The effect of temperature and salinity on the survival of *Mytilopsis leucophaeata* larvae: The search for environmental limits. *Journal of Experimental Marine Biology & Ecology.*

In the general discussion "New insights in biofouling control of *Mytilopsis leucophaeata*" (**CHAPTER VIII**), key issues and considerations concerning invasion capacities, biofouling capacities and possible biofouling control of *Mytilopsis leucophaeata* are deduced from the main results of the different chapters. Future perspectives for further research in the field of biofouling control and invasion patterns of *M. leucophaeata* are provided and the need for invasive species awareness is emphasized.

CHAPTER II

Mytilopsis leucophaeata: The brackish water equivalent of *Dreissena polymorpha*?

A review

Paper accepted as:

VERWEEN A, VINCX M, DEGRAER S (IN PRESS)

Mytilopsis leucophaeata: the brackish water equivalent of *Dreissena polymorpha*?

In: Van der Velde, G, Rajagopal, S, Bij de Vaate, A (Eds)

Zebra Mussels in Europe.

Backhuys Publishers, The Netherlands.

ABSTRACT

European brackish waters have recently been invaded by the brackish water mussel, *Mytilopsis leucophaeata*. Although the genus *Mytilopsis* originated from Europe more than 60 million years ago, it disappeared out of Europe after its expansion to Central America. *Mytilopsis leucophaeata* now has its natural habitat along the southern coast of the U.S. and ranges from Tampico, Mexico to the Hudson River estuary. In the early 19th century, *M. leucophaeata* re-invaded Europe with a first record in the harbour of Antwerp, Belgium, but it is only when the species became a biofouling problem in the 1990s that attention was brought back to this relatively unknown species. The systematic classification, evolution, ecology and biogeographical expansion of *M. leucophaeata* in European waters are discussed. Because of the morphological resemblance with the freshwater zebra mussel, *Dreissena polymorpha*, and the fact that both species have overlapping habitat tolerances, both species were compared whenever possible and a clear identification guide is proposed. Although invasion by *M. leucophaeata* in Europe seems rather slow, its fouling problems are even more severe than those of *D. polymorpha*, underpinning the statement that *M. leucophaeata* is becoming the brackish water equivalent of *D. polymorpha* in Europe. Expansion along European brackish waters is still taking place and speeding up, especially by means of ballast waters and hull fouling of ships, with very recent discoveries in the Black Sea, the Guadalquivir in Spain and the Baltic Sea in Finland.

KEY WORDS

Mytilopsis leucophaeata, systematics, morphology, ecology, biogeographical expansion, Europe, *Dreissena polymorpha*

INTRODUCTION

The brackish water mussel *Mytilopsis leucophaeata* (Conrad, 1831), also known as Conrad's false mussel (Mondadori, 1980) or the dark false mussel, is a mytiliform bivalve, which originates from the U.S. and was first detected in European waters in 1835 in the harbour of Antwerp (Belgium). *Mytilopsis leucophaeata* is a typical estuarine species, and thus resistant to a wide range of oligo- to mesohaline conditions (Siddall, 1980).

In the 1990s, *M. leucophaeata* was detected as a robust fouling species in industrial cooling water systems along estuarine rivers, and as such became an economic problem. This fact, and the apparent rapid spread of *Mytilopsis* throughout Europe brought the attention back to this relatively unknown species.

In this paper, current information on the biology of *M. leucophaeata* is reviewed and because of its resemblance to the much better known *Dreissena polymorpha* (Pallas, 1771), comparison with this freshwater zebra mussel is considered in all aspects of this review.

To maximize the accessibility of the review, this paper can be approached from different angles in point of view. Readers interested in baseline information on *M. leucophaeata* on e.g. biogeography, developmental biology or identification will find this information in the different sections, which provide an overview of existing knowledge. Readers interested in new insights (on biofouling) on *M. leucophaeata* resulting from this literature review are redirected to the last, concluding section, in which an overview of the lessons learned is provided.

SYSTEMATIC CLASSIFICATION AND EVOLUTION

The genus *Mytilopsis* (Bivalvia, Veneroida, Dreissenidae) was generally considered to include nine species: *Mytilopsis adamsi* (Morrison, 1946); *M. africana* (Van Beneden, 1835); *M. allyneana* (Hertlein and Hanna, 1949); *M. cochleata* (Kickx, 1835); *M. domingensis* (Récluz, 1852); *M. leucophaeata* (Conrad, 1831); *M. sallei* (Récluz, 1849); *M. trautwineana* (Tryon, 1866) and *M. zeteki* (Hertlein and Hanna, 1949) (**Fig. 1**). However, Marelli and Gray (1983) concluded that *M. cochleata* and *M. leucophaeata* are synonyms. All members of the genus *Mytilopsis* inhabit tropical brackish waters, except for *M. leucophaeata*, which also occurs in temperate regions.

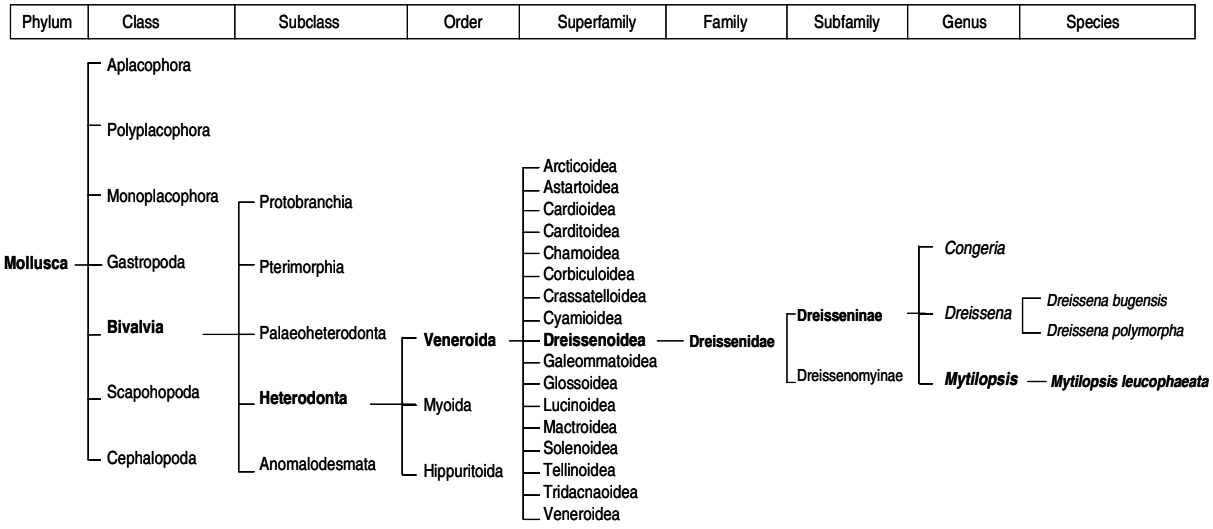

Fig. 1: Taxonomical tree of *Mytilopsis leucophaeata* after Nuttall (1990).

The superfamily Dreissenoidea arose in Europe more than 60 million years ago through the genus *Mytilopsis* (**Fig. 2**) and underwent a broad Eurasian expansion during the next 50 million years. About 5 million years ago, the genus disappeared completely out of Europe. The invasion of the New World occurred more than 30 million years after its first appearance in Europe and *Mytilopsis* spread throughout North and Central America. The apparent lack of fossils from the eastern coast of North America supports the fact that man-made introduction is the reason of this extended distribution into the New World (Nuttal, 1990). The currently living species *M. leucophaeata*, sometimes referred to as *Congeria cochleata* (Kickx, 1835) is not known before 5 million years ago. By means of *M. leucophaeata*, *Mytilopsis* re-invaded European waters again in recent times.

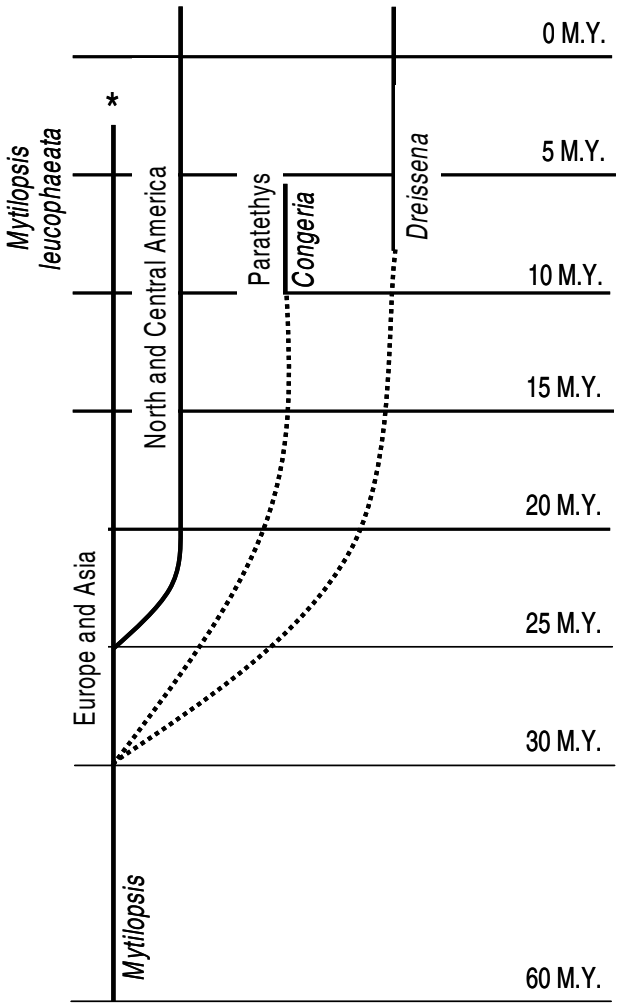

Fig. 2: Evolutionary review of the appearance and spread of the Dreissenids, based on fossil records by Steininger et al. (1985).
(*: reintroduction in Europe after extinction).

Dreissena did not exist in Europe until 7 million years ago (Steininger et al., 1985). Much of its dispersal throughout Europe, North and Central America occurred since the late 19th century. Because *Mytilopsis* was considered a subgenus of *Congeria*, it was suggested that *Dreissena* and *Mytilopsis* evolved from extincted branches of the genus *Congeria* (Babak, 1983; Mackie et al., 1989). The evolutionary development according to Nuttall (1990), who elevated *Mytilopsis* to the genus level, now shows that *Dreissena* and *Congeria* both arose from *Mytilopsis* (Marelli, 1994; Therriault et al., 2004).

The history of the Dreissenids illustrates the success of the high salinity-tolerant *Mytilopsis*, which has survived since the early Tertiary. Nevertheless, its invasion of the New World during the Oligocene appears to have been luck and its natural disappearance from Europe, about 5 million years ago, shows its vulnerability to changing conditions. Both *Mytilopsis* and *Dreissena* seem to be very slow colonizers, unless influenced by man (Nuttall, 1990).

IDENTIFICATION

The basic developmental pattern of all gastropod and bivalve molluscs shows a large uniformity, whether they inhabit marine or freshwater environments (Conn, 1991). Specifically for bivalves, the life cycle can be roughly divided into two periods: (1) from egg till settling larva, they are pelagic and only protected by a larval soft shell and (2) after settlement, the individuals become benthic and develop a hard mytiliform shell.

Much confusion in identification arises between *M. leucophaeata* and *D. polymorpha*. Since *D. polymorpha* is far better known than *M. leucophaeata* and both species are very much alike, *M. leucophaeata* is often misidentified as *D. polymorpha*. The life history patterns are quasi identical for both species and distinguishing between both in their larval phase is more difficult than for the adult mussels. Therefore a good identification tool for adults and larvae is summarized and differences between both species are emphasised.

1. ADULTS

The shell morphology of *M. leucophaeata* was originally described by Conrad (1831) as follows (Marelli and Gray, 1983):

"Shell incurved, white, with very rugose epidermis; anterior side much depressed; hinge margin excavated, with the teeth obsolete; on the posterior side, under the beaks is a pointed laminar tooth directed inwards. Cab. Academy, No. 1453. Inhabits the southern coast of the U.S."

This poor description made it impractical to distinguish *M. leucophaeata* from other Dreissenidae.

In 1835, Nyst redescribed the species, which he named *Mytilopsis cochleatus* Kickx, much clearer with detailed drawings of the shell:

"Oblong, subcylindric shell curved, a little depressed posteriorly, a little compressed towards the upper edge and slightly dilated at the posterior end of the cardinal ligament, covered with cob-webby threads which make it appear finely and transversely striated and which meet with the age in some species of curved lammelibranchs.

The beaks are pointed and slightly curved, the shell is covered inside with a septiformous lamina, such as in several species of this kind, but it is moreover provided with an appendix in the shape of a spoon, placed under the septiformous lamina on the side of the upper edge.

The right valve of this species is larger than the left; this character is most perceptible on the lower edge. This shell is usually brown, ashen, and crossed by whitish zones; the young individuals appear sometimes striped."

From current detailed knowledge on conchological redescriptions of *M. leucophaeata*, we can summarize that the shell is mytiliform and byssate (**Table I**). The exterior periostracum is creamlike coloured in young *M. leucophaeata* to dark brown in adults, with fine to medium rough concentric lines. For juveniles, it is very common to have the same distinct stripes or zigzag patterns as *D. polymorpha.* General appearance is long and wide, ventrally rounded and dorsally flattened, although juveniles seem more elongated and rectangular because of very smoothly curving margins. Body shape of *D. polymorpha* is much broader with flattened ventral margin and more rounded dorsally (MacNeill, 1992) (**Fig. 3**).

Fig. 3: Exterior left valve of (1) *Dreissena polymorpha* and (2) *Mytilopsis leucophaeata.*

Much of the species confusion between *M. leucophaeata* and *D. polymorpha* occurs because of overlapping shell colouring. *Dreissena polymorpha* has in general a brownish-white zigzag pattern, but not all zebra mussels are striped (Pathy and Mackie, 1993).

The interior shell of *M. leucophaeata* is coloured grey, with porcelain pallial and extra-pallial regions, and a narrow septum (myophore plate). The pallial line is very short and a pallial sinus is present, though very weakly developed (**Fig. 4**). The posterior retractor muscle scar is situated directly besides the posterior limit of the nymph. The main and most reliable distinguishing characteristic however is the presence of an apophysis near the umbo, a small triangular or rounded tooth, which serves as an attachment point for anterior retractor muscles, which is quite large in *M. leucophaeata* and visible with a binocular or even with the bare eye in larger individuals.

Dreissena polymorpha has a white shell interior with a broad septum with an entire pallial line, but without pallial sinus. In *D. polymorpha*, both anterior adductor and anterior retractors attach to the septum and an apophysis is absent. The fact that *Dreissena* would have evolved from *Mytilopsis* could indicate a reduction of the apophysis during the late Miocene (Panã, 1962). However, there is no evolutionary explanation for the disappearance of this characteristic, since *Mytilopsis*, with an apophysis, appeared 40 million years before *Dreissena* and is still successful (Nuttall, 1990).

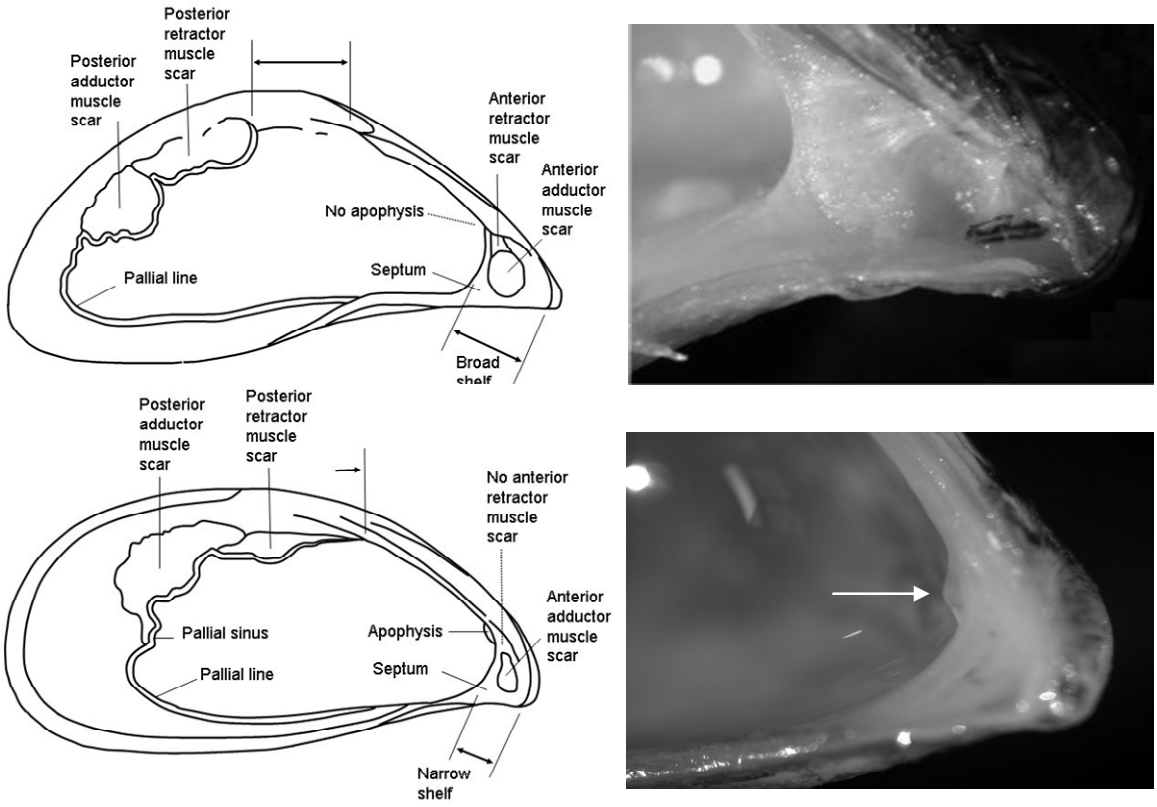

Fig. 4: Interior left valve of (1) *Dreissena polymorpha* and (2) *Mytilopsis leucophaeata* with a detailed view of the apex (1) without and (2) with apophyse.

There is also a difference in length between both species. *Mytilopsis leucophaeata* is a rather small mussel species with adult size ranging between 10 – 20 mm, while adult *D. polymorpha* has an average length of 40 mm (Chase and Bailey, 1999).

Table I: Comparison of characteristics of adult *Mytilopsis leucophaeata* and *Dreissena polymorpha* (according to Pathy and Mackie, 1993) (+: present; -: absent).

	Mytilopsis leucophaeata	***Dreissena polymorpha***
Exterior		
shell colouring	adult: dark brown, possible stripes juvenile: striped patterns	striped patterns, all black or white sometimes without stripes
ventral margin	convex, rounded ventro-lateral shoulder	concave, acute ventro-lateral shoulder
dorsal margin	flattened	rounded
umbo	rounded	pointed
posterior end	rounded	angled
Interior		
septum	narrow	broad
apophyse	+	-
pallial line	indented posterior in pallial sinus	entire, rounded
pallial sinus	+ (very weak)	-

2. LARVAE

Within 24 hours after the external fertilisation of gametes, released in the water column, a short-living trochophora stage is identified. Although rarely seen in plankton samples, this stage is frequently observed in laboratory induced fertilisation studies (**Fig. 5**). The trochophora has a ring of cilia, a prototroch, important for a direct swimming motion, and transforms into a veliger stage. Veligers already have a soft bilateral symmetric bivalve shell, but they feed and move with their larval organ, a ciliated velum. Early veligers have a straight, dorsal hinge and rounded ventral margins. These first larval shells (prodissoconch I) are free of ornaments and D-shaped in profile, which is referred to as D-shaped veligers. Later a second, more ornamented larval shell (prodissoconch II) is secreted and gives the larva a clam-like profile: veliconcha, which is the last larval pelagic stage. Just before the larvae will become benthic, they grow considerably and develop new organs, like a muscular foot used for swimming near the bottom and crawling on surfaces. This pediveliger stage also forms gill filaments in the mantel cavity, which do not reach maturity until after metamorphosis. Primary settlement occurs by secreting a byssal thread onto a filamentous surface and is, once anchored, accompanied by loss of the velum: postveliger. Young individuals, morphologically similar to adult mussels, but still immature, are referred to as juveniles.

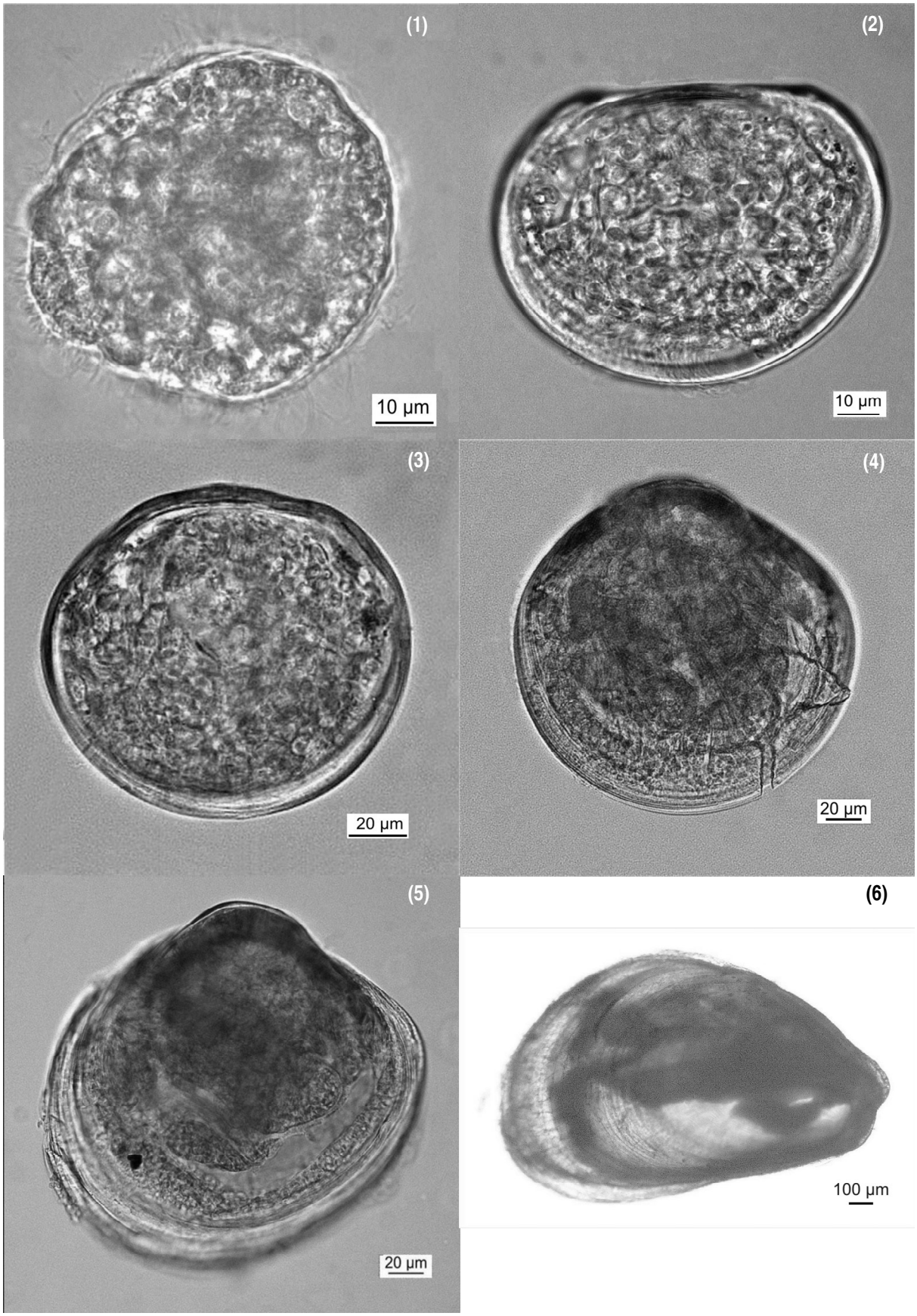

Fig. 5: Larval stages of *Mytilopsis leucophaeata*: (1) trochophora; (2) D-shaped veliger; (3) veliconcha; (4) pediveliger; (5) postveliger; (6) juvenile.

Since there is some synonymy in literature concerning the larval stages of mussels, an overview of the possible names is given in **Table II**.

Table II: Synonymy in life history stages of *Dreissena polymorpha* and *Mytilopsis leucophaeata* (Ackerman, 1995).

Pelagic larvae	**trochophora**	
	veliger	**D-shaped** or straight-hinged veliger **veliconcha** or umbonal veliger **pediveliger** or settling veliger
Benthic mussels	**postveliger** or spat or plantigrade mussel	
	juvenile of siphon-forming mussel	
	adult or branchi-siphonal mussel	

Although adult Dreissenids become much larger than *M. leucophaeata* adults, size is not a distinguishing larval characteristic (Conn et al., 1993; Ackerman et al., 1994; Verween, unpublished data) (**Table III**). The overall size of each developmental stage for each of the two species is overlapping, and these sizes differ for different individuals of one species. There is however a difference in shape which becomes more explicit as the individual develops.

Table III: Developmental sizes of *Mytilopsis leucophaeata* and *Dreissena polymorpha* (Rajagopal, pers comm; Verween, unpublished data; Conn et al. 1993; Ackerman et al., 1994).

	Mytilopsis leucophaeata	***Dreissena polymorpha***
egg	> 32 µm	40 – 96 µm
trochophora	< 63 µm	57 – 121 µm
D-shaped veliger	50 – 91 µm	70 – 160 µm
veliconcha	109 – 150 µm	120 – 280 µm
pediveliger	145 – 220 µm	167 – 300 µm
postveliger	220 – 480 µm	158 – 500 µm
juvenile	> 580 µm	> 500 µm

No comparative study has been conducted for the trochophora stage. The other stages however have been studied intensively and differences between both species were summarized per species.

The D-shaped veliger in *M. leucophaeata* has a typical straight dorsal hinge and rounded ventral valve margins (**Fig. 6**). The hinge has a subtle rounded lateral profile and becomes angular at the ends. The veliconcha develops an umbo, which makes it look clam-like and shows a uniform pigmentation. The

asymmetry of the shell becomes clearer as the anterior side is slightly elongated and less rounded compared to the posterior side. The body shape of the pediveliger is highly asymmetric with the anterior being markedly elongated and less rounded than the posterior. A high concentration of pigment is visible near the umbonal region. Development and growth of the larval shell continues in posteroventral direction in the postveliger stage. The anterior shoulder is more pronounced and dorsally is a well developed umbo visible. The pigment stays concentrated near the umbo and a pigment spot is posteroventrally visible. The juveniles have a rounded posterior and a rather pronounced anterior shoulder and will continue developing mytiliform. The pigmentation is less concentrated and fills almost the entire shell.

In general, the shell valves of the D-shaped veliger and the veliconcha of *D. polymorpha* are more ovoid. The veliconcha has a velum pigment at the anterior side (**Fig. 7**). The pediveliger has a highly pronounced anterior side, which is rather angulated. The pigment is rather randomly spread in the larvae. The velum pigment is mostly still present, but not in all pediveligers. The postveliger is randomly pigmented. Development occurs in a rather ventral way, with a much longer and straighter anterior shoulder. The anterior margin in juveniles is straight. The umbo is well developed, but less pronounced than in *M. leucophaeata*. No specific pigmentation is present. In **Table IV** the main distinguishing characteristics of larval and postlarval stages between *M. leucophaeata* and *D. polymorpha* are summarized. However, it needs to be emphasised that gut content can easily been misidentified as pigment spots, so figures 6 and 7 should be additionally used to distinguish between both species.

Table IV: Comparison of characteristics of larval and postlarval stages of *Mytilopsis leucophaeata* and *Dreissena polymorpha* (according to Conn et al., 1993) (+: present; -: absent).

	Mytilopsis leucophaeata	***Dreissena polymorpha***
gut pigment	+	+
velar pigment	-	+
posterior-ventral pigment	+	-
general shape	rounded	ovoid
anterior vs posterior shoulder	slightly lower	markedly lower

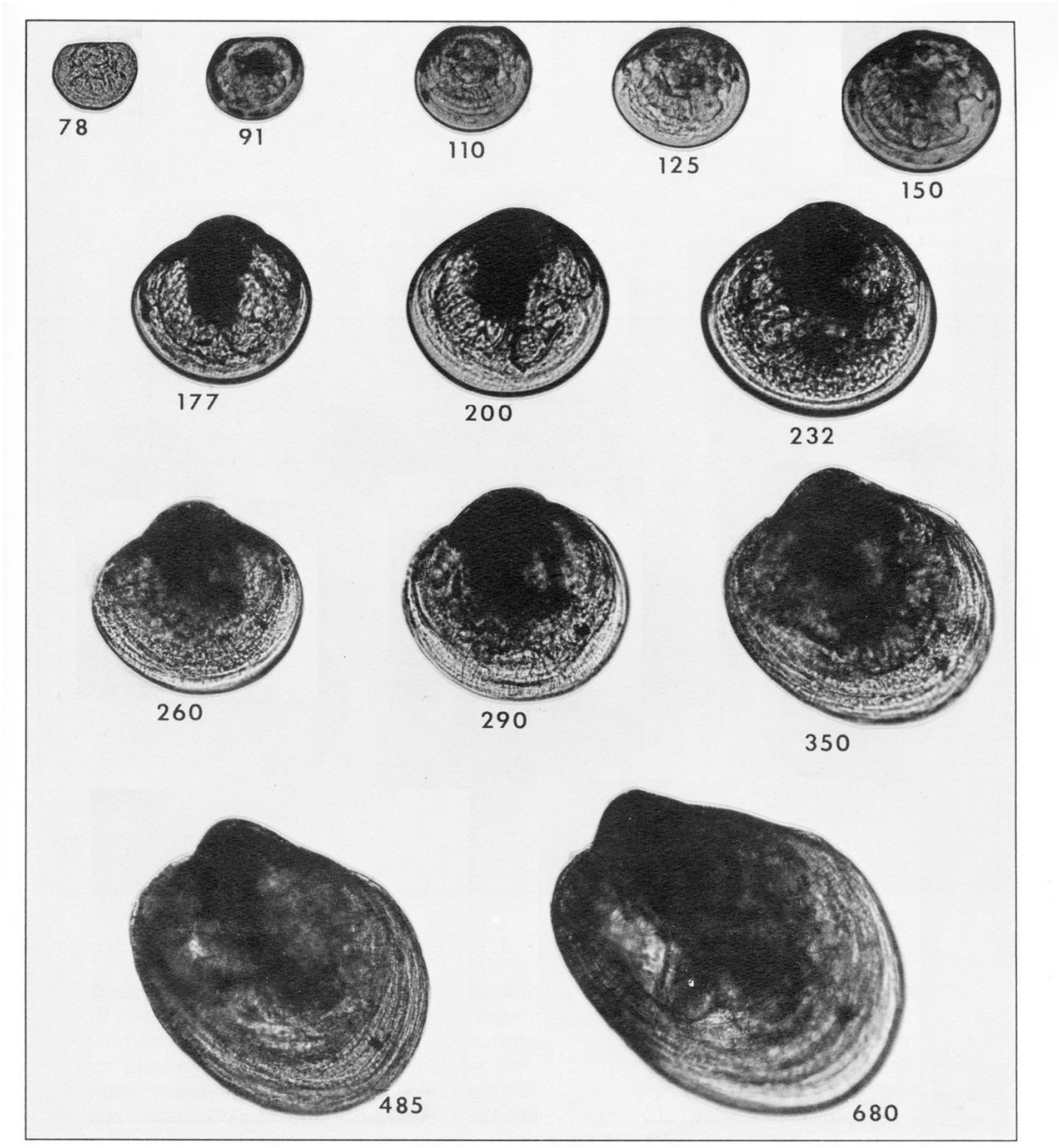

Fig. 6: Photomicrographic sequence showing lateral views of various developmental stages of *Mytilopsis leucophaeata* and shell length (µm) (Conn et al., 1993).

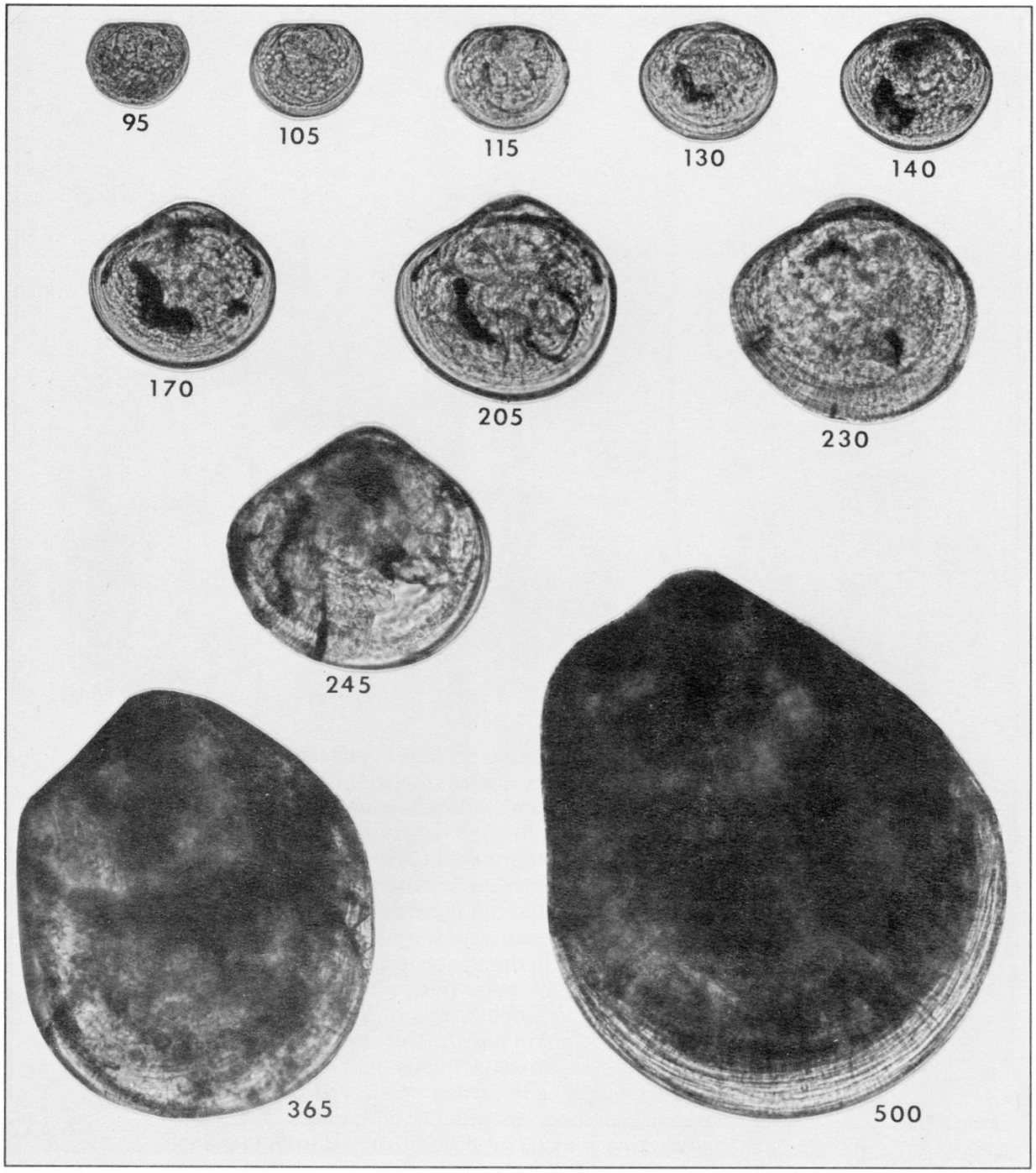

Fig. 7: Photomicrographic sequence showing lateral views of various developmental stages of *Dreissena* spp. and shell length (µm) (Conn et al., 1993).

ECOLOGICAL CHARACTERISTICS

Mytilopsis leucophaeata has been detected in rather isolated waters, like the Black Sea (Therriault et al., 2004) as well as in very open regions, like the river Schelde (Verween et al., 2005) at very different ranges in temperature and salinity. This leads to the hypothesis that the habitat preferences and environmental limits of this species are very broad, and that the species may tolerate a wide variety of environmental circumstances in its newly invaded habitat.

Overlapping tolerances of *M. leucophaeata* and *D. polymorpha* in habitat and food preferences, temperature and salinity allow a sympatric distribution of both species, especially in estuaries. An estuary is a semi-enclosed coastal water body in which sea water mixes with fresh water. The consequent highly dynamic gradient in salinity and temperature leads to possible sympatric distribution of both species, as already observed in the estuarine delta of the Rhine, Meuse and Schelde Rivers (Wolff, 1969; Marelli and Gray, 1983) and the North Sea Channel (Van der Velde et al., 1998).

1. SUBSTRATUM PREFERENCES

One of the most principal factors affecting the distribution and abundance of *D. polymorpha* is a suitable substrate for attachment (Karatayev et al., 1998). Primary settlement merely happens on filamentous structures, such as byssus threads of adults. Secondary settlement occurs mostly on hard surfaces, particularly rocks and stones, and macrophytes. Highest abundances however have been recorded on artificial substrates.

Although no specific research has been conducted on habitat preferences of *M. leucophaeata*, the species has been found merely attached on artificial substrates such as the conduits of the cooling water installation in Holland and Antwerp (Rajagopal et al., 1995; Verween et al., 2005), large, stone walls at Cardiff Docks (Oliver et al., 1998) and wooden posts and piling in the River Thames (Bamber and Taylor, 2002). In the Delta area in Holland, mussels have been found attached to naturally occurring stones and wood, but only in low densities (Wolff, 1969).

2. FOOD RESOURCES

Mussels filter-feed primarily on planktonic algae and zooplankton but other nutritional sources are bacteria, detritus, and organic matter. The filtration rate of *D. polymorpha* is affected by size, turbidity, temperature and certain concentrations of specific sizes and kinds of algal and bacterial cells (Mackie and Schloesser, 1996). *Mytilopsis leucophaeata* is a filter feeder with phytoplankton as major food source. They are able to ingest particles as small as 4 µm, and can feed on the flagellate *Isochrysis galbana* Parke in laboratory cultures (Verween, unpubl data).

3. SALINITY TOLERANCES

The salinity tolerance limits of *M. leucophaeata* are mostly described in combination with data on *D. polymorpha*. Because the hyperosmotic regulation of body fluids, which is a universal adaptation of brackish water animals, can enlarge the salinity tolerance of a species, literature data concerning *M. leucophaeata* vary greatly (**Table V**).

Table V: Overview of observed salinity ranges where presence of *Mytilopsis leucophaeata* is recorded, according to literature.

Min. salinity (PSU)	Max. salinity (PSU)	Optimal salinity (PSU)	Region	Reference
	26.4		the Netherlands	Otto and Wielinga, 1933
0		12.66	laboratory	Castagna and Chanley, 1973
				Deaton et al., 1989
0.21	2.74	0.75	the Netherlands	Janssen and Janssen-Kruit, 1967
3.48	9.42		the Netherlands	Janssen and Janssen-Kruit, 1967
0.57	14.9		Rhine, Meuse, Schelde	Wolff, 1969
0	31.6			Wolff, 1969
8	22		Virginia Keys, U.S.	Siddall, 1980
0.5	2		Southern England, U.S.	Smith and Boss, 1996
		15	Cardiff Docks, England	Oliver et al., 1998
6	14		Thames, England	Bamber and Taylor, 2002
0.5	5		Black Sea, Ukrain	Therriault et al., 2004
		20.9	Gaudalquivir, Spain	Escot et al., 2003
0.1	11.7		Schelde, Belgium	Verween et al., 2005
development of larvae				
10	32			Siddall, 1980

It has been stated that *M. leucophaeata* can live and establish in salinities ranging from almost freshwater (0.1 PSU) over oligo- to mesohaline conditions, with a maximum of 26.4 PSU, which indicates that this species can be found across nearly the whole estuarine gradient. Although *M. leucophaeata* can survive even in freshwater (0 PSU) and in highly saline water (31 PSU), these are well above the levels preferred for propagation (Wolff, 1969). Only true seawater (35 PSU) is outside its reach of survival. There is however still disagreement about the optimal salinity level for *M. leucophaeata*, ranging in literature from 0.75 PSU to 20.9 PSU, which could indicate that *M. leucophaeata* is able to adapt to circumstances more derogatory from its original habitat.

The larvae and postlarvae of *M. leucophaeata* are capable of development at even higher salinities, ranging to 32 PSU. This characteristic makes it possible for *M. leucophaeata* to cross the oceans as larvae in ballast water with high salinities and as such colonise a new, isolated estuarine habitat (Siddal, 1980).

Since *D. polymorpha* is a typical freshwater species, its capability to adapt to different salinity levels is much smaller than for *M. leucophaeata*. The euryhaline capacities of *M. leucophaeata* however lead to a possible habitat overlap in salinity between the two species. According to MacNeill (1991), salinity tolerances appear to overlap greatly between 0.2 and 3 PSU. Both species are dominant in sessile communities of the North Sea Channel connecting Amsterdam harbour with the North Sea, where salinity ranged form 1.7 to 9.2 PSU (Van der Velde et al., 1998). As a general rule it is accepted that *D. polymorpha* tolerates salinities up to 6 PSU, which leads to a possible habitat overlap for both species between 0.1 and 6 PSU.

4. TEMPERATURE TOLERANCES

Although almost all members of the genus *Mytilopsis* inhabit tropical and subtropical waters, *M. leucophaeata* is restricted to warm, more temperate waters (Marelli and Gray, 1983). At its place of origin, the Gulf of Mexico, yearly water temperature fluctuates between 24 and 27 °C, but in European waters, the species endures much lower temperatures (**Table VI**).

Its resistance to low temperatures makes *M. leucophaeata* a potentially very suitable inhabitant of all temperate waters throughout Europe. *Dreissena polymorpha* tolerates temperatures up to 29°C, which

makes temperature not a limiting characteristic to distinguish between possible *M. leucophaeata* and *D. polymorpha* habitats.

Table VI: Overview of observed temperature ranges where presence of *Mytilopsis leucophaeata* is recorded, according to literature.

Min. temperature (°C)	Max. temperature (°C)	Region	Reference
13	30	Miami, U.S.	Siddall, 1980
11	26	North Sea Channel, The Netherlands	Rajagopal et al., 1995
6.8	25.8	Schelde, Belgium	Verween et al., 2005

However, temperature is an important, species-specific factor in the initiation of spawning (de Vooys, 1999). Spawning in *Mytilus edulis* only occurs when the water temperature exceeds 10 °C (Chipperfield, 1953; Wilson and Sees, 1974). For *D. polymorpha,* 12 °C is the minimum temperature allowing gonad maturation and as a general rule is accepted that no veligers will appear in the water column at lower temperatures (Ram et al., 1996), although Mantecca et al. (2003) found a spawning population at 25 m depth, where temperature stayed below 10 °C most of the year. Monitoring data show that for *M. leucophaeata*, this threshold temperature for gamete maturation may be 13 ± SE 1 °C (Verween et al., 2005). Other studies indicate that reproduction usually starts at a temperature higher than 15 °C (Schütz, 1969) or even higher than 20 °C (Rajagopal et al., 1995).

LIFE HISTORY[1]

Since almost no literature is available on the life history patterns of *M. leucophaeata*, population dynamics of *M. leucophaeata* in relation to environmental factors were investigated in the cooling water system of an industrial installation along the River Schelde during the period 2000 – 2004, determining (1) the yearly period of larval presence in the water column and (2) the growth rate of juvenile and adult mussels.

[1] Since research was not yet finished at the moment of acceptance of the manuscript, this part of the review was more extensively published in CHAPTER III and V.

1. RECRUITMENT

In all years spawning began end of May – early June and lasted for about four to five months (**Fig. 8**). Temperature at first detection ranged between 16.2 – 19.5 °C, salinity ranged from 2.6 to 4.9 PSU. Two or more distinct larval peaks could be observed per year. Different peaks were probably due to different spawning periods (Borcherding, 1991), but although major differences in densities between months and years were found, the period of larval occurrence was markedly similar (Verween et al., 2005).

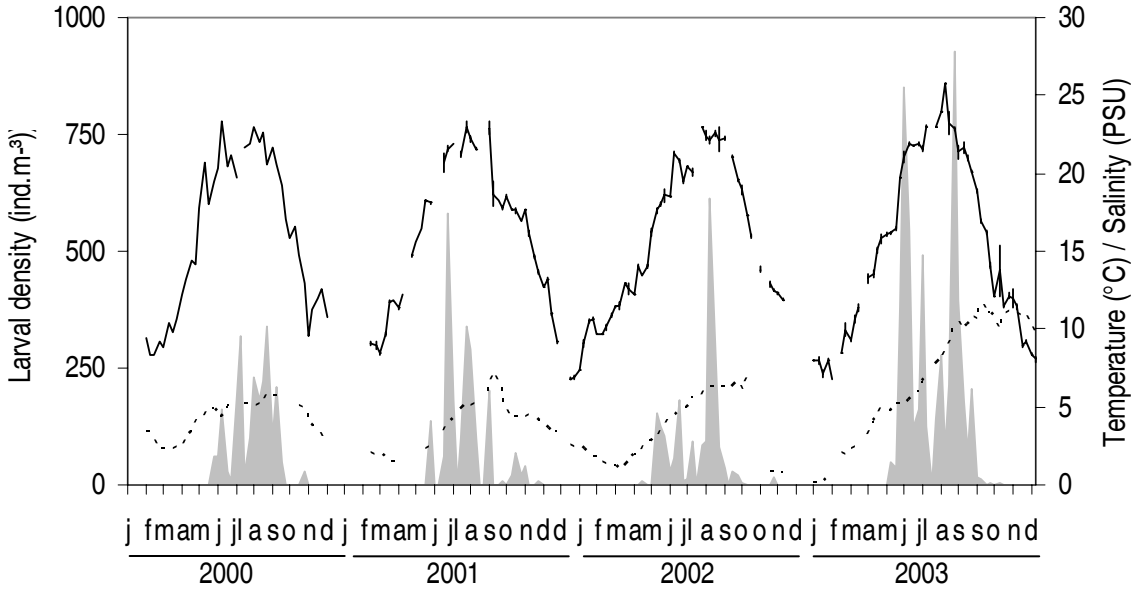

Fig. 8: Seasonal variation in larval arrival of *Mytilopsis leucophaeata* in the cooling water system in the harbour of Antwerp (full line: temperature (°C), dashed line: salinity (PSU), grey shaded: larval density (ind./m³)) (adapted from Verween et al., 2005).

Dreissenidae are sequential spawners (Borcherding, 1991), and 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, thereby creating periodic synchronisation in larval production, resulting in different larval peaks. For *D. polymorpha* interannual differences in recruitment success are most likely influenced by environmental factors, e.g. weather conditions, abundance and timing of phytoplankton blooms, wind currents, etc. (Garton and Haag, 1993), although the specific influence on variation in larval abundance in the water column is still poorly understood (Nichols, 1996). However, an increase in adult density could lead to increasing recruitment success (Sprung, 1993), as monitored for 2003, which could indicate that the adult stock of *M. leucophaeata* in the harbour dock of Antwerp is still expanding.

The release of gametes in *D. polymorpha* is highly variable (Nichols, 1996) throughout its range in Europe, Russia and North America and can be a very synchronized event, focused over 1 or 2 weeks, or can be completely non-synchronized, occurring throughout the year, depending on the site where the species is found. In some localities, zebra mussels start spawning at water temperatures of 12 °C, but do not start until water reaches 22 °C at other areas. On average, peak larval densities in the water column are reached very rapidly and then gradually decline over a 6 - 8 week period.

2. GROWTH

By means of growth cages, shell growth was followed in the Schelde river during 2003 and 2004. Monitoring several length classes allowed to model shell growth of a modal mussel, settled at the end of February over a period of nearly 5 years (**Fig. 9**).

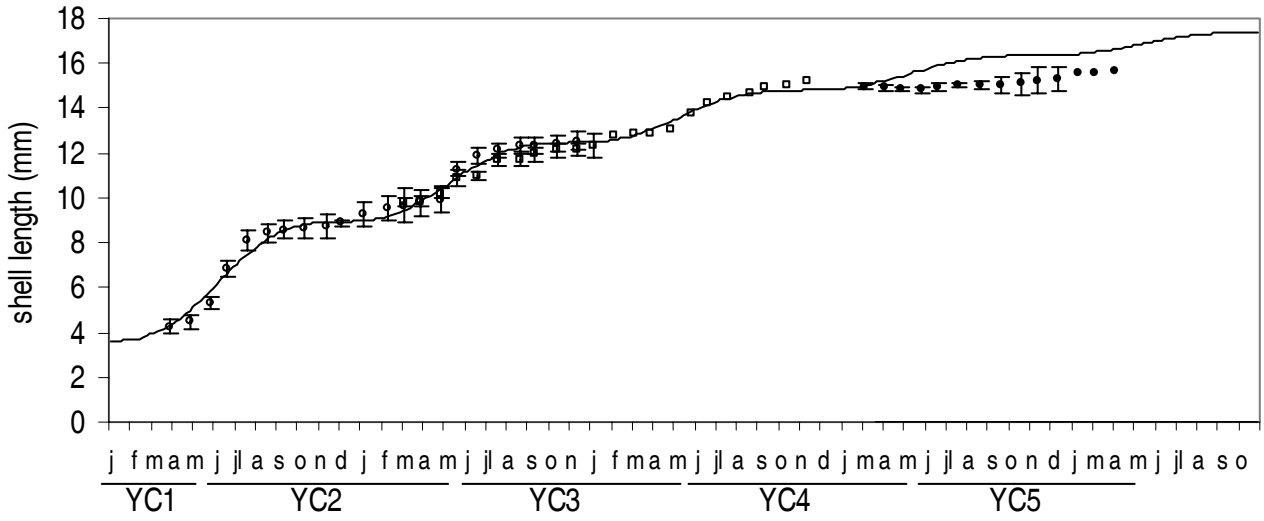

Fig. 9: The extrapolated graphical presentation of the von Bertalanffy growth function over four years (adapted from Verween et al., 2006).

Mytilopsis leucophaeata followed an oscillatory growth trajectory similar to many species living in environments with a distinct annual cycle in temperature and/or light conditions (Crisp, 1984), with one summer growing season a year. A negative, linear relation between shell length and shell growth rate was detected. The parameters of the Von Bertalanffy growth curve were estimated and gave an asymptotic length (L_∞) of 17 mm, although empty shells larger than 20 mm have been found, and a growth constant K of 0.56 per year (Verween., unpubl data).

Table VII: Overview of maximum shell length measurements of *Mytilopsis leucophaeata*, according to literature.

Max. length (mm)	Region	Reference
10 - 20	U.S.	Abbott, 1974 Emerson and Jacobson, 1976 Pennak, 1978
22	Miami, U.S.	Siddall, 1980
14	North Sea Channel, The Netherlands	Rajagopal et al., 1995
27	The Netherlands	Gittenberger et al., 1998
20	Cardiff Docks, England	Oliver et al., 1998
15.2	Thames, England	Bamber and Taylor, 2002
22	Schelde, Belgium	Verween et al., 2006

Historic American identification guides on shells describe a size range from 1 to 2 cm for *M. leucophaeata* (Abbott, 1974; Emerson and Jacobson, 1976; Pennak, 1978). More detailed information on the species (**Table VII**) shows that the average maximal length indeed is about 20 mm, but generally, smaller individuals (10-15 mm) are found in the field. The larger sizes like 27 mm, described by Gittenberger et al. (1998) can be considered exceptionally large for this species.

The duration of the growing season of *M. leucophaeata* is mainly restricted to the summer, similar to that of *D. polymorpha*, when growth also occurs primarily during the summer with little or no growth during wintertime (Bij de Vaate, 1991). In contrast, *D. polymorpha* seems to grow faster (K = 0.808) and much larger (L_∞ = 40.5 mm), but has a shorter life span between 2 and 4 years (Conides et al., 1997; Chase and Bailey, 1999).

BIOGEOGRAPHICAL DISTRIBUTION

All Dreissenidae have recently spread throughout the world by means of shipping activities (Nuttall, 1990). Obviously, the ever expanding industrial development, hull fouling and ballast water discharges from transoceanic shipping together with the creation of canals link isolated water bodies with each other and create an ideal passive transferring method for both aquatic and terrestric species from one region to another. These phenomena are believed to be responsible for the recent spread of these and other non-indigenous species throughout the world (Coutts, 1999; Therriault et al., 2004). More specifically, it is assumed that the ballast water transfer and hull fouling of industrial ships are very important vectors for the spread of *M. leucophaeata*. Other human-mediated vectors for dispersal of

mussels include hull fouling on recreational boats trailered from infested to uninfested water bodies, commercial bait transport, hatchery stocking activities, aquaria releases and navigation and irrigation canals (O'Neill, 1996). However, since European brackish water bodies are seldom recreational and not naturally connected to one another, these other vectors seem unlikely for the spread of *M. leucophaeata*. In Europe, mussels may also disperse naturally by being transported passively as planktonic larvae in water currents and by attaching to other organisms such as crayfish and turtles (Carlton, 1994). They may also attach to the legs and feet of shorebirds, but these are only low-level factors (Johnson, 1994). Water currents can disperse *M. leucophaeata* larvae further downstream in estuaries, but because of saline boundaries, it will never reach the high dispersal capacity of *Dreissena* larvae in freshwater ecosystems. The presence of brackish water step stones throughout Europe might however improve the dispersal of *M. leucophaeata* by means of transportation through birds, although no specific research has been conducted.

Mytilopsis leucophaeata originates along the southern coast of the U.S. and ranges from Tampico, Mexico to the Hudson River estuary (Marelli and Gray, 1983). It re-invaded Europe with a first record in 1835 in the harbour of Antwerp in Belgium (Nyst, 1835). The species, which Nyst addressed as "original and at least new to Belgian fauna" was found attached in great abundance to piles in a ship repair dock. Since the mussels were accompanied by barnacles and corals, Nyst concluded that they were probably brought there by transoceanic ships. However, it needs to be emphasised that no details on the type of barnacles and corals were mentioned, and as such they do not completely confirm the trans-oceanic crossing theory, although it is most likely it happened this way. In the early 19th century the species was also found in France and the Netherlands (Récluz, 1849; Reeve, 1858; Fisher, 1858, all in Marelli and Gray, 1983).

After a period of apparent absence of the species in European waters with no new records during almost 100 years, *M. leucophaeata* was detected in Dunkerque and the Canal of Caen in France (Germain, 1931). In 1960, Adam (1960) shortly described *M. leucophaeata* again in Belgian waters, near Nieuwpoort, as part of listing of Belgian molluscs, and the species also invaded the Rhine River as mentioned by Wolff (1969).

It is only when *M. leucophaeata* became an economic problem in the 1990s as an important industrial fouler, that attention was brought back to this, until then, relatively unknown species. In 1994, *M. leucophaeata* was causing fouling problems in the cooling water installation of the Velsen and Hemweg power station in the Netherlands (Rajagopal et al., 1995), and was later detected in the North Sea

Channel (Van der Velde et al., 1998) and the Waal River (Kelleher, 1997). In 1996, *M. leucophaeata* was recorded for the first time from British waters. Clumps of polychaetes, together with *Mytilus* and *Mytilopsis* were found on the walls of Roath Basin, Cardiff Docks in South Wales (Oliver et al., 1998). Given the long time that *M. leucophaeata* has inhabited nearby countries like Belgium and France, it is surprising that no previous records in England exist. The presence of the North Sea Channel with its high salinity acts as a barrier for the natural dispersal of the species. Since ballast water is its main transfer method, and this is less used by the smaller Channel crossing ships, it has been almost impossible for *M. leucophaeata* to cross this barrier. The presence of the rare American crab *Rhithropanopeus harrisii* might support the hypothesis that *Mytilopsis* invaded the Roath Basin from North America instead of from European waters (Oliver et al., 1998), although *Rhithropanopeus* has also been found in the Schelde River (Ysebaert et al., 2000). In 1998, the brackish water mussel was identified for the second time in England, in Cliff Fort Lagoon, first as a high density of dead valves in the sediment, but in 1999, live specimens were found on wooden posts and piles (Bamber and Taylor, 2002). Recently (Therriault et al., 2004), *M. leucophaeata* is identified from the Dniester Liman Black Sea Basin (Moldavia – Ukrain) for the first time. Again ballast water discharges are probably responsible for this invasion since canal development opened corridors between previous disjunct regions of the Black Sea and the Caspian Sea. In 2003, a large population of *M. leucophaeata* was found in the River Guadalquivir of the Iberian Peninsula (Spain) (Escot et al., 2003), where it is causing massive fouling problems in an industrial cooling water system, together with *Cordilophora caspia* and *Corbicula fluminea* among others. Very recently, October 2005, *M. leucophaeata* has been detected in the northern Baltic Sea in Finland, in an area affected by cooling waters from a power plant (Laine et al., 2006) (**Fig. 10**).

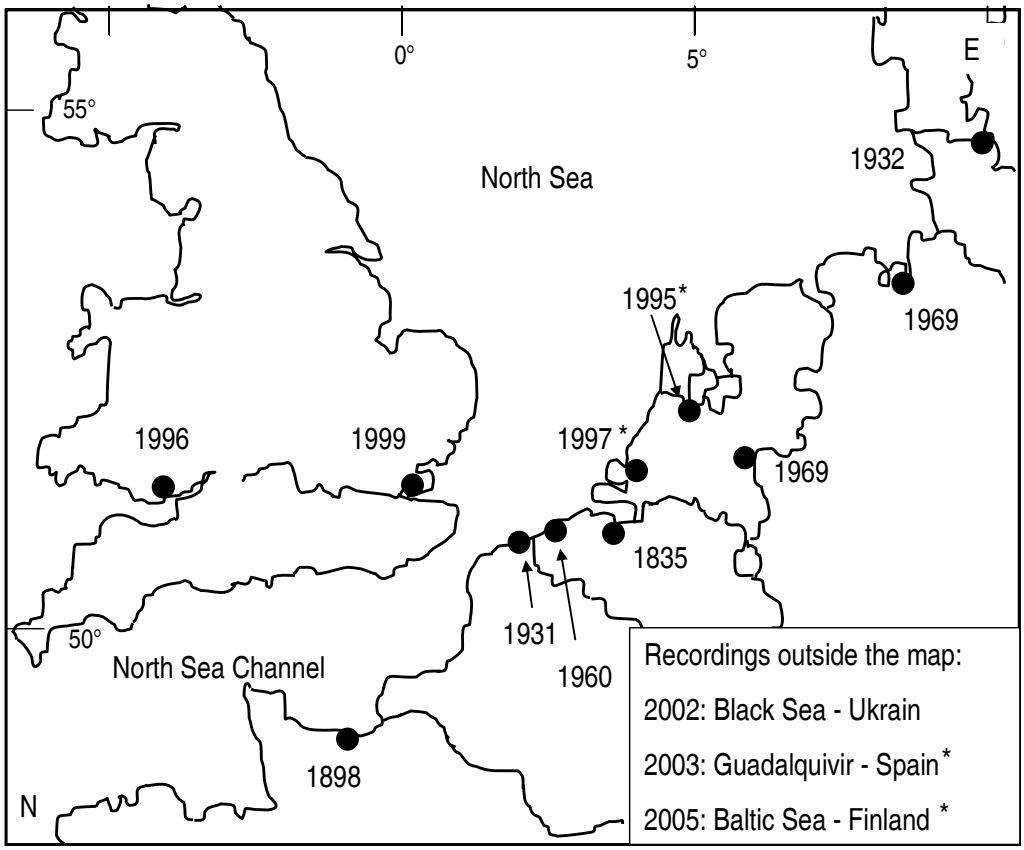

Fig. 10: Distribution of *Mytilopsis leucophaeata* in Europe, with data of first recordings. * = first recordings that co-occurred with industrial fouling problems.

It took almost 30 million years for *Mytilopsis* to expand its range from Eurasia to North America during the Eocene, but considerably less time to re-invade Europe during recent times (Therriault et al., 2004). The fact that the invasion of British waters from mainland Europe seemed almost impossible suggests that *M. leucophaeata* is not an efficient active invader (Oliver et al., 1998). However, the species has found a powerful invasion tool in the use of ballast water from transoceanic ships. This means that as long as shipping activities keep expanding, the invasion of the brackish water mussel will speed up. Although awareness programs are already in full development in North America, and ballast water use is restricted carefully, it is only in 2008 that the European Strategy on Invasive Alien Species (Council of Europe, 2003) will be implemented by the majority of the European member states (Council of Europe, 2004). A worldwide agreement will be needed to restrict or minimise this invasion by *M. leucophaeata* and many other aquatic invaders.

IN CONCLUSION: LESSONS LEARNED

Former chapters summarize literature information on the biology and ecology of *M. leucophaeata*. In this chapter, attention is brought back to the fundamental questions concerning this relatively unknown species. Can *M. leucophaeata* really become the brackish water equivalent of *D. polymorpha*, or is it, although invasive, a rather harmless species?

1. INVASION CAPACITIES

The habitat preferences and environmental limits of *M. leucophaeata* are very broad, which means that, theoretically, we can expect this species in all European brackish water bodies. Yet, to invade into a new area, *M. leucophaeata* has to overcome a major obstacle: although the species has a broad tolerance to salinity, survival with reproduction is impossible in fully fresh or seawater. This makes it almost impossible for the bivalve to cross these natural salinity barriers and as such to naturally invade into new areas. In contrast to *D. polymorpha*, who can expand very rapidly as soon as a new freshwater basis is colonized, *M. leucophaeata* is a rather slow natural colonizer with low dispersal capacities, who is restricted to brackish water bodies.

Transfer from one place to another is thus mainly human-induced. By means of transport as larvae in ballast water or as adults attached to the hull, shipping traffic is the most important vector for dispersal of *M. leucophaeata* (Therriault et al., 2004). The presence of stepping stones of estuaries all over Europe however leaves the possibility of minor dispersal by means of transportation of juveniles through attachment to the legs and feet of waterfowl and shorebirds.

No clear invasion pattern throughout time can be found in Europe (**Fig. 10**), which might indicate that *M. leucophaeata* is present in Western Europe since the early 19th century, but has not been identified before because of misidentification of the species as *D. polymorpha* in the past and the lack of interest in the species.

To reverse the spreading of *M. leucophaeata*, public awareness is important, just like for every other invasive species. Especially in Europe, almost no implementations have yet been made to raise the awareness of industrial initiatives and public governments.

2. BIOFOULING CAPACITIES

Although *M. leucophaeata* is a slower colonizer than *D. polymorpha*, it is definitely an even severe fouling species. Though, only few biofouling problems have been reported so far for a couple of reasons:

- Misidentification of the species. Along the River Schelde, fouling problems with *M. leucophaeata* have not been reported, because of confusion with *D. polymorpha* (Verween, pers observations). Because of this fact, we can be sure that there are more spots where *M. leucophaeata* fouling is misidentified, and as such not reported and treated in the proper way. This problem can easily be solved through an enhanced communication between science and industry. However, distinguishing between adults of both species is not difficult at all. The most important feature is the apophysis, a small triangular or rounded tooth, only present in *M. leucophaeata* (**Fig. 4**). Identification on larvae-level is more problematic, so in this case, searching for the adult population nearby the problem zone is recommended.

- The unequal proportion between fresh and brackish water. Hundreds of rivers and their tributaries cross the European continent, with only a couple of estuaries (e.g. Schelde and Solway Firth estuary) and brackish water seas (e.g. Baltic and Caspian Sea). Add the fact that little industry is present along these brackish water bodies in contrast to the freshwater rivers, and the few known *M. leucophaeata* fouling problems are a fact.

- No legal framework on biocide dosage. Biofouling is prevented by treatment with biocides, of which chlorination is the most effective and cheap control measure (IPPC, 2000). The use of biocides is only recently being restricted in Belgium and a lot of European countries still lack the legislation with respect to discharges of chemicals in cooling water. Because of that, concentrations of used biocides are so high that mussels are killed completely and no observation of species has happened in the past.

3. RESISTANCE TO ANTI-FOULING TECHNIQUES

Although Pathy and Mackie (1993) posed that the ecological and economical threat is less severe than that of the zebra mussel, the fact that they inhibit brackish waters makes them far more resistant to environmental changes than freshwater species (Siddall, 1980), which makes them potentially an even more robust fouler than *D. polymorpha*. Therefore, *M. leucophaeata* is more resistant to anti-fouling techniques than the freshwater *D. polymorpha*, as proven by Rajagopal et al. (1997; 2003; 2005b). A comparison of chlorine toxicity data with *M. leucophaeata, D. polymorpha* and *M. edulis* showed even that *M. leucophaeata* is the most tolerant species (Rajagopal et al., 2002b).

M. leucophaeata is smaller than the *D. polymorpha* adults, which makes the fouling problems less severe in density. On the other hand, *M. leucophaeata* is a long-lived species in comparison to *D. polymorpha*, which means that the adult population will remain a problem in the conduits for a longer time, indicating more severe problems in time.

Knowledge on the cyclic presence of mussel larvae provides a basis for an ecologically and economically proper use of biocides (Relini, 1984). The strict timing of *M. leucophaeata* larvae in the harbour of Antwerp is an indication that to prevent new biofouling, a targeted dosage of biocides during the period of larval presence will be as effective as a continuous dosage throughout the year. This saving can lead to the exploration on the use of ecologically less harmful, but more expensive biocides. The long lifespan on the other hand states that even though larvae may be effectively combated, the adult population in the conduits will remain a non-combatable source of larvae for a long time.

So in summary, *M. leucophaeata* is a slower invader in Europe than *D. polymorpha* in the U.S.. However once invaded, he is an even more severe fouling species than *D. polymorpha*, and as such it has to be taken in account that *M. leucophaeata* has most definitely the potential of becoming the brackish water equivalent of *D. polymorpha* in Europe.

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.

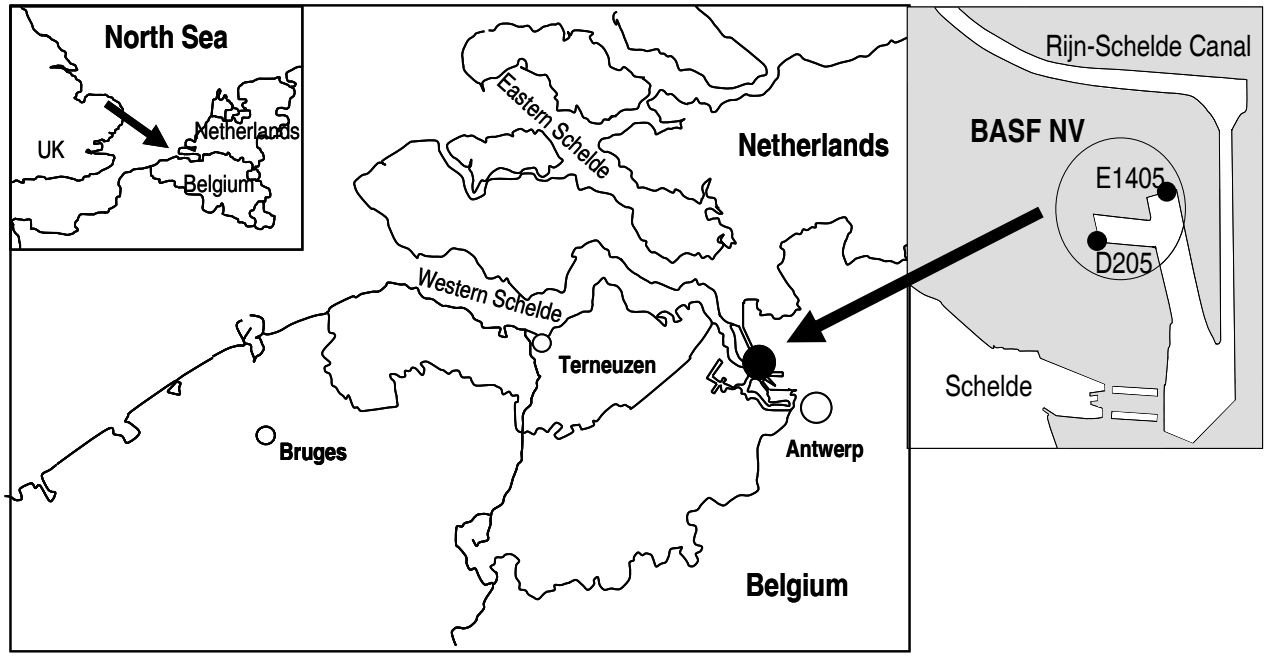

Fig. 1: Location of BASF, N.V. in the harbour of Antwerp.

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 m³), each with an average water flow of 1 m³/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.

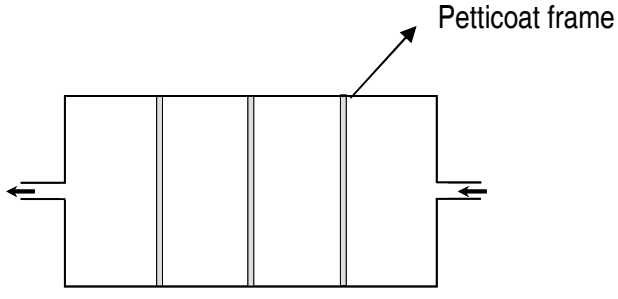

Fig. 2: PVC-tank with petticoat nets.

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

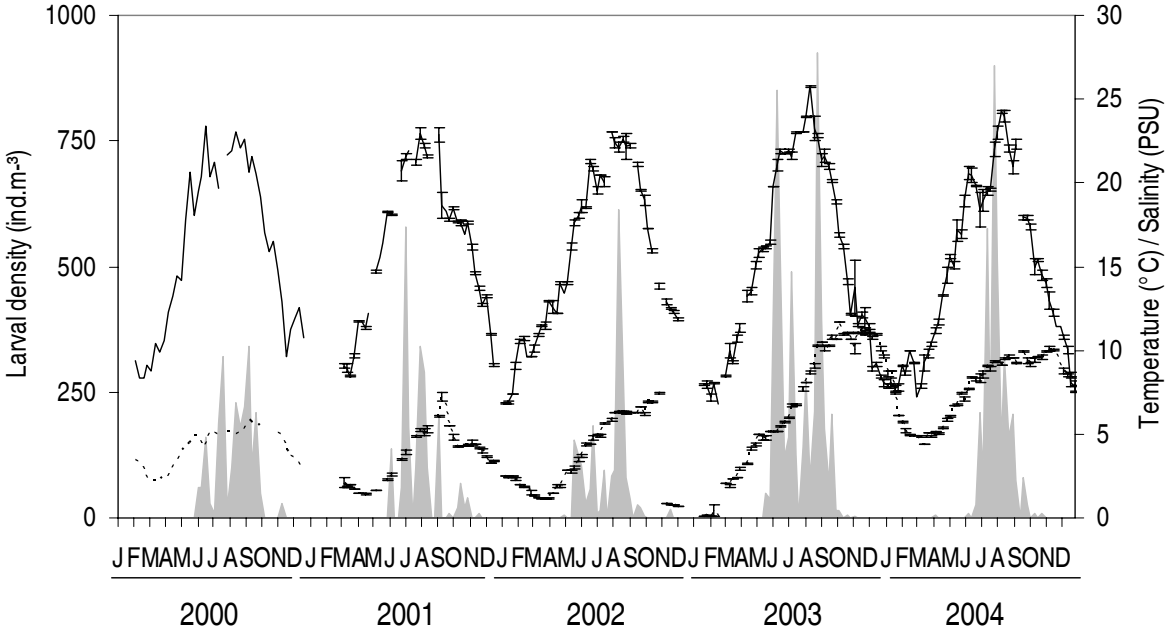

Fig. 3: Seasonal variation in larval arrival of *M. leucophaeata* in the simulated natural water system at BASF, Antwerpen N.V. (full line: temperature (°C), dashed line: salinity (PSU), grey shaded: larval density (ind./m³)).

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).

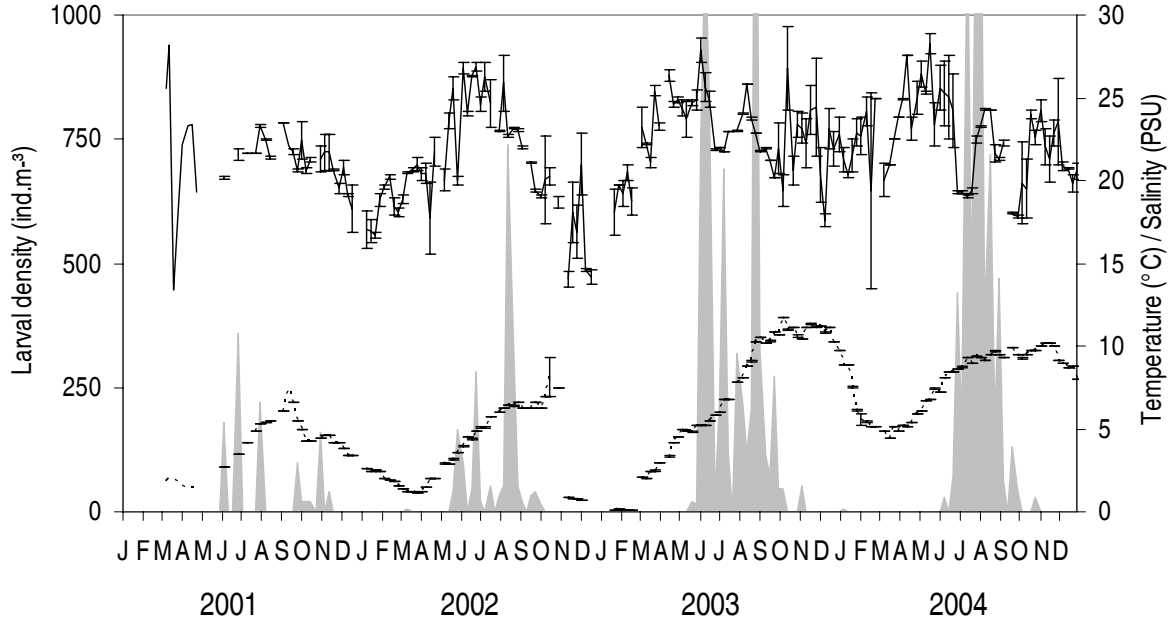

Fig. 4: Seasonal variation in larval arrival of *M. leucophaeata* in the simulated warm cooling water system at BASF, Antwerpen N.V. (full line: temperature (°C), dashed line: salinity (PSU), grey shaded: larval density (ind./m³)).

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 m³ in the simulation system and 1580 larvae per m³ in the natural system.

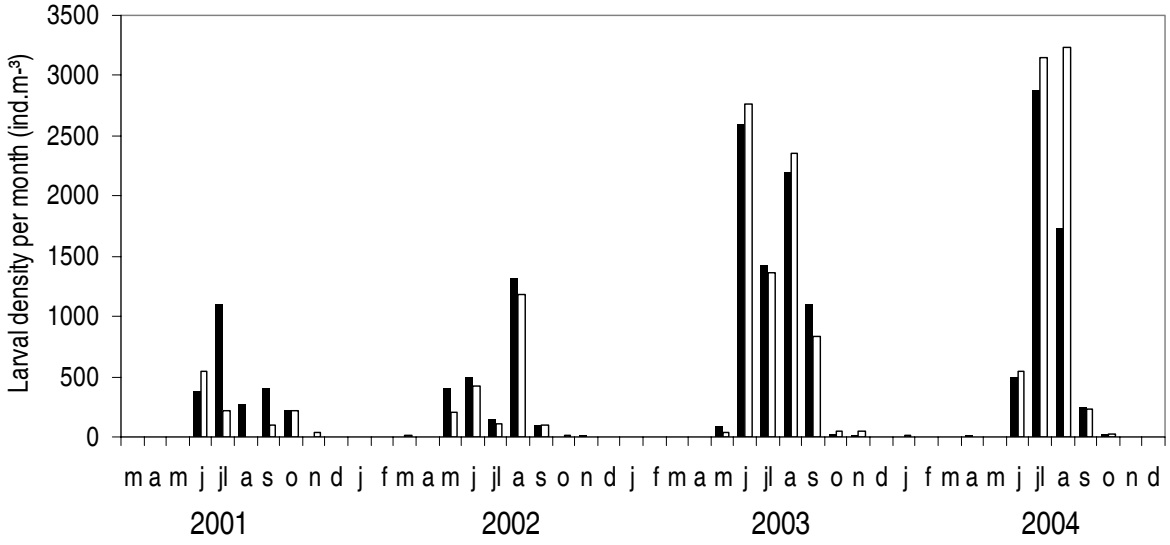

Fig. 5: Monthly comparison of larval abundance of *M. leucophaeata* in the simulated natural and heated conditions. (■ = natural environment; □ = heated system).

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.

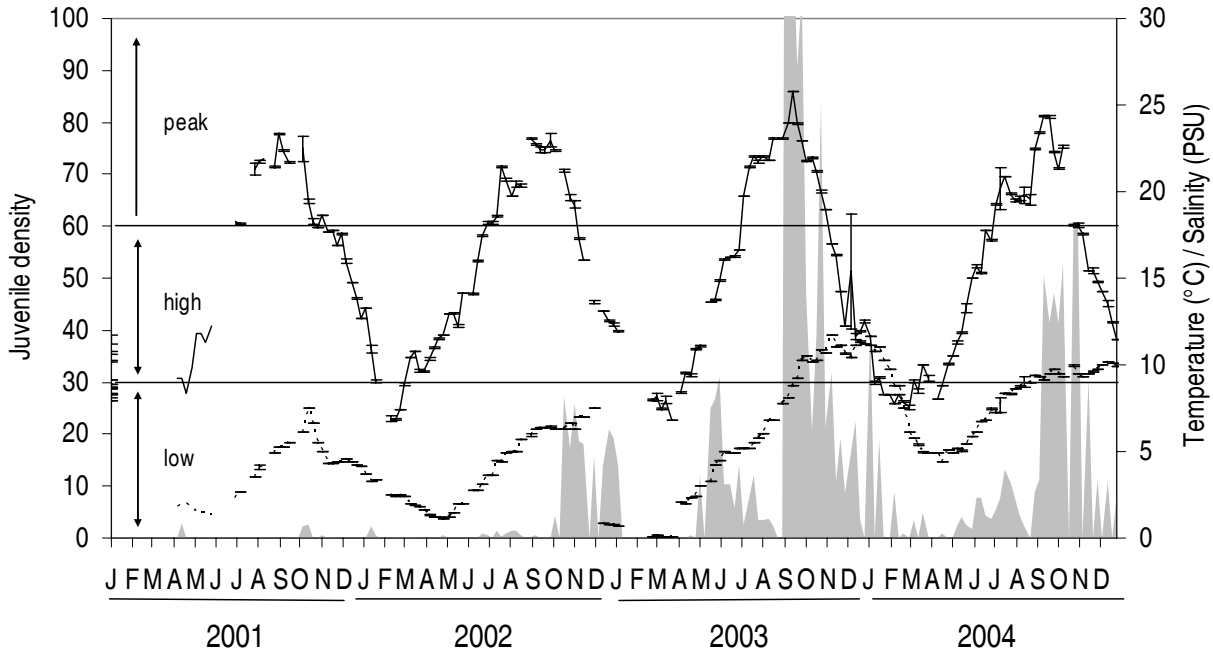

Fig. 6: Seasonal variation in presence of *M. leucophaeata* settlement in the natural water system at BASF, Antwerpen N.V. with indication of the density classes (full line: temperature (°C), dashed line: salinity (PSU), grey shaded: juvenile density).

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.

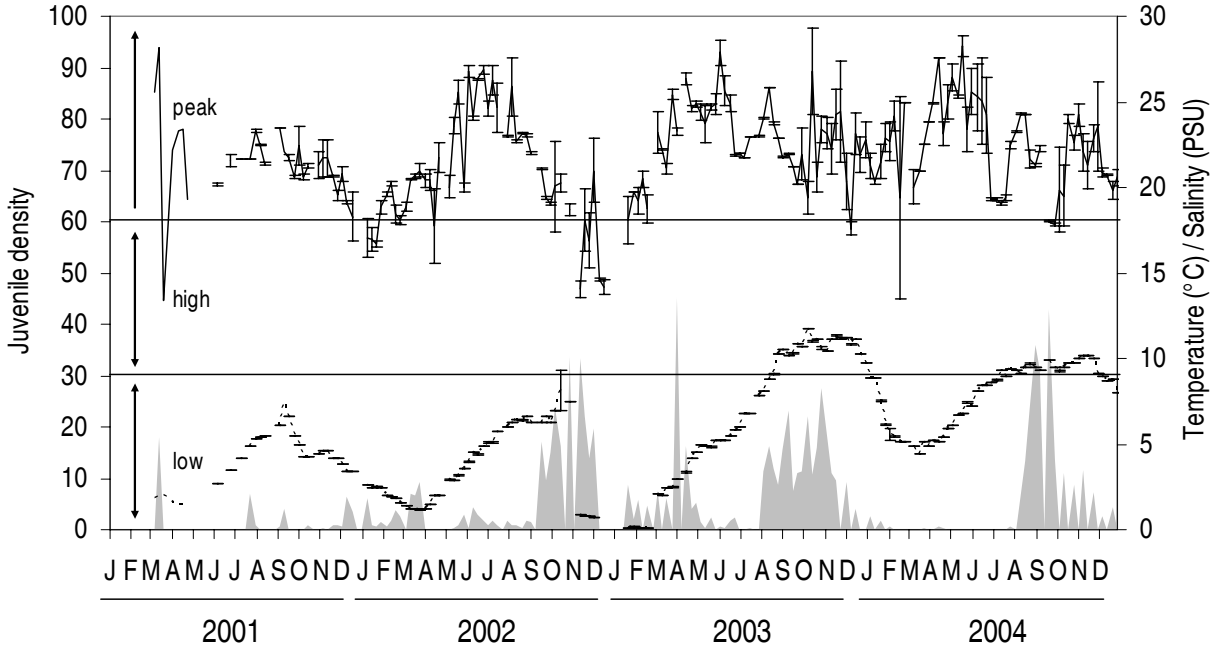

Fig. 7: Seasonal variation in presence of *M. leucophaeata* settlement in the simulated warm cooling water system at BASF, Antwerpen N.V. with indication of the density classes (full line: temperature (°C), dashed line: salinity (PSU), grey shaded: juvenile density).

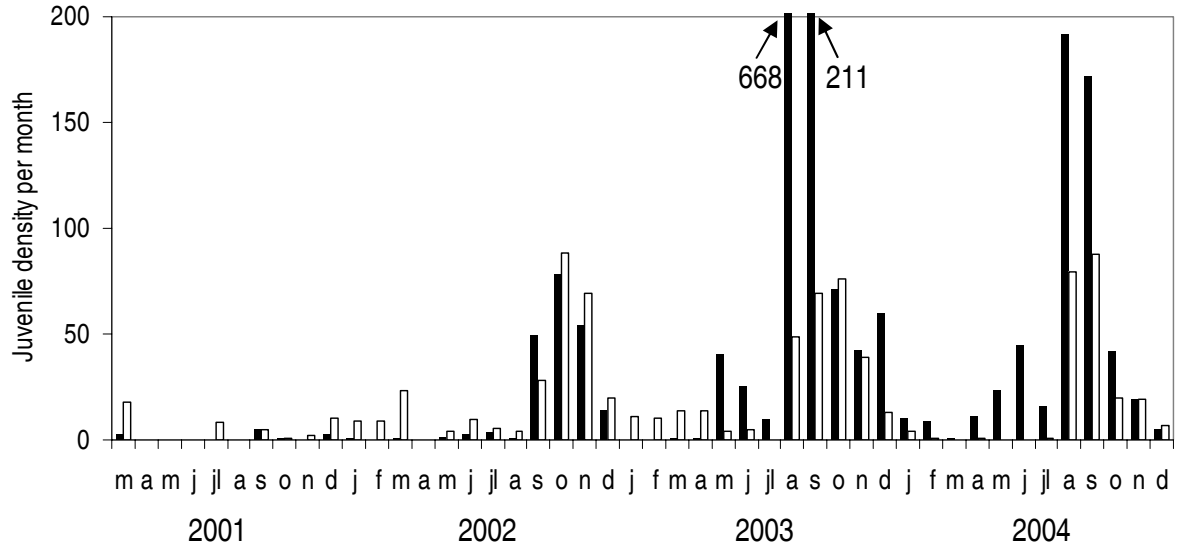

Fig. 8: Monthly comparison of abundance of *M. leucophaeata* settlers in the simulated natural and heated conditions. (■ = natural environment; □ = heated system).

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*.

CHAPTER IV

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

Paper submitted as:

VERWEEN A, VINCX M, DEGRAER S

Seasonal variation in gametogenesis and spawning of *Mytilopsis leucophaeata*,

an invasive bivalve in Europe

Journal of Molluscan Studies

ABSTRACT

This study is the first to report on the reproductive cycle of the invasive brackish water mussel *Mytilopsis leucophaeata*, an important biofouling species in Europe. The habitat preferences and environmental limits of adult *M. leucophaeata* are very broad, which means that, in theory, we can expect this species to invade all brackish water bodies (Verween et al., in press). However, to successfully establish itself in a new habitat, entering this new habitat is not enough; the invader has to be able to reproduce in a successful way.

By means of a histological study of the gonads, seasonal variability in gametogenesis was monitored over two years in the harbour of Antwerp, Belgium. Gametogenesis slowly started late winter (January) and accelerated in spring during the spring plankton bloom until a main spawning period was reached in summer (August). The single uninterrupted spawning period lasted from June to December, during which more than 50 % of all individuals were in an early or late spawning state. This pattern differs from the one observed for the closely related zebra mussel, *Dreissena polymorpha*, which usually displays two separate, but short spawning periods.

As was the case in other mussel species, temperature was found to be highly correlated with gametogenesis in *M. leucophaeata*. The existence of a temperature threshold of 13°C, governing the reproductive cycle in *M. leucophaeata*, was hypothesized since it synchronized well with the onset of the spawning season. Additionally, food concentration might be an important environmental factor governing the timing of gametogenesis and spawning.

KEYWORDS

Mytilopsis leucophaeata, gametogenesis, Westerschelde, temperature, food concentration

INTRODUCTION

Mytilopsis leucophaeata (Conrad, 1831), the brackish water mussel, is a brackish water species with a typically high resistance to environmental changes (Verween et al., in press). The species invaded European waters in the 19th century (Nyst, 1835), but has never been subject to much attention, neither in its place of origin, along the southern coast of the U.S., nor in its newly invaded habitat. However, since the 90s, *M. leucophaeata* has manifested itself as a severely fouling species (Rajagopal et al., 1995; Verween et al., 2005), clogging European industrial cooling water systems, and as such has attracted the attention of the industrial and scientific communities.

The appearance of a non-native species offers an opportunity to study the adaptation of an organism to new environments. In order to be successful, an organism must be able to adapt to appropriate seasonal cues regulating important life history processes, such as reproduction (Garton and Haag, 1993). Assessing potential consequences of an invader and developing successful control strategies thus requires a thorough understanding of its basic life patterns in its new environment. A literature review on *M. leucophaeata* (Verween et al., in press) however showed that knowledge on its life history is almost completely lacking, both within its original habitat and its newly invaded environment.

The seasonal occurrence of *M. leucophaeata* larvae in the water column in the harbour of Antwerp has been shown to be consistent throughout the years, with larvae typically appearing in May – June, showing one or more peaks of abundances in August – September, and disappearing in late autumn (Verween et al., 2005). Until now however, no data were present on the process of gametogenesis, preceding the actual spawning. In the case of European lakes, many studies indicated that populations of the closely related *Dreissena polymorpha* (Pallas), the zebra mussel, demonstrate an annual gametogenic cycle with one or more spawning events during summer and fall and a high degree of gametogenic synchronization (Borcherding, 1991). However, little information is available on the timing of the phases of gametogenesis and how they are affected by the abiotic environment. Numerous studies have identified temperature as a key environmental factor governing the timing of both gametogenesis and spawning in dreissenid mussels (Borcherding, 1991; Fong et al., 1995; Ram et al., 1996; Nichols, 1996; Claxton and Mackie, 1998), such as *M. leucophaeata* and *D. polymorpha*, and also food availability has been suggested as an important regulator (Borcherding, 1991; Nichols, 1996; Ram et al., 1996). For *Mytilus edulis* L. in European waters, repeated spawning has been reported from early

spring to late summer, occurring earlier in the year in warmer waters and progressively later at higher latitudes. These observations can be interpreted as evidence that temperature acts as a principal determining factor in controlling the reproductive cycle (Seed, 1969a; 1975).

Based on data on the larval presence of *M. leucophaeata* in the water column (Verween et al., 1995), we hypothesized that *M. leucophaeata* has only one spawning period in the Schelde river, and as such does not display the bimodal pattern found in other mussel species such as *M. edulis* and *D. polymorpha*. In addition, we investigated the correlation between environmental variables such as temperature, salinity and food availability and the process of gametogenesis.

MATERIAL AND METHODS

All data originated from an industrial site in the harbour of Antwerp, along the Schelde river (51°21.37' N; 4°17.30' E). The Schelde estuary is 160 km long, extending from the mouth at Vlissingen (The Netherlands) to the inland city of Gent (Belgium) (Ysebaert et al., 1993), as such covering the whole salinity gradient from salty to fresh water. The study area is situated in the oligohaline zone where *M. leucophaeata* causes fouling problems. Individual specimens were scraped from the walls of a test-installation, built specifically to allow biological monitoring of *M. leucophaeata* population dynamics in its natural environment. Water temperature (°C) and salinity (PSU) of the incoming water were monitored weekly, by means of field sampling devices. A Profiline conductivity meter LF 197 was used for salinity and temperature measurements, while oxygen content was measured by an Oxi 320 microprocessor oxygen meter. Chlorophyll a concentration (µg/l) was measured from a 500 ml water sample through filtering onto a Whatman GF/F filter and analysing with a HPLC-sampler according to Jeffrey et al. (1997).

Mussels were collected on a weekly basis from April to December 2003 and on a monthly basis from November 2005 to September 2006. All samples were placed on ice and immediately transported to the lab, where they were prepared for histological study. The gonads were removed, fixated in Bouin's fluid and stored in 70 % ethanol. The tissues were embedded in paraffin (60 °C) and sections cut at 5 - 10 µm were stained with toluidin blue (Pearce, 1985). In winter 2003, when gonads were limited in size, the mussels were fixated as a whole, and subsequently decalcified and sectioned. In 2003 the number of monthly successful slides ranged from 10 to 27. In 2005 - 2006, an average of 25 individuals was

brought into slides every month. The slides were analysed using a Leica DMLB microscope at 200x and 400x magnification. A total of 419 mussel slides were examined in this study; the mussel length averaged 13.58 mm ± SD 1.79.

1. CLASSIFICATION OF THE GONAD CONDITION AND MEAN GONAD INDEX

Perhaps the most useful and reliable information concerning seasonal trends in gametogenesis is the information obtained from histological preparations of the gonads (Seed and 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. A classification system of Seed (1969a) described for *M. edulis,* was used to study the gametogenesis in *M. leucophaeata*.

The scale of gametogenic development for both male and female mussels was determined using an original arbitrary classification system, described by Seed for *M. edulis* (1969a). This system has already been successfully applied for *M. edulis* (Kautsky, 1982), *D. polymorpha* (Borcherding, 1991) and *Perna canaliculus* (Gmelin) (Buchanan, 2001). The 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 objectivity. To make the classification as objective as possible, Seed (1969a) used multiple criteria in the assessment of each stage and pictures of each stage in both sexes were compared between *M. edulis* and *M. leucophaeata* (**Fig. 1**) to refine the stages. The system classified the gonad morphological condition in ten reproductive stages. The classification stages included the resting or spent condition (stage 0), the gamete development period (stage 1 – 4), the maximal gonad maturity (stage 5) and the spawning period (stage 6 – 9). The major characteristics of each stage are given in **Table I**.

The Mean Gonad Index (MGI), defining the breeding condition of any sample (Seed, 1969a), was determined by multiplying the number of individuals in each stage by its numerical score (deduced from the arbitrary rating of the stages 0 - V) 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 5, when all individuals are sexually mature. An increase of the MGI indicates a period of development in gonadal tissue, while a decrease of the MGI indicates a period of active spawning (Seed, 1975).

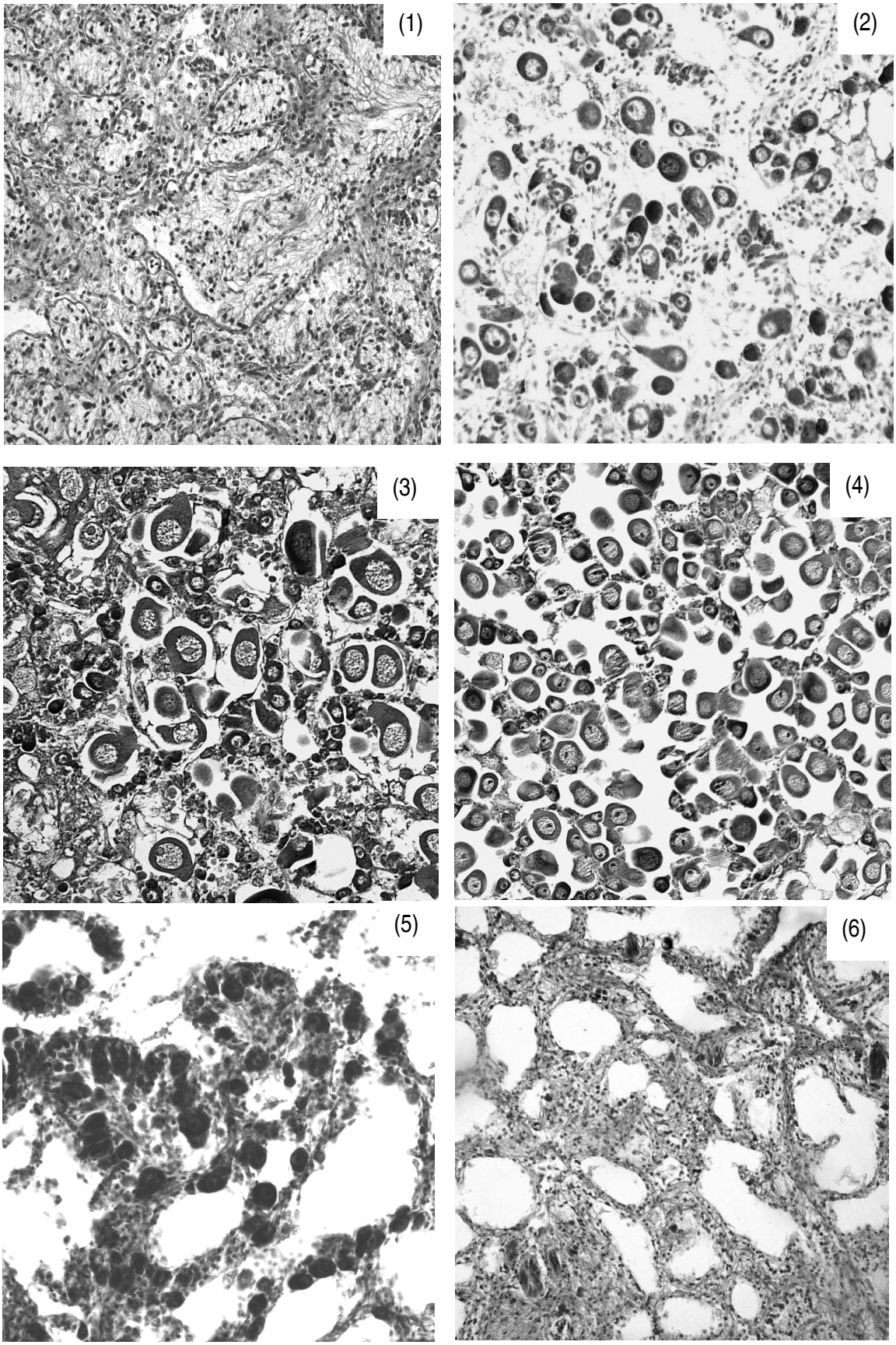

Fig. 1a: Micrographic photos of female gonads of *Mytilopsis leucophaeata* at various stages in the gametogenic cycle (10x20x): (1) Developing I; (2) Developing III; (3) Developing V; (4) Spawning III; (5) Spawning I; (6) Spent.

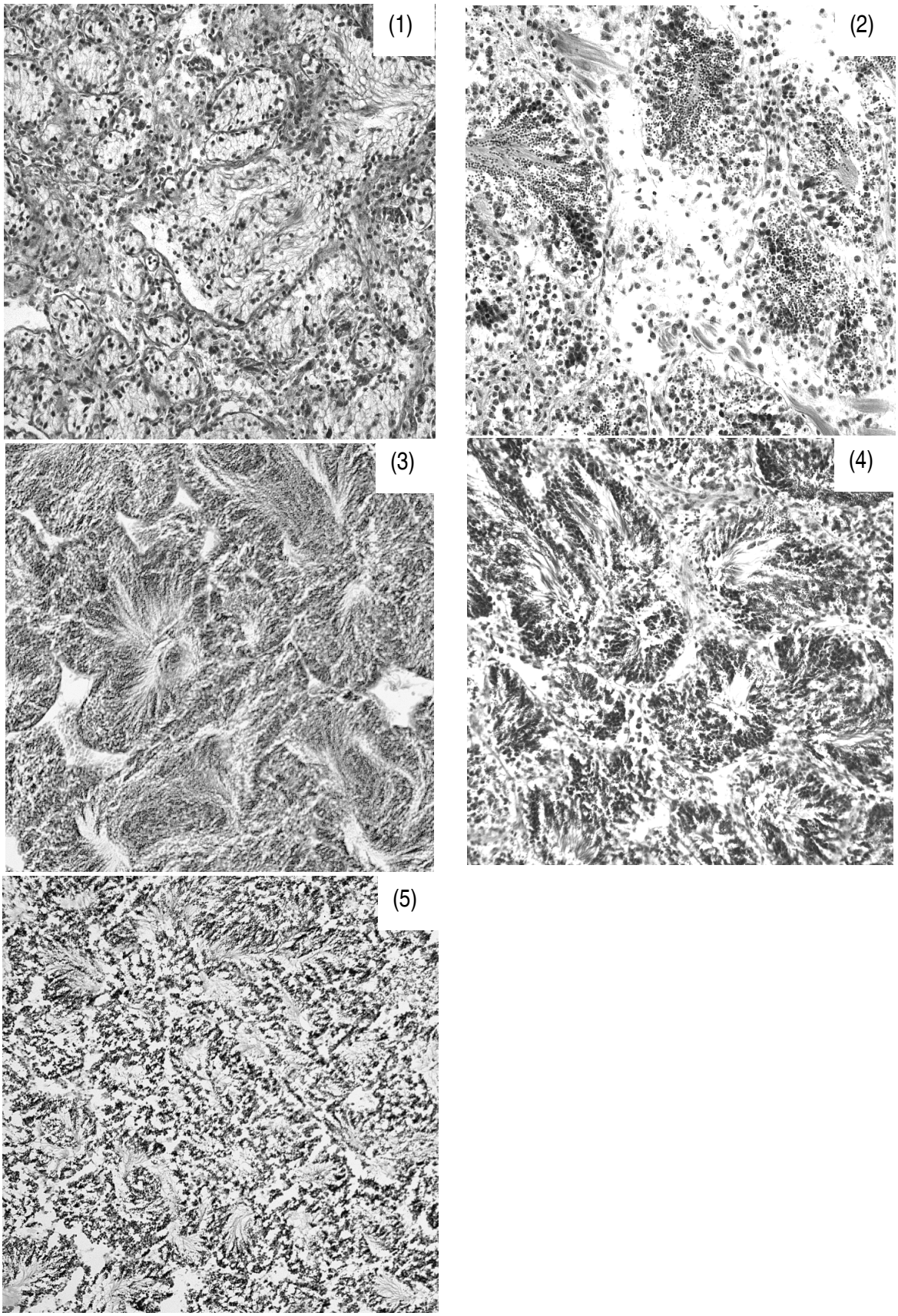

Fig. 1b: Micrographic photos of male gonads of *Mytilopsis leucophaeata* at various stages in the gametogenic cycle (10x20x): (1) Developing I; (2) Developing III; (3) Developing V; (4) Spawning III; (5) Spawning I.

2. DATA ANALYSIS

In this study, data of two years were combined to investigate the seasonal variability within the gametogenic process of *M. leucophaeata*, taking into account annual variation. Maximal overlap of the data was chosen to estimate the yearly error margin of the gametogenic cycle as accurate as possible. Homogeneity of variance and normality were tested using the Bartlett's test and the Shapiro-Wilk's W-test, respectively. Monthly and yearly differences in gametogenic stages of *M. leucophaeata* were tested by analysis of variance (ANOVA). Statements of significant differences were based on accepting $p < 0.05$. Univariate Pearson correlation matrices were used to determine correlations between the environmental variables and the MGI. Multiple regressions were conducted to examine the relationship between the environmental variables and the MGI. Regression and correlation analyses were done using the statistical software SAS 9.1 (SAS Institute Inc., 2004).

Table I: Microscopic classification system for *Mytilopsis leucophaeata* gonads, according to Seed (1969a) and Buchanan (2001).

	Stage	General description
0	Resting or spent	No trace of sexuality; stores of fat and glycogen are accumulated in the connective tissue, frequently obscuring genital canals.
1	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.
2	Developing II	Ripe gametes appear in centre of follicles, although mainly occupied by early stages of gametogenesis.
3	Developing III	Follicle size increases; follicles half filled with ripe gametes, half with early stages.
4	Developing IV	Almost maximal proliferation of follicles; general reduction of early stages of gametogenesis and increase of ripe gametes.
5	Developing V	Fully ripe gonad; ova compacted into polygonal configuration and male gonads distended with ripe sperm arranged in compact laminae.
6	Spawning IV	Active discharge of gametes in progress, but follicles still relatively full; reduction of follicle pressure induces rounding of ova and loss of laminae appearance in males
7	Spawning III	Follicles approximately half full with mature gametes but with relatively few early development stages; in females, eggs are rounded rather than polygonal.
8	Spawning II	Follicles are considerably less than half full with mature gametes.
9	Spawning I	Follicles collapsing; only residual gametes remain, sometimes undergoing cytolysis; centre of follicles can be filled with yellow-brown matrix of cytolysis.

RESULTS

1. SEASONAL PATTERN IN GAMETOGENESIS OF *MYTILOPSIS LEUCOPHAEATA*

Factorial ANOVA (year and month) indicated no significant differences in MGI development between 2003 and 2006 (p = 0.15). Therefore, the MGI and relative dominance of the developmental conditions of both years could be combined to investigate the average seasonal variation in gametogenesis of *M. leucophaeata* in the harbour of Antwerp.

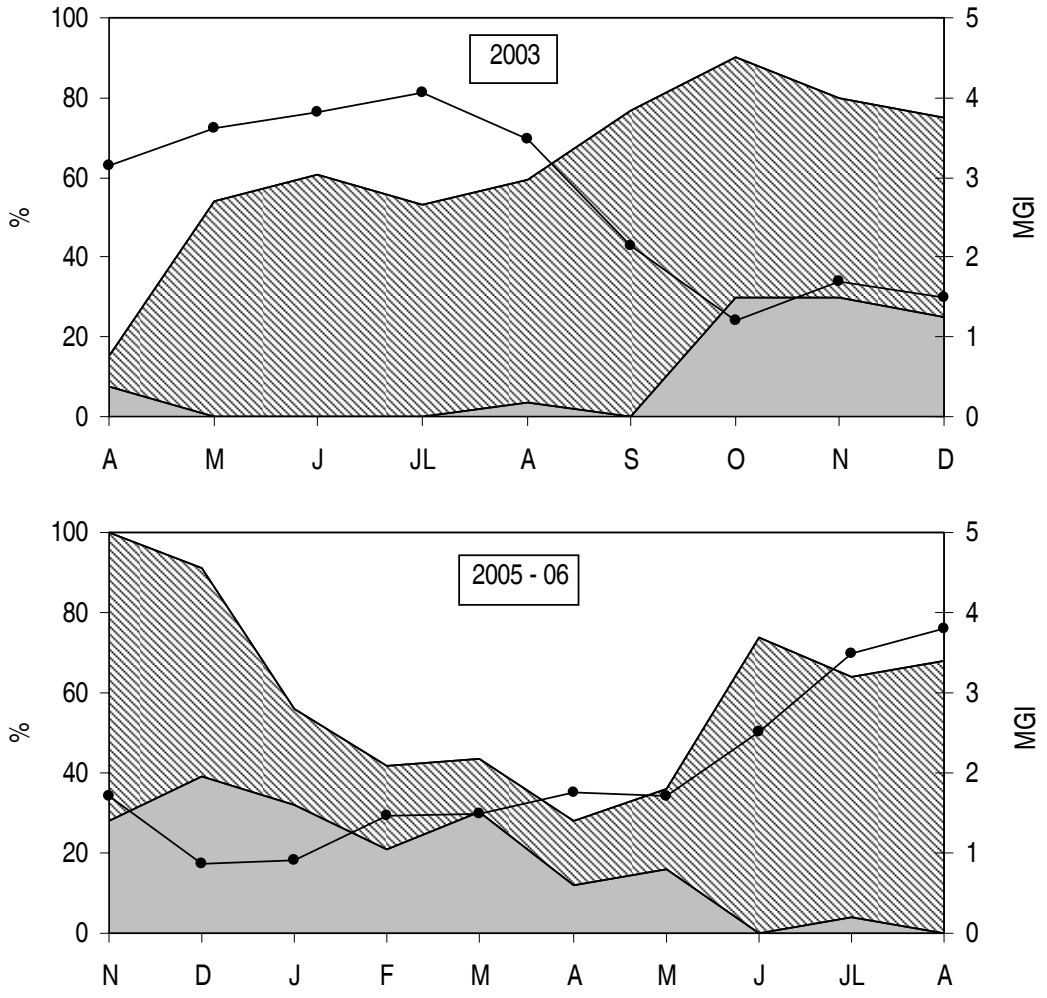

Fig. 2: Breeding cycle of *Mytilopsis leucophaeata*, as defined by the average MGI over the two monitored periods, with total percentages in the spawning, developing and spent conditions (● = average MGI; white surface = developing; striped = spawning; grey = spent).

The gametogenic cycle was subject to interannual variation, however with a similar seasonal pattern throughout the years (**Fig. 2**). No significant monthly year-to-year variation in the total percentages of the three gonad conditions were found in either one of the monitored periods (p = 0.92). The MGI showed the lowest values in winter (December MGI = 1.5 in 2003 and 0.87 in 2006; January MGI = 0.92 in 2006), indicating that most individuals were spent, and increased slowly in early spring (March MGI = 1.48) and than faster in late spring – early summer, especially clear in 2006 (March - April MGI 2006 = 1.62 ± SE 0.1 to June MGI 2006 = 2.52). The highest MGI was reached in July 2003 and August 2004 with respectively MGI = 4.07 and 3.80. This period could be interpreted as being the main spawning period for *M. leucophaeata*. In early autumn, the MGI rapidly decreased again towards the winter minimum as can be observed for 2003.

The analysis of the gonadal conditions confirmed this pattern of gametogenesis and provided more details. In 2006, the redevelopment of the spent or resting gonads started in January – February, with an average of 51.2 % ± SE 7.2 of the individuals under developing conditions, but still 26.4 % ± SE 5.6 as resting or spent. In the following months, the percentage of individuals with resting gonads decreased and the percentage of developing gonads reached it maximum at 72 % in April. In 2003 as well as in 2006, from May on, the redevelopment percentages decreased, and many individuals began to spawn. The percentage of spawning individuals reached a maximum in June (67.4 % ± SE 6.5), but values still exceeded 50 % until December. Only a small part of the individuals were already spent or resting in the summer period (average 2.4 % ± SE 1.5). In 2003, from October on, a high percentage of individuals was spent (30.4 %), with only little gonads being in development (10.0 %).

The most significant feature of the annual spawning season of *M. leucophaeata* was its duration; the period over which more than 50 % if the individuals were spawning extended over 6 months. Thus although only a single spawning period was detected, it lasted for a very long time.

2. CORRELATION WITH THE ENVIRONMENT

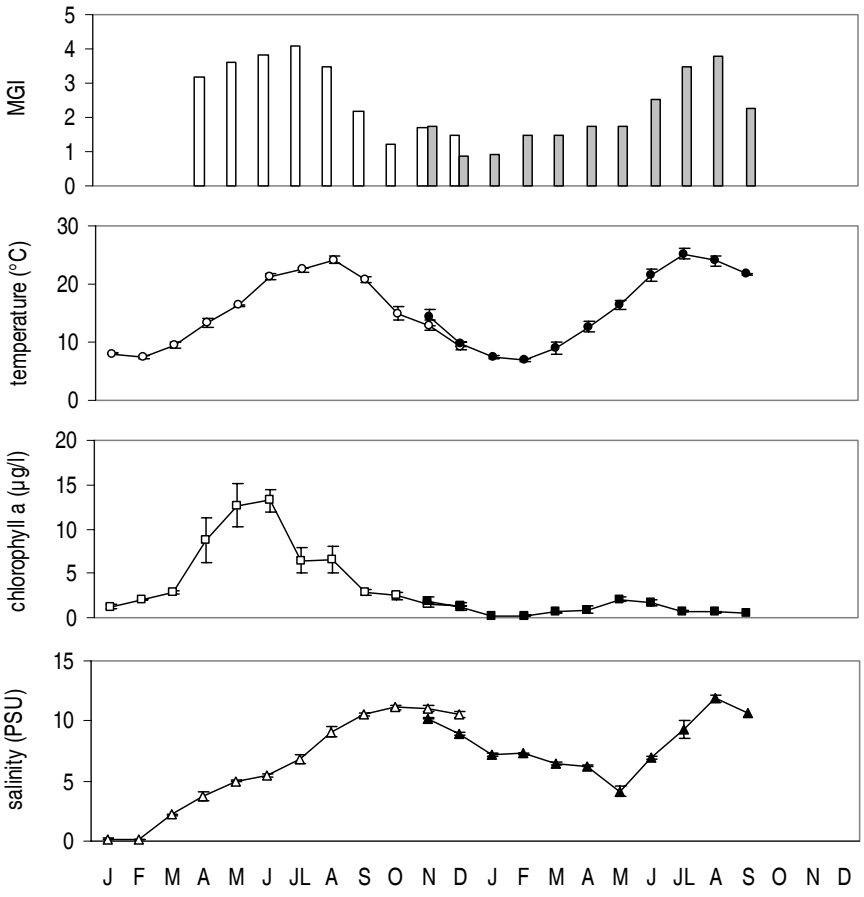

Fig. 3: Seasonal changes in MGI over the two monitored years in *Mytilopsis leucophaeata* and the corresponding changes in environmental factors ± SE (Δ = 2003; ▲ = 2006).

Since there was a significant difference in environmental variables between both monitoring periods (ANOVA $p < 0.05$), the correlation analyses between MGI and the environmental variables were separated between both years. Comparison of the results of the MGI with the average environmental conditions of the water in both years (**Fig. 3**) revealed that the rise in MGI clearly coincided with a rise in temperature, in both monitoring periods. The temperature gradually rose from minima of 7.2 ± 0.3 °C in February to maxima of 24.2 ± 0.6 °C in August 2003 and 25.2 ± 0.9 °C in July 2006. The highest MGI (MGI = 3.77 ± 0.3), however, did not coincide with maximal temperatures, but occurred somewhat earlier in 2003 (July) and later (August) in 2006. When the average MGI was compared with the water conditions, highly significant positive correlations between MGI and temperature (Pearson r = 0.81; $p < 0.0001$) and between MGI and chlorophyll a concentration (r = 0.65; $p = 0.002$) were detected (**Table III**).

Table III: Results of Pearson correlation analysis with indication of the significance level (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$).

	Temperature	Chlorophyll a	Salinity	MGI
Temperature		0.33	0.13	0.81 ***
Chlorophyll a			-0.49 *	0.65 **
Salinity				-0.19

A significant difference in the chlorophyll a concentration was found between both monitoring periods (ANOVA $p = 0.004$) although the same general pattern was observed in both years; the maximal MGI was not reached before the spring chlorophyll a peak was observed in the water column (maximum of 13.2 µg/l in June 2003; maximum of 2.1 µg/l in May 2006). The chlorophyll a concentration intercorrelated with salinity ($r = -0.49$; $p = 0.03$). For salinity, no clear pattern nor a correlation with MGI ($r = -0.19$; $p = 0.45$) was found. The multiple regression (*Y = 0.85 * temperature - 0.30 * chlorophyll a*) was highly significant ($R = 0.86$; $p < 0.000$) with temperature and chlorophyll a being significantly contributing variables (temperature: $p = 0.0000$; chlorophyll a: $p = 0.033$).

DISCUSSION

1. SEASONAL PATTERN IN GAMETOGENESIS OF *MYTILOPSIS LEUCOPHAEATA*

In the harbour of Antwerp, *M. leucophaeata* displayed a strong seasonal pattern in its reproductive cycle. The annual variation was limited: no significant differences in patterns were observed between both monitoring periods. The gametogenesis started in January with a slowly increasing trend in late winter, accelerating through spring until the main spawning period for *M. leucophaeata* was reached in June. The data on larval abundances of *M. leucophaeata* (Verween et al., 2005) endorse this pattern with larvae appearing in the water column from June onwards, 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 approximately 3 – 4 weeks (Mackie and Schloesser, 1996). The Mean Gonad Index (MGI) decreased rapidly after this spawning season, reaching a minimum in early winter. Remarkable in this MGI pattern is the occurrence of only one spawning period. In many mussel species, the 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). This pattern of repeated spawning periods, recognized as multiple peaks in MGI, was recorded in European waters for *M. edulis* (Seed, 1975). Also *D. polymorpha* generally has multiple spawning periods in Europe (Borcherding, 1991; Nichols, 1996; Jantz and Neumann, 1998), usually coinciding with two distinct peaks in larval densities.

Although only one spawning period occurred in *M. leucophaeata*, its duration was extremely long. During more than half of the year, from June to December, over 50 % of all individuals were in an early or late spawning state. This pattern of prolonged spawning season deviates from the general patterns found in other mussels. In *D. polymorpha*, the release of gametes is a highly synchronized event, which is focused on a 1 – 2 week period (Nichols, 1996). Most studies concerning this species concluded that spawning can occur any time during a 1 to 2 month period when gametes are fully developed, depending on environmental conditions (Sprung, 1987; Borcherding, 1991). However, the process of spawning can exceptionally be completely non-synchronous in *D. polymorpha*, occurring throughout the year (Nichols, 1996). In *M. edulis*, short spawning seasons have also been recorded (Chipperfield, 1953; Seed, 1975), but extended spawning periods from direct gonad examinations of *M. edulis* were occasionally found (Johnstone, 1898; Battle, 1932, both in Seed, 1969a).

2. CORRELATION WITH THE ENVIRONMENT

Because of the highly significant positive correlation between temperature and the MGI in this study (Pearson r = 0.81; $p < 0.0001$), it should be concluded that temperature might be an important factor governing gametogenesis in *M. leucophaeata*. This pattern supports the theory that temperature is the main regulator of gametogenesis in mussels (Sprung, 1983; Seed and Suchanek, 1992). Gametogenic index increased in early summer with increasing water temperature. Following spawning, gametogenesis commenced 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 like in *D. polymorpha* (Garton and Haag, 1993) and *M. edulis* (Lubet, 1959; Kautsky, 1982).

Literature indicated the presence of a threshold temperature of 12 °C in *D. polymorpha*, governing the onset of the spawning season (Walz, 1978a; Sprung, 1987; Borcherding, 1991; Ram et al., 1996; Jantz

and Neumann, 1998). The existence of a temperature threshold has been accepted for many bivalve species (e.g. 10 – 12 °C in *Mytilus edulis* and *Mya arenaria*; 15 – 16 °C for *Ostrea edulis* and *Pecten irradians*; 20 °C for *Crassostrea virginica*; see review by Mackie 1984 in Borcherding 1991). Previous studies already suggested a threshold temperature of 13 ± 1 °C for gamete maturation in *M. leucophaeata* (Verween et al., 2005). This was confirmed by the analysis of the gametogenic process; it was only after a temperature of 13 ± 0.3 °C was reached in April that the percentage of spawning individuals rapidly increased (**Fig. 2**), indicating the onset of the synchronized spawning season.

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 temperature was still low at that time. In this study, the MGI also increased rapidly during the spring phytoplankton bloom after a very slow rise in winter. The maximal MGI was reached only after this bloom. Food quantity and quality, expressed as the chlorophyll a concentration, might be an important determinant in *M. leucophaeata* gametogenesis ($r = 0.65$; $p = 0.002$), although less important than temperature. Numerous studies 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 et al., 1985; Seed and Suchanek, 1992). Multiple regression of all contributing factors endorsed this pattern, with temperature ($p = 0.000$) as well as chlorophyll a ($p = 0.033$) being significantly contributing factors. It can be suggested that both factors, the presence of a temperature threshold and the food concentration, may be more or less simultaneously, although not equally important, responsible for the onset of spawning.

Although literature data on the impact of salinity on the mussel reproduction are very scarce, we also incorporated salinity in this study. Yet, no significant correlation between salinity and the MGI could be found ($r = - 0.19$; $p = 0.45$). This absence of a 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. Yet, the lack of information on the effect of salinity on gametogenesis in other mussel species can also indicate the insignificance of this variable in the reproductive cycle, independent of the species' nature.

CONCLUSIONS:

IMPLICATIONS FOR FUTURE INVASIONS BY *MYTILOPSIS LEUCOPHAEATA*

The habitat preferences and environmental limits of adult *M. leucophaeata* are very broad, which means that, in theory, we can expect this species to invade all brackish water bodies (Verween et al., in press). However, to successfully establish itself in a new habitat, entering a new habitat is not enough; the invader has to be able to reproduce in a successful way.

The presence of a prolonged spawning period in the life cycle of *M. leucophaeata* is an advantageous feature for successful invasion. Species which spawn more or less continuously might be expected to have a more stable adult population than seasonally breeding species, since damage caused by unsuccessful recruitments is likely to be reduced (Seed and Brown, 1977). Therefore, we can hypothesize that *M. leucophaeata* has the capacities to become a better invader on a long term than *D. polymorpha*.

Temperature seemed to be an important factor limiting the colonization of new invaders. The onset of the spawning season and consequently also the timing of the whole reproductive cycle depends on the rise of the temperature above a temperature threshold (Borcherding, 1991). The temperature threshold of 13 °C for the onset of the gametogenic processes in *M. leucophaeata* can confine the limits of successful establishment. However, even with this disadvantageous characteristic, the species will still develop higher establishment capacities than its relative, *D. polymorpha*, which has a slightly lower temperature threshold of 12°C.

ACKNOWLEDGEMENTS

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CHAPTER V

Growth patterns of *Mytilopsis leucophaeata*, an invasive biofouling bivalve in Europe

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Growth patterns of *Mytilopsis leucophaeata*, an invasive biofouling bivalve in Europe

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ABSTRACT

For the first time, growth in *Mytilopsis leucophaeata* (Conrad), an important fouling species in Europe, was investigated. By means of growth cages, individual shell growth of three cohorts - with respectively initial shell length ≤ 5 mm, 10 mm and 15 mm - was monitored in the harbour of Antwerp (Belgium) during 2003 – 2004. *Mytilopsis leucophaeata* followed an oscillatory growth pattern with a single summer growing period per year (May – August). Growth decreased during wintertime, but never ceased completely. *Mytilopsis leucophaeata* has an average growth rate of less then 3 to 6 mm/year. Temperature was found to be the main environmental factor affecting growth. Von Bertalanffy growth function was used to model growth of individuals ≤5 mm, resulting in L_∞ = 16.7 mm and K = 0.56. Based on a combination of growth of all three cohorts, hypothetical growth of an average individual mussel could be modeled over a 5 year period, resulting in a maximum length > 19 mm with a growth constant of 0.41. Its longevity (more than five years) and positive effect of higher water temperatures on growth, combined with its high resistance to chlorination, provides *M. leucophaeata* a high potential for severe and long-lasting biofouling.

KEYWORDS

Mytilopsis leucophaeata, growth, Westerschelde, temperature

INTRODUCTION

The brackish water mussel, *Mytilopsis leucophaeata* (Conrad, 1831) (syn. *Congeria cochleata* Kickx in Nyst, 1835), a mytiliform bivalve (Mollusca, Bivalvia, Veneroida, Dreissenidae), is resistant to a wide range of oligo- to mesohaline conditions (1 – 18 PSU (Practical Salinity Units)). *Mytilopsis leucophaeata* originates from the southern coast of the U.S. to Tampico, Mexico and was first detected in European waters in 1835 in the harbour of Antwerp (Belgium). In North America the species invaded the Hudson River in New York in the 1930s (Rehder, 1937) and was recently found in the Upper Mississippi River (Koch, 1989) and Southern New England (Smith and Boss, 1996). In Europe, it has been found in brackish water bodies along the North Sea coasts from Germany (Therriault et al., 2004) through the Netherlands (Rajagopal et al., 2002b) and Belgium (Verween et al., 2005) into France (Bamber and Taylor, 2002) and in the UK (Oliver et al., 1998) and has recently been reported in the Guadalquivir river in Spain (Escot et al., 2003), the Black Sea basin (Therriault et al., 2004) and the northern Baltic Sea (Laine et al., 2006) (**Fig. 1**). Although *M. leucophaeata* is known in Europe for over 170 years, it is recently spreading rapidly throughout this part of the world mainly by means of ballast water discharges from transoceanic shipping (Oliver et al., 1998). *Mytilopsis leucophaeata* is invading Europe more slowly than *Dreissena polymorpha* in the U.S. (Verween et al., in press) with dispersal being mainly human-induced.

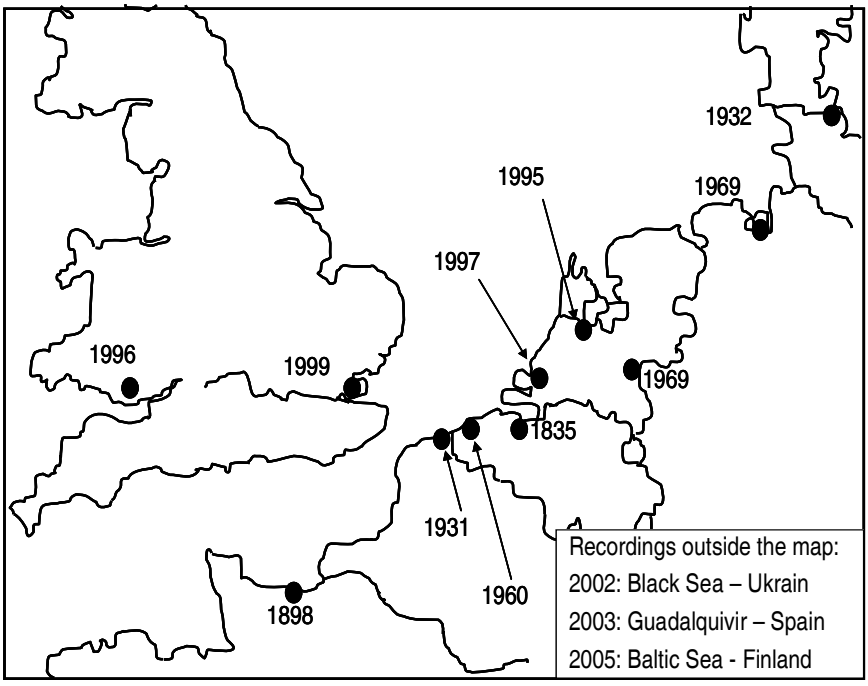

Fig. 1: Distribution of *Mytilopsis leucophaeata* in Europe, with data of first recordings.

Mytilopsis leucophaeata and the freshwater zebra mussel, *D. polymorpha* (Pallas, 1771) are both Dreissenidae with a very similar life cycle. Especially the free-swimming larvae are an important invasion tool for both species. The planktonic D-shaped veligers stay in the water column for about two weeks before settlement (Ackerman et al., 1994) and as such can easily survive transportation from one region to another. Furthermore, being a brackish water species, *M. leucophaeata* larvae and post-larvae are euryhaline and capable of development to metamorphosis at 10 to 32 PSU (Siddall, 1980). After settlement, the post-larvae develop a protective shell, which grows stronger as the individuals become larger. Because of this protective shell, they become highly resistant to external circumstances, including anti-fouling agents. The fact that they inhabit brackish waters makes them far more resistant to environmental changes than freshwater species, being a significant fouling pest in cooling water systems of numerous industrial plants (Rajagopal et al., 1997; Verween et al., 2005) and in potable and service-water pipes (Bamber and Taylor, 2002). Since individuals become more resistant to anti-fouling agents, such as chlorine, as they grow larger (Rajagopal et al., 2002b), knowledge on the growth of *M. leucophaeata* as a function of environmental conditions is indispensable in developing efficient control measures.

Next to its economic impact, the invasion of pest bivalves, such as *M. leucophaeata* can also have important ecological consequences. *Dreissena polymorpha* invasions for instance may lead to decreased plankton biomass resulting in decreased turbidity and increased growth of benthic plants, while some benthic invertebrates may be adversely affected, with an altered food web and shifted ecosystem as result (MacIsaac, 1996).

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. We hypothesize that *M. leucophaeata* has definitely the potential of becoming the brackish water equivalent of *D. polymorpha* in Europe.

The aims of this study were therefore:

- To investigate growth rate of different size classes of *M. leucophaeata;*
- To analyse the influence of environmental factors on the growth of *M. leucophaeata;*
- To model shell growth of *M. leucophaeata*.

MATERIALS AND METHODS

1. STUDY SITE AND EXPERIMENTAL SETUP

All field work was conducted at the site of BASF, Antwerpen N.V. (Belgium), along the Schelde river. The industrial plant is situated at the right bank in the harbour of Antwerp, near the Dutch border (**Fig. 2**), and receives water of intermediate salinity (1 – 12 PSU) coming from the Westerschelde river and from the Rijn-Schelde channel.

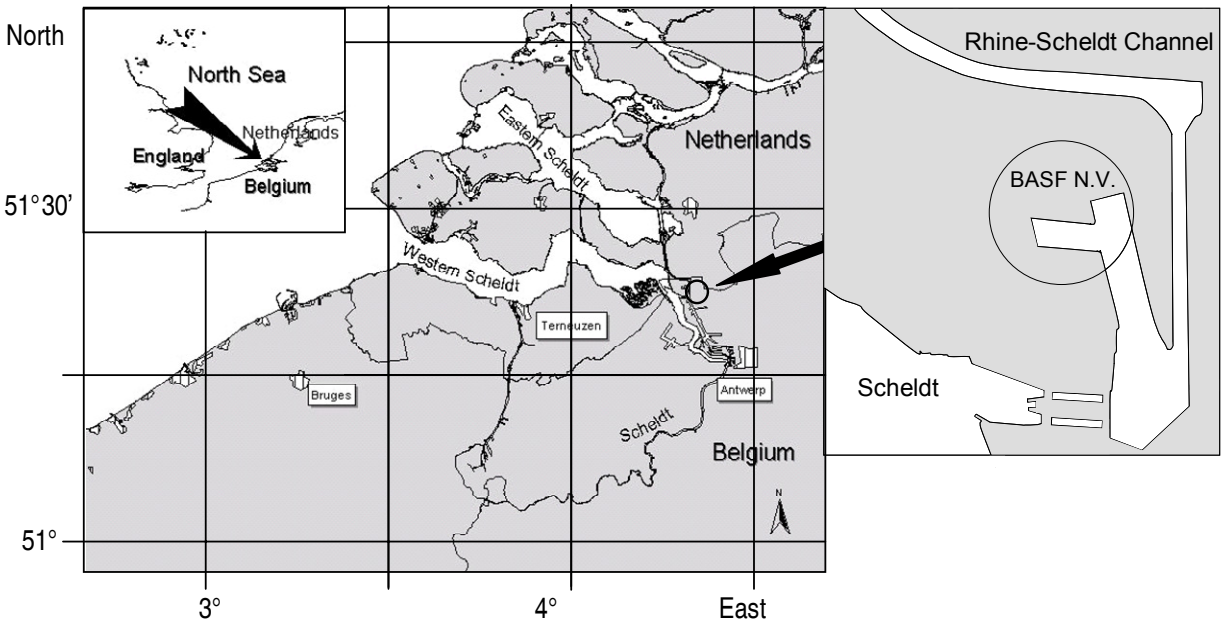

Fig. 2: Map of Westerschelde river with indication of the study site.

Biofouling problems with the brackish water mussel at BASF Antwerpen N.V. were first detected in 1998. During summer, the cooling water conduits of the industrial plant take in up to 80 000 m³ of 1 mm-filtered, but untreated river water per hour and in wintertime, an average flow of 30 000 m³ of water per hour occurs. Larvae can thus enter the system together with the extracted water, where they may attach onto substrates, such as the heat exchangers and the tubes in the conduits. Peak larval densities exceeded 1500 individuals per m³ (Verween, unpublished data).

Water is pumped into the cooling water system at two intake points, at a distance of one kilometre from each other. At each intake point, a control installation for biofouling hazard was built. These installations received water from the dock, but were disconnected from the biocide-usage in the rest of the system, as such making it possible to study growth of *M. leucophaeata* under non-toxicological circumstances.

Each installation consisted of three PVC-tanks in which an average water flow of 1 m^3 h^{-1} was generated. In each of the PVC-tanks, growth cages were suspended in such a way that full water circulation through the cages was allowed. Each growth cage was divided into twenty individual growth chambers. To prevent individuals from escaping and to allow water circulation through the cage, the top and bottom of each chamber consisted of a plastic net with mesh size of 1 mm.

The use of growth cages could reduce growth as a result of a reduced water circulation due to clogging of the cage perforation and as such limiting food (Karatayev et al., pers comm). In the present study, meshes were cleaned biweekly to avoid clogging and ensuring a continuous water flow, similar to that outside the cages. Garton and Johnson (2000) have proven that changing the mesh size and the design of the growth cages had no significant effect on the individual shell growth. This implies that the mussels in the cages are not negatively influenced by their cloistering and that they experience about the same circumstances as 'free' individuals.

2. GROWTH MEASUREMENTS

To determine growth and the influence of the environment, two factors need to be considered: (1) size dependency of shell growth rate and (2) individual variation of shell increment (Jantz and Neumann, 1998). To include this variation, mussels from three different cohorts were collected. In February 2003 mussels with various sizes were collected from the dock and gently rinsed. The mussels were kept in a mesh bag suspended in the control installation, before being placed in the growth cages. Shell length (i.e. maximum dimension along the anterior-posterior shell axis) was measured to the nearest 0.01 mm using an electronic vernier calliper. Based on their initial shell length (ISL), individuals were allocated to three size classes: smaller than or equal to 5 ± 1 mm ($ISL_{\leq 5}$), equal to 10 ± 1 mm (ISL_{10}) and equal to 15 ± 1 mm (ISL_{15}). Growth was monitored for one hundred twenty mussels: two installations, each with three PVC-tanks, leading to six growth cages, each filled with 20 mussels. Each cohort was initially represented by 40 individuals. Length of all individuals was measured monthly between February 28th 2003 and December 21st 2004. Growth is expressed as shell length increment (SLI) and calculated from following equation:

$$SLI\ (\mu m/day) = (SL_{t+1} - SL_t) / dt \quad \text{(Jantz and Neumann, 1998)} \qquad (1)$$

with SL_{t+1} as the final shell length (µm), SL_t the initial shell length (µm) and dt the time (in days) between t and $t + 1$. Average SLI within each size class was expressed as the average of the individual SLIs of the size class (µm/day).

Water temperature (°C), salinity (PSU) and oxygen content (mg/l) of the incoming water were monitored weekly, by means of field sampling devices. A Profiline conductivity meter LF 197 was used for salinity and temperature measurements, while oxygen content was measured by an Oxi 320 microprocessor oxygen meter. Chlorophyll a concentration (µg/l), from 2003 on was measured from a 500 ml water sample through filtering onto a Whatman GF/F filter and analysing with a HPLC-sampler according to Jeffrey et al. (1997). Chlorophyll a data from 1999 until 2002 were derived from Schaar van Ouden Doel, nearby the monitored dock (chemical measuring network MWTL). ANOVA indicated no significant difference in chlorophyll a concentrations between both sample points.

3. GROWTH ANALYSIS

Growth (SLI) between years and installations was compared using the Mann-Whitney U-test, between mussel groups by means of Kruskal-Wallis test. Regression analysis between SLI and ISL was conducted and univariate Pearson correlation analysis was used to search for correlations between environmental variables at time t and SLI at time $t+1$. Multiple regression was conducted to examine the relationship between the environmental variables at time t and SLI at time $t+1$. Because of its highest growth rates and consequent most obvious growth pattern, the growth class with $ISL_{\leq 5}$ was considered to determine the influence of the environmental factors on the individual growth rate. Regression and correlation analysis were conducted with the statistical software SAS 9.1 (SAS Institute Inc., 2004). Because of the same reasons, the maximal length and growth rate were determined for growth class with $ISL_{\leq 5}$. The length increment for *M. leucophaeata,* as for other species showing seasonal oscillations in growth, was described by the Von Bertalanffy growth function (VBGF):

$$L_t = L_\infty * (1 - e^{-K(t-t0) + (CK/2\pi) \sin [2\pi (t-ts)]}) \quad \text{(Gayanilo et al., 1989, modified from von Bertalanffy, 1938)} \quad (2)$$

where L_t is the predicted length at age t, L_∞ is the asymptotic length, K is the growth rate, C determines the amplitude of the seasonal growth oscillation, t_s is the starting point of the oscillation and t_0 the theoretical age at zero length. The values of the five parameters were estimated by means of non-linear

estimation with the least squares method as provided by the statistical software package STATISTICA 5.5 (Statsoft, 2000).

An independent estimate of L_∞ and K was obtained using the Ford-Walford method in which length and age were rearranged as data pairs consisting of length at a specific time $t = n$ and length at a succeeding time $t = n + 1$ (Gulland, 1983). The time difference between the length measurements (dt) was kept constant. Based on the linear regression equation $y = ax + b$, L_∞ and K were estimated as $L_\infty = b / (1 - a)$ and $K = (-1 / dt) * ln(a)$.

Since there was no significant difference in growth rate between years (see results), hypothetical shell growth of an average individual mussel could be modeled over five year classes (last 6 months of year class 0 to year class 5), in which $ISL_{\leq 5}$ was considered to represent growth in the last 6 months of the year classes 0 to year class 2, ISL_{10} the last 6 months of year classes 1 to year class 3 and ISL_{15} the last 6 months of year classes 3 to year class 5. The first 6 months of year class 0 could not be included because our investigation was initiated in February, while year class 0 individuals can only be found from July onwards. To avoid a bias due to the minor growth and high mortality in ISL_{15} (see results), Von Bertalanffy parameters were also calculated without this cohort.

RESULTS

1. GROWTH RATE

Mann-Whitney U-test indicated no significant difference in mussel growth between the two test-installations ($p = 0.11$) and between 2003 and 2004 ($p = 0.37$). Kruskal-Wallis analysis showed a high significant difference in SLI between growth classes ($p = 0.002$).

For cohorts $ISL_{\leq 5}$ and ISL_{10}, the SLI performed a seasonal oscillation, in which a yearly period of peak growth, with average SLI higher than 10 µm/day, could be distinguished. In 2003, this growing season started in May and ended in August (average $SLI_{\leq 5}$ = 30 µm/day, average SLI_{10} = 14 µm/day), while in 2004, peak growth took place from May until July (average $SLI_{\leq 5}$ = 23 µm/day, average SLI_{10} = 15 µm/day). During wintertime, almost no growth was observed, with minimum shell growth of 1 µm/day during November 2004 (2003: average $SLI_{\leq 5}$ = 7 µm/day, average SLI_{10} = 2 µm/day; 2004: average

$SLI_{\leq 5}$ = 4 µm/day, average SLI_{10} = 6 µm/day). Maximum shell growth was recorded in June 2003, with $SLI_{\leq 5}$ = 58 ± 11 µm/day and SLI_{10} = 28 ± 11 µm/day.

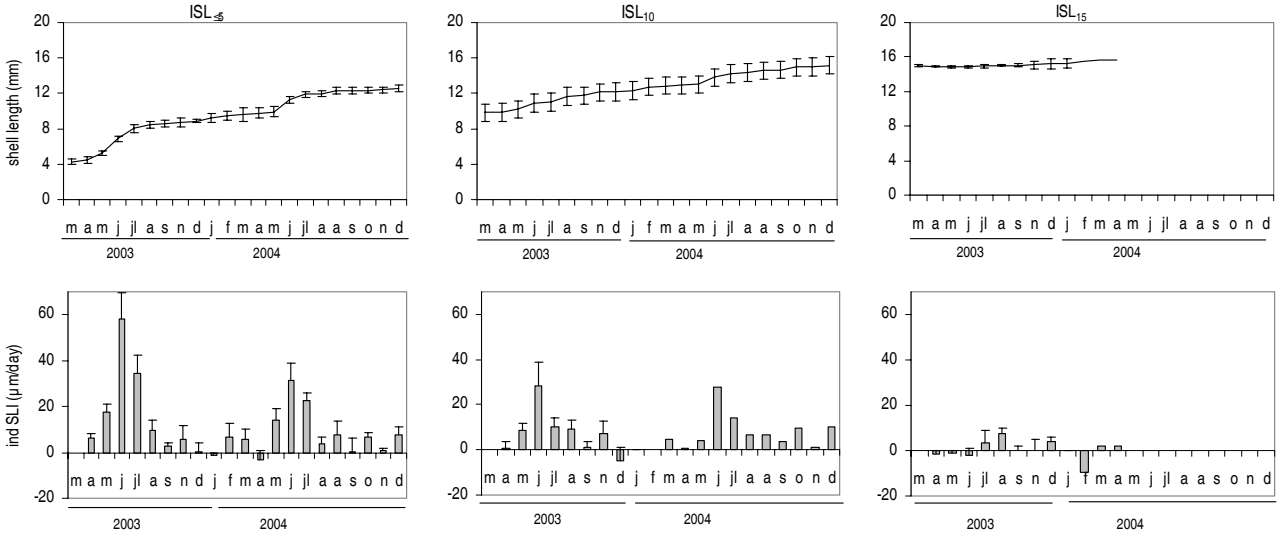

Fig. 3: Seasonal changes in shell length ± SE and SLI ± SE in 2003 – 2004 in three size classes (SLI = shell length increment).

During the entire growth monitoring period individuals with $ISL_{\leq 5}$ grew from an average of 4.2 mm to 12.6 mm in 22 months with an average shell increment (SLI) of 11.4 µm/day, whereas individuals with ISL_{10} grew from 9.8 mm to 15.2 mm with an average SLI of 7.5 µm/day (**Fig. 3**). The larger individuals (ISL_{15}) died within a year. In this length class, no distinct growing periods could be observed, and almost no growth occurred (average SLI_{15} = 0.39 µm/day; maximum value = 3.5 µm/day in June 2003).

Correlation analysis resulted in a negative relationship between ISL and SLI (r = 0.98; *p* = 0.09), indicating a size-dependency of shell growth. The decrease in shell growth with increasing shell length was linear with the linear regression model explaining 99.9 % of variation in shell growth for the growing season (May – August) and 97.1 % for the yearly average growth.

2. CORRELATION WITH THE ENVIRONMENT

Comparing the results of the shell length increment with the environmental conditions of the water (**Fig. 4**), the start of the growing season (May – June) coincided with the maximal monthly increase in temperature and salinity. Temperature and salinity rose from 16 °C to 21 °C and from 4.8 PSU to 6.0 PSU in May 2003 and from 15 °C to 21 °C and from 6.7 PSU to 8.0 PSU in June 2004. Highest SLI

however did not coincide with the maxima of these variables, but occurred earlier (June). Maximal temperature occurred in August (23.2 °C in 2003, 23.0 °C in 2004), maximal salinity in September – October (11.0 PSU in 2003 and 9.6 PSU in 2004).

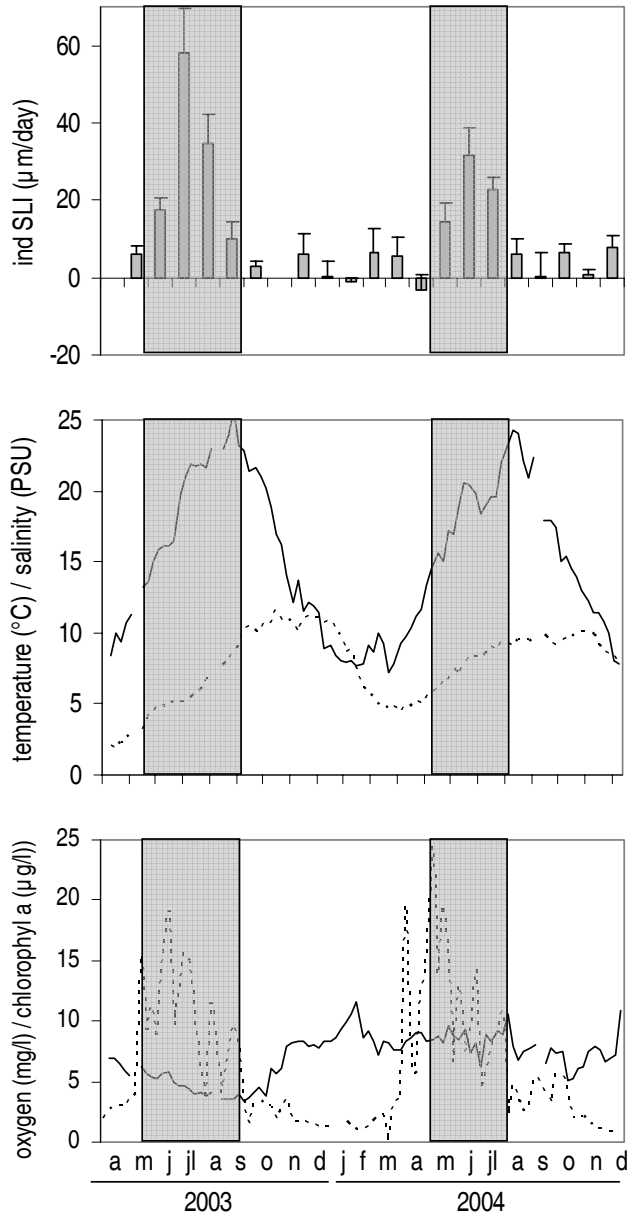

Fig. 4: Seasonal changes in SLI in *Mytilopsis leucophaeata* ± standard errors and the corresponding changes in environmental factors (dark squares: growing season; full line: temperature and oxygen content; dashed line: salinity and chlorophyll a).

When statistically comparing the average individual growth rate with the conditions of the water, a highly significant positive correlation of SLI with temperature (Pearson r = 0.69; p = 0.0008) was detected (**Table I**), however not with salinity (r = - 0.01; p = 0.97) nor with the oxygen content of the water (r = -

0.39; p = 0.09). The duration of the growing season further seemed to be correlated with the quantity of algal food in the water: growth did not occur before a first chlorophyll a peak in the water column (maximum of 15.6 µg/l on 15th April 2003; maximum of 24.4 µg/l on 27th April 2004) and ceased directly after the last chlorophyll a peak (7.6 µg/l on 19th August 2003; 10.9 µg/l on 27th July 2004). However, there was no significant relationship between SLI and chlorophyll a content (r = 0.22; p = 0.34) and none of the variables were significantly intercorrelated (max. correlation coefficient: 0.39). Multiple regression was significant (p = 0.02) with one significantly contributing variable, temperature (p = 0.006).[1]

Table I: Results of Pearson correlation analysis with indication of the significance level (*** $p < 0.001$).

	Temperature	Salinity	Oxygen content	Food	SLI
Temperature		0.27	- 0.30	0.39	0.69***
Salinity			0.20	- 0.36	- 0.01
Oxygen content				- 0.30	- 0.39
Chlorophyll a					0.22

3. SHELL GROWTH MODELING

The Von Bertalanffy growth function, modeled on the growth cohort $ISL_{\leq 5}$ gave a maximum length (L_∞) of 16.7 ± 1.2 mm with a growth constant (K) of 0.56 ± 0.09. Seasonal growth oscillation is rather small (C = 0.11). The function coincided well with the observed data points: 98.7 % of the variance in data points of the cohort was explained by the growth function. All parameters of the Von Bertalanffy growth function were highly significant ($p < 0.0001$). The Ford-Walford method yielded slightly different values for L_∞ and K for growth cohort $ISL_{\leq 5}$ (L_∞ = 14.5 mm; K = 1.09) (**Fig. 5**) and explained 96.9 % of the variance in the data points.

Because after 12 months (March 2004) growth of cohort $ISL_{\leq 5}$ showed a great overlap with cohort ISL_{10} at the start of the monitoring (March 2003), and no significant difference in length between both years was detected (Mann-Whitney U-test: p = 0.36), both cohorts could be merged and growth could be modelled continuously over a period of 34 months. Since a gap is present when merging cohorts ISL_{10} and ISL_{15} and cohort ISL_{15} showed little or no growth and these individuals died within a year, merging

[1] Multiple regression with only one significant variable does not give any extra information in this study and is thus unnecessary.

this cohort was approached with prudence. As such, we reproduced the hypothetical minimal growth of *M. leucophaeata* spanning 5 year classes of its life cycle.

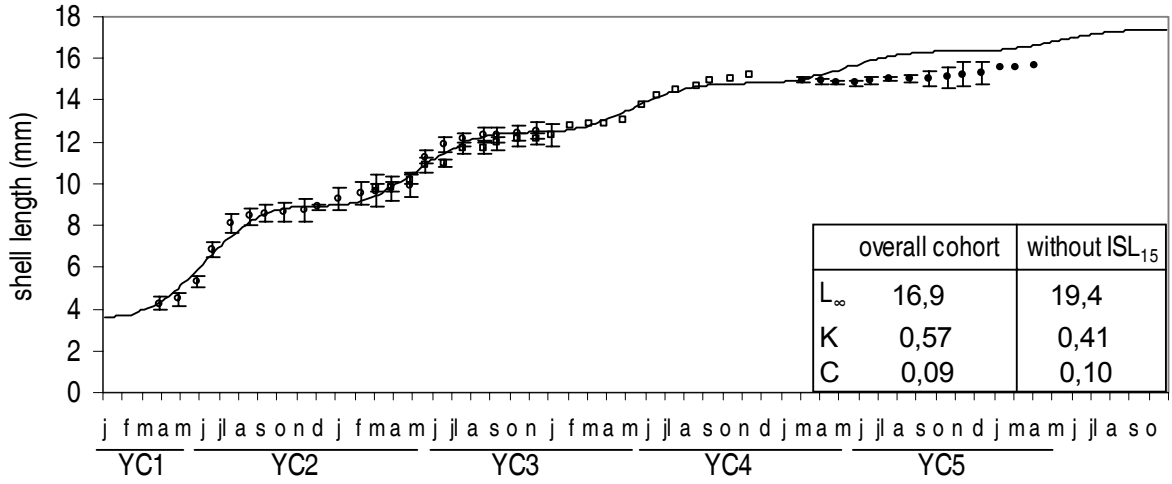

Fig. 5: Graphical presentations of the von Bertalanffy growth function excluding ISL_{15} and measured data points over five year classes ± SE. L_∞: asymptotic length, K: growth constant, C: amplitude of seasonal growth oscillation. The VBGF parameters have been calculated for the overall cohort and the cohort excluding ISL_{15}.

The Von Bertalanffy growth function of the overall benthic growth of *M. leucophaeata* coincided very well with the observed data points, as 98.8 % of the variance in data points of the overall cohort was explained by the growth function. The cohort led to a maximum length (L_∞) of 16.9 ± 0.24 mm with a growth constant (K) of 0.57 ± 0.03. The seasonal growth oscillation is small (C = 0.09). Excluding cohort ISL_{15}, a maximum length of 19.4 mm was calculated with a growth constant of 0.41. The seasonal growth oscillation remained small (C = 0.10) and variation was attributed for 98.8 % by the model.

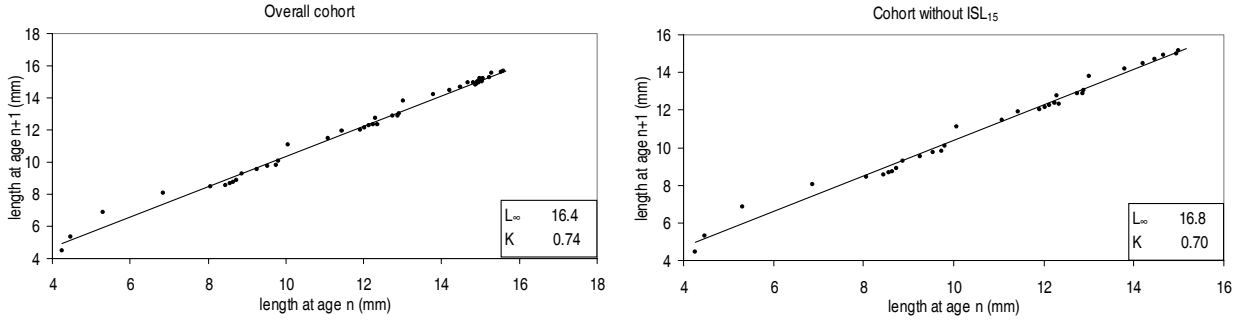

Fig. 6: Graphical presentation of the Ford-Walford method for the estimation of L_∞ and K. L_∞ = intercept / (1 – slope); K = (-1/dt)*ln (slope).

The Ford-Walford method yielded comparable values for L_∞ and K for the overall growth cohort (L_∞ = 16.4 mm; K = 0.74) (**Fig. 6**) and explained 99.2 % of the variance in the data points. Excluding ISL_{15}, estimates showed only minor changes: L_∞ = 16.8 mm, K = 0.70 with 98.7 % of the variance explained.

DISCUSSION AND CONCLUSIONS

1. GROWTH

Growth in mussels is particularly seasonal, with little or no growth during winter (Seed, 1969b), as for numerous species living in environments with a distinct annual cycle in temperature and/or light conditions (Brey, 1999). Similarly in this study, growth of *M. leucophaeata* also occurred oscillatory, comprising a single period of major growth (late spring till summer), identified here as growing season, and a period of minor growth (autumn and winter) per year. During the growing season, SLI showed a unimodal distribution, with maximum growth in June and July. This observation falls well within the range of variation in growth patterns, observed for *D. polymorpha*, in which maximum growth rate was found to vary from the very beginning of the growing season (May-June) (Smit et al., 1992; Garton and Johnson, 2000) to the end of the growing season (Morton, 1969).

Yearly average growth rate for *M. leucophaeata*, measured here, varied from about 3 to 6 mm/year, which is quite low compared to *D. polymorpha* (yearly average growth rate: 15 to 20 mm/year) (Mackie, 1991; Mackie and Schloesser, 1996).

Although *M. leucophaeata* tended to grow more slowly on a yearly basis, it never ceased growing completely, whereas a full growth stop had already been demonstrated for *D. polymorpha* (Morton, 1969; Bij de Vaate, 1991). Because 6 °C (Bij de Vaate, 1991) to 10 °C (Mackie, 1991; Jantz and Neumann, 1992; Smylie, 1994) is considered to be the lower temperature limit for growth and development of the closely related *D. polymorpha*, a full growth stop in this species can be expected during North-western European winters. Being a subtropical species, the slow, though continuous growth in *M. leucophaeata* in winter, when temperature dropped as low as 6.3 °C, should thus be interpreted as a result of its eurytopic nature. Indeed, habitat preferences and environmental limits of *M.*

leucophaeata are proven to be very broad (Smith and Boss, 1996; Oliver et al., 1998; Bamber and Taylor, 2002; Escot et al., 2003).

During our monitoring period, the onset of spawning in *M. leucophaeata* was detected in May 2003 and June 2004 (Verween et al., 2005). The present study indicated that the growing season of *M. leucophaeata*, identified here as a period of growth with SLI higher than 10 µm/day, started after this onset of spawning. This can be explained by availability of an energy-surplus for somatic growth after a period of gametogenesis. Add the high temperature during summer and the large quantity of food available in the water column, and enough energy can be present to induce a (somatic) growth spurt (Dorgelo, 1993).

Because (1) shell growth was positively correlated with temperature ($r = 0.69$; $p = 0.0008$), (2) no significant correlation between growth rate and chlorophyll a concentration was found ($r = 0.22$; $p = 0.34$) and (3) chlorophyll a concentration was only weakly correlated to temperature ($r = 0.39$; $p = 0.08$), it should be concluded that mainly temperature is regulating shell growth of *M. leucophaeata*. Multiple regression, showing a significant effect, endorsed this pattern with only temperature as significantly contributing variable ($p = 0.006$) in shell growth. Next to temperature, food quantity and quality can however be considered to probably represent the second most important determinants of mussel growth, as already demonstrated for the bivalves *Mytilus provincialis* (Ceccherelli and Rossi, 1984) and *M. edulis* (Frechette and Bourget, 1987). A similar pattern of environmental (somatic) growth control could also be demonstrated for *D. polymorpha*: temperature as the most important controlling variable (Griffiths et al., 1991; Karayücel, 1996; McMahon, 1996) and generally a positive relationship between growth and chlorophyll a concentration (Walz, 1978b; Jantz and Neumann, 1992; Sprung, 1995).

Shell growth rate (SLI) of *M. leucophaeata* was strongly dependent on the initial shell length (ISL), with (1) smaller individuals growing faster than larger individuals and (2) a near complete cessation of growth for individuals larger than 15 mm. Such length-dependency of growth, also detected for other bivalves, such as *D. polymorpha* (Neumann et al., 1993) and *M. edulis* (Seed, 1969b), is believed to be caused by (1) a lower metabolic activity in older individuals or (2) a relatively stronger increase in body mass over body length, which would require longer periods of feeding in order to maintain enough energy to grow (Seed, 1969b).

2. SHELL GROWTH MODELING

Modeling of shell growth cohort $ISL_{\leq 5}$ according to the seasonal Von Bertalanffy growth function indicated a maximum theoretical length of *M. leucophaeata* of 16.7 mm with an average growth constant K of 0.56. Although this L_∞ corresponds with the somewhat lower maximum length of 14 mm of *M. leucophaeata*, found in the nearby North Sea Channel near the Hemweg and Velsen power station (The Netherlands) (Rajagopal et al., 1995), many larger individuals were found in the harbour of Antwerp (personal observations). The different values of the Ford-Walford model (L_∞ = 14.5 mm; K = 1.04) can be explained by the fact that Ford-Walford is a linear model-fitting technique (Sun et al., 2001), being less suitable in modelling seasonal growth. Szypula (1987) also proved that the Ford-Walford model yielded the least reliable results in modelling length growth in various fish species in comparison to other models.

Because only small individuals have been taken into account in this Von Bertalanffy growth modeling, a surplus value was given to the study by means of modeling a hypothesized shell growth of an average individual mussel over 5 year classes in the Schelde river near Antwerp. The overall Von Bertalanffy growth function indicated (1) a maximum theoretical length of 17 mm and (2) an average growth constant K of 0.57. However, when excluding the cohort ISL_{15} from the model, a higher maximal length (19.4 mm), but lower growth constant (0.41) was predicted. Both predictions seem reliable, because measurements of large individuals, collected in the harbour of Antwerp, revealed several individuals with a length of about 17 mm up to a maximum, though rare length of 26 mm.

Table II: Overview of maximum shell length measurements of *Mytilopsis leucophaeata*, according to literature

Max. length (mm)	Region	Reference
10 - 20	U.S.	Abbott, 1974 Emerson and Jacobson, 1976 Pennak, 1978
22	Miami, U.S.	Siddall, 1980
14	North Sea Channel, The Netherlands	Rajagopal et al., 1995
27	The Netherlands	Gittenberger et al., 1998
20	Cardiff Docks, England	Oliver et al., 1998
15.2	Thames, England	Bamber and Taylor, 2002
17-22	Schelde, Belgium	Verween et al., in press

Historic American identification guides on shells describe a size range from 1 to 2 cm for *M. leucophaeata* (Abbott, 1974; Emerson and Jacobson, 1976; Pennak, 1978). More detailed information on the species (**Table II**) shows that the average maximal length indeed is about 20 mm, but generally, smaller individuals (10-15 mm) are found in the field. The larger sizes like 27 mm, described by Gittenberger et al. (1998) can be considered exceptionally large for this species. Compared to *D. polymorpha* ($K = 0.808$; $L_\infty = 40.5$ mm) (Conides et al., 1997), *M. leucophaeata* in the harbour of Antwerp thus seem to grow slower and smaller. However, the growth constant K is not extremely low in comparison to many populations of other bivalves, with K-values ranging from 0.07 to 0.97 (Bachelet, 1980, 1981; Urban and Campos, 1994; Walker and Heffernan, 1994; Ramón et al., 1995), and is average when compared to other mussel species, as *Mytilus edulis* ($0.02 \leq K \leq 1.46$ in Dolmer, 1998), *Perna perna* ($0.31 \leq K \leq 0.7$ in McQuaid and Lindsay, 2000) and *Limnoperna fortunei* ($0.33 \leq K \leq 0.38$ in Maroñas et al., 2003). *Mytilopsis leucophaeata* is however a very small mussel species, respective to other mussels, with L_∞ being 32 - 283 mm, 66 - 117 mm and 36 mm for respectively *M. edulis* (Dolmer, 1998), *P. perna* (McQuaid and Lindsay, 2000) and *L. fortunei* (Maroñas et al., 2003).

The modeling study at hand of the overall growth cohort renders an estimate of the minimum longevity of *M. leucophaeata* in the Schelde river of almost 5 years, when growth starts to decrease dramatically and individuals become rare. Cessation of growth however does not necessarily indicate the maximum longevity: slow growth in larger individuals – almost impossible to measure - together with their rareness thus makes it difficult to estimate the true longevity of *M. leucophaeata* in the harbour of Antwerp. Estimation of mussel longevity has also proven to be very difficult: for *D. polymorpha*, life span estimates varied from 3 to 19 years (Karatayev et al., pers comm). Recent data on European populations however report a maximum longevity between 2 and 4 years (Conides et al., 1997; Chase and Bailey, 1999), while North American populations have a shorter life span of 1.5 to 2 years (Mackie and Schloesser, 1996). It is because of these phenomena that data consisting maximum life span of *M. leucophaeata* presented here are just a first indication, and that further research would be needed to come to definite proof.

3. BIOFOULING CONSEQUENCES

Rajagopal (1997, 2003) carried out shell valve movement experiments using three species of mussels: a freshwater mussel *D. polymorpha*, a marine mussel *M. edulis* and a brackish water mussel *M. leucophaeata*. Adult mussels were subjected to continuous or intermittent chlorination at different concentrations. Their lethal and sublethal responses were compared to those of control mussels and

shell valve activity was monitored. These studies have proven that *M. leucophaeata* is much more tolerant to chlorination than the other mussel species, as such endorsing the fact that *M. leucophaeata* can become a major fouling problem once established. Again, being a brackish water species with broad habitat preferences raises its defensibility against external conditions, such as environmental changes (Verween et al., personal observations) as well as biocides.

This study on the population dynamics of *M. leucophaeata* extends the number of arguments for a severe biofouling potential of *M. leucophaeata*;

1. This study and many others demonstrate that temperature is the most important factor in determining (shell) growth in mussels, with in general, an increase in growth with a rise in temperature over the ecological range of the species (Bayne and Worrall, 1980). By definition, the water temperature within cooling water systems is (slightly) higher than in the surrounding water, resulting in cooling water systems inducing a higher growth rate of *M. leucophaeata*. Similarly, according to Karatayev (1995) the growth of *D. polymorpha* was significantly higher in heated cages than in the unheated zone. Hence, higher temperature waters should be considered as an advantageous environment, implying the presence of an energy surplus for any physiological process in *M. leucophaeata* (Sprung, 1995; Walz, 1978b). This energy surplus should not only be used for somatic growth, but also for gametogenesis, indicating a raise in reproductive capacity in the installation in comparison with the surrounding water. As a result of this increased reproductive capacity, an increased recruitment success, along with its biofouling consequences, might be hypothesized.

2. The study at hand shows that *M. leucophaeata* is a small, slowly growing, invasive bivalve, which seems, by means of these characteristics, a rather harmless fouling species, especially in comparison to the larger, quickly growing *D. polymorpha*. However, very little growth is required before mussels reach the size equal to the interpolate gap of plate heat exchangers, at which point blockage can occur (Jenner et al., 1998). Point blockage is considered a major problem, caused by biofouling mussels. Direct interference with the functioning of the system (cf. definition of biofouling) should thus not be considered strictly correlated with size

3. Its high longevity (more than five years, compare to *D. polymorpha*: 2 – 4 years) indicates that once fouling problems have arisen, these problems might persist for a long time: an adult *M.*

leucophaeata is very tolerant to chlorination, not easily removable and, once established, will keep on producing offspring for a long period of time.

We can conclude that *M. leucophaeata* definitely has the potential of becoming the brackish water equivalent of *D. polymorpha* in Europe. Although fouling problems caused by *M. leucophaeata* are different in nature in comparison to those instigated by *D. polymorpha*, they may be as severe and even more difficult to solve, as already observed in power stations in the Netherlands (Rajagopal et al., 1995), Belgium (Verween et al., 2005), the central Gulf of Finland (Laine et al., 2006) and possibly upcoming in the U.K. (Bamber and Taylor, 2002) and other brackish water related industries.

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CHAPTER VI

Larval presence prediction through logistic regression: An early warning system against *Mytilopsis leucophaeata* biofouling

Paper adapted from:

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Biofouling

ABSTRACT

Mytilopsis leucophaeata is a biofouling bivalve causing major problems in the cooling water system of several large water-using industrial facilities in the harbour of Antwerp (Belgium). This study aimed at developing a statistical model to predict larval response of *M. leucophaeata* larvae to environmental conditions in estuarine ecosystems. Multiple logistic regression, taking into account temporal autocorrelation, was applied on a large dataset allowing us to predict the probability of occurrence of *M. leucophaeata* larvae at BASF N.V. Antwerpen as a response to the environmental variables. The final model made it possible to predict larval presence in the water column just by monitoring water temperature. Results from subsampling indicated that the model was stable. The model was fitted on the data of 2005 demonstrating a 98 % precise prediction of larval occurrence o*f M. leucophaeata* in the water column, with a sensitivity of 100 % and a specificity of 97 %, even though autumn 2005 was exceptionally warm, which lead to an extended presence of larvae.

KEYWORDS

Mytilopsis leucophaeata, biofouling control, population dynamics, larvae, logistic regression, predictive statistics

INTRODUCTION

Mytilopsis leucophaeata (Conrad, 1831), the brackish water mussel, is a mytiliform bivalve (Mollusca, Bivalvia, Veneroida, Dreissenidae) and a typical estuarine species (Siddall, 1980) which occurs mostly in brackish waters (Boettger, 1932). The species originates from the southern coast of the U.S. through Tampico, Mexico (Marelli and Gray, 1983), is becoming an important biofouling species in Europe (Rajagopal et al., 1994, 1995; Verween et al., 2005) and is a rapidly expanding intruder outside its natural habitat in the U.S. (Christmas, pers comm).

Biofouling is defined as the disfunctioning of a technical installation because of interactions between natural biological processes and the functioning of the installation itself (Jenner et al., 1998). Worldwide, biofouling problems yearly pose an enormous economic cost, especially in cooling water systems, with mussels being the most hazardous fouling species (Rajagopal et al., 1996), next to Hydrozoa, Bryozoa, Cirripedia and other Bivalvia.

The most effective and inexpensive mussel control measure in power stations is the use of chlorination. However, there are disadvantages in the use of chlorine as an antifouling agent in a once-through cooling system. Chlorine by-products are potential pollutants of receiving water bodies and its non-specific toxicity can impact non-target organisms (Jenner et al., 1998). A total residual chlorine level of 1 mg/l is normally used for mussel control in Europe during the breeding season while during non-breeding periods considerably lower chlorine levels, between 0.2 and 0.5 mg/l, are used (Rajagopal et al., 2002a). However, the resistance of *M. leucophaeata* to chlorination is higher than for other common biofouling mussel species, such as *Mytilus edulis* L. and *Dreissena polymorpha* (Pallas) (Rajagopal et al., 2002b).

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), including biocide-passage. In contrast, the planktonic larvae and plantigrades are the most vulnerable life stages, without protective valves, and thus susceptible to biocides. Hence, knowledge of the presence of mussel larvae provides a basis for an ecologically and economically proper use of these detrimental chemicals (Relini, 1984).

Until now, larval presence at BASF Antwerpen N.V. throughout the year was detected by means of intensive monitoring through weekly replicate quantitative plankton samples. The observed 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 (Verween et al., 2005). To prevent new biofouling by *M. leucophaeata*, a targeted dosage of biocides during the period of larval presence would be as effective as a continuous dosage throughout the year. A pointed dosage would also decrease the amount of biocides needed, allowing facilities to: (1) meet the Belgian biocide draining criteria (VLAREM II, 4.2.4., VLAREM II, annex 2.3.1.) and (2) explore the use of ecologically less harmful and more expensive, but still effective biocides.

The aims of this study were: (1) to find the most important candidate environmental variables determining larval presence of *M. leucophaeata* and (2) to quantify the relationship between these variables and the presence of *M. leucophaeata* larvae to as such create a logistic regression model, predicting the larval presence.

MATERIAL AND METHODS

1. STUDY AREA

The study area is situated at the site of BASF, Antwerpen N.V. (Belgium), along the Schelde river. BASF, Antwerpen N.V. is the largest chemical production center of Belgium, producing mineral fertilizers, complex and simple synthetics and their pre-products, synthetic fibers, basic chemicals and refinement products. The industrial plant is located at the right bank in the harbour of Antwerp, near the Dutch border (**Fig. 1**), and receives water of intermediate salinity (1 – 12 PSU) coming from the Westerschelde river and from the Rijn-Schelde channel. The Schelde estuary measures 160 km from the mouth near Vlissingen (The Netherlands) to Gent (Belgium), and is one of the longest estuaries in North-Western Europe with a still complete salinity gradient.

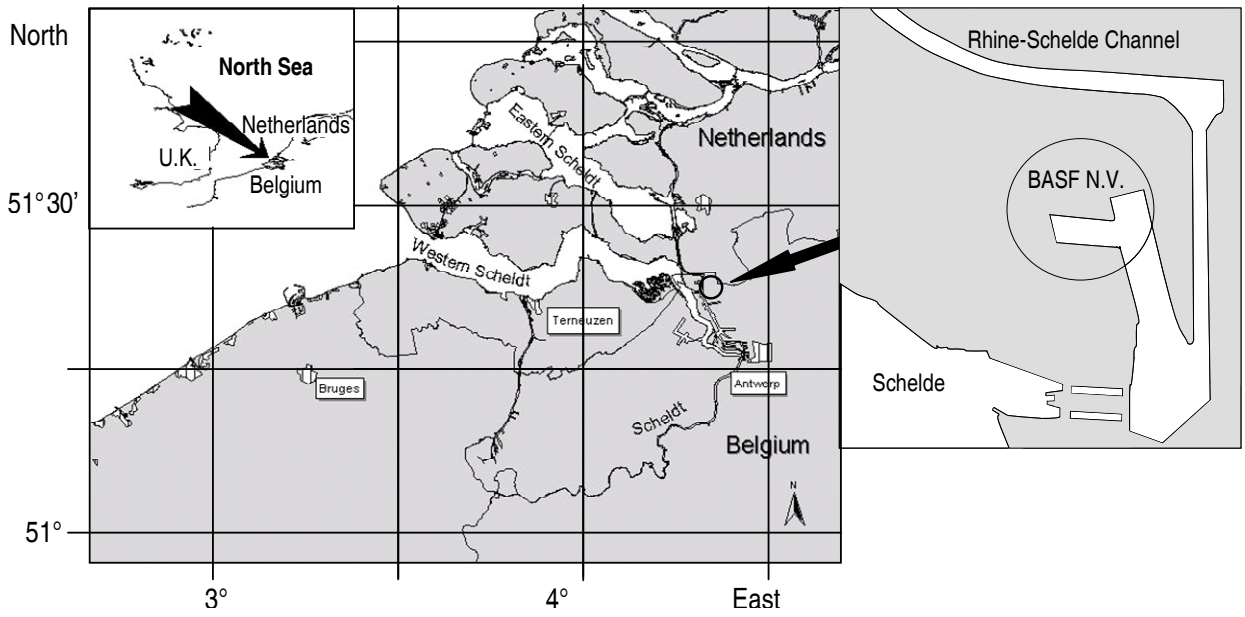

Fig. 1: Map of Westerschelde river with indication of the study site.

2. DATA COLLECTION

Biofouling problems with *Mytilopsis leucophaeata* at BASF Antwerpen N.V. were first detected in 1998 (Verween et al., 2005). The cooling water conduits of the industrial site take in up to 80 000 m³ of 1 mm-filtered, but untreated river water per hour. Larvae can thus enter the system together with the extracted water, where they may attach onto substrates, such as the heat exchangers and the tubes in the conduits. Peak larval densities exceeded 1500 individuals per m³ (Verween et al., 2005). Growth conditions in the cooling water system are ideal for sessile organisms: the steady water flow assures abundance of nutrients and oxygen, while access for predators is limited. As a consequence of this undesired biological growth, restriction in water flow, blockage of the heat exchangers, increased rate of corrosion and loss of heat transfer may occur. All these have negative environmental and economical consequences.

Mytilopsis leucophaeata larval presence and density at BASF N.V. have been monitored weekly during 2000 – 2005 by sieving 50 liter plankton samples through a 63 µm mesh sieve. For more detailed information on the sampling methods and the monitoring program, see Verween et al. (2005). For each sampling occasion, temperature, salinity and oxygen content were measured. Water temperature (°C) and salinity (PSU) of the incoming water were monitored weekly, by means of field sampling devices. A Profiline conductivity meter LF 197 was used for salinity and temperature

measurements, while oxygen content was measured by an Oxi 320 microprocessor oxygen meter. Chlorophyll a concentration (µg/l) was measured from a 500 ml water sample through filtering onto a Whatman GF/F filter and analysing with a HPLC-sampler according to Jeffrey et al. (1997). Chlorophyll a data from 2000 until 2002 were provided by the chemical measuring network MWTL. From 2003 onwards, chlorophyll a was measured by the University of Ghent.

3. STATISTICAL ANALYSIS

In this study, larval presence/absence was chosen as the target response variable since for biocidal control, the amount of larvae to be combated is unimportant: biocides will be used in equal concentration from the first notice of larval presence in the water column until the last, regardless its amount.

Data collected between 2000 and 2004 were used to create and evaluate the predictive model while data from 2005 were used to test the predictive capacity of the model. As the binary larval presence/absence data do not perform a Gaussian distribution, logistic regression was used to model the response of the occurrence of *M. leucophaeata* larvae to one or more abiotic environmental predictors. Logistic regression (Cox, 1970) falls within the framework of generalized linear models (GLM) and is used to describe the relationship between a binomial distributed response variable and one or more explanatory variables (Hosmer and Lemeshow, 1989). Hence, the logistic regression model takes the form $Y_i \sim B (p_i , n_i)$ with response variable Y_i showing a binomial distribution with probability of larval occurrence p_i and size of sample size n_i being 1. The response variable is related to the predictor variables through the logit link function:

$$Logit (p_i) = log [p_i / (1+p_i)] = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + ... + \beta_k X_{ki} + ... \quad (1)$$

With β_0 being the intercept, $\beta_1, \beta_2, ...$ the regression parameters and $X_1, X_2, ...$ the values of the independent variables.

Equation (1) can be rewritten to define the estimated probability p_i as:

$$p_i = \{ e^{(\beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + ... + \beta_k X_{ki} + ...)} \} / \{ 1 + e^{(\beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + ... + \beta_k X_{ki} + ...)} \} \quad (2)$$

which is bound between 0 and 1.

However, for some variables (e.g. time), a unimodal (i.e. a response that is increasing and then decreasing), rather than a linear relationship can be expected between the logit (p_i) and the linear predictor. This was countered by the inclusion of a quadratic term in the regression equation.

The use of logistic regression models to predict likely occurrence or distribution of species has been applied in many ecological studies (Pearce & Ferrier, 2000), except for the marine and estuarine environment (Ysebaert et al., 2002). Most models are however predicting the probability of occurrence of a species on a spatial instead of a temporal scale. Since larval presence of *M. leucophaeata* on time t might be dependent on the environmental variables on time t, but also of those on time t-1, t-2, etc., there is a need for taking serial autocorrelation into account. This implies that residual errors of samples taken close to each other in time might be correlated and samples, more distant from each other in time can be assumed to be independent. Hence, the correlation of residuals was modeled as a decaying function of time distance. As sample points were equally distant in time, a first order autoregressive residual covariance matrix was chosen:

$$cov[\xi_i, \xi_j] = \sigma^2 * \begin{pmatrix} 1 & \rho & \rho^2 & \rho^3 \\ \rho & 1 & \rho & \rho^2 \\ \rho^2 & \rho & 1 & \rho \\ \rho^3 & \rho^2 & \rho & 1 \end{pmatrix}$$

with ξ_i, ξ_j as generic random variables, σ^2 being the variance and ρ the correlation coefficient between two adjacent samples.

Models were fitted by the GLIMMIX-procedure (Generalized Linear Mixed Models) in the SAS software Version 9.1 (SAS Institute Inc., 1989).

Sampling year was incorporated into the model as a fixed covariate. As such, average probabilities of occurrence are allowed to differ between years. Besides response curves for each single abiotic variable separately, all variables were simultaneously used in a stepwise backwards multiple logistic regression analysis to derive a model that would predict the presence or absence of *M. leucophaeata* larvae through time. The significance of the independent variables was tested using the type III-test ($p < 0.05$) for fixed effects. To visualize the predictive capacity of each model, a graphical presentation was used.

A decision threshold value was chosen, indicating a limit value above which is assumed that *M. leucophaeata* larvae are present in the water column. If the predicted value is lower than the threshold value, it is accepted that larvae are absent in the water column. The threshold probability value was specified, based partially on knowledge of prior probability of occurrence and partly on value judgments regarding the consequences of various kinds of correct and incorrect decisions (Metz, 1986; Fielding & Bell, 1997): while the most obvious decision threshold value would be 0.5, you may well want to choose a different decision rule given the relative seriousness of making one type of error. For example if predictions from a model tend to overestimate the occurrence of a species, then the predicted distribution will include not only areas of suitable circumstances (true positives) but also substantial areas of unsuitable circumstances (false positives). If on the other hand a model will underestimate the occurrence of a species, areas with potentially suitable circumstances will remain unidentified (false negatives) (Pearce and Ferrier, 2000).

Evaluating the predictive performance of models using independent data is a vital step in model management (Pearce and Ferrier, 2000). Such evaluation is often measured by cross-classifying actual observations and predictions. A species is predicted to be present or absent at a site or time based on whether the predicted probability is higher or lower than a specified threshold probability value (**Table I**). The table was used to calculate the accuracy, sensitivity and specificity of the model. Sensitivity (or true positive fraction) is here defined as the proportion of true positives that are correctly identified by the test (i.e. $A / (A + C)$). Specificity (or true negative fraction) is defined as the proportion of true negatives that are correctly identified by the test (i.e. $D / (B + D)$). Using these indices, the accuracy (i.e. the percentage of the sample correctly predicted) can be calculated as $(A + D) / (A + B + C + D)$.

Table I: Classification table describing the agreement between observed and predicted presence or absence of a species. Each of the values A, B, C and D represent numbers of observations, so that their sum equals the sample size of the evaluation sample (Pearce and Ferrier, 2000).

		Observed data: Presence	Observed data: Absence	
Predicted data	Presence	A	B	A + B
	Absence	C	D	C + D
		A + C	B + D	A + B + C + D

Statistical resampling was used to evaluate the predictive ability of the final model. The dataset was split randomly into two equal groups, building the model with the chosen variables using half of the data. Ten runs with ten different splits of the data were conducted. We then generated predicted p-values for the ten models and tested the predictions with the other half of the datasets by examining the percentage correctly predicted, sensitivity and specificity.

To obtain an estimate of the predictive capacity of a model, evaluation is best undertaken with independent data other than those used for creating the model. Therefore, the ability of the predictive model was tested on the dataset of larval presence of *M. leucophaeata* at BASF, Antwerpen N.V. in 2005.

RESULTS

1. CHARACTERIZATION OF THE ABIOTIC ENVIRONMENT

The incoming water at the industrial plant showed a clear yearly cyclic pattern in environmental variables (**Fig. 2**).

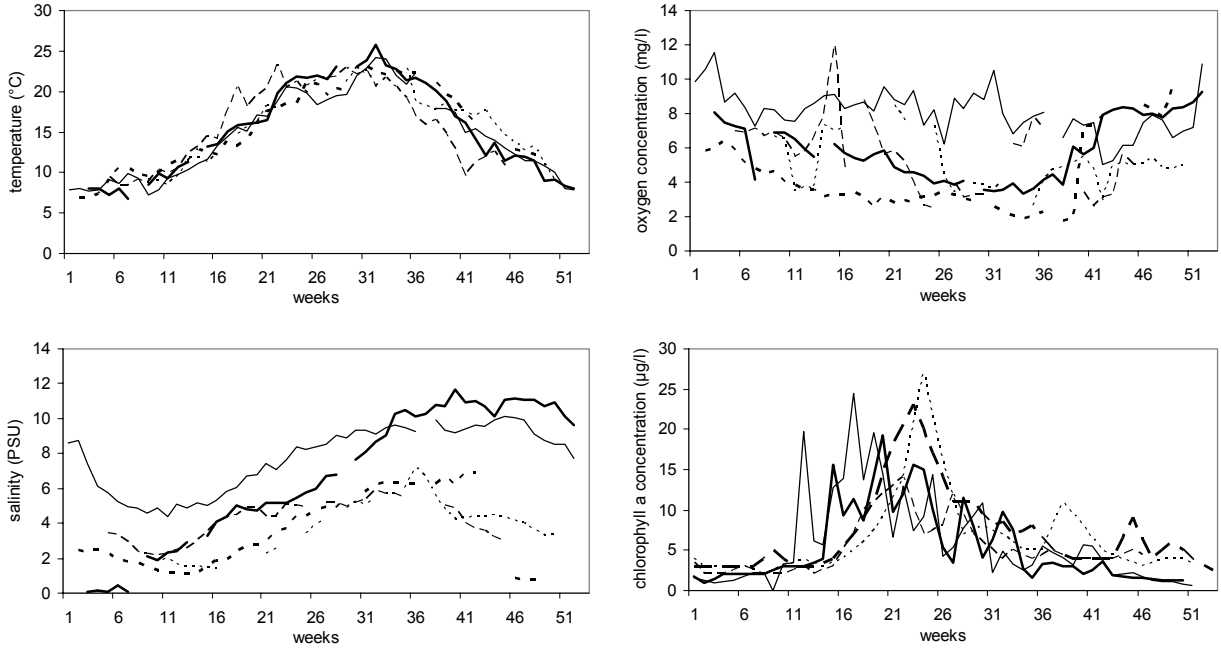

Fig. 2: Seasonal variation throughout the years in environmental variables in the cooling water system at BASF, Antwerpen N.V. (– – - 2000; - - - - 2001; **– –** 2002; **——** 2003; —— 2004)

Temperature was low in winter and spring and reached a maximum in late summer. Almost no difference between years was detected. Average temperature varied between 6.8 and 25.8 °C throughout the study period, with an average temperature of 15.4 °C (**Table II**). Salinity reached minimal values in early spring and increased throughout the year with maxima in late autumn. Salinity levels varied between 0.1 and 11.7 PSU, with an average of 5.4 PSU. Interestingly, salinity kept rising during winter in 2003 in contrast to other years, leading to an overall increase of salinity in 2004. Although the general salinity pattern was equal throughout all years, a difference between years was detected. Oxygen content varied between 0.8 and 12 mg/l with a mean of 5.9 mg/l and did not show a clear seasonal pattern. In 2004, highest overall oxygen concentrations were found. Food concentration, measured as chlorophyll a content, showed a typical phytoplankton pattern with high values in spring and summer to a maximum value of 27 µg/l, and very low values in wintertime, reaching 0 µg/l.

Table II: Environmental variables in the harbour of Antwerp from 2000-2005 with numbers of observations (n), median, minimum and maximum values.

Variable		n	Median	Min.	Max.
Temperature	(°C)	218	15.4	6.8	25.8
Salinity	(PSU)	214	5.4	0.1	11.7
Oxygen content	(mg/l)	211	5.9	0.8	12.0
Chlorophyll a	(µg/l)	250	6.1	0.0	27.0

Pearson's correlation coefficients among environmental variables were calculated, and highly significant correlations ($p < 0.0001$) were detected between temperature and each of the other variables; salinity ($r = 0.46$), chlorophyll a ($r = 0.42$) and oxygen concentration ($r = -0.28$).

2. OBSERVED AND MODELED DISTRIBUTION OF *MYTILOPSIS LEUCOPHAEATA* LARVAE ALONG ENVIRONMENTAL GRADIENTS

Although major differences in larval densities between months and years were found, the period of larval occurrence was markedly similar (**Fig. 3**): in 2000 larvae first appeared in the plankton on 3 June, in 2001 on 6 June, in 2002 on 21 May, in 2003 on 20 May and in 2004 on 8 June. Taking a weekly sampling into account, a yearly difference of at least 18 and possibly even 25 days can appear.

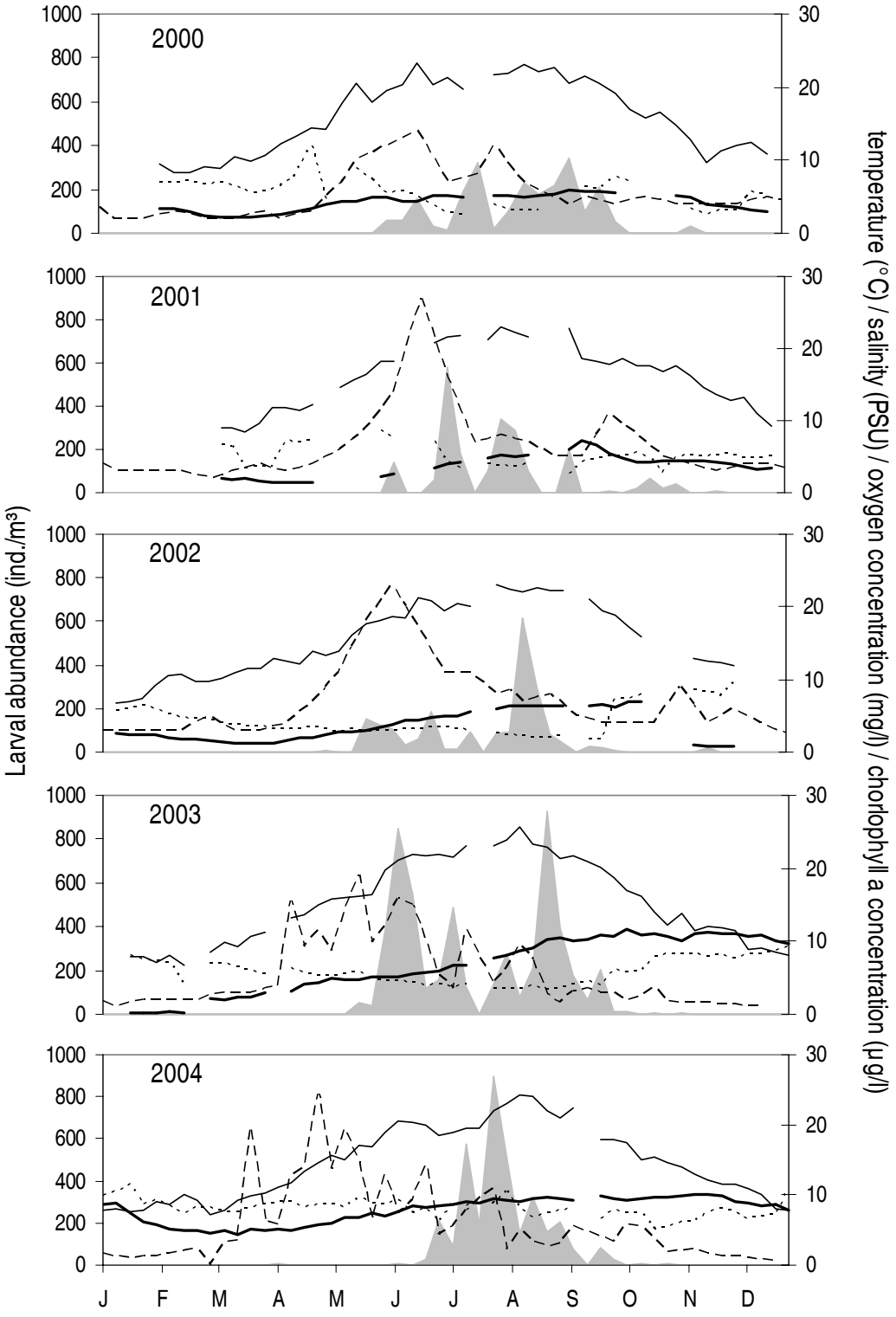

Fig. 3: Seasonal variation in larval arrival of *Mytilopsis leucophaeata* in the cooling water system at BASF, Antwerpen N.V.

(grey shaded: larval abundance; —— temperature; ····· salinity; ▬ oxygen concentration; ····· chlorophyll a concentration)

Larvae occurred mainly within a narrow range in temperature (18 – 22 °C), salinity (5 – 9 PSU) and food concentration (4 – 11 µg/l) (**Fig. 4**), although larval presence occurred across a wide range of oxygen levels.

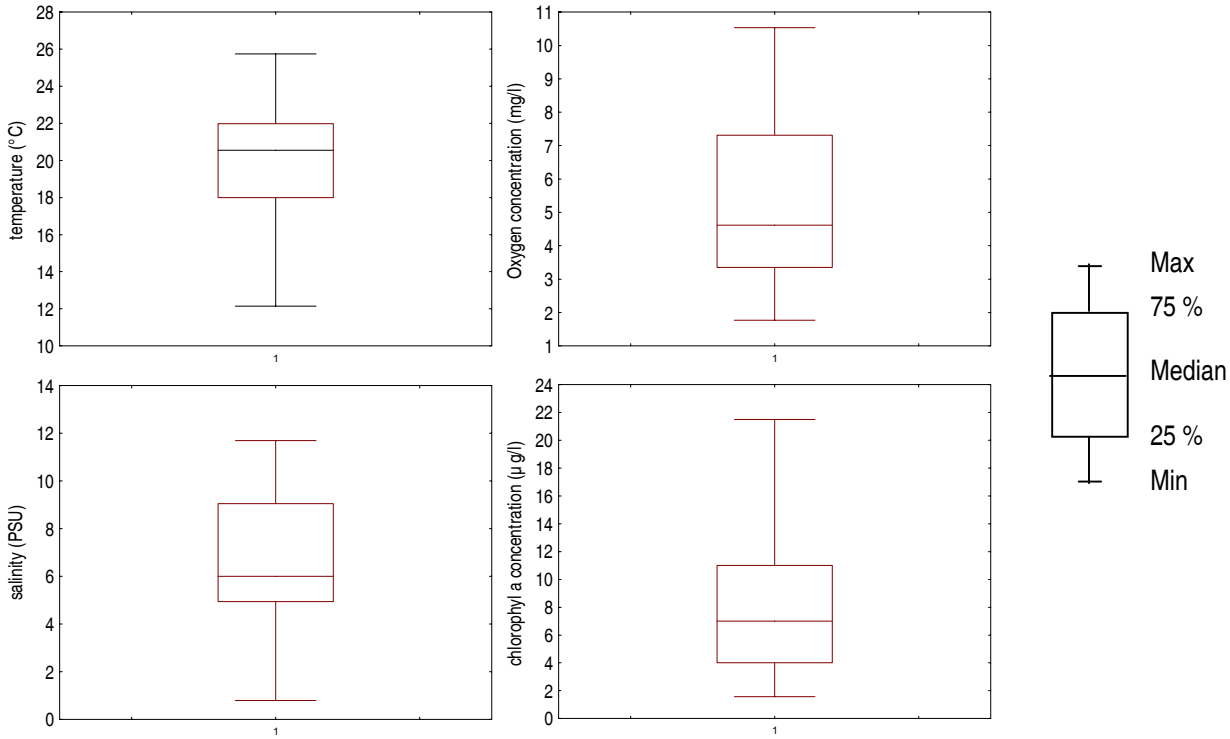

Fig. 4: Box-and-whisker plots for the presence of *Mytilopsis leucophaeata* larvae in relation to measured environmental variables in 2000-2005 at BASF, Antwerpen N.V.

The obtained *M. leucophaeata* response curves for each single abiotic variable (**Fig. 5**) were in general agreement with the observed larval distributions from Fig. 4. In all cases, *M. leucophaeata* larvae showed highest probability of occurrence at maximal temperature, salinity and chlorophyll a values. The increase in the curve, observed at maximal values, indicated that the larvae probably can occur at conditions even higher than those recorded in the present study. The steep increase of the curve with increasing temperature suggested that the probability of larval occurrence is very temperature dependent. For salinity and chlorophyll a concentration, a much smoother increase with increasing values was detected, indicating a much broader tolerance to these variables, with preference of occurrence at higher values. Furthermore, a smooth decrease of probability of occurrence with increasing oxygen rate was detected.

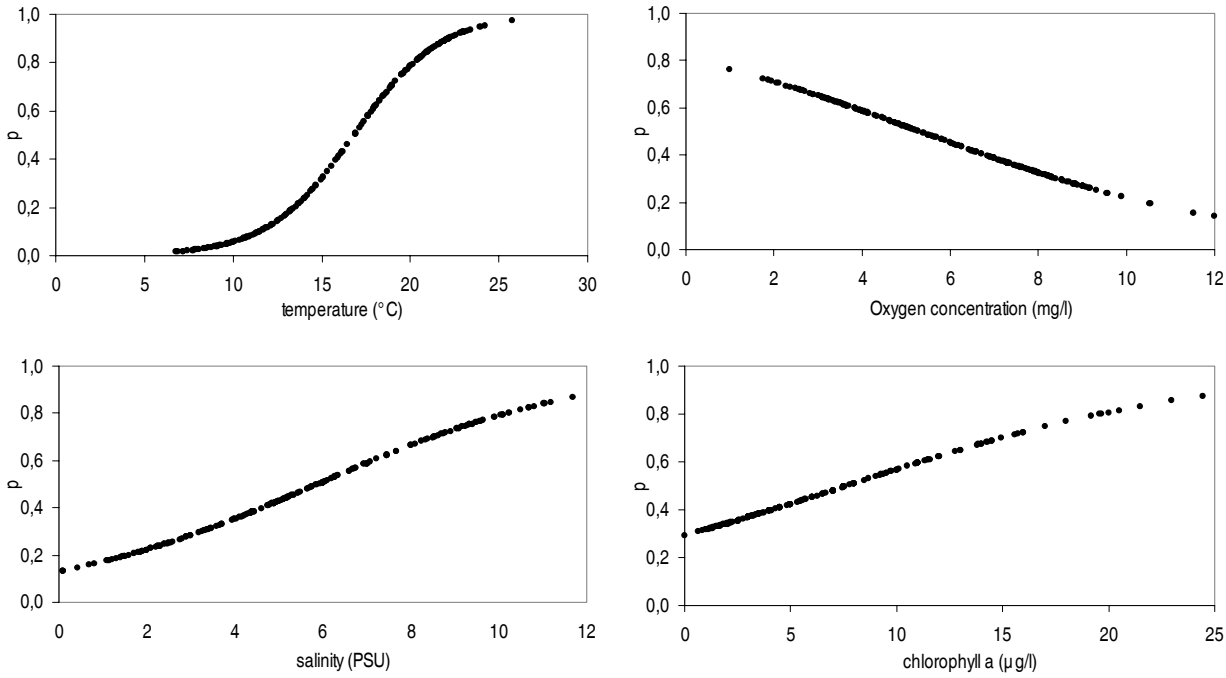

Fig. 5: Fitted Gaussian logit curves showing probability of occurrence (P) of *Mytilopsis leucophaeata* larvae in relation to model temperature, salinity, oxygen and chlorophyll a concentration.

3. MULTIPLE LOGISTIC REGRESSION

Different stepwise multiple logistic regressions with autocorrelation were run with all abiotic variables together (**Table III**). Sampling year had a significant effect in all the tested models ($F > 2.19$; $p < 0.09$). It was however, not added in the predictive model since it is impossible to predict year-effect for the next year.

Table III: Estimates of logistic regression statistics and accuracy, sensitivity and specificity percentages for different models relating *Mytilopsis leucophaeata* presence to environmental and/or temporal variables.

Models	β_i	SE	*p*-value	accuracy (%)	sensitivity (%)	specificity (%)
Intercept	-6.85	1.185	<0.0001	83.3	75.9	94.9
Temperature	0.40	0.072	<0.0001			
Salinity	0.06	0.108	0.610			
Chlorophyll a concentration	-0.02	0.047	0.642			
Intercept	-14.75	4.136	0.001	84.8	76.8	96.2
Week	0.84	0.368	0.027			
Week²	-0.012	0.0058	0.039			
Temperature	0.15	0.134	0.260			
Salinity	0.05	0.138	0.739			
Oxygen concentration	-0.20	0.131	0.123			
Chlorophyll a concentration	0.04	0.071	0.555			
Intercept	-14.95	4.069	0.0006	84.8	76.8	96.2
Week	0.87	0.361	0.020			
Week²	-0.013	0.0057	0.031			
Temperature	0.15	0.132	0.245			
Oxygen concentration	0.19	0.121	0.127			
Chlorophyll a concentration	0.04	0.069	0.587			
Intercept	-14.17	3.559	0.0002	85.3	77.5	96.3
Week	0.85	0.342	0.015			
Week²	-0.013	0.0055	0.023			
Temperature	0.15	0.127	0.231			
Oxygen concentration	0.19	0.116	0.110			
Intercept	-15.99	3.129	< 0.0001	84.8	76.8	96.2
Week	1.18	0.207	< 0.0001			
Week²	-0.018	0.0032	< 0.0001			
Oxygen concentration	-0.19	0.109	0.081			
Intercept	-15.55	3.471	< 0.0001	85.3	77.5	96.3
Week	0.89	0.333	0.009			
Week²	-0.013	0.0053	0.014			
Temperature	0.14	0.121	0.246			

Although the model without time effect predicted the larval presence with high accuracy of 86 %, it lacked precision in time of arrival and disappearance of the larval cohort on a lower threshold scale. When calculating the predictive capacity at a threshold level of P = 0.05, the model without time effect had an accuracy of 64.4 %, with a sensitivity of 56.7 %. The predictive capacity was far more accurate with the addition of time effect (**Fig. 6**), with an accuracy of 75.9 % and a sensitivity of 98.3 %. Specificity did not differ greatly between both models being 100 % without and 98.3 % with time effect.

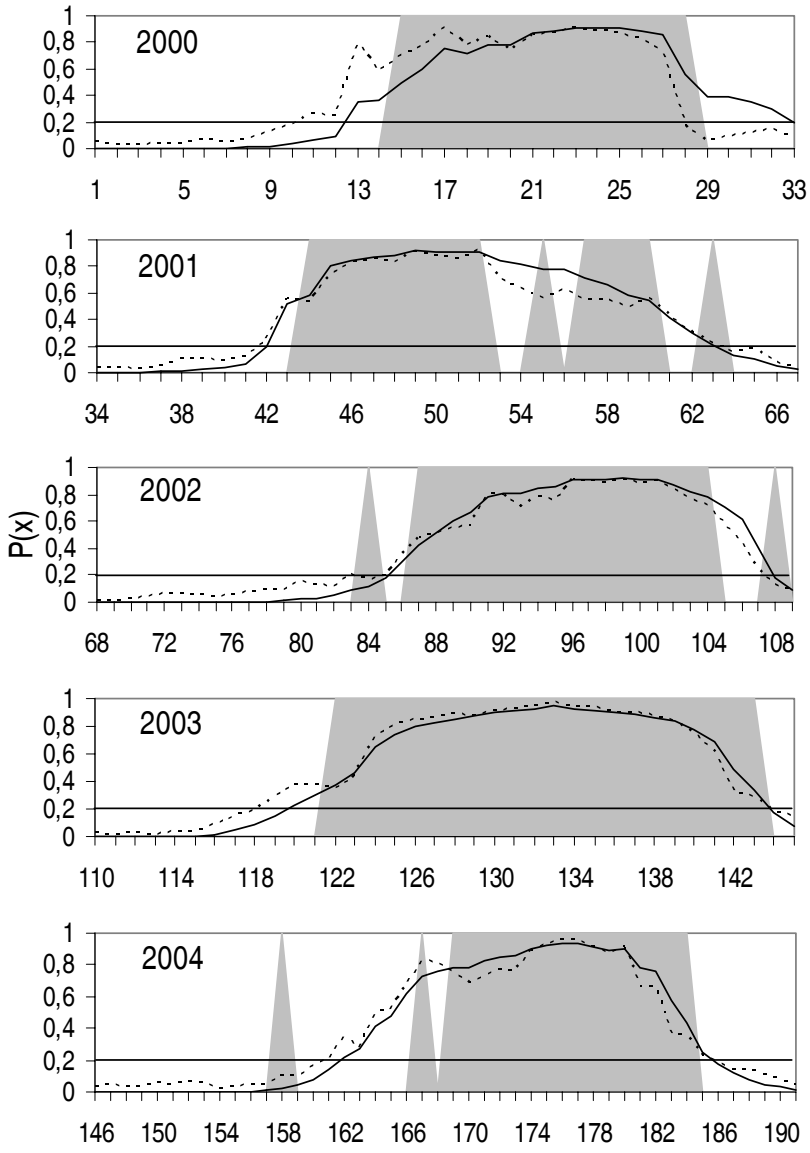

Fig. 6: Temperature model (dashed line) and temperature-time model (solid line) performance relative to subsequent observations throughout the monitoring period (larval presence: grey shaded). P(x) above threshold value P(x) = 0.2: modeled larval presence.

The predictive performance of the different models with time effect did not show much difference with average accuracy of 85.1 ± 0.3 %, average sensitivity of 77.1 ± 0.5 % and specificity of 96.3 ± 0.1 %.

Stepwise deletion of the least significant variable ($p > 0.25$) (Hosmer and Lemeshow, 1989) resulted into two final models; (1) including week, week² and oxygen concentration, referred to as oxygen-time model, and (2) including week, week² and temperature as predictive variables, referred to as temperature-time model. The simplest model with best predictions on *M. leucophaeata* larval presence, however, proved to be the temperature-time model, including only temperature as predictive variable:

$logit\ (P_i) = -15.547 + 0.887 * week - 0.0133 * week^2 + 0.141 * temperature$

with P_i the probability of occurrence of *M. leucophaeata* larvae.

A decision threshold value of P(x) = 0.2 was chosen to predict presence of *M. leucophaeata* larvae, indicating that P(x) $\geq$0.2 means that larvae are present in the cooling water system. This temperature model showed an accuracy of 85.3 %, with a sensitivity of 77.5 % and a specificity of 96.3 %.

4. EVALUATING THE PREDICTIVE PERFORMANCE

The final temperature-time model appeared to be very stable. Ten model runs (based on the random selections of 50 % of the data) gave an average accuracy of 82.7 ± 0.3 %, with an average sensitivity of 73.1 ± 0.6 % and a specificity of 96.4 ± 0.6 %, indicating a good predictive performance of the model.

The predictive capacity was also tested on the dataset of larval presence of *M. leucophaeata* at BASF, Antwerpen N.V. in 2005 (**Fig. 7**). Temperature was monitored throughout the year, and predictions were checked with plankton samples.

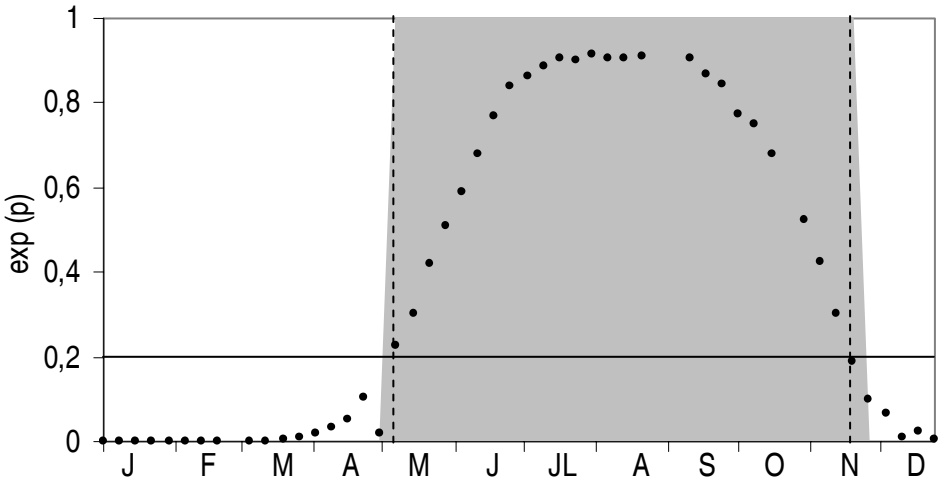

Fig. 7: Final temperature logistic regression model performance on the dataset of 2005. (grey shaded: monitored presence of larvae; black points: predicted presence of larvae). The dashed line indicates the identical timing of monitoring and prediction of first and last presence of *Mytilopsis leucophaeata* larvae.

Autumn 2005 was much warmer than previous years, with an average temperature of 12.3 °C instead of the normal 10.4 °C leading to higher water temperature and expanded presence of mussel larvae in the water column. Even in these exceptional conditions, it appeared that the temperature-time model was highly predictable: an accuracy of 98 % was measured, with a sensitivity of 100 % and a specificity of 97 %, confirming the efficiency of this relatively simple model.

Predicting larval appearance for 2005 with the model consisting of week, week² and oxygen concentration as predictive variables showed a less precise predictive capacity: accuracy amounts 96 %, with a sensitivity of 96 % and a specificity of 95 %.

DISCUSSION

1. ECOLOGY OF *MYTILOPSIS LEUCOPHAEATA*

Biofouling of industrial cooling water systems by *M. leucophaeata* is an increasing problem in Europe (Verween et al., in press) and a possible threat for the U.S.. Since the larval plankton phase is the most vulnerable phase in the life cycle of mussels, new biofouling can be avoided by combating this life stage. Mussels are sequential spawners, releasing gametes over a period of several weeks to a couple of months (Borcherding, 1991), indicating that larvae will be present in the water column during a fixed time of the year. This strict timing of arrival of *M. leucophaeata* larvae at incoming cooling water of the Schelde river was used as a tool against new biofouling by this species. However, a maximal yearly difference in larval arrival of 25 days (i.e. almost one month) could appear. This demonstrates the utility of an appropriate predictive model in biofouling control, as it would have allowed the saving of one month's unnecessary biocide application if mussel larvae appeared in intake water one month later than they typically arrived. A trustworthy model would limit the use of antifouling agents to the minimum to obtain a maximal *M. leucophaeata* larval mortality.

Larval response curves of *M. leucophaeata* based on abiotic environmental variables were in general agreement with the observed larval distributions and revealed that temperature was a major determining factor of larval presence. Also for other bivalves water temperature is generally believed to be the prime factor in triggering gamete release (Chipperfield, 1953; Nichols, 1996; de Vooys, 1999) and it has been shown that the overall duration of larval production is responsive to seasonal water temperature regimes

(Nichols, 1996). The intensity and duration of bivalve reproduction on the other hand are controlled by an interaction between several environmental factors, such as temperature, salinity and food availability (Kautsky, 1982). The larval response curve on salinity indicated that up to 10 PSU and even higher values are preferred by *M. leucophaeata* larvae. Indeed, larvae and postlarvae of *M. leucophaeata* are capable of development at even higher salinities, ranging to 32 PSU (Siddall, 1980). It is this characteristic that makes it possible for *M. leucophaeata* to cross oceans by means of ballast water and as such colonize new estuarine habitats. The importance of food availability is a logical feature for growing larvae; food abundance is the primary controlling factor for gonad growth in *Mytilus edulis* (Bayne, 1965), resulting in the amount of larvae produced. The smooth decrease of probability of occurrence with increasing oxygen rate would mean that *M. leucophaeata* larvae preferred lower oxygen levels. However, a very different oxygen pattern measured throughout the different years (**Fig. 2**) needs to be emphasized in explaining this pattern, making it possibly unreliable. The erratic, non-seasonal pattern of fluctuation in ambient oxygen concentration across years makes it difficult to predict the oxygen preferences of *M. leucophaeata* larvae. Sprung (1987) concluded that *D. polymorpha* larvae survived at 18 °C for short periods at oxygen levels as low as 20 % saturation (< 2 mg/l), with an increased mortality at higher temperatures. In general, only severely stressed aquatic systems would have oxygen levels low enough to inhibit larval dreissenids (McMahon and Alexander, 1991).

Although it is difficult to ascertain whether all of the relevant factors for the distribution of (the larval phase of) a species have been taken into account (Ysebaert et al., 2002), scientific knowledge on the species contributes in choosing between variables. Other potential important factors such as light regimes and water currents (Ram et al., 1996) have not been taken into account.

2. PREDICTIVE MODEL

A significant correlation was detected between temperature and each of the other variables, so it could be useful to take multicollinearity problems into account in the modeling. However, the possibility exists that although intercorrelated, both variables still will have a significant impact on larval presence. Therefore, when one variable is entered into the model, a second that is correlated with it may still explain variation in the probability of occurrence.

In choosing the best logistic regression model in predicting larval presence of *M. leucophaeata*, several arguments were considered. An important feature was the future use of the model in anti-fouling

studies. To incorporate this modeling technique in industrial monitoring technologies, it is preferable that the complexity of the model is minimal, without reducing its predictive capacity. Ultimately, a simple but very good predictive model taking into account week, week² and temperature as predictive variables (temperature-time model) was chosen. The time-effect, here incorporated as week, was added because larvae were present in the water column for a limited time period throughout the year, arriving in May - early June and staying in the water until September - October. Statistical modeling improved significantly after adding this time effect. The developed temperature-time model made it possible to predict larval presence in the water column just by monitoring water temperature. This is an advantage in industrial use as a simple automatic monitoring system is efficient enough in determining the period of biocide-usage.

In applying this temperature-time model to combat *M. leucophaeata* larvae, dosing of biocides needs to coincide with the period of larval presence. Accuracy values alone do not fully asses the reliability of the model since its interpretation depends on knowledge of the prior probability of larval occurrence. All these measures depend on the choice of a decision threshold value, here P(x) = 0.2. Although the choice of this decision threshold is rather subjective and can differ according to different practitioners, it is well chosen according to the stated problem. From industrial point of view, it is better to predict some false positives (predicting presence, while larvae are absent) but still include the total period of larval presence, than to predict false negatives (predicting absence, while larvae are present) but as such start dosing too late or stopping too soon. Therefore, sensitivity percentage may be more important than specificity in delimiting the dosing period.

Predictive models are needed to manage the ecological and technological impacts of species invasions (Byers et al., 2002). Logistic regression is a powerful tool in many ecological (e.g. Bini and Thomaz, 2005) and economical (e.g. Bielza et al., 2003) studies, and has recently become a predictive tool in marine and estuarine animal ecology (Ysebaert et al., 2002). Yet, although commonly used in assessing and predicting potential occurrence and ecological impacts of introduced species and as such prioritize future invasion threats (e.g. Ramcharan et al., 1997; Ricciardi, 2003), industrial systems suffering severe biofouling problems lack the benefits of a good predictive model. This research program is the first predictive study related to biofouling problems, where knowledge on the biological processes initiating biofouling is used to prevent new problems. Nevertheless, predictive models on larval arrival can become an important tool in biofouling control programs as it combats new mussel fouling

proactively in an economically advantageous way; biocide-usage can be restricted to a couple of months, reducing the amount of chemicals needed.

Future research will investigate the possibility of using this temperature-time model within other cooling water systems along the Schelde estuary, facing the same fouling problems by *M. leucophaeata*. Since *M. leucophaeata* is a potential fouling organism in many European brackish waters, the presented model can be extended and adapted to other brackish water bodies. The simplicity of the model, using only temperature as an external measurable predictive factor, can be its strength in extending its future use as a data-driven model. However, a weak point in the modeling of *M. leucophaeata* presence is the fact that the exact role of the different environmental variables on the reproductive cycle of *M. leucophaeata* is still poorly understood, and needs to be carefully examined. The relatively unstudied fouling species *M. leucophaeata* has never been subject to much attention in the past, leading to a lack in the physiological knowledge of the species. Although closely related to *D. polymorpha*, not all biological facts and figures are applicable to *M. leucophaeata*. It is also possible that *M. leucophaeata* populations will eventually evolve genetic adaptations to local ecological conditions, as found in *M. edulis* (Gartner-Kepkay et al., 1980; Dickie et al., 1984). Prudence is advised in expanding the model to other regions with *M. leucophaeata* fouling problems, but a first ecological tool in avoiding new biofouling by *M. leucophaeata* is designed and effective and is open for adaptation to other conditions.

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CHAPTER VII

The effect of temperature and salinity on the survival of *Mytilopsis leucophaeata* larvae: the search for environmental limits

Paper submitted as:

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The effect of temperature and salinity on the survival of *Mytilopsis leucophaeata* larvae:

The search for environmental limits

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ABSTRACT

The brackish water mussel, *Mytilopsis leucophaeata*, is a rapidly expanding invasive bivalve in Europe with great biofouling capacities. Being a typical brackish water species with very broad habitat preferences and environmental limits, adults are extremely tolerant to temperature and salinity. The lifecycle of mussels however, consists of two phases: (1) from fertilization until the larvae are settling, they are pelagic, only protected by a larval soft shell and (2) after settlement, the individuals become benthic and develop a hard mytiliform shell. The fact that adult mussels can close their protective valves is the major reason why they are important fouling species, difficult to remove once settled. Therefore, vulnerability of different larval life stages of *M. leucophaeata* to temperature and salinity was investigated during standardized acute 48-h experimental tests. In addition, the survival limits of the most vulnerable larval life stage were determined at different temperature–salinity combinations. Results indicated that even in the larval phase of *M. leucophaeata* not all stages are equally vulnerable. A clear distinction could be made between 4 h old embryos and 2 days old larvae, with these latter being already extremely resistant to changes in temperature and salinity. Optimal condition for development of 2h old embryos showed to be 22°C at 15PSU. Surrounding this optimum, conditions stayed good for development in a rather wide range: only salinities of 0 and 25PSU and temperatures below 10°C or above 30°C caused high embryonic mortality. Thus, even the most vulnerable larval phase in the life cycle of *M. leucophaeata* can be considered resistant to environmental conditions, particularly considering the lack of a hard protective shell.

KEYWORDS

Static acute 48-h tests, D-shaped larvae, embryo, *Mytilopsis leucophaeata,* salinity, temperature

INTRODUCTION

Mytilopsis leucophaeata (Conrad, 1831), the brackish water mussel, is a mytiliform bivalve (Mollusca, Bivalvia, Veneroida, Dreissenidae) and a typical brackish water species (Boettger, 1932) with very broad habitat preferences and environmental limits. The species originates from the southern coast of the US to Tampico, Mexico (Marelli and Gray, 1983) and is becoming an important biofouling species, rapidly expanding in Europe (Rajagopal et al., 1994, 1995; Verween et al., in press).

Worldwide, biofouling problems yearly pose an enormous economic cost, especially in cooling water systems, with mussels being the most hazardous fouling species (Rajagopal et al., 1996) because 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. Bayne et al. (1976) stated that the earliest stages are the most sensitive in the life cycle of a bivalve, and as the larva develops into a benthic juvenile, its tolerance limits for various environmental conditions increases. This has led to the theoretical distinction of two important phases in a mussel life cycle (Conn et al., 1993): (1) from fertilization until the larvae are settling, they are pelagic, only protected by a larval soft shell and (2) after settlement, the individuals become benthic and develop a hard mytiliform shell. It can thus be suggested that the first, larval phase is the vulnerable one, and thus possibly most susceptible to changes in external environment (Claudi and Evans, 1993). Therefore, we hypothesize that although *M. leucophaeata* is a typical brackish water species, highly resistant to environmental conditions, their larval phase will be vulnerable to changes in the surrounding environment, lacking a protective shell, similar to other mussel species.

The capacity of an organism to survive in its environment is restricted by its limits of tolerance to abiotic factors. The lethal effects of temperature, salinity and salinity-temperature combinations on the survival of embryos and larvae of *M. leucophaeata* lead to a large amount of biological information of the species since temperature and salinity are primary abiotic variables affecting survival, activity and distribution of marine organisms (Kinne, 1964). The thermal range within which growth and normal physiological development of a species can occur is usually narrower than the tolerance limits (Kinne, 1970; Newell and Branch, 1980) and environmental tolerance to salinity may not be the same for gametes as for adults (Fong, 1998), indicating that more knowledge is necessary than only information on adults, to predict the possible establishment of a species in a new habitat.

The specific aims of this study were:

- To investigate the vulnerability of different stages of *M. leucophaeata* larvae to changes in the current environment (temperature and salinity);
- To determine the limits of survival of *M. leucophaeata* larvae, in order to define a possible range where the species can induce fouling problems in the future.

MATERIAL AND METHODS

The influence of temperature and salinity on the survival of D–shaped and 4h-old larvae of *M. leucophaeata* was investigated. The lethal effects of temperature, salinity and salinity-temperature combinations were examined in the laboratory through standardized acute 48-h tests (ASTM, 1999).

1. BROOD STOCK

The research was conducted at Ghent University during summer-autumn 2005 and 2006. Each year, in the beginning of May, before the start of the spawning season (Verween et al., 2005), approximately 400 adults (> 10 mm) were collected from the cooling water installation of an industrial plant in the harbour of Antwerp (51° 21.37' N, 4° 17.30' E). These adults had never been in contact with the biocides used to control biofouling in the cooling water system, at any moment in their lifecycle.

Mussels were thoroughly scrubbed and rinsed to remove epifaunal organisms and maintained in a flow-through broodstock tank at a temperature lower than that measured in the field (12 ± 1°C) as to prevent spawning of ripe animals (Stanyczykowska, 1977; Stoeckel et al., 2004). Natural, non-filtered brackish Schelde-water was used and mussels were additionally fed three times a week with live micro-algae, being the flagellate *Isochrysis galbana* Parke (3×10^5 cells.ml^{-1}) (Guillard, 1975), as to make sure that food was *ad libidum* (Helm et al., 2004). Water was changed twice a week and the tank was cleaned and rinsed with fresh water once a week to remove possible attachment of algae and tubeworms. Dead individuals were removed from the broodstock daily.

2. SPAWNING AND FERTILIZATION

The day before the experiment, eighty adults were stored overnight at 4 °C. Spawning was induced by placing them individually into 50 ml beakers containing 25 ml aerated artificial brackish water (Instant Ocean®, Aquarium Systems, France) with a salinity of 8 PSU and a temperature of 20 °C. After thirty minutes, when the siphons were extracted, 0.25 ml 10^{-3} $M.l^{-1}$ fluvoxamine was injected near the inhalating siphon (Ram et al., 1993; Fong, 1998). Thirty minutes after this injection, the water was changed with fresh aerated brackish water. Fluvoxamine is a selective serotonin reuptake inhibitor, ensuring a longer activity of serotonin (5-hydroxytryptamine; 5-HT), which is a neurotransmitter important in the gametogenesis and the induction of spawning in mussels (Ram et al., 1993). The longer serotonin is active, the better spawning is regulated. Fluvoxamine is the most powerful spawning inducer in any bivalve (Fong, 1998).

Males began releasing sperm within 30 to 60 minutes, while females began releasing ova within 60 to 90 minutes. Once spawning was detected, adults were placed in new beakers with artificial brackish water, but without fluvoxamine. Each 30 minutes the water was changed until all animals stopped spawning. Bayne (1965) stated that sperm and eggs of *Mytilus edulis* L. should be less than one hour old for fertilization. To ensure that eggs would be exposed to viable sperm, mussels were activated to spawn in two batches of 40 individuals. The second batch was exposed to fluvoxamine 1 - 1.5 h after the first batch. In this way, we induced an overlap of females, spawning in the first batch, and males, spawning in the second batch (Stoeckel et al., 2004).

Fertilization occurred with the eggs and sperm of a minimum of three individuals each. A 2 ml suspension of sperm was added to the egg suspension in a measuring cylinder and gently stirred with a plunger for 30 seconds. After two hours of rest, the solution was stirred again, three 1 ml aliquots were separated and embryos already developed into a 2-cell or older stage were counted under a Leica MZ 16 binocular microscope. The embryos in the aliquots were discarded to reduce contamination risk. The mean number of fertilized eggs was determined and the solution was left to rest for two more hours.

3. STATIC ACUTE 48-H TESTS

Standardized static 48h acute tests were conducted on 4h-old embryos and two day old larvae of *M. leucophaeata* to test the effect of changes in temperature and salinity on the survival rate. The

American Society for Testing and Materials (ASTM, 1999) recommended 4h-old embryos (maximum time after fertilization) for the test. The duration of the test was fixed at 48 hours because embryos in the control treatment usually develop into straight hinge D-shape larvae with completely developed shells in 20 to 30 hours. However, in order to investigate the vulnerability of different larval phases, 48h tests were in the beginning also conducted on two day old D-shaped larvae of *M. leucophaeata*.

3.1. Larval vulnerability of *M. leucophaeata*

Four hours after fertilization, embryos were added in a random order to the test solutions at a concentration of ± 10 embryos/ml in a 50 ml glass cylindrical vial, already containing the test solution. The variation in concentration of embryos in the various test solutions was minimized by keeping the embryo suspension well mixed with a plunger and using a high precision automatic pipette. All treatments occurred under the same light conditions consisting of continuous lighting of two 8 W TL-lights. The organisms were not fed during the test because they do not feed during embryonic development into D-shaped larvae (first 72 hours) (Honkoop, 1999): uneaten food could decrease the amount of dissolved oxygen and as such influence the test results. Test solutions were also not aerated, because the bubbles can collect within the mantle cavity of the larvae (Helm et al., 2004). Vials were covered to keep out extraneous contaminants and bacteria and to minimize evaporation. Test solutions were made one day in advance so they would be oxygen saturated at the beginning of the experiments.

The procedure for the tests with two day old D-shaped larvae was similar. However, a concentration of ± 5 larvae/ml in 50 ml was used, the organisms were fed (3.10^5 cells *Isochrysis galbana*/ ml) during the test and test solutions were aerated in order to keep the larvae in suspension (Stoeckel et al., 2004).

For both stages, salinity-dependent mortality rate at salinities of 5, 10, 15, 20 and 25 PSU was tested at a constant temperature of 20 °C and temperature-dependent mortality rate at temperatures of 5, 10, 15, 20 and 25 °C was tested at a constant salinity of 8 PSU. As a universal control for all experiments, embryos and D-shaped larvae were exposed to artificial water with the same characteristics (20 °C and 8 PSU) of that where fertilization occurred. This combination temperature-salinity approaches the field conditions, at the beginning of larval presence in the water column (Verween et al., 2005). Different salinities were obtained by using different concentrations of artificial salts (Instant Ocean®, Aquarium Systems, France), while different temperatures were obtained by using different small climate chambers for the experimental setups.

Since *M. leucophaeata* 2-d larvae showed high resistance to the tested conditions, all further experiments were only conducted with 4-h embryos as to determine the limits of the mussel's most vulnerable larval phase.

3.2. Temperature-salinity tolerance of *M. leucophaeata* embryos

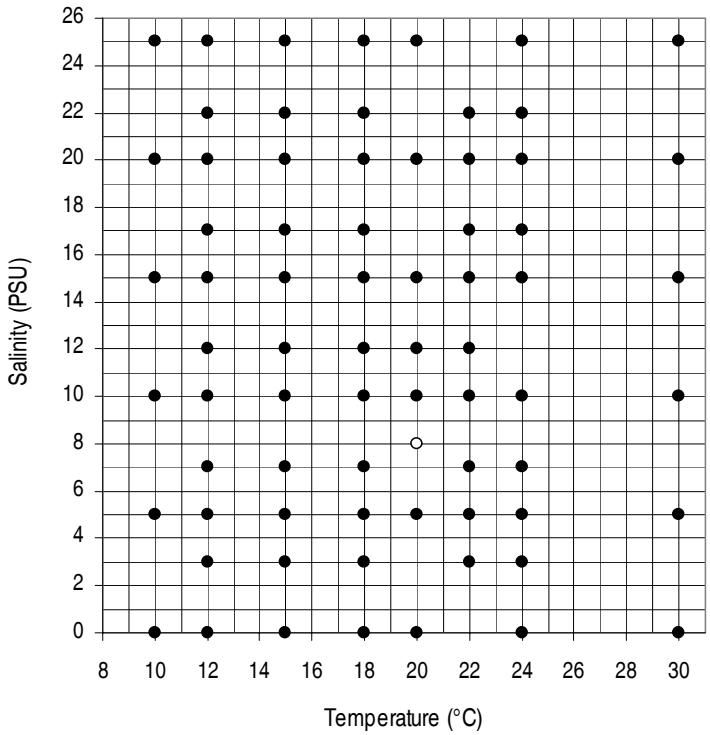

Fig. 1: Tested combinations of salinity and temperature. The white point indicates the universal control; 20 °C at 8 PSU.

Only the procedure for four hour old embryos was used in this experimental setup. Over the two years, six experiments were conducted, ranging in temperature between 8 and 30 °C and in salinity between 0 and 25 PSU (**Fig. 1**). Seventy-two combinations were tested, each consisting of three replicates. As a universal control for all experiments, embryos were exposed to artificial water with the same characteristics (20 °C and 8 PSU) of that where fertilization occurred.

Embryo-larval development was stopped after 48 hours by adding 2.5 ml 4 % buffered formaldehyde and colored with Rose Bengal. All larvae were counted by means of a binocular microscope; a distinction was made between larvae shells containing meat and empty shells (ASTM, 1999).

For each test chamber in each treatment, the percentage of embryos/larvae that did not result in live larvae *A* has to be calculated as follows (Stephan, 1977):

$$A = 100 * (N - B) / N$$

with *B* the number of live larvae at the end of the test and *N* the total number of counted individuals, alive or dead.

M is the average percentage of embryos/larvae that did not result in live larvae for the control treatments.

4. STATISTICAL ANALYSIS

Homogeneity of variance and normality were tested using Levene's and Shapiro-Wilk's W-test, respectively. Larval vulnerability to temperature and salinity was tested by analysis of variance (ANOVA). Although in the majority of 48h test cases on embryos (with exception of data used to test larval vulnerability) ANOVA assumptions were not fulfilled (not even after arcsinus-transformation) statistical differences on raw data were examined by Main effects and Factorial ANOVA (SAS 9.1). The large sample size allows the statistics to follow a normal distribution (Central Limit Theorem) (Sokal and Rohlf, 1981). Statements of significant differences were based on accepting $p < 0.05$.

RESULTS

All control treatments in the different experiments showed a survival percentage ≥70 %. This number lies between 71.8 and 86.8 %, i.e. the boundaries recommended by the ASTM (1999).

1. LARVAL VULNERABILITY OF *MYTILOPSIS LEUCOPHAEATA*

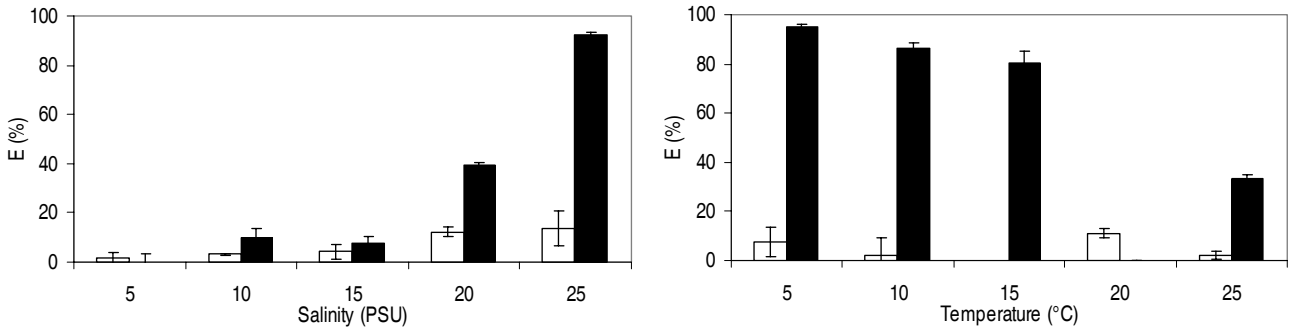

Fig. 2: Mortality rates (%) ± SE of *Mytilopsis leucophaeata* 4-h embryos (■) and 2-d larvae (□) at 20°C with different salinities and at 8 PSU with different temperatures.

Overall one-way ANOVA showed no significant difference in mortalities of 2-d D-shaped larvae at different salinities (p = 0.125) and different temperatures (p = 0.626). In all tested combinations, the highest mortality amounted only 13.7 ± 7.0 % (**Fig. 2**), indicating a high resistance to abrupt changes in the environment. Four hour embryos, however, were more vulnerable, with both salinity and temperature significantly reducing survival (p < 0.005). A significantly higher mortality was detected at higher salinities (p < 0.001), ranging between 39.5 ± 0.7 % at 20 PSU and 92.1 ± 1.5 % at 25 PSU. A significantly lower embryonic mortality was found at high temperatures 20 and 25 °C (p < 0.001), whereas highest mortalities were observed at lowest temperature, i.e. 5 °C with 95.1 ± 1.3 %.

2. TEMPERATURE-SALINITY TOLERANCE OF *MYTILOPSIS LEUCOPHAEATA* EMBRYOS

Since *M. leucophaeata* 2-d larvae showed high resistance to the tested conditions, all further experiments were only conducted with 4-h embryos as to determine the limits of the mussel's most vulnerable larval phase.

The optimal conditions, in which quasi all embryos developed to D-shaped larvae (E = 3.3 ± 0.2 %), were reached at 22 °C and 15 PSU (**Fig. 3a**). Surrounding this optimum, conditions stayed good for development in a rather wide range: temperature could vary between 15 and 24 °C and salinity between 15 and 22 PSU with mortality only ranging between 0 and 58 %. The limits of survival were found only at extreme temperatures of 10 and 30 °C and salinities of 0 and 25 PSU, indicating a broad tolerance of embryos to variation in temperature and salinity.

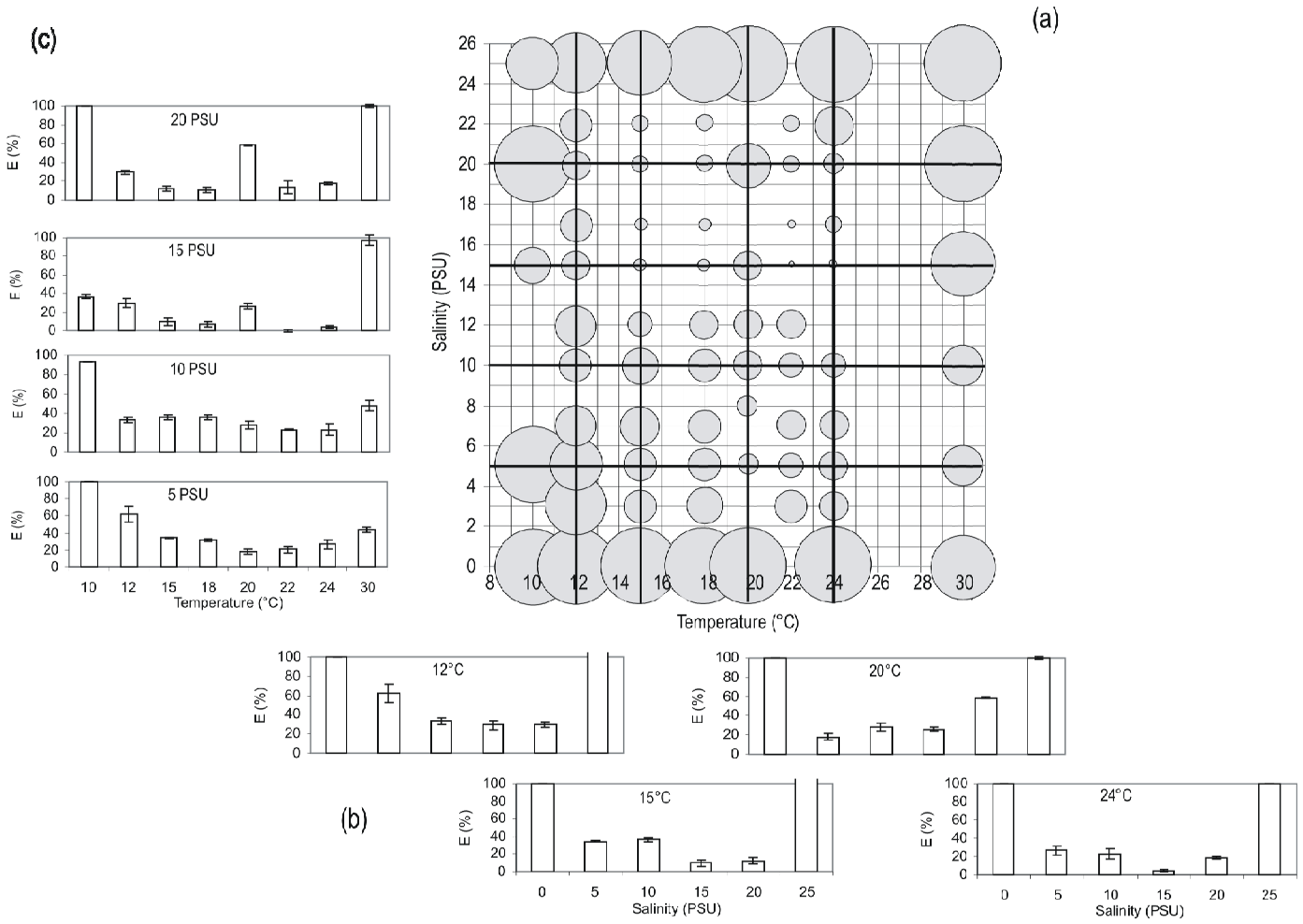

Fig. 3: Mortality rates (%) of *Mytilopsis leucophaeata* 4-h embryos at the tested combinations of temperature and salinity. (a) Surface plot of all tested combinations. The larger the surface of the circle the higher the mortality rate, ranging from 0 to 100%. (b) Mortality rates ± SEs at temperatures 12, 15, 20 and 24 °C. (c) Mortality rates ± SEs at salinities 5, 10, 15 and 20 PSU.

2.1. Temperature tolerance

Mytilopsis leucophaeata embryos were susceptible only to extreme temperatures: at a temperature of 10 °C, all embryos died at salinities of 0, 5 and 20 PSU and mortality was above 60 % at 25 PSU. At 30 °C, a similar pattern was distinguished, with mortalities ranging between 96 and 100 % at 0, 15, 20 and 25 PSU. Whereas no embryos survived a 48h exposure to extreme salinities 0 and 25 PSU at either tested temperature, embryos had a high tolerance of intermediate temperatures (12 – 24 °C) at intermediate salinities (5 – 20 PSU) with a mortality ranging between 0 and 76 % (**Fig. 3b**).

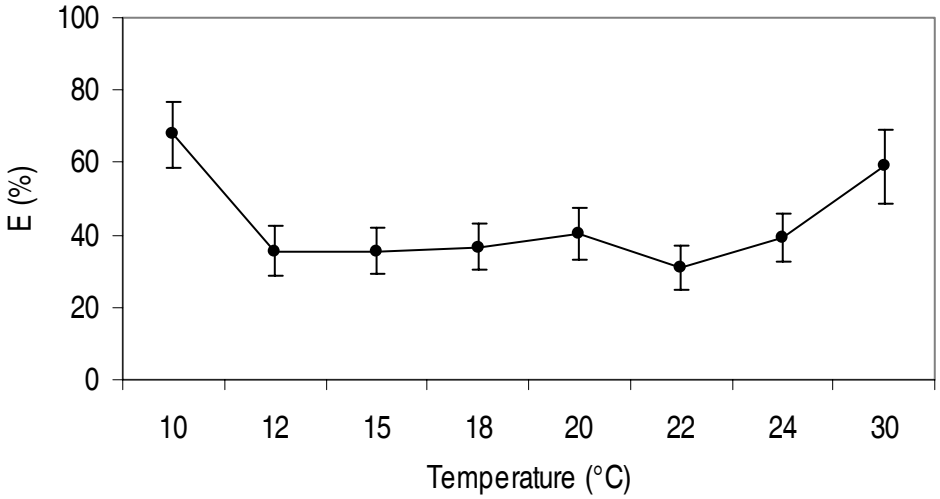

Fig. 4: Mean mortality (E %) ± SEs at tested temperatures.

Two-way ANOVA showed an overall highly significant effect of temperature ($P < 0.001$) on embryonic mortality of *M. leucophaeata* with 10 and 30 °C inducing a highly significantly higher mortality ($P < 0.001$) than the other temperatures (**Fig. 4**). Maximum mortalities, however, did not reach 100 % (E = 81 ± 7 %).

2.2. Salinity tolerance

Again, embryonic development should be considered resistant to changes in salinities: independent of temperature (except at extreme temperatures) survival rates were high with mortality ranging between 0 and 62 %.

Two-way ANOVA showed an overall highly significant effect of salinity ($P < 0.001$) on embryonic mortality of *M. leucophaeata* with 0 and 25 PSU inducing a highly significant higher mortality ($P < 0.001$) than the other salinities (**Fig. 5**). The effect of extreme salinities on mortality (96.0 ± 1.4 %) was significantly higher ($P = 0.016$) than the effect of extreme temperatures (82.4 ± 3.9%).

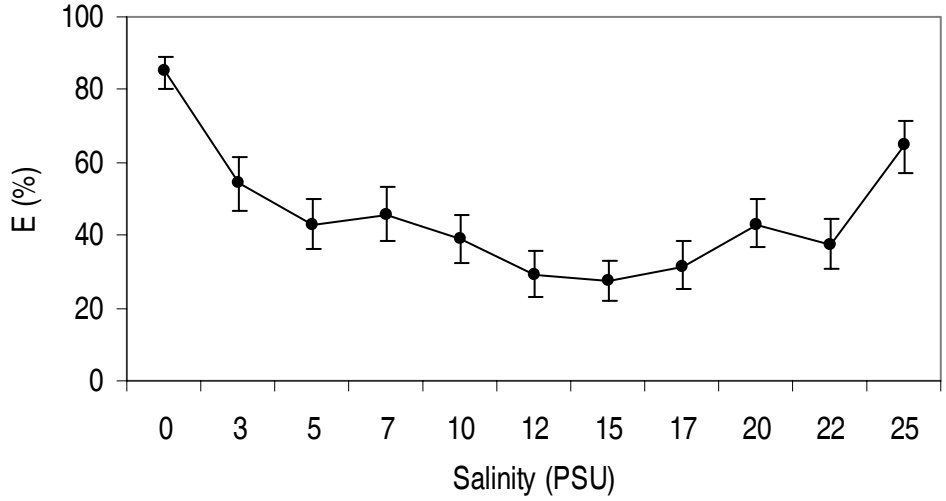

Fig. 5: Mean mortality (E %) ± SEs at tested salinities.

2.3. Temperature-salinity tolerance

Data insinuated that embryos were more tolerant to low salinities (5 – 10 PSU) at high temperatures (30 °C) than at low temperatures (10 °C), proven by factorial ANOVA ($P < 0.001$). The trend of higher tolerance of embryos to high salinities (15 – 20 PSU) at lower temperatures (15 – 18 °C) than at lower salinities (5 – 10 PSU) at lower temperatures (**Fig. 3c**) was not statistically proven (factorial ANOVA $P = 0.134$).

DISCUSSION

1. LIFE STAGE DEPENDENT TOLERANCE OF *M. LEUCOPHAEATA*

This research indicated that in the larval phase, not all stages of *M. leucophaeata* are equally vulnerable as hypothesized. A clear distinction could be made between 4 h old embryos and 2 days old larvae, with the latter being already extremely resistant to variation in temperature and salinity. Therefore, for *M. leucophaeata*, the theoretical shift from the vulnerable, larval phase to the highly resistant, benthic phase needs to be nuanced with emphasis on the fact that the first, very young embryos are most vulnerable: a gradient in resistance might thus be expected from the early life stage to the benthic stage.

In marine invertebrates, the degree of tolerance to environmental variations often varies during ontogeny (Kinne, 1970; 1971). Developing eggs and newly hatched larvae of some invertebrates, such as *M. leucophaeata*, may already tolerate extreme wide ranges of salinity or temperature. However, early ontogenic stages of most invertebrates exhibit lesser tolerances than the respective later stages or adults. For *Dreissena polymorpha*, the resistance to both salinity and temperature raises with age (Wright et al., 1996). Gametes and larvae of the mussel *Mytilus californianus* die in diluted seawater in which adults can survive 'indefinitely' (Fox, 1941). Larvae of *Mytilus edulis* survive at salinities of 15 - 40 PSU with temperatures of 5 - 20 °C (Brenko and Calabrese, 1969), but adult mussels are more tolerant to a wide variety of environmental variables (Seed and Suchanek, 1992).

2. TEMPERATURE-SALINITY TOLERANCE OF *M. LEUCOPHAEATA*

2.1. Temperature-tolerance

At its place of origin, *M. leucophaeata* is restricted to warm, more temperate waters (Marelli and Gray, 1983) but in Europe, it endures much lower temperatures; the species has been found in fluctuating water temperatures ranging from minima of 5 °C in Finland (Laine et al., 2006) up to 30 °C in Miami (Siddall, 1980). For most bivalves, temperature is not the main restricting environmental variable. Adult *D. polymorpha* can survive easily in temperatures up to 29 °C (Karatayev, 1995) while *Cerastoderma edule* is widely distributed in European estuaries, and thus well adapted to a wide temperature range (Boyden and Russell, 1972). Also *Crassostrea gigas* has a broad temperature tolerance (Leffler and Greer, 1991) while *M. edulis* has an upper sustained thermal tolerance limit of about 29°C (Almada-Villela et al., 1982).

Table I: Temperature-tolerance for adult and larval bivalves (°C) in comparison to *Mytilopsis leucophaeata*.

	adult	larvae	references
Mytilopsis leucophaeata	5 - 30	10 - 30	Laine et al., 2006; Siddall, 1980
Dreissena polymorpha	0 - 29	12 - 24	Karatayev, 1995; Sprung, 1993
Cerastoderma edule	3 - 20		Boyden and Russell, 1972
Crassostrea gigas	-1.8 - 35	wide range	Leffler and Greer, 1991; Fabioux et al., 2005
Mytilus edulis	< 29	10 - 25	Almada-Villela et al., 1982; Brenko and Calabrese, 1969
Ostrea edulis		wide range	Fabioux et al., 2005

Temperature, however, is an important species-specific factor for spawning initiation (de Vooys, 1999) and monitoring data show that for *M. leucophaeata*, the threshold temperature for gamete maturation may be 13 ± 1 °C (Verween et al., 2005), comparable with the threshold of 12 °C in *D. polymorpha* (Ram et al., 1996). Other studies indicate that reproduction in *M. leucophaeata* usually starts at a temperature higher than 15 °C (Schütz, 1969) or even higher than 20 °C (Rajagopal et al., 1995).

Optimal temperature for embryonic development in *M. leucophaeata* appeared to be 22 ± 2°C, although salinity rather than temperature seemed the limiting factor in this development process. Since spawning in *M. leucophaeata* is situated in summer period, a fluctuating temperature regime with high enough summer temperature will be sufficient to secure establishment of *M. leucophaeata*. In this experimental setup, maximal mortalities at the extreme temperature levels 10 and 30 °C amounted only 81 ± 7 %, indicating that the true limits of embryonic tolerance to temperature may even be more extreme. Also larvae of *D. polymorpha* and *M. edulis* show a broad larval tolerance to temperature (Sprung, 1993; Almada-Villela et al., 1982), as also applying for the larval phase of *Ostrea edulis* and *C. gigas*, both surviving a wide range of temperature (Robert et al., 1988; Diederich, 2006).

2.2. Salinity tolerance

Mytilopsis leucophaeata is a typical euryhaline species, with adults being able to survive in salinities ranging from 0.1 PSU to 31 PSU, indicating that this species can be found across nearly the whole estuarine gradient with only true seawater (35 PSU) outside its reach of survival (Verween et al., in press). However, these levels are well above the levels preferred for propagation (Wolff, 1969). The same goes for e.g. *C. gigas*, which can occur below 10 PSU and survive above 35 PSU although it is unlikely to breed at such extreme salinities (Fabioux et al., 2005). Also adult *C. edule* and *M. edulis* adults are tolerant to a wide range of salinities (Boyden and Russell, 1972; Almada-Villela, 1984).

Table II: Salinity-tolerance for adult and larval bivalves (PSU) in comparison to *Mytilopsis leucophaeata*.

	adult	larvae	references
Mytilopsis leucophaeata	0.1 - 31	3 – 22	Verween et al., in press
Cerastoderma edule	18 - 40 (optimum)	20 – 50, but 30 - 35 (optimum)	Boyden and Russell, 1972; Kingston, 1974
Crassostrea gigas	10 - 35		Fabioux et al., 2005
Mytilus edulis	15 - 40 (optimum)	15 – 40	Almada-Villela, 1984; Brenko and Calabrese, 1969
Ostrea edulis		28 - 32 (optimum)	Robert et al., 1988

Although Siddall (1980) stated that the larvae and postlarvae of *M. leucophaeata* are capable of development at very high salinities ranging to 32 PSU, this study showed that embryos of *M. leucophaeata* are not that tolerant to changes in salinity with upper survival limit only at 22 PSU. However, this very young larval stage – only 4 hours after fertilization – should be considered as already extremely tolerant to changes in salinity, considering its complete lack of protection against external influences. Normal embryonic development of *M. leucophaeata* is possible in the salinity range of 3 to 22 PSU. Kingston (1974) found that also *C. edule* larvae survived a broad salinity range of 20 – 50 PSU but grew optimally only in a smaller range, with frequent deformations at 20 PSU and no metamorphosis at 45 PSU. Also *O. edulis* and *M. edulis* larvae survived in a wide range of salinity (Robert et al., 1988; Brenko and Calabrese, 1969).

2.3. Temperature-salinity tolerance

Thermal responses may be modified by other concomitantly effective environmental variables such as salinity, which has received the greatest attention. Several aquatic invertebrates living in habitats with greatly fluctuating temperature and salinity conditions can tolerate subnormal temperatures better at the lower end of their salinity range and supranormal temperatures better at the upper end of their salinity range (Kinne, 1970). Beneficial effects of such low/low and high/high combinations have been found in the amphipod *Gammarus duebeni* (Kinne, 1952), the crab *Rhithropanopeus harrisii* (Kinne and Rotthauwe, 1952) and the colonial hydroid *Cordylophora caspia* (Kinne, 1958). In *M. leucophaeata*, however, the opposite pattern is found with a higher tolerance of suboptimal temperature at the upper end of the tested salinity range and vice-versa. This pattern was also detected in other mussels such as *M. edulis,* where survival of larvae at salinities of 15 to 40 PSU is uniformly good at 5 to 20 °C but is reduced drastically at 25 °C, particularly at high and low salinities (Branko and Calabrese,1969). Wright et al. (1996) found that also survival of *D. polymorpha* larvae was negatively influenced by the combination high temperature / high salinity with percentage survival decreasing abrupt at the high/high combination of 26 °C – 8 PSU.

CONCLUSIONS: IMPLICATIONS FOR POSSIBLE FUTURE INVASION

Although embryos and larvae of *M. leucophaeata* lack a protective shell, they are already remarkably resistant to variation in temperature and salinity; only salinities of 0 and 25 PSU or higher at the moment of fertilization, independent of the prevailing temperature, cause mortalities high enough to ensure the prevention of a successful introduction of *M. leucophaeata*. Regarding the temperature

range, at temperatures of 10 and 30 °C independent of the prevailing salinity, maximal mortality has never been reached. This information has important implications regarding further spread of the species.

The accidental or deliberate release of non-native species into new habitats by shipping and aquaculture activities is an increasing phenomenon all over the world (Reise et al., 1999; 2006). Invasion success of an exotic species is not only dependent on the species' environmental tolerances but also on the invaded habitat. If the new species arrives in a continuous, widespread habitat, such as freshwater rivers, barriers of dispersal are an exception, and the species can expand its newly invaded habitat easily on a natural way. This explains the rapid spreading of *D. polymorpha* in a newly invaded habitat (e.g. Great Lakes) (Ram and Mc Mahon, 1996). Estuaries however, are mostly discrete, non-connected habitats, and can be identified as islands surrounded by fresh or seawater, posing as such a limited habitat to invade naturally. Since fresh and seawater are outside the range for survival of *M. leucophaeata*, it makes it almost impossible for the bivalve to cross these natural salinity barriers and as such to naturally invade into new areas. Transfer from one place to another is thus most probably mainly human-induced. By means of transport as larvae in ballast water (Conn et al., 1993) or as adults attached to the hull, shipping traffic is the most important vector for dispersal of *M. leucophaeata* (Therriault et al., 2003). Therefore, harbours and industrial installations are perfectly operating bases for *M. leucophaeata* spreading.

The more commercial species, such as *C. gigas* and *O. edulis* arrive in their new habitats by means of commercial introduction; they are cultivated for commercial use in aquaculture hatcheries and often spread naturally from there on (Andrews, 1980; Chew, 1990). As for *M. leucophaeata*, high temperatures are required for spawning and larval development of the oysters, somehow limiting the invasion capacities of the species.

The habitat preferences and environmental limits of adult *M. leucophaeata* are very broad, which means that, theoretically, we can expect this species in most brackish water bodies. *Mytilopsis leucophaeata* is known to reproduce once a year, with spawning between end of May and September-October in Europe, somewhat later in the US (Verween et al., 2005). In this period, the most vulnerable phase in larval development – the embryos - needs good development conditions, mainly characterized by a high temperature, somehow limiting the potential locations for future invasion. Therefore, especially colder regions could give rise to doubt possible invasion success by *M. leucophaeata*. If even summer temperature stays below 10 °C, it is theoretically impossible for the mussel to establish. However, even

there the risk can not fully be excluded; man-made facilities, such as large harbours or cooling water systems, can artificially instigate higher water temperatures. This can promote conditions for species introduction, even in habitats where it normally would not establish.

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CHAPTER VIII

General discussion:

New insights in biofouling control of *Mytilopsis leucophaeata*

OUTLINE

Although the major aim of this doctoral thesis was to find an ecologically sound biofouling control method for *Mytilopsis leucophaeata*, the thesis yielded much more information concerning this rather unknown species. Good knowledge on the autecology of a biofouling species is a necessary step for successful biofouling treatment; therefore, chapter II to V dealt with specific biological processes of *M. leucophaeata*. Chapter VI and VII treated the development of specific control methods.

In chapter VIII, the problem of *M. leucophaeata* biofouling is discussed in more detail and information is assembled over the three following aspects: (1) the invasive capacities of *M. leucophaeata*, (2) the biofouling capacities of *M. leucophaeata* in newly invaded habitats and (3) possible ecologically sound control measures for *M. leucophaeata* biofouling. Future perspectives are briefly unfolded as well.

INVASIVE CAPACITIES OF *MYTILOPSIS LEUCOPHAEATA*

1. DEFINITION OF AN INVASIVE SPECIES

The scientific topic on alien invasive species is very popular nowadays, but what is really an invasive species? Official U.S. definitions regarding invasive species were provided in Executive Order 13112 (1999). "Invasive species" means an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health. An “alien species” is defined as a species, subspecies or lower taxon occurring outside of the historically known range it occupies naturally and outside its dispersal potential as a result of direct or indirect introduction or care by humans. An alien species includes any part, gametes or propagule that might survive and subsequently reproduce. Synonyms are non-native, non-indigenous, foreign, and exotic. So, although a large number of non-native, alien species are regularly introduced worldwide, only a limited number of them are considered invasive. *Mytilopsis leucophaeata* is without any doubt an invasive species in Europe, since its biofouling problems cause economic harm.

An alien species can come from any place in the world; a species native to one part of the world may be transported to another part by human activities or natural causes. If this species is able to become

established at the new location - i.e. developing offspring in more than one season or being present longer than the expected lifetime of an individual - it can become invasive. Man has distributed aquatic and terrestrial species over millennia, yet only since the 90s the problem has been addressed with great alert. The main reason is that over the last century, many new and more effective dispersal vectors such as intensified shipping, canals, aquaculture and aquaria (Reise et al., 2006) have become active, increasing the invasion rate (Minchin, 2004). Although the hypothesis exists that invasions are part of nature and would happen anyway, most scientists are of opinion that human-mediated processes are doing more than just speeding up the process (Gollash, pers comm); the Atlantic Ocean e.g. acts as a migration barrier as the duration of the larval phase of marine organisms is too short to enable a distribution with natural means and human-mediated vectors are essential. The same arguments state for fresh and brackish water species; these organisms would not be able to migrate through fully saline water by natural means.

2. *MYTILOPSIS LEUCOPHAEATA*: A SLOW THOUGH RESISTENT INVADER

The invasion history of *M. leucophaeata* in European brackish waters (**Chapter II**) does not fully answers the question if the species is a good invader; although the species has been present in Western Europe since the early 19th century, no clear invasion pattern can be deduced. However, we hypothesize that mainly the misidentification of the species as *D. polymorpha* is responsible for this blurry pattern and that *M. leucophaeata* indeed has been present and spreading through European waters ever since it first establishment in 1835 (Nyst, 1835).

Invasion starts with one or more incidents of arrival, followed by an establishment of a small group of successfully reproducing individuals which may proceed in an expansive phase, eventually turning into a phase of adjustment to their new environment (Reise et al., 2006). *Mytilopsis leucophaeata*'s life cycle has some specific features, advantageous in the process of successful establishment. (1) As most Dreissenids, *M. leucophaeata* can carry ripe gametes for a very long time (**Chapter IV**), being reflected by the seasonal flexibility in larval production patterns and (2), in contrast to most other bivalve species, *M. leucophaeata* has only one yearly spawning period, but of extremely long duration; from June to December, over 50 % of all individuals are in a spawning stage (**Chapter III – IV**). This combination of prolonged ripeness of gametes with elongated spawning period protects the newly invaded bivalve from sudden events. When an invaded population is still in an early stage, establishment can fail because of sudden changes in environmental factors, e.g. an abrupt temperature rise can wipe out all larvae present at that time. The first larval batch is also possibly subject to unknown predators or other

negative influences in the new waters, making it rather difficult to develop as such immediately a successful reproducing offspring. The expanded availability of ripe gametes together with the prolonged presence of newly hatched larvae in the water column avoids the sudden elimination of a complete recruitment season and as thus a failing of successful establishment. (3) *Mytilopsis leucophaeata* is a rather long-lived species with a minimum life span of 5 years (**Chapter V**). This gives the newly arrived individuals the opportunity of several trials to successfully produce offspring. Few individuals can as such suffice to create an expansive established population, minimizing the risk of an unsuccessful establishment in a newly invaded habitat. So most of the biological characteristics of *M. leucophaeata* make it a good invading species; once the species is arrived in a new habitat, it will most likely establish quite easily.

Although *M. leucophaeata* has very broad habitat preferences and environmental limits, to invade into a new area, it has to overcome a major obstacle: although the species has a broad tolerance to salinity, survival with reproduction is impossible in fully fresh or seawater. Since brackish waters are mostly isolated islands surrounded by a barrier of sea or fresh water, it is almost impossible for the bivalve to cross these natural salinity barriers and as such to naturally invade into new areas. In contrast to *D. polymorpha*, who can expand very rapidly as soon as a new freshwater basis is colonized, *M. leucophaeata* is a rather slow natural colonizer with low dispersal capacities, who is restricted to brackish water bodies. Therefore, its dispersal is almost completely dependent on human-induced transport. By means of transport as larvae in ballast water or as adults attached to the hull, shipping traffic is the most important vector for dispersal of *M. leucophaeata* (Therriault et al., 2004) (**Chapter II**).

3. BRACKISH WATERS: VULNERABLE TO INVASIONS

Most of the arrivals of new alien species become established in brackish waters (Reise et al., 1999). The fact that they are in general species-poor with broad environmental conditions and often subject to intensive international shipping makes them sensitive to new invasions (Wolff, 1999; Paavola et al., 2005; Nehring, 2006), with harbours being excellent arrival and operating bases for alien species. It has been estimated that up to 4000 pelagic and bethic species are being transported between continents by ships every day (Minchin and Gollash, 2003). The high infection rate can also be explained by the fact that genuine brackish water species are mostly highly tolerant for changing environmental conditions (Wolff, 1999), and can thus adapt more easily to new conditions than marine or freshwater species (Nehring, 2006). Reise et al. (1999, 2006) calculated that ratios for non-native to

native species may be 1:40 in European marine waters, 1:20 at open coasts, and even 1:5 in estuaries or lagoons. More than half of all alien species are benthic invertebrates with macroalgae ranking on second place.

Mytilopsis leucophaeata is a resistant brackish water species, with very broad environmental tolerances. Only true seawater (35 PSU) and fresh water (0 PSU) are out the borders of adult survival (**Chapter V**). Therefore we could expect *M. leucophaeata* to invade in all brackish waters when able to enter (**Chapter II**). However, the lifecycle of a mussel consists of two phases: (1) when the mussel has developed a protective shell, it is almost invulnerable but (2) when it is still in its larval phase without a hard shell, the individual is much more vulnerable to external influences (Conn et al., 1993), possibly limiting the survival range of the species. Although embryos and larvae of *M. leucophaeata* lack a protective shell, they are already remarkably resistant to variation in temperature and salinity (**Chapter VII**); survival of the newly hatched embryos is only fully impossible in very cold ($\leq$10°C) water. This somehow limits the potential locations for future invasion. *Mytilopsis leucophaeata* is known to reproduce once a year, with spawning between end of May and September – October in Europe, somewhat later in the U.S. (**Chapter III**). In this period, the most vulnerable phase in larval development - the embryos - needs optimal development conditions, mainly characterized by a high temperature. Therefore, especially colder regions could give rise to doubt about invasion capacities by *M. leucophaeata*. In natural brackish waters with summer temperature below 10 °C, it is impossible for the mussel to establish. However, man-made facilities, such as large harbours or cooling water systems, can artificially instigate higher water temperatures. This can promote conditions for species introduction, even in habitats where it normally would not establish.

BIOFOULING CAPACITIES OF *MYTILOPSIS LEUCOPHAEATA*

1. DEFINITION OF A GOOD BIOFOULER

Any surface exposed to natural waters provides an opportunity for the settlement and subsequent growth of organisms; together with the cooling water of an industrial installation, numerous organisms get pumped up and become dispersed in the system. The industrial cooling water system provides an ideal habitat for a lot of these species, since the majority are sessile suspension feeders: they enter a system with a constant water flow assuring a continuous supply of food and oxygen, but without predators, since these are kept out by means of the screening system. Giving these perfect conditions, settlement will occur quickly and growth can be rapid (**Chapter I**). An important feature of a cooling water system is the arrival in a system with a higher temperature than the surrounded natural environment; as a consequence of the use of the water to cool down electrical processes, the water in its turn gets heated.

To become an industrial biofouler, a species needs at least two specific characteristics: (1) it must have planktonic larvae which are able to enter the industrial system (Kovalak et al., 1993) and (2) the adults must attach to artificial hard surfaces and be able to obtain food out of the water flow (Railkin, 2004). However, this only gives a species the opportunity to invade and survive in the infested system but it is not a guarantee for successful establishment and survival against biocides. The effectiveness of a biofouler strongly increases when (3) it is able to produce a large offspring, which in turn can also attach onto the available substrate, as such expanding the problem, (4) adults can protect themselves in some way from the used industrial control measures such as biocides, which makes it difficult to remove them and (5) adults preferentially attach to the delicate structures of the system, e.g. onto heat exchangers, narrow transition pipes and small mesh filters, which are difficult to clean and costly in repair.

2. *MYTILOPSIS LEUCOPHAEATA* : A SEVERE AND PERSISTENT BIOFOULER

The biological studies accomplished in this thesis all revealed temperature as being the key factor governing *M. leucophaeata* life processes. Since temperature in a cooling water installation is higher than in the surrounding environment, this has advantageous consequences for the biofouling capacities of the species. The existence of a temperature threshold for gametogenesis and spawning has been accepted for many bivalve species (Mackie, 1984), governing the onset of the spawning season.

Mytilopsis leucophaeata has a temperature threshold of 13 ± 1 °C for gamete maturation and spawning, resulting in the start of the spawning season by the beginning of the summer, when water temperature in the environment exceeds the threshold value (**Chapter III – IV**). In the cooling water system however, an average constant temperature of 20 °C prevails, exceeding this threshold value all year long. The hypothesis can be raised that this continuous possibility of spawning by adult mussels in the cooling water system can become an unceasing source of new larvae into the natural environment. However, also food availability is a major controlling factor in gonad development and both factors may be more or less simultaneously responsible for the onset of spawning. This would reduce the risk of continuous spawning in the industrial system although a prolonged spawning in comparison to the environment is believed. Temperature is also the main factor in regulating shell growth in mussels, with in general an increase in growth with rise in temperature over the ecological range of the species (Bayne and Worral, 1980). The same applies for *M. leucophaeata*, with an average temperature of 20 °C being well within the limits of survival of the species (**Chapter V**). Hence, warmer waters should be considered as an advantageous environment for this original subtropical species (Marelli and Gray, 1983), implying the presence of an energy surplus for any physiological process in *M. leucophaeata* (Sprung, 1995; Walz, 1978b) such as growth. This indicates the beneficial effect of the cooling water on *M. leucophaeata* growth, enlarging as such caused fouling problems.

But even without the advantageous effect of the warmer environment, *M. leucophaeata* has some characteristics that make the species a severe fouling species. *Mytilopsis leucophaeata* is a small, slowly growing, invasive bivalve (**Chapter V**) which seems by means of these features a rather harmless fouling species, especially in comparison to the larger, quickly growing *D. polymorpha*. However, little growth is required before mussels reach the size equal to the interplate gap of plate heat exchangers (4-10 mm), at which point blockage can occur (Jenner et al., 1998). So, direct interference with the functioning of the system (cf. definition of biofouling) should not be considered strictly correlated with size. Its high longevity indicates that once fouling problems have arisen, these problems might persist for a long time: an adult *M. leucophaeata* is very tolerant to chlorination (Rajagopal et al., 2002b), not easily removable and, once established, will continue producing offspring for a long period of time.

Although Pathy and Mackie (1993) posed that the ecological and economical threat of *M. leucophaeata* is less severe than that of the zebra mussel, the fact that they inhibit brackish waters makes them far more resistant to environmental changes than freshwater species (Siddall, 1980), which makes them potentially very robust foulers (**Chapter II**), even more severe than the world-wide known *D.*

polymorpha. Also Rajagopal et al. (2005b) stated that *M. leucophaeata* presents a considerably more formidable fouling problem compared to *M. edulis* and *D. polymorpha*.

BIOFOULING CONTROL OF *MYTILOPSIS LEUCOPHAEATA*

Mytilopsis leucophaeata is a rather slow natural colonizer needing human-mediated vectors for its dispersal, but once established the species has all advantages to become a severe fouling species. Until now, the control of *M. leucophaeata* biofouling aimed only at killing the adult mussels, which pose the existing problem. However, not only do mussels have their own defensive strategy against the use of biocides, i.e. closing their valves, *M. leucophaeata* is also far more resistant to these chemicals than other mussel foulers like *M. edulis* and *D. polymorpha* (Rajagopal et al., 2002a; 2005b) **(Chapter V)**. Scientific research concerning adult *M. leucophaeata* fouling control measures is already extensive (Rajagopal et al., 1994; 1997; 2002; 2003; 2005a,b), therefore this thesis aimed at developing control measures against *M. leucophaeata* biofouling in another way. It is generally believed that there are two important phases in a mussel life cycle (Conn et al., 1993): (1) from fertilization until the larvae are settling, they are pelagic, only protected by a larval soft shell and (2) after settlement, the individuals become benthic and develop a hard mytiliform shell. It can thus be concluded that the first, larval phase is possibly most susceptible to changes in external environment (Claudi and Evans, 1993). Hence, knowledge of the presence of mussel larvae provides a basis for an ecologically and economically proper use of these detrimental chemicals (Relini, 1984).

1. AUTECOLOGICAL BASELINE KNOWLEDGE AS A FIRST STEP IN BIOFOULING CONTROL

Biological monitoring showed that the yearly period of larval occurrence of *M. leucophaeata* in the harbour of Antwerp was markedly similar, even though major differences in densities were found between months and years (**Chapter III**). This 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 targeted dosage of biocides during the period of larval presence would be as effective as a continuous dosage throughout the year. Another advantage is that adults tend to be weaker after spawning when they have little energy reserves (Bayne et al, 1976), so it is very obvious that effective control measures are best adopted during spawning periods (Rajagopal et al., 2005a). A targeted dosage would decrease the amount of biocides needed, allowing facilities to: (1)

meet the (inter)national biocide draining criteria (Directive 98/8/EC; Directive 2000/60/EC; VLAREM I; VLAREM II) (**Chapter I**) and (2) explore the use of ecologically less harmful, but more expensive biocides. The almost all year round presence of secondary settlers (**Chapter III**) 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 pointed combat. However, this strategy is just a first step in the right direction; treatment still works reactively, i.e. larvae must be monitored first before action can be undertaken, and a maximal yearly difference in larval arrival of 25 days (i.e. almost one month) could appear, possibly resulting in one month's unnecessary biocide application if mussel larvae appeared in intake water one month later than they typically arrive (**Chapter VI**) or allowing them to enter the system unharmed if larvae appeared one month earlier.

2. AN EARLY WARNING TOOL FOR BIOFOULING THREAT

The second aim in the search for ecologically sound biofouling control of *M. leucophaeata* was to refine the previous method. By means of the development of a predictive model (**Chapter VI**) the larval presence of *M. leucophaeata* could be predicted with high accuracy. To incorporate this modeling technique in industrial monitoring technologies, it was preferable that the complexity of the model was minimal, without reducing its predictive capacity. The developed temperature-time model made it possible to predict larval presence in the water column just by monitoring water temperature. This is an advantage in industrial use as a simple automatic monitoring system is efficient enough in determining dosage of biocides. Yet, although commonly used in assessing and predicting potential occurrence and ecological impacts of introduced species and as such prioritize future invasion threats (e.g. Ramcharan et al., 1997; Ricciardi, 2003), industrial systems suffering severe biofouling problems lack the benefits of a good predictive model. This research program is the first predictive study related to biofouling problems, where knowledge on the biological processes initiating biofouling is used to prevent new problems. Nevertheless predictive models on larval arrival can become an important tool in biofouling control programs as it combats new mussel fouling proactively in an economically advantageous way; biocide-usage can be restricted to a couple of months, predicted with high accuracy, reducing the amount of chemicals needed without increasing the risk of over- or under-dosage.

3. TOWARDS A NATURAL PREVENTION OF *MYTILOPSIS LEUCOPHAEATA* BIOFOULING

In search for a more ecologically sound biofouling control for *M. leucophaeata* we tried to go one step further; could we eliminate *M. leucophaeata* larvae just by using environmental variables as a 'killing' tool? Being a brackish water species, *M. leucophaeata* is a very euryhaline species (Siddall, 1980), rather insensitive to changes in salinity. The fact that *M. leucophaeata* has a subtropical origin makes the species also relatively resistant to temperature changes (Marelli and Gray, 1984). Yet, since a mussel's life cycle consists of a larval, vulnerable and an adult, invulnerable stage, we searched for a mortal combination of the prevailing environmental variables that made it technically possible to combat *M. leucophaeata* larvae without the use of detrimental chemicals. For *M. leucophaeata* however, the theoretical shift from the vulnerable, larval phase to the highly resistant, benthic phase needs to be nuanced with emphasis on the fact that only the first, very young embryos are vulnerable: a gradient in resistance might be expected from the early life stage to the benthic stage (**Chapter VII**). Even the very first life stages, i.e. 4h old embryos, are already remarkably resistant to abrupt changes in temperature and salinity, despite their lack of a protective shell (**Chapter VII**); only in fully fresh (0 PSU) or seawater (> 25 PSU), nor in cold ($\leq$10 °C) or very warm ($\geq$30 °C) water, development of the newly hatched embryos was impossible. These limits are beyond the range that could be artificially adjusted in an industrial cooling water system, indicating that a fully ecological solution against *M. leucophaeata* biofouling is at the moment still not feasible.

RESEARCH OUTLOOK

1. LIFE STAGE DEPENDENT VULNERABILITY TO BIOCIDES

Reduction of the amount of biocides needed to control *M. leucophaeata* biofouling leaves room for exploring the use of ecologically less harmful, but more expensive biocides. Toxicity is generally measured for adults alone and results are commonly applied on al bivalve life stages (Sprung, 1993; Stoeckel and Garton, 1993), as has also been the case with *M. leucophaeata* (Rajagopal et al., 1994; 1997; 2002; 2005a,b). It is however advisable to evaluate the toxicity of any candidate toxicant against the life stage for which the treatment will be used in order to ensure that appropriate levels of chemicals are used (Fisher et al., 1994), especially for *M. leucophaeata* where the vulnerability of life stages increases rapidly with age. Therefore, a first research expansion should be to test specific toxicity levels of biocides such as chlorine or sodium hypochlorite (Rajagopal et al., 1997; 2002) on early life stages of

M. leucophaeata, as to minimize the amount of chemicals to appropriate levels. The effect of co-occurring variation in temperature and salinity will be examined as to determine the minimal lethal threshold concentration of biocides at technologically feasible changes in abiotic environment. Also the lethality of other, more expensive and less detrimental chemicals might be tested.

2. GENETIC FINGERPRINTING AS A TOOL FOR DISENTANGLING *MYTILOPSIS LEUCOPHAEATA* DISPERSAL THROUGHOUT EUROPE

Mytilopsis leucophaeata is an invasive bivalve, having the potential of becoming the brackish water equivalent of *D. polymorpha,* but still many uncertainties exist on its European invasion pattern. A complete AFLP DNA-fingerprinting for *M. leucophaeata* in Europe and the U.S. is already present (Rajagopal, pers comm), but more detailed information is needed to reveal population genetics and phylogeographical relationships of this invasive species. Microsatellite markers (Astanei et al., 2005) can be used to analyze genetic variability in different European and American populations and as such identify the source population and true invasion pattern of *M. leucophaeata* in Europe. The invasion history of a species is a valuable guide for predicting the consequences of its introduction into a new environment (Ricciardi, 2003) needed to manage ecological and technological impacts of species' invasions (Beyers et al., 2002).

3. INCREASING THE INDUSTRIAL AWARENESS TO BIOFOULING OF NEW INVADERS

Thousands of alien terrestrial, fresh water, brackish water and marine species have already been intentionally or unintentionally introduced in Belgium (Branquart et al., 2006). Many of these were able to establish themselves and some became invasive. Trials to combat problematic biological invasions are often difficult, expensive and not always successful, making prevention the most cost-efficient solution against problematic invasions. The Belgian Forum on Invasive Species (http://www.biodiversity.be/thematic-forums/invasive-alien-species) is an informal expert group aiming to stimulate scientific research on the ecology of invasive alien species as a support to develop efficient monitoring and management strategies. It regularly updates the reference list of alien species invading terrestrial, freshwater and marine ecosystems in Belgium and acts as the national node of the IUCN Invasive Species Specialist Group, but does only reach a small group of mainly specialists. However, to prevent alien species from entering, awareness is an indispensable tool; not only scientists, but all relevant sectors have to be sensibilized.

Public awareness on alien and invasive species in Belgium is in full development. Via the Belgian Biodiversity Platform, brochures are spread concerning the march of exotic species (http://www.natuurwetenschappen.be/biodiversity). The Flanders Marine Institute (VLIZ) is currently working on a list of exotic species on the Belgian part of the North Sea (BPNS), which will be available to public through their website to emphasise the problem of alien invasions (http://www.vliz.be/NL/Infoloket/Infoloket_Gevaren_van_de_zee). Although these awareness initiatives are very basic in comparison to e.g. U.S. (Habitattitude – www.habitattitude.net) or international initiatives (Global Invasive Species Program – www.gisp.org), they are a very good start in raising consciousness among the public.

No initiatives however have yet been taken from an industrial perspective. Harbours are excellent arrival and operating bases for alien and invasive species. Much of the harbours are directly surrounded by industrial activities, which may use the natural water in one of their processes, e.g. as cooling water. So, not only are these facilities perfect watch-towers for new arrivals, they are often also the first human activities being hampered by these invasive species, e.g. a population of *Rangia cuneata* (G.B. Sowerby I, 1831), an estuarine bivalve new to the European brackish water fauna, has been by coincidence recorded at BASF, Antwerpen N.V. (**Appendix I**). After initially finding only a few small individuals in August 2005, *R. cuneata* was encountered frequently in the pipes of the cooling water system of the industrial plant from February 2006 onwards. Before this record, *R. cuneata* was only known from the Gulf of Mexico and the Atlantic coast of North America. This new invader, already causing biofouling problems, emphasizes the importance of a regularly biological monitoring of the waters in the nearness of harbours. Early detection is needed to be able to eradicate if possible or to minimise the problem before it has completely manifested itself in its new habitat: prevention is better than cure. Intervention in an early stage of establishment of non-native species is also of advantage for the industrial facilities, since it can seriously reduce future problems with invasive species. To join these perfect operating bases for detection of new species into biological monitoring programs, awareness is necessary in the industrial sector. Therefore, a community platform with presentations dealing with and explaining the seriousness of the problem of alien and invasive species should be developed.

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APPENDIX I

First European record of the invasive brackish water clam *Rangia cuneata* (G.B. Sowerby I, 1831) (Mollusca: Bivalvia)

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Aquatic Invasions 1 (4): 198-203

ABSTRACT

A population of *Rangia cuneata* (G.B. Sowerby I, 1831), an estuarine bivalve, has been recorded in the harbour of Antwerp, Belgium. This species is new to the European brackish water fauna. After initially finding only a few small individuals in August 2005, *R. cuneata* was encountered frequently in the pipes of the cooling water system of an industrial plant from February 2006 onwards. Before this present record, *R. cuneata* was only known from the Gulf of Mexico and the Atlantic coast of North America.

INTRODUCTION

During an intensive monitoring study of the biology of the invasive bivalve, *Mytilopsis leucophaeata* (Verween et al. 2005), a test-installation for biomonitoring was installed at an industrial plant in the harbour of Antwerp (51°21.37' N 4°17.30' E), along the Schelde River, Belgium (**Fig. 1**). Monitoring was undertaken on a weekly basis from February 2000 onwards. In August 2005, some small individuals (10-15 mm) of an unknown bivalve were found in the test-installation and were later identified as *Rangia cuneata* (G.B. Sowerby I, 1831) (Mollusca, Mactricae). This species had never been encountered during previous samplings.

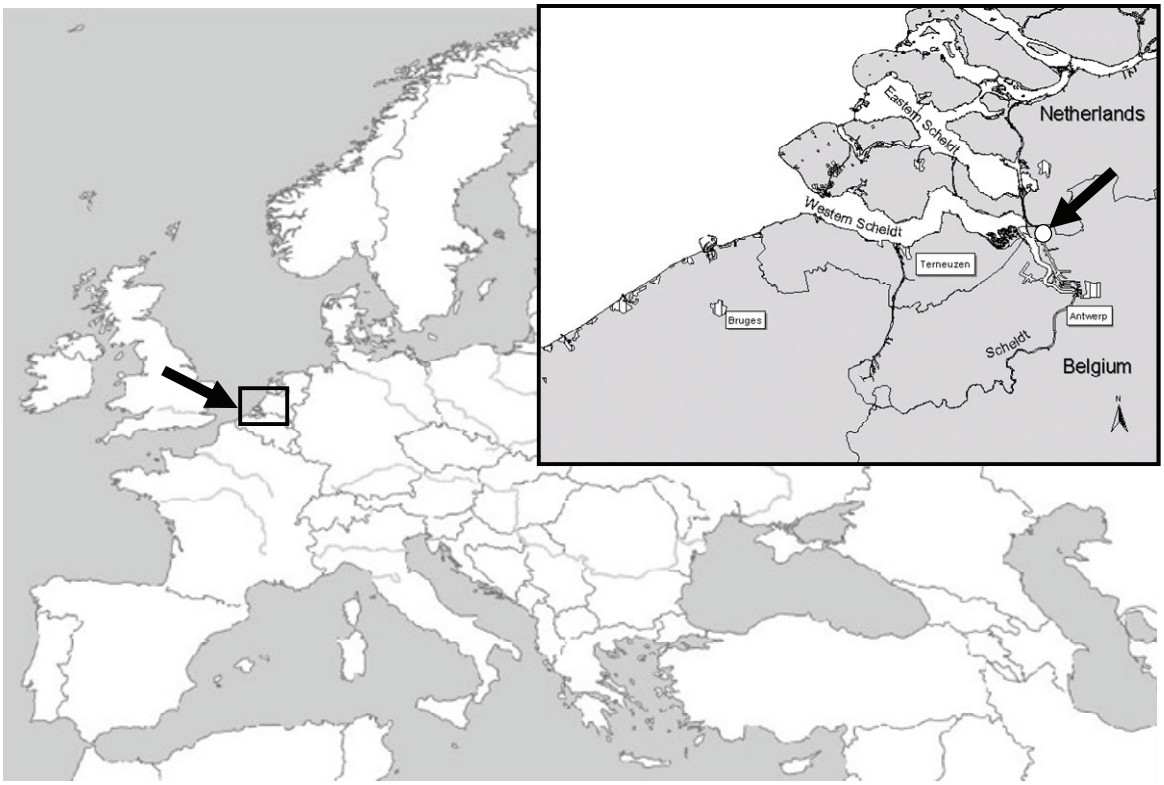

Fig 1: Harbour of Antwerp, Belgium, location where *Rangia cuneata* was collected.

The identification was hampered by the fact that we were dealing with young individuals not fully exhibiting all morphological characters of the adults. Several typical characters such as the heavy shell, the specifically bended

umbos and the heavy chestnut periostracum were thus not apparent in this material. The first specimens collected resembled the indigenous surf clam *Spisula subtruncata* but this species does not occur in brackish waters. Larger specimens (20-25 mm), collected in September 2005 had a more robust shell with a specific bended umbo, a clear pallial sinus - both characteristics not present in *Spisula* - and transversally striated lateral teeth. Therefore these specimens could be referred to the Mactridae, but not to any European species.

As the weekly sampling programme continued, more and larger individuals were found in the test installation, thus facilitating its identification as *Rangia cuneata*. Early in 2006, a dense population of *R. cuneata* was finally detected in the silt in the inlet pipes of an industrial cooling water system (current velocity: 6-6.5 m/s). Although these pipes, more than 2 m in diameter, consist of concrete, a layer of soft substrate is present at the bottom, as such creating an ideal habitat for the clams. In this particular case, the inlet pipes had to be cleaned to restore an optimal water flow, indicating that *R. cuneata* can be identified as a true nuisance fouling species. The first and to our knowledge only report of *R. cuneata* causing biofouling problems in pipes was in the Getty oil refinery in Delaware City DE, USA, where it clogged fire hoses (Counts 1980).

IDENTIFICATION

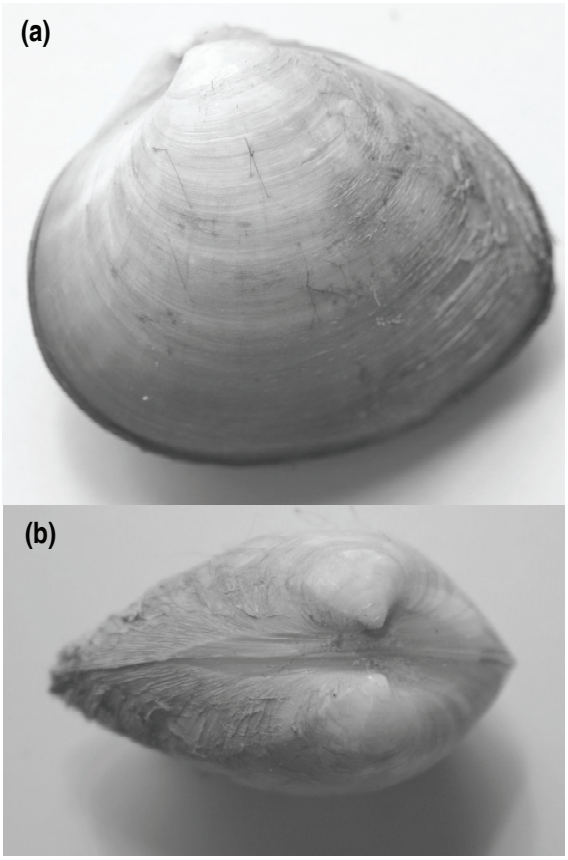

Fig. 2: *Rangia cuneata*, (a) lateral and (b) dorsal outside view. Attention to the anteriorly curved umbo.

The identification of *R. cuneata* (Wedge clam, Atlantic Rangia, common Rangia) was based on characteristics presented in Abbott (1974) and LaSalle and de la Cruz (1985). In addition, specimens were compared with samples contained in the collections of the Royal Belgian Institute of Natural Sciences and the National Museum of Natural History in The Netherlands. The valves are thick and heavy, with a strong, rather smooth pale brown periostracum (**Fig. 2a**). The shells are equivalve, but inequilateral with the prominent umbo curved anteriorly (**Fig. 2b**). An external ligament is absent or invisible, but the dark brown internal ligament lies in a deep, triangular pit immediately below and behind the beaks. Both valves have two cardinal teeth, forming a Λ-shaped projection (**Fig. 3b**). The upper surface of the long posterior lateral teeth (LaSalle and de la Cruz 1985) is serrated. The inside of the shell is glossy white, with a distinct, small pallial sinus, reaching to a point halfway below the posterior lateral (**Fig. 3a**). The pallial line is tenuous (Garcia-Cubas 1981).

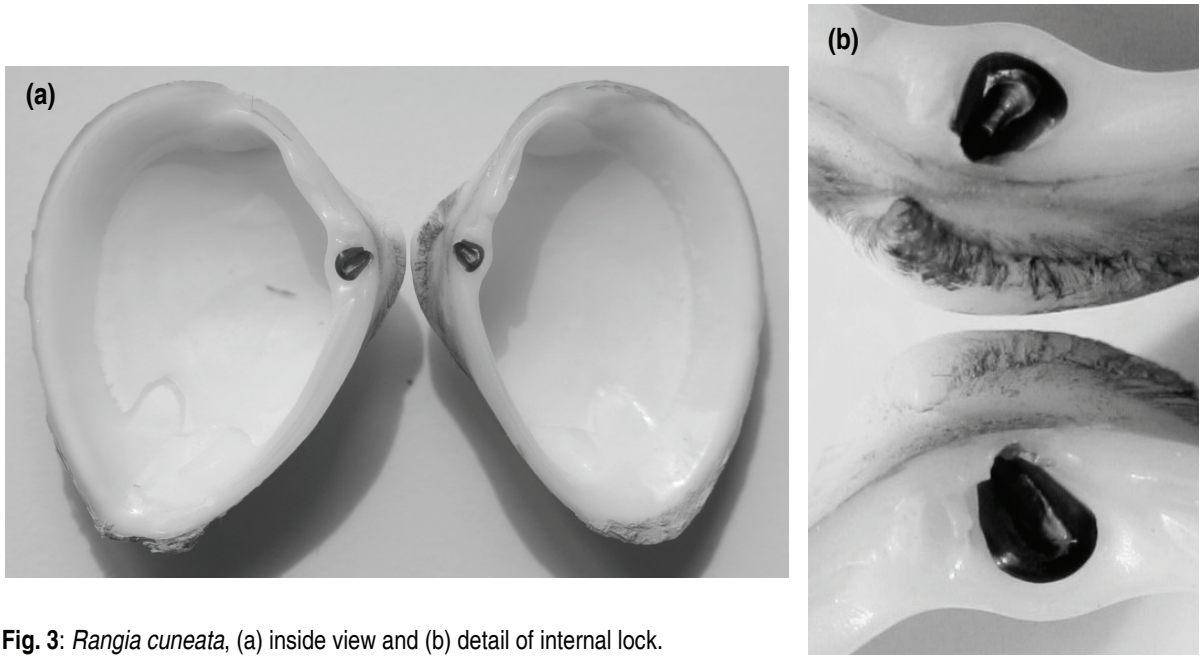

Fig. 3: *Rangia cuneata*, (a) inside view and (b) detail of internal lock.

To facilitate the identification of *R. cuneata*, a key for the identification of all current European Mactridae is presented. This key combines the diagnostic features, as provided by Tebble (1966) (native European mactrids) and LaSalle and de la Cruz (1985):

1. Lateral teeth smooth; external and internal ligaments separated by a very small calcareous septum…….. 2
- Upper and lower surfaces of the lateral teeth of the left valve and upper surfaces of the right valve laterals serrated; external and internal ligaments not separated by a calcareous septum…… 3

2. Anterior cardinal tooth of right valve almost parallel with the hinge line; adult shells normally < 6.35 cm in length…….. *Mactra corallina*
- Anterior cardinal tooth of right valve not parallel with the hinge line; adult shells normally > 6.35 cm in length …… *Mactra glauca*

3. Pallial sinus comparatively deep, reaching to a point below and in front of the middle of the posterior lateral tooth or teeth …….. 4
- Pallial sinus comparatively shallow, reaching to a point below and behind the middle of the posterior lateral tooth or teeth ……..... *Spisula subtruncata*

4. Beaks in the midline and turned inwards and slightly forward……. 5
- Beaks in front of the midline clearly turned inwards and forward……. *Rangia cuneata*

5. In the left valve the projecting Λ-shaped cardinals reach more than half-way down the hinge plate; dorsal areas about the beaks with fine concentric lines …….. *Spisula elliptica*
- In the left valve the projecting Λ-shaped cardinals reach no more than half-way down the hinge plate; dorsal areas about the beaks with fine concentric grooves …….. *Spisula solida*

Misidentifications are possible with other young Mactrids living in low salinity areas such as *Rangianella* (native range from Louisiana to Terminos Lagoon) and *Mulinia* (native in Florida, Texas and the Gulf of Campeche) superficial resembling each other and *Rangia* (Dall 1894). Montagna and Kalke (1995) noted that *Mulinia lateralis* is morphological similar to juvenile *R. cuneata* and that numerous misidentifications have occurred. However, in *Mulinia* the lateral teeth are not crenulated and *Rangianella flexuosa* lacks the pallial sinus.

HABITAT CHARACTERISTICS

Rangia cuneata inhabits low salinity estuarine habitats (Parker 1966) and is as such most commonly found in areas with salinities from 5-15 PSU (Swingle and Bland 1974). Along the Mexican Gulf coast, they form the basis for an economically important clam fishery (Wakida-Kusunoke and MacKenzie 2004). *Rangia cuneata* possesses both extracellular (blood and body fluid) and intracellular mechanisms of osmoregulation, which enables it to respond to sudden salinity changes in many estuaries (Bedford and Anderson 1972). They can cross the 'horohalinicum', the 5-8 PSU salinity boundary which usually divides fresh and salt-water invertebrates, making them one of the few freshwater clams to become established in brackish water (Ladd 1951) as such thriving in a zone unfavourable for many animals. Competition and predation may explain its scarcity in high salinity environments (Cooper 1981).

A combination of low salinity, high turbidity and a soft substrate of sand, mud and vegetation appears to be the most favourable habitat for *R. cuneata* (Tarver 1972). Although larvae prefer coarser sediment for settlement, adults are often found in muddy sediments (Fairbanks 1963; Cain 1975; Jordan and Sutton 1984). In the harbour of Antwerp, salinity ranged in 2005 from 4.3 to 10.3 PSU, ideal for the species, and temperature varied seasonally between 6.4 and 23.5 °C.

Rangia cuneata is a non selective filter-feeder, turning large quantities of plant detritus and phytoplankton into clam biomass (Darnell 1958) but the species also appears to obtain organic matter and phosphate from the sediment by direct ingestion or by feeding on bacteria associated with these materials (Tenore et al. 1968). Having a low mobility, its shell provides hard substrate for epifaunal taxa (Hoese 1973). Possible predators are fish, crabs, gastropods and ducks (LaSalle and de la Cruz 1985).

LIFE HISTORY

In the USA, *Rangia cuneata* has two spawning periods ranging from March-May and late summer-November in Louisiana (Fairbanks 1963) and February-June and September-November in Mexico (Rogers and Garcia-Cubas 1981), although in both areas, spawning may be continuous. Cain (1975) found that gametogenesis was initiated when the water temperature rose above 15 °C and with salinities above 0 PSU or below 15 PSU (Hopkins 1970).

Despite the broad tolerance of adults to environmental changes, embryos are much more vulnerable; they do not develop at 0 PSU, with 18-29 °C and 6-10 PSU being optimal conditions. Larvae however, tolerate 8-32 °C and 2-20 PSU (Cain 1973). Growth of larvae was best at high salinities and high temperatures as the survival of the larvae is reduced by the interaction of temperature and salinity at low salinity – high temperature and high salinity - low temperature combination (Cain 1973). Larvae are capable of selecting substrates, based on physical, biological and chemical factors (Sundberg and Kennedy 1993) but although they prefer soft sediment, this is not necessary for settlement and metamorphosis.

In the harbour of Antwerp, individuals of different lengths were found, ranging from 4 to 40 mm. In Mexican waters, adults range from 25 to 60 mm in length. Minimum length for mature adults ranges between 24 mm in Louisiana (Fairbanks 1963) and 14 mm in Virginia (Cain 1972). Wolfe and Petteway (1968) modeled clam growth with an L_∞ of 75.6 mm, which would represent a 10 year old individual. In Louisiana however, a maximum length of 94 mm was reported for *R. cuneata* (LaSalle and de la Cruz 1985). In fact these clams grow to their largest size in slightly brackish water that is too fresh to allow them to reproduce.

DISTRIBUTION AND INVASION HISTORY

The known geographic distribution of *R. cuneata* ranges in the Gulf of Mexico from Laguna de Terminos, Campeche, Mexico in the east to north western Florida in the north and along the Atlantic coast of North America from Florida up to the lower portion of the Hudson River, New York (Dall 1894; Andrews 1971; Ruiz 1975; Carlton 1992; Wakida-Kusunoke and MacKenzie 2004). *Rangia cuneata* is considered to be native to the Gulf of Mexico and introduced to the NW Atlantic, where it is predominantly found in estuaries.

There were no sightings along the U.S. Atlantic coast until 1956, where *R. cuneata* was thought to be extinct since the Pleistocene (Hopkins and Andrews 1970). Along the Atlantic coast the species was first observed again in the Chesapeake Bay in the 1960s (Pfitzenmeyer and Drobeck 1964; Hopkins and Andrews 1970) where it might have been present before 1955 (Hopkins and Andrews 1970). Prior to the 1960s *R. cuneata* was not

observed, while in the 1960s *R. cuneata* was extremely abundant and widespread in many estuaries on the U.S. Atlantic coast, reoccupying the whole distibutional range occupied during the Pleistocene or warmer recent times (Hopkins and Andrews 1970). *Rangia* may owe its reappearance on the U.S. Atlantic coast to the transportation of *Crassostrea virginica* from the Gulf of Mexico to Chesapeake Bay (Pfitzenmeyer and Drobeck 1964) or to the transportation as larvae in ballast water from the Gulf of Mexico. However, it is also possible that the species is not a recent invader and had always been present in its historically range, but was overlooked. Thus, small populations of this species may always have been present in the Potomac drainage system but restricted to the headwaters of the small tributaries. Some unknown ecological change may have sparked the resurgence of a small undiscovered population surviving since the Pleistocene (Pfitzenmeyer and Drobeck 1964). The first observation further north, in the lower portion of the Hudson River, New York dates from 1988, where the species has probably been introduced through ballast water (Carlton 1992).

DISCUSSION

Rangia cuneata has some history as an invasive species and specific environmental conditions may trigger a sudden outburst of the species, thus its sudden presence in the harbour of Antwerp is not really a surprise.

Similar to the recent invasion along the northern part of the U.S. Atlantic coast, the way of introduction in European waters is most likely as larvae in ballast water. The species has not been previously observed in Belgian waters, not even in the whole European brackish water scene. Two questions can be raised (1) why *R. cuneata* was first found along the Schelde estuary and (2) why it did not appear or has been detected in European waters earlier.

Nehring (2006) hypothesized that estuaries, often being a combination of brackish water with its unsaturated niches and intensive international shipping, have the highest potential infection rate with alien species of all aquatic systems. Brackish water species also have, due to specific physiological characteristics making them more resistant to external circumstances, a better chance of being transported alive than marine or freshwater species and they probably have a higher establishment potential after release (Wolff 1999). Indeed, in the oligohaline region of estuaries, *R. cuneata* occupies an otherwise rather “open niche” and as the species diversity is usually low, species adapted to this environment such as *R. cuneata* and the co-occurring *M. leucophaeata* (Verween et al. in press) may occur in large numbers (Cain 1975).

The species may have been misidentified in the past. Harbours are often difficult to sample biologically and infected industries mainly focus on destroying the species without fully identifying the species. There is no doubt about the new arrival of the species in the industrial plant of Antwerp harbour because of the ongoing monitoring

study, but it cannot be excluded that *R. cuneata* was already present in the harbour prior to its current detection, but had never been found or identified as such.

Rangia cuneata should thus be considered a hazardous biofouling species. The presence of a layer of soft substrate however is expected to be a *conditio sine qua non* to initiate fouling of the inlet pipes of an industrial cooling water system. We hypothesize that the process of *Rangia* fouling may occur as follows: (1) *Rangia cuneata* settles into the (thin) layer of substrate, (2) once present the species will create hydrodynamically benign conditions, leading to an increased accumulation of substrate and (3) this extension of *Rangia*'s habitat leads to an increase of *Rangia* invasion. As such, *R. cuneata* infestation is hypothesized to progress through a vicious circle and will finally lead to a malfunctioning of the cooling water system, as observed in the study site. To avoid such problems, a more frequent cleaning of the pipes might be advisable: the lack of suitable soft sediment for settlement in the tubes is believed to be an effective measure against *R. cuneata* biofouling.

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APPENDIX II

Publication list Annick Verween

(As on 12 January, 2007)

A1 PUBLICATIONS

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.

VERWEEN, A, VINCX, M, DEGRAER, S (2006) Growth patterns of *Mytilopsis leucophaeata*, an invasive biofouling bivalve in Europe. Biofouling 22 (4): 221 – 231.

VERWEEN A, HENDRICKX, F, VINCX M, DEGRAER S (IN PRESS) Larval presence prediction through logistic regression: An early warning system against *Mytilopsis leucophaeata* biofouling. Biofouling.

VERWEEN A, VINCX M, DEGRAER S (SUBMITTED) The effect of temperature and salinity on the survival of *Mytilopsis leucophaeata* larvae: The search for environmental limits. Journal of Experimental Marine Biology & Ecology.

VERWEEN A, VINCX M, DEGRAER S (SUBMITTED) Seasonal variation in gametogenesis and spawning of *Mytilopsis leucophaeata*, an invasive bivalve in Europe. Journal of Molluscan Studies.

A2 PUBLICATIONS

VERWEEN A, KERCKHOF, F, VINCX M, DEGRAER S (2006) First European record of the invasive brackish water clam *Rangia cuneata* (G.B. Sowerby I, 1831) (Mollusca: Bivalvia). Aquatic Invasions 1 (4): 198-203.

B2 PUBLICATIONS

VERWEEN, A, VINCX, M, DEGRAER, S (IN PRESS) *Mytilopsis leucophaeata*: the brackish water equivalent of *Dreissena polymorpha*? A review. In: Van der Velde, G, Rajagopal, S, Bij de Vaate, A (Eds) The Zebra Mussel in Europe. Backhuys Publishers, The Netherlands.

C1 PUBLICATIONS

VERWEEN, A, DEGRAER, S, VINCX, M (2002) Reducing the economic impact of an invasive bivalve, *Mytilopsis leucophaeata*, in the harbour of Antwerp. In: Mees, J, Seys, J (Eds) VLIZ Young Scientists' Day, Brugge, Belgium 13 March 2002: book of abstracts. VLIZ Special Publication 7: 53.

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