CHAPTER VII

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

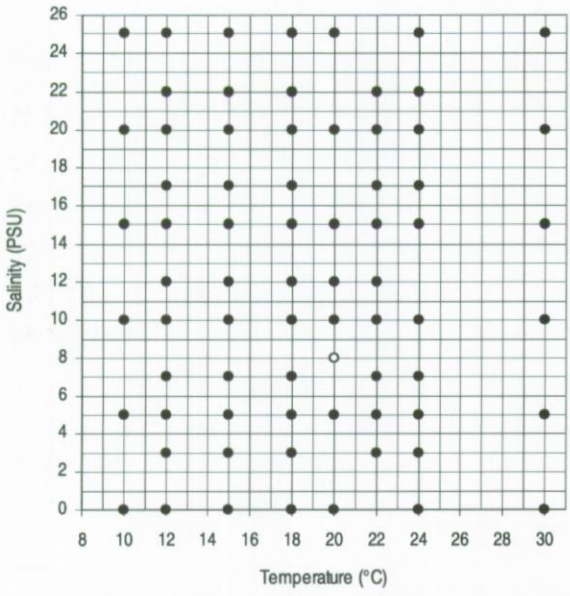

Fig. 1: Tested combinations of salinity and temperature. The white point indicates the universal control; 20 °C at 8 PSU.

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*

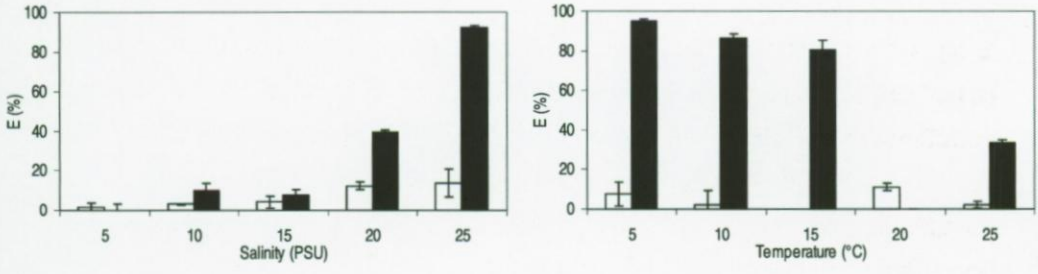

Fig. 2: Mortality rates (%) ± SE of *Mytilopsis leucophaeata* 4-h embryos (■) and 2-d larvae (□) at 20°C with different salinities and at 8 PSU with different temperatures.

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.

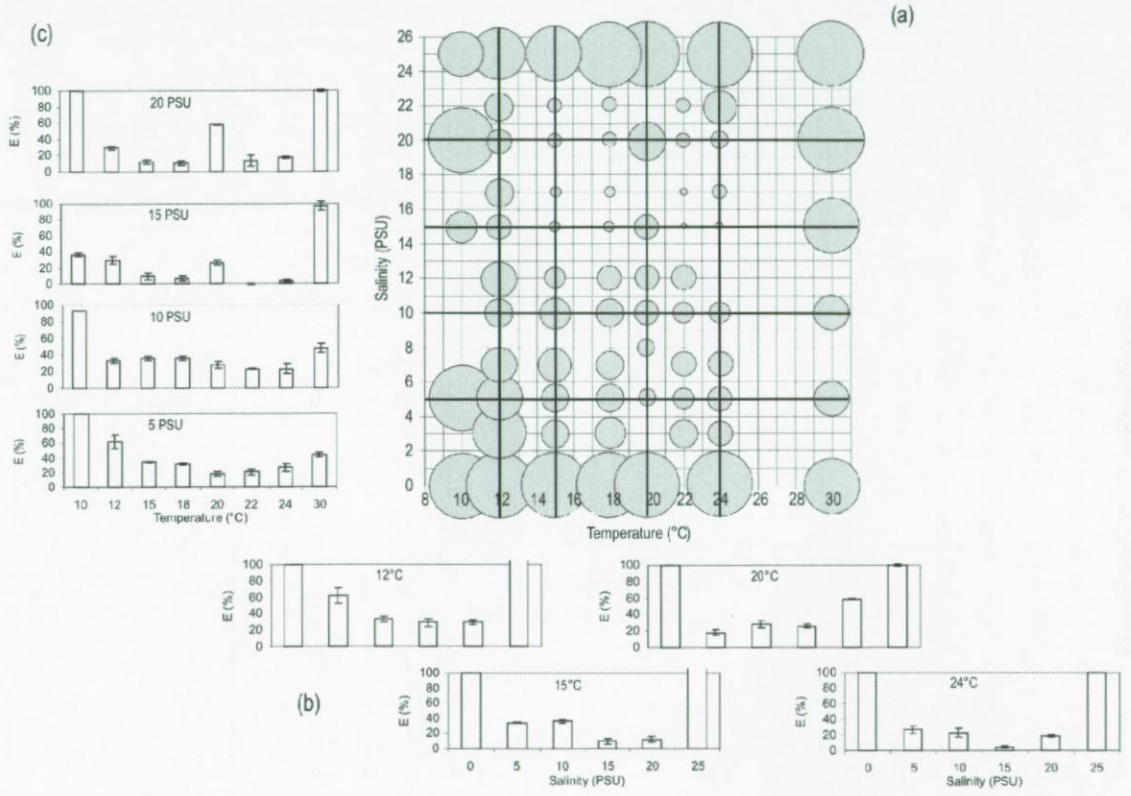

Fig. 3: Mortality rates (%) of *Mytilopsis leucophaeata* 4-h embryos at the tested combinations of temperature and salinity. (a) Surface plot of all tested combinations. The larger the surface of the circle the higher the mortality rate, ranging from 0 to 100%. (b) Mortality rates ± SEs at temperatures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

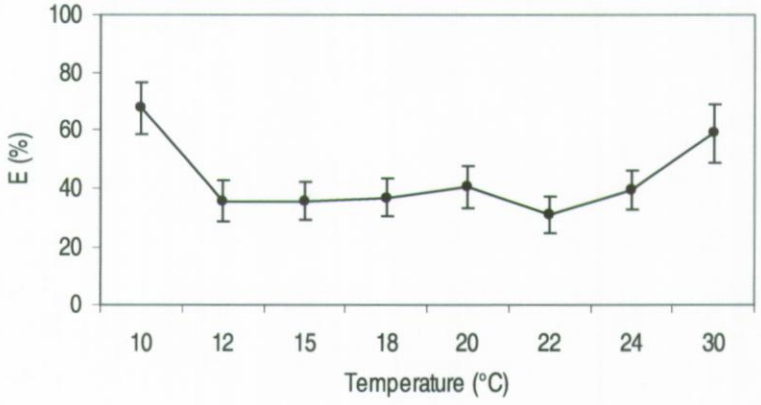

Fig. 4: Mean mortality (E %) ± SEs at tested temperatures.

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%).

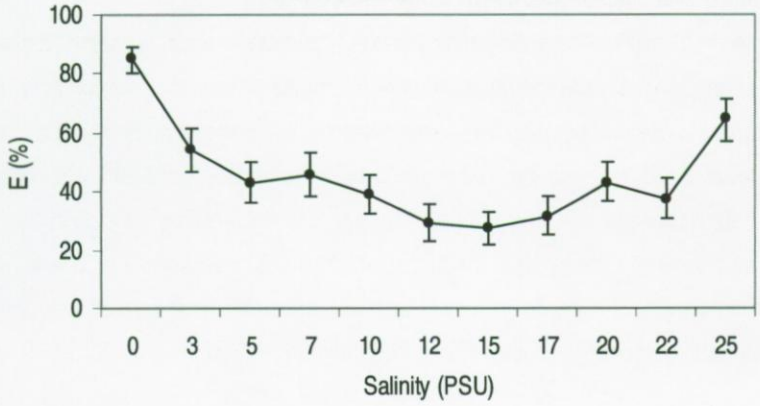

Fig. 5: Mean mortality (E %) ± SEs at tested salinities.

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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