The effect of temperature and salinity on the survival of *Mytilopsis leucophaeata* larvae (Mollusca, Bivalvia): The search for environmental limits

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Received 11 September 2006; received in revised form 20 April 2007; accepted 20 April 2007

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 fluctuations in temperature and salinity. The life cycle of mussels however, consists of two phases: (1) from fertilization until larval settlement 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 and are 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 larval stages show a differential vulnerability: 4 h old embryos were more vulnerable to changes in temperature and salinity than 2 day old larvae. Maximal survival of 4 h old embryos was found at 22 °C at salinity 15. Surrounding this optimum, conditions stayed good for survival in a rather wide range: only salinities of 0 and 25 and temperatures below 10 °C or above 30 °C caused high embryonic mortality. Thus, even the most vulnerable larval stage in the life cycle of *M. leucophaeata* can be considered highly resistant to environmental conditions. Considering the broad environmental limits of adult as well as larval *M. leucophaeata*, we can expect this species to appear many brackish water bodies worldwide, with only colder regions potentially limiting its invasion success.

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Keywords: Acute 48 h tests; D-shaped larvae; Embryo; *Mytilopsis leucophaeata*; Salinity; Temperature

1. 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. Mussels are considered the most hazardous
fouling species (Rajagopalan et al., 1996) because adult mussels can shut their protective shell valves and stop byssus production to isolate their body from the external environment (Khalanski and Bordet, 1981), for instance in case of biocide passage. Bayne et al. (1976) stated that the earliest life stages are the most sensitive in the life cycle of a bivalve and, as the larva develops into a benthic juvenile, its tolerance towards 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 larval settlement 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 most vulnerable one and thus possibly most susceptible to changes in environmental conditions. This has led to the theoretical distinction of two important phases in a mussel life cycle (Conn et al., 1993): (1) from fertilization until larval settlement 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 most vulnerable one and thus possibly most susceptible to changes in environmental conditions. 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.

The capacity of an organism to survive in its environment is restricted by its tolerance limits towards various abiotic factors. The 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 on 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 its 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 larval stages of M. leucophaeata to the 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.

2. Material and methods

The influences of temperature and salinity on the survival of D-shapes (i.e. 2 day old larvae, D-shaped in profile) and 4 h old larvae of M. leucophaeata were investigated. The lethal effects of temperature, salinity and salinity–temperature combinations were examined in the laboratory through standardized acute 48 h tests (ASTM, 1999).

2.1. Brood stock

The research was conducted at the Ghent University during summer–autumn 2005 and 2006. Each year in early 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. These adults had never been in contact with the biocides, used to control biofouling in the cooling water system, at any moment in their life cycle.

Mussels were thoroughly scrubbed and rinsed to remove epifaunal organisms and maintained in a flow-through brood stock tank at a temperature lower than that measured in the field (12±1 °C) to prevent spawning of ripe animals (Stanyczykowska, 1977; Stoeckel et al., 2004). Natural, non-filtered brackish water from the river Schelde was used and mussels were additionally fed three times a week with live micro-algae, being the flagellate Isochrysis galbana Parke (3×10⁵ cells ml⁻¹) (Guillard, 1975), assuring the ad libitum availability of food (Helm et al., 2004). The 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 tube worms. Dead individuals were removed from the brood stock daily.

2.2. Spawning and fertilization

The day before the experiment, 80 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 salinity of 8 and temperature of 20 °C. After 30 min, when the siphons were extracted, 0.25 ml 10⁻³ M ¹⁻¹ fluvoxamine was injected near the inhalant 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 min, while females began releasing ova within 60 to 90 min. Once spawning was detected, adults were placed in new beakers with artificial brackish water without fluvoxamine. The water was changed every 30 min until no further animals started spawning. Bayne (1965) stated that sperm and eggs of *Mytilus edulis* should be less than one hour old for fertilization to succeed. To ensure that eggs would be exposed to viable sperm, mussels were induced to spawn in two batches, each containing 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 eggs and sperm from at least three individuals. A 2 ml suspension of sperm was added to the egg suspension in a container and was gently stirred with a plunger for 30 s. After two hours of rest, the solution was stirred again, three 1 ml aliquots were separated and embryos already developed to a 2-cell stage or beyond were counted using a Leica MZ 16 binocular microscope. These counts allowed estimation of the number of fertilized eggs in the solution. The embryos in the aliquots were discarded. The solution was left to rest for two more hours.

### 2.3. Static acute 48 h tests

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

#### 2.3.1. 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 the 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 (1) they do not feed during embryonic development into D-shaped larvae (first 72 h) (Honkoop, 1999) and (2) undigested 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 5, 10, 15, 20 and 25 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 8. As a universal control for all experiments, embryos and D-shaped larvae were exposed to artificial water with the same characteristics (20 °C and salinity 8) as that in which fertilization occurred and similar to 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 day old larvae showed high resistance to the test conditions, all further experiments were conducted only with 4 h old embryos to determine the limits of the mussel’s most vulnerable larval stage.

#### 2.3.2. Temperature-salinity tolerance of embryos

Only the procedure for 4 h old embryos was used in this experiment. Over the two years, six experiments were conducted, with temperature ranging between 8 and 30 °C and salinity between 0 and 25 (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 salinity 8) of that in which fertilization occurred.

Embryo-larval development was stopped after 48 h 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 filled and empty larval shells (ASTM, 1999).

For each test chamber in each treatment, the mortality $E$ was calculated as follows (Stephan, 1977):

$$E = 100 \times \frac{(N - B)}{N}$$

with $B$ as the number of filled larval shells at the end of the test and $N$ as the total number of shells.

2.4. 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 one-way analysis of variance (ANOVA). Although in the majority of 48 h test cases on embryos (with exception of data used to test larval vulnerability) ANOVA assumptions were not fulfilled (not even after arcsin-transformation) statistical differences on raw data were examined by multiple 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 $P<0.05$.

3. Results

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

3.1. Larval vulnerability of $M$. leucophaeata

Overall one-way ANOVA showed no significant difference in mortalities of 2 day old D-shaped larvae at different salinities ($F=0.67; \text{df}=4; P=0.125$) and temperatures ($F=2.35; \text{df}=4; P=0.626$). In all tested combinations, the highest mortality amounted up to only 13.7\%±7.0 (Fig. 2), indicating a high resistance to abrupt changes in the environment. Four hour old embryos, however, were more vulnerable, with both changes in 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 salinity 20 and 92.1\%±1.5 at salinity 25. A significantly lower embryonic mortality was found at high temperatures 20 and 25 °C ($P<0.001$) whereas the highest mortalities were observed at lowest temperature, i.e. 5 °C with 95.1\%±1.3.

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

Since $M$. leucophaeata 2 day old larvae showed a high resistance to the test conditions, all further experiments...
were conducted only with 4 h old embryos to determine the limits of the mussel’s most vulnerable larval phase.

The optimal conditions, in which almost all embryos developed to D-shaped larvae ($E = 3.3\% \pm 0.2$), were reached at 22 °C and salinity 15 (Fig. 3a). Surrounding this optimum, mortality was low (maximum 58%) in a rather wide range of temperature and salinity: 15 to 24 °C and salinity 15 to 22. The limits of survival were found only at extreme temperatures of 10 and 30 °C and salinities 0 and 25, indicating a broad tolerance of embryos to variation in temperature and salinity.

### 3.2.1. Temperature tolerance

*M. leucophaeata* embryos were susceptible only to extreme temperatures: at a temperature of 10 °C, all embryos died at salinities 0, 5 and 20 and mortality was above 60% at salinity 25. At 30 °C, a similar pattern was determined, with mortalities ranging between 96 and 100% at salinities 0, 15, 20 and 25. Whereas no embryos survived a 48 h exposure to extreme salinities 0 and 25 at either tested temperature, embryos were highly tolerant towards intermediate temperatures (12–24 °C).

![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±S.E.s at temperatures 12, 15, 20 and 24 °C. (c) Mortality rates±S.E.s at salinities 5, 10, 15 and 20.](Image)

![Fig. 4. Mean mortality ($E\%$)±S.E.s at tested temperatures.](Image)

![Table 1 Results of the two-way main effects and factorial ANOVA](Table)
at intermediate salinities (5–20) with a mortality ranging between 0 and 76% (Fig. 3b).

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 significantly higher mortality ($P<0.001$) than the other temperatures (Fig. 4). Maximum mortalities, however, did not reach 100% ($E=81\%\pm7$) (see Table 1).

### 3.2.2. Salinity tolerance

Again, embryos 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 salinities 0 and 25 inducing a significantly higher mortality ($P<0.001$) than the other salinities (Fig. 5). The effect of extreme salinities on mortality (96.0% ±1.4) was significantly lower ($P=0.016$) than the effect of extreme temperatures (82.4% ±3.9).

#### 3.2.3. Temperature-salinity tolerance

Data indicated that embryos were more tolerant towards low salinities (5–10) at high temperatures (30 °C) than at low temperatures (10 °C) (factorial ANOVA $P<0.001$). The trend of higher tolerance of embryos towards high salinities (15–20) at lower temperatures (15–18 °C) than at lower salinities (5–10) at lower temperatures (Fig. 3c) could however not be underpinned statistically (factorial ANOVA $P=0.134$).

### 4. Discussion

#### 4.1. Life stage dependent tolerance of *M. leucophaeata*

This research indicated that during 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 day old larvae, with the latter being already extremely resistant to variations 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 phase.

In marine invertebrates, the degree of tolerance to environmental conditions often varies during ontogeny (*Kinne, 1970, 1971*). Developing eggs and newly hatched larvae of some invertebrates, such as *M. leucophaeata*, may already tolerate extremely wide ranges of salinity or temperature. However, early ontogenic stages of most invertebrates exhibit lower tolerances than the respective later stages or adults. Similarly, for *Dreissena polymorpha*, the resistance to

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**Table 2**

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

<table>
<thead>
<tr>
<th>Bivalve Species</th>
<th>Adult</th>
<th>Larvae</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mytilopsis leucophaeata</em></td>
<td>5–30</td>
<td>10–30</td>
<td>(Laine et al., 2006; Siddall, 1980)</td>
</tr>
<tr>
<td><em>Dreissena polymorpha</em></td>
<td>0–29</td>
<td>12–24</td>
<td>(Karataev, 1995; Sprung, 1993)</td>
</tr>
<tr>
<td><em>Cerastoderma edule</em></td>
<td>3–20</td>
<td></td>
<td>Boyden and Russel (1972)</td>
</tr>
<tr>
<td><em>Crassostrea gigas</em></td>
<td>–1.8–35</td>
<td>Wide range</td>
<td>(Jeffler and Greer, 1991; Fabioux et al., 2005)</td>
</tr>
<tr>
<td><em>Mytilus edulis</em></td>
<td>&lt;29</td>
<td>10–25</td>
<td>(Almada-Villela et al., 1982; Brenko and Calabrese, 1969)</td>
</tr>
<tr>
<td><em>Ostrea edulis</em></td>
<td></td>
<td>Wide range</td>
<td>(Fabioux et al., 2005)</td>
</tr>
</tbody>
</table>

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**Table 3**

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

<table>
<thead>
<tr>
<th>Bivalve Species</th>
<th>Adult</th>
<th>Larvae</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mytilopsis leucophaeata</em></td>
<td>0.1–31</td>
<td>3–22</td>
<td>Verween et al. (in press)</td>
</tr>
<tr>
<td><em>Cerastoderma edule</em></td>
<td>18–40 (optimum)</td>
<td>20–50, but 30–35 (optimum)</td>
<td>(Boyden and Russel, 1972; Kingston, 1974)</td>
</tr>
<tr>
<td><em>Crassostrea gigas</em></td>
<td>10–35</td>
<td></td>
<td>Fabioux et al. (2005)</td>
</tr>
<tr>
<td><em>Ostrea edulis</em></td>
<td>28–32 (optimum)</td>
<td></td>
<td>Robert et al. (1988)</td>
</tr>
</tbody>
</table>
both salinity and temperature is known to increase with age (Wright et al., 1996). Gametes and larvae of the mussel *Mytilus californianus* die in diluted seawater, in which adults can easily survive (Fox, 1941). Larvae of *M. edulis* survive at salinities 15–40 with temperatures of 5–20 °C (Brenko and Calabrese, 1969), while adult mussels can tolerate a wider variety of environmental variables (Seed and Suchanek, 1992).

### 4.2. Temperature-salinity tolerance of *M. leucophaeata*

#### 4.2.1. 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 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* for example can easily survive temperatures up to 29 °C (Karatayev, 1995). Also *Cerastoderma edule*, widely distributed in European estuaries, is well adapted to a wide range of temperatures (Boyden and Russel, 1972). Other examples are the broad temperature tolerance of *Crassostrea gigas* (Leffler and Greer, 1991) and an upper thermal tolerance limit of about 29 °C for *M. edulis* (Almada-Villela et al., 1982) (see Table 2).

Temperature, however, remains an important species-specific factor for spawning initiation (de Voors, 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 however 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 survival in *M. leucophaeata* appeared to be 22 ± 2 °C, although salinity rather than temperature seemed to be the limiting factor for survival. Since spawning in *M. leucophaeata* is situated in the summer period, a fluctuating temperature regime with ambient summer temperatures might thus be sufficient to allow establishment of *M. leucophaeata*. Also in our experiments, maximum mortalities at the extreme temperatures of 10 and 30 °C amounted up to only 81% ± 7, indicating that the true limits of embryonic tolerance to temperature may be even more extreme. Also larvae of *D. polymorpha* and *M. edulis* show a broad temperature tolerance (Sprung, 1993; Almada-Villela et al., 1982), as do the larval phases of *Ostrea edulis* and *C. gigas* (Robert et al., 1988; Diederich, 2006).

#### 4.2.2. Salinity tolerance

*M. leucophaeata* is a typical euryhaline species, with adults being able to survive in salinities ranging from 0.1 to 31, indicating that this species can be found across nearly the whole estuarine gradient with only true seawater (salinity 35) 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 *C. gigas*, which can occur at salinities below 10 and survive above 35 although it is unlikely to breed at such extreme salinities (Fabiozzi et al., 2005). Also, *C. edule* and *M. edulis* adults are tolerant of a wide range of salinities (Boyd and Russel, 1972; Almada-Villela, 1984) (see Table 3).

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

#### 4.2.3. Temperature–salinity tolerance

Thermal responses may be modified by other effective environmental variables such as salinity, which has received the greatest attention. Several aquatic invertebrates living in habitats with strongly fluctuating temperature and salinity conditions can tolerate subnormal temperatures better at the lower end of their salinity range and supernormal 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 harrissii* (Kinne and Rotthauwe, 1952) and the colonial hydroid *Cordylophora caspia* (Kinne, 1958). In *M. leucophaeata*, however, the opposite pattern was found with a greater 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 15 to 40 is uniformly good at
5 to 20 °C but is reduced drastically at 25 °C, particularly at high and low salinities (Brenko 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 — salinity 8.

5. 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 0 and 25 shortly after fertilization, independent of the prevailing temperature, cause mortalities high enough to ensure the prevention of a successful introduction of *M. leucophaeata*. When considering the temperature tolerance of *M. leucophaeata* embryos, at temperatures of 10 and 30 °C, independent of the prevailing salinity, maximal mortality has never been reached. This information has important implications regarding the further spread of the species.

The accidental or deliberate release of non-native species into new habitats by e.g. shipping and aquaculture activities is an increasing problem 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 geographic spread of 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 easily colonize its newly invaded habitat in a natural way. This explains the rapid spreading of *D. polymorpha* in a newly invaded habitat (e.g. Great Lakes) (Ram and McMahon, 1965). Estuaries however, are mostly discrete, non-connected habitats, and can be identified as islands surrounded by fresh or seawater, as such posing a limited habitat to invade naturally. With fresh and sea water being outside the range for survival of *M. leucophaeata*, crossing these natural salinity barriers and as such the naturally invasion into new areas is largely inhibited. Transfer from one place to another is thus most probably mainly mediated through human activity. By means of transport as larvae in ballast water (Conn et al., 1993) or as adults attached to the hull, shipping traffic is considered the most important vector for dispersal of *M. leucophaeata* (Therriault et al., 2003). Therefore, harbours and industrial installations are perfect operating bases for the spread of *M. leucophaeata*.

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. *M. 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 developmental conditions, mainly characterized by a relatively high temperature, somehow limiting the potential locations for future invasion. Therefore, the invasion success of *M. leucophaeata* might especially be limited in colder regions. If summer temperatures stay below 10 °C, it is theoretically impossible for the mussel to establish itself. However, since man-made facilities, such as large harbours or cooling water systems, can artificially induce higher water temperatures, the risk of invasion cannot fully be excluded. This can promote conditions for *M. leucophaeata* introduction, even in habitats where it normally would not establish.

Acknowledgements

The first author was financially supported by a BOF-project (contract 011D13503) of Ghent University and especially appreciates the logistic and financial support of BASF, Antwerp N.V. and ONDEO Nalco (contract d.d. 21/10/2001). This research is part of the UGent project GOA 01G00705.

We would also like to thank the three anonymous referees and the editor for providing constructive comments and valuable corrections on earlier versions of the manuscript. [RH]

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