Rapid Communication

First records of Conrad's false mussel, *Mytilopsis leucophaeata* in the southern Bothnian Sea, Sweden, near a nuclear power plant

Ann-Britt Florin[1]*, Kerstin Mo[1], Filip Svensson[2], Ellen Schagerström[2], Lena Kautsky[2] and Lena Bergström[1]

1 *Institute of Coastal Research, Department of Aquatic Resources, Swedish University of Agricultural Sciences, Sweden*
2 *Department of Ecology, Environment and Plant Science, Stockholm University, Sweden*

E-mail: ann-britt.florin@slu.se (ABF),kerstin.mo@slu.se (KM), filip.svensson@su.se (FS), ellen.schagerstrom@su.se (ES), lena.kautsky@su.se (LK), lena.bergstrom@slu.se (LB)

*Corresponding author

Abstract

The invasive, biofouling, Conrad's false mussel *Mytilopsis leucophaeata* was first recorded in Sweden during spring 2011 in the cooling water system of the power plant of Forsmark in the southern Bothnian Sea. The cooling water discharge area offers a favourable environment for growth, survival, and reproduction of *M. leucophaeata* and may provide a stepping stone for further spread. We present three different studies in the area, revealing a rapid increase in mussels in the artificially heated area, with densities of the magnitude of thousands of individuals m^{-2}, as well as mussels living in surrounding waters, indicating an on-going expansion in the region.

Key words: cooling water; biofouling; invasive mussel

Introduction

Conrad's false mussel *Mytilopsis leucophaeata* (Conrad, 1831), also known as dark false mussel or brackish water mussel, is native to the southern Atlantic coast of North America from Mexico to the Hudson River, USA (Kennedy 2011). In European waters, it was first recorded in the harbour of Antwerp (Belgium) in 1835 (Nyst 1835, cited in Verween et al. 2010), and was subsequently reported from additional European locations (Verween et al. 2010; Kennedy 2011). However, the species did not attract much interest until it became a biofouling problem in the 1990s (Verween et al. 2010; Rajagopal et al. 2012). The first observations of *M. leucophaeata* in the Baltic Sea were made in the Gulf of Finland in 2003, in the vicinity of a Finnish nuclear power plant (Laine et al. 2006). In 2006, it was recorded close to another Finnish nuclear power plant in the Gulf of Bothnia (Laine and Urho 2007).

Mytilopsis leucophaeata is a mytiliform bivalve (Mollusca, Bivalvia, Veneroida, Dreissenidae) that produces strong byssus threads by which it attaches to hard substrates. It is a filter feeder with phytoplankton as a major food source (Verween et al. 2010). It is similar to the fresh-water zebra mussel, *Dreissena polymorpha* (Pallas, 1771) and likely has often been misidentified as this better-known species. A review of differences in the morphology of adult and juvenile specimens of both species is given by Verween et al. (2010).

Mytilopsis leucophaeata is a typical brackish-water species with wide salinity tolerances, capable of establishing viable populations in almost fresh water up to salinities above 20. It can survive, but not reproduce, in more saline conditions; however it can not survive in full seawater (salinity 35) (Verween et al. 2010; Kennedy 2011). *Mytilopsis leucophaeata* is a warm-temperate species typically found in water temperatures between 5 and 30°C, with a lower limit for reproduction of 13°C, and a lethal upper temperature limit of 40°C (Laine et al. 2006; Verween et al. 2010; Kennedy 2011). Verween et al. (2005) found that spawning in Antwerp

Harbour, Belgium, started in late May to early June and

Figure 1. Map of the sampling area and sampling stations. P denotes position of artificial substrates (AS) using Plexiglas plates while S indicates AS using paving stones. T indicates the location of the automatic temperature logger in the Biotest basin. Sampling was also made in the inlet and outlet of the cooling water tunnels. For additional information on sampling stations see Appendices 1 and 2.
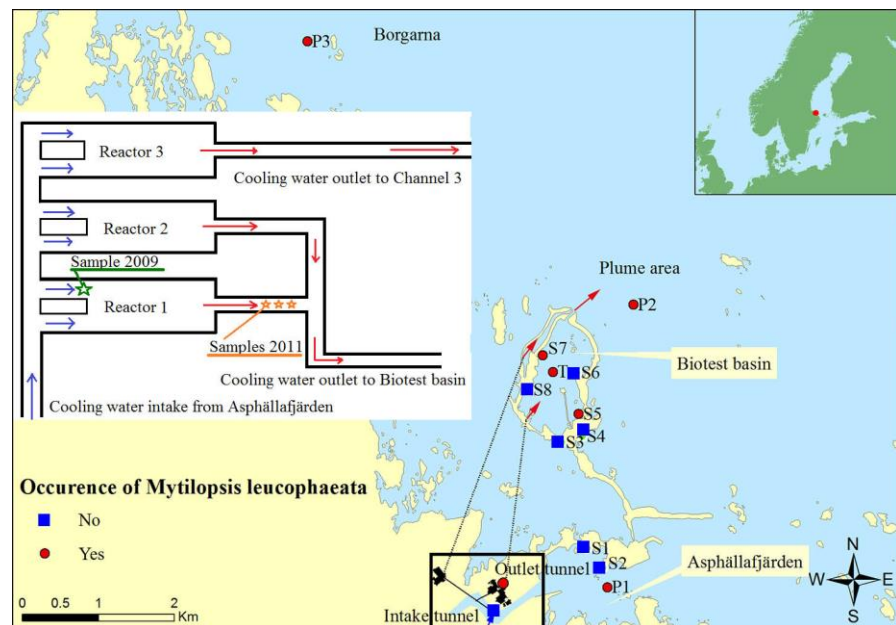
lasted for about 5 months. Growth was positively correlated with temperature and followed a distinct annual cycle, with growing season from May to September (Verween et al. 2006). Although there was no direct correlation between food and growth, the time of start and finish of the growing season seemed to be related to a threshold level of food availability (Verween et al. 2006). Adult *M. leucophaeata* normally do not exceed 20 mm length; however, there are records of a few individuals up to 27 mm (Verween et al. 2010; Kennedy 2011).

Despite its wide salinity tolerance, reproduction is not possible in either fully freshwater or fully marine environment. This makes *M. leucophaeata* a relatively slow colonizer with low dispersal capacity, restricted to brackish water. Thus, shipping may be an important vector for transportation, both for larvae in ballast water and for adults attached to the hull. The species is also found on artificial substrates. Because of high temperature, a relatively constant food supply, and generally good oxygen levels in cooling water systems, these are often an ideal habitat for *M. leucophaeata* (Verween et al. 2005; 2006). The rapid reproduction rate possible in this type of environment may result in dense populations, which can clog water intakes and may damage or cause failure to cooling-water systems (Rajagopal et al. 2012). Dense populations of *M. leucophaeata*

may also have similar ecological effects as *D. polymorpha,* creating new substrate for other organisms, increasing water transparency by filter feeding, etc. (Sousa et al. 2009). In contrast, *M. leucophaeata* in its native range is typically considered to be uncommon and does not cause any noteworthy damage (Kennedy 2011).

Material and methods

The study area

The current observations are from studies conducted in the vicinity of the nuclear power plant of Forsmark in the southern Bothnian Sea, Sweden (Figure 1). The power plant uses about 130 m^3/s of brackish water (salinity just below 5) in the cooling water system, taken from the shallow bay of Asphällafjärden. The water is transported through separate tunnels to and from three reactors. At irregular intervals, the reactors are shut off and the tunnels are emptied for cleaning. Visual inspection and sampling in the intake tunnel to reactor 1 were made on 7 July 2009. On 20 April 2011, quantitative samples were collected at three sites in the outlet tunnel from reactor 1, which had not been emptied for 6 years, by scraping off the mussels growing within a 15 × 15 cm quadrat frame placed arbitrarily at each site (N = 3 per site). The material was brought to the

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laboratory, identified, counted, and a subsample of mussels was measured.

After use, the cooling water from reactor 1 and 2 (about 90 m^3/s) is transported through a 2.4 km tunnel to the Biotest basin (Figure 1), which is a 0.9 km^2 enclosure with 2.5 m mean depth. The cooling water is heated about 10°C during its passage through the power plant and the temperature in the basin is elevated by about 8°C compared to surrounding waters when all systems are running. The water from the Biotest basin and from the third reactor, which is run through a separate channel, is mixed with ambient sea water in the Plume area. The surface area of surrounding seawater that exhibits an elevated temperature of at least 1°C covers an area of 5-10 km^2.

Colonisation experiments with artificial substrates

Colonisation experiments using artificial substrates (AS) were performed from 2010–2012 to survey the presence of hard bottom invertebrates close to the cooling water outlet and from 2010–2011 to study composition of benthic communities in different temperature regimes.

In the first study, each AS consisted of Plexiglas plates (20 × 20 × 0.5 cm) mounted in layers two cm apart along a metal rod. In the first two years, five layers were used per AS, and the AS were anchored with rocks to the sea-floor. In the third year, the number of layers was reduced to three, and a 10 kg concrete plate was used as an anchor. The AS were placed in water 2 to 4 m deep in an area close to the cooling water intake (Asphällafjärden), and within the discharge area of the cooling water (Plume area, Figure 1). In 2010, the AS (3 per area) were in place from 13 July to 1 December. Unfortunately, all plates from the Plume area were lost, probably due to a heavy storm a few weeks before the end of the experiment. In 2011, the AS (5 per area) were in place from 26 August to 22 November. All AS from Asphällafjärden were retrieved and three AS from the Plume area. From 19 June to 1 October 2012, 10 AS were set in three areas: the intake area, the ~~plume~~ Plume area, and a third area (Borgarna) that is located 8 km from the power plant and is not affected by the cooling water outlet (Figure 1). All AS were recovered and all but five of the AS from the intake area were analysed.

A second study design used AS made of granite paving stones that were placed in the Asphällafjärden (intake) area and in the Biotest basin (Figure 1). The stones (21.5 × 13.5 × 4.5 cm) were tied to 8 mm ropes and connected with each other in groups of five. The AS were set at 1m depth at four locations in the Biotest basin, two locations just outside the basin, and at two locations in the Asphällafjärden. The substrates were set in summer 2010 and 5 stones per location were lifted in July 2011 for determination of species composition and abundance of colonizing organisms.

In addition, a set of 20 stones from each of three of the locations (S-1, S-5 and S-8, Figure 1) were transferred for further cultivation in laboratory experiments. The stones were kept submerged in brackish water, with constant air supply and at temperatures matching the site of collection. After 48 days with treatment of different nutrient loads the experiment was terminated and all macrofauna collected. A remaining set of three stones from locations S-2, S-3, S-5 and S-7 were retrieved in September 2011 and later analysed as described above.

Results and discussion

Observations

Mytilopsis leucophaeata was first recorded in Sweden during spring 2011 in the cooling water outlet tunnel of reactor 1 (Figure~~s~~ 1 ~~and 2~~). Mean density at the three sites studied, i.e. the innermost wall of the cooling tunnel, a pillar inside the tunnel, and the lower part of the tunnel wall, was about 70 m^{-2} and mean (± SD) length of the mussels was 19.5 ± 2.0 mm. Although no mussels were detected in the intake tunnel during the inspection in 2009, the presence of large adult mussels in the outlet tunnel suggests that the environment in the outlet tunnel was more favourable for survival of larvae than the environment in the intake tunnel. One likely factor would be the elevated temperature in the outlet compared to the intake.

In the colonisation experiments, *M. leucophaeata* was not recorded in 2010, nor in July 2011 when the first set of paving stones were collected. However, in September 2011, numerous specimens (6-16 mm) were found on the paving stones as well as on the ropes used for their attachment in the Biotest basin but not in the intake area (Figures 1 and 2). The presence of adult mussels in September 2011, despite the absence of juveniles two months earlier, is hard to explain. Possibly, the mussels were present in the Biotest basin earlier in the summer but not on the

AS. Alternatively, adult mussels may have been introduced through the cooling water tunnels later in
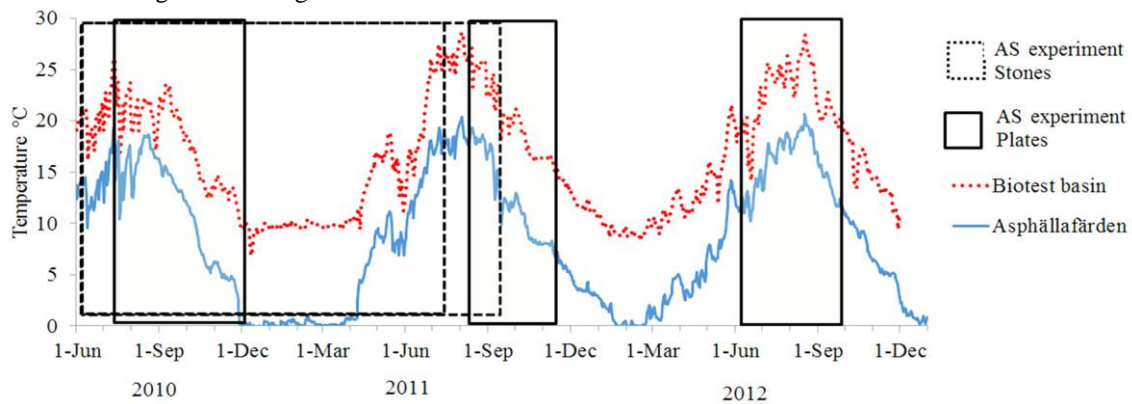

Figure 2. Temperature in Asphällafjärden (blue solid line) and Biotest basin (red dotted line) recorded by automatic data loggers from 1 June 2010 to 31 December 2012. Time of artificial substrate experiment using stones is shown by the dashed-line ~~solid~~ block~~s~~ while experiments conducted using ~~Pp~~lexiglas~~s~~ plates~~sheets~~ is indicated by solid-line blocks.
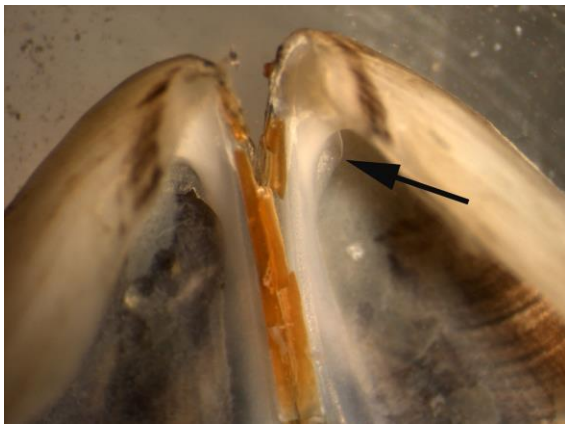

Figure 3. Adult specimen of *M. leucophaeata* found attached to a temperature data logger in the Biotest basin 29 November 2012. The characteristic apophysis is clearly visible (arrow). Photograph by Yvette Heimbrand.
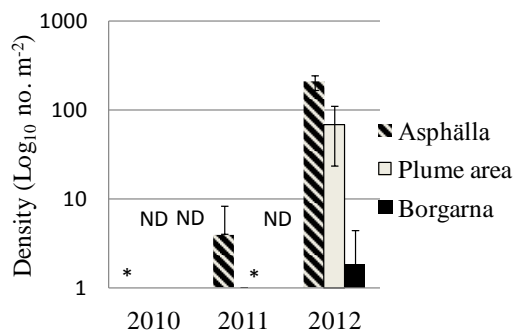

Figure 4. Increasing density over time of *M. leucophaeata* on artificial substrate plates deployed at three sites (mean ± SD). * = No mussels present. ND = no data.

summer. When the outlet tunnel was emptied (10 April to 22 June) mussels may have fallen off the tunnel walls and been flushed into the basin when cooling water was turned on again.

The first juveniles were collected in November 2011. Three juvenile specimens (1–2 mm) were found on the AS plates placed in the intake area. A year later, numerous juveniles (1–4 mm long; mean ± SD density: 208 ± 40 m^{-2}) were recorded in the same area, and also in the Plume area (1–2 mm; 68 ± 45 m^{-2}). One juvenile specimen (1 mm long) was also found at Borgarna, 8 km from the power plant. No colonization experiments were made in the Biotest basin in 2012. However, numerous adult mussels were observed on stones and field equipment in relation to other studies in November 2012 (Figure 3). In an area of < 15 cm^2, 12 adult mussels were observed, attached to an automatic temperature data logger, corresponding to a density of 8 000 m^{-2}. This density is approaching the same magnitude as the population causing biofouling problems in the Loviisa ~~Nuclear~~ nuclear ~~Power~~ power plant, i.e., 28 000 individuals m^{-2} (Laine et al. 2006). The rapid increase in abundance between 2011 and 2012 in the Biotest basin as well as in surrounding areas (Figure 4) suggests a rapidly on-going expansion and a potential further invasion of *M. leucophaeata* in the region in the future.

Growth

The cooling water tunnels and the Biotest basin probably offer a habitat with optimal growth

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conditions for *M. leucophaeata,* due to elevated water temperature, increasing both metabolism and food quantity, and high water flow providing constant food supply (Verween et al. 2005; 2006). The specimens found in the tunnels varied between 17 and 23 mm in size while those in the Biotest basin were 6–16 mm in the autumn (Figure 5). According to a von Bertalanffy growth model presented by Verween et al. (2006), mussels in similar temperature conditions, but more fluctuating salinity, were 12 mm at 3 years, 14 mm at 4 years, and 14–17 mm at 5 years of age. In contrast, Vorstmann (1933; cited in Kennedy 2011) found mussels in Amsterdam harbour, which did not seem to be more than a year and a few months old, were up to 24 mm in size. Examining the shells found in the cooling tunnels revealed three to four distinct growth rings suggesting an age of 3–4 years. The ages of the specimens observed on the data logger (put out in December 2011, retrieved in November 2012) are unknown, and they may represent both resettled adult mussels and original settling of larvae during the year. The latter would suggest a yearly growth rate of between 6 and 16 mm. Overall our results show that *M. leucophaeata* in the vicinity of the power plant both grew rapidly and reached an unusually large size compared to most previous studies (Verween et al. 2010; Kennedy 2011). The reason for the higher growth rate in this study compared to Verween et al (2006) might be due to the stable low salinity in this system in contrast to the fluctuating salinity in the previous study.

Probable dispersal routes and the role of coastal cooling water systems

The closest other known locality of *M. leucophaeata* in the region is Olkiluoto, Finland. As this is 200 km away, dispersal by means of larval drift seems unlikely, and it is more likely that the mussels we observed were introduced by shipping. In 2012, *M. leucophaeata* was also found in the ports of Turku and Naantali, Archipelago Sea, Finland (Lehtinen and Urho 2013), indicating a rapid dispersal of this species in the northern Baltic Sea. Introduction by ballast water or hull fouling could also explain why most specimens were encountered in Asphällafjärden, where the harbour belonging to the power plant company is located. Larvae transported by the ballast water or juvenile mussels attached to the hull may then be transported by the cooling water system into the Biotest basin.

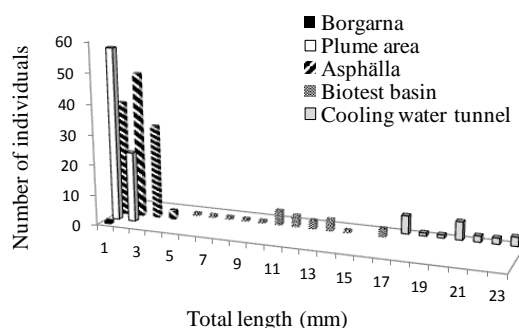The fact that adult specimens have so far only been found in the vicinity of cooling water

Figure 5. Length distribution of the *M. leucophaeata* encountered in vicinity of the Swedish nuclear power plant in the Southern Bothnian Sea.

systems in the northern Baltic Sea indicates that the species is normally restricted by water temperature during winter at this latitude, and is unlikely to reproduce outside of areas of elevated water temperature. This might explain why no mussels were observed on the AS placed by the cooling water intake in 2010. The water temperature when collecting the AS in December 2010 was close to 0°C. It is unlikely that any *M. leucophaeata* could have survived at this temperature, even if they had been there earlier. In 2011 and 2012 the AS were collected earlier in the year, when the temperature was not so low (i.e. 6 and 11°C, Figure 2).

The cooling water discharge area can offer a favourable environment for the survival and reproduction of *M. leucophaeata* and act as a stepping stone towards further spread (Gollasch and Leppäkoski 2007). This is especially true for the Biotest basin, which holds a temperature on average 8°C above that of surrounding waters and in which the average daily temperature is rarely below 10°C (Figure 2). The salinity level in the southern Bothnian Sea (4–6) is also favourable for *M. leucophaeata* survival and reproduction. Considering temperature requirements for reproduction reported previously ($13–20^\circ$C; Verween et al. 2010), and that predicted climate warming occur (c.f. Neumann 2010), it is likely the species will expand further in the Baltic Sea.

Acknowledgements

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Supplementary material

The following supplementary material is available for this article.

Appendix 1. Geographic coordinates and environmental data for studied locations near a nuclear power plant in Sweden.

Appendix 2. Records and abundance of *Mytilopsis leucophaeata* near a nuclear power plant in Sweden.

Appendix 1. Geographic coordinates and environmental data for studied locations near a nuclear power plant in Sweden.

| Area code | Area name | Coordinates | | Experiment | Environmental variables | | | | |
		Lat (DD.DD)	Long (DD.DD)		Found on substrate	Natural substrate in the area	Depth (m)	Salinity	Temp. (°C)
P1	Asphälla	60.403	18.200	Plexiglas plates	Plexiglas plates	Soft bottom & sand	2.25	4.6	10.8
P2	Plume area	60.437	18.209	Plexiglas plates	Plexiglas plates	Gravel & stone	4.25	4.6	11.4
P3	Borgarna	60.469	18.133	Plexiglas plates	Plexiglas plates	Gravel & stone	3	4.6	11.4
T	Biotest basin	60.429	18.189	Temperature logg	Electronic device, rope	Gravel & stone	2.5	4.4	10.5
S1	Asphälla	60.408	18.196	Paving stones		Soft bottom & sand			
S2	Asphälla	60.406	18.199	Paving stones		Soft bottom & sand			11.9
S3	Outside Biotest basin	60.421	18.190	Paving stones		Soft bottom & sand			12.8
S4	Outside Biotest basin	60.421	18.195	Paving stones		Soft bottom & sand			
S5	Biotest basin	60.424	18.195	Paving stones	Paving stones, ropes	Gravel & stone			17.0
S6	Biotest basin	60.429	18.196	Paving stones		Gravel & stone			
S7	Biotest basin	60.431	18.185	Paving stones	Ropes	Gravel & stone			19.5
S8	Biotest basin	60.427	18.182	Paving stones		Gravel & stone			
Intake	Cooling water intake tunnel	60.400	18.170	Inspection of tunnel		Cooling water conduct			
Outlet	Cooling water outlet tunnel	60.410	18.170	Inspection of tunnel	Cooling water conduct	Cooling water conduct			

Appendix 2. Records and abundance of *Mytilopsis leucophaeata* near a nuclear power plant in Sweden.

| Area code | Total number of individuals | | | | | | Abundance, no. m^{-2} (mean± SD) | | | |
	2009 July	2010 Dec	2011 April	2011 July	2011 Sep/Nov	2012 Nov	2009	2010	2011	2012
P1		0			3	125		0	3 (4)	208 (40)
P2					0	81			0	68 (45)
P3						1				0.8 (3)
T						12				8000
S1				0						
S2				0	0				0	
S3				0	0				0	
S4				0						
S5				0	3				16(16)	
S6				0						
S7				0	numerous					
S8				0						

Intake	0		0	
Outlet		numerous		70 (?)