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Hot Water Treatment (Chronic Upper Lethal Temperature) Mitigates Biofouling by the Invasive Asian Mussel *Limnoperna fortunei* in Industrial Installations

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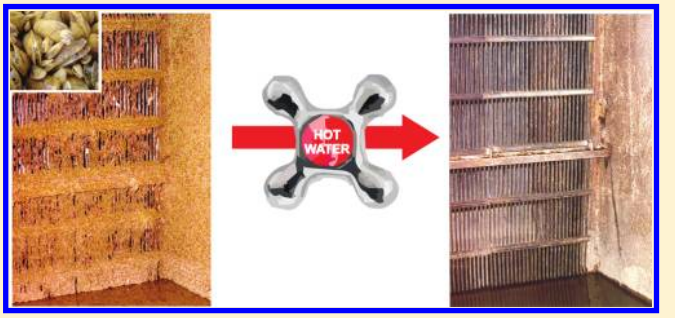
ABSTRACT: Since its introduction in South America around 1990, the freshwater mussel *Limnoperna fortunei* has become a major fouling pest for most industrial plants that use raw river or lake water, chiefly for cooling purposes. We assessed the tolerance of the mussel to upper lethal temperatures as an economical and environmentally innocuous method of controlling its fouling in industrial installations. Survival of juvenile (7 ± 2 mm in length) and adult (21 ± 2 mm) individuals, acclimated to 12 and 28 °C, was evaluated under laboratory conditions. At 38−43 °C, all mussels die after 0.7 to 17.5 h, regardless of acclimation temperature and size class. At 34−36 °C, total mortality takes 25.0 to 644.3 h, regardless of the size of the animals, but mussels acclimated at 12 °C die significantly faster that those acclimated at 28 °C. Comparison of these results with the range of conditions currently used in the industry indicates that heat treatment is a viable alternative for an efficient control of this Asian mussel in fouled systems.



INTRODUCTION

The golden mussel, *Limnoperna fortunei* Dunker (1857), is a freshwater bivalve native to Southeast Asia. It was introduced in Hong Kong, Taiwan, Japan, and South America between 1965 and 1990.[1] From its first detection along the coasts of the Río de la Plata estuary in Argentina (ca. 35°S) in 1991,[2] *Limnoperna* has been swiftly spreading north and west. Currently, it is the dominant macroinverebrate and a major fouling pest in the Paraná-Uruguay basin, with reported population peak densities in excess of 200 000 mussels m^{-2}.[1] While still restricted to the southern half of South America, its fast northward dispersion, associated with its peculiar biological and ecological traits, suggest that invasion of Central and North America in the near future is very likely.[1,3,4]

L. fortunei attaches to any hard surface, as well as to some less firm substrates. The growth of *Limnoperna* populations in raw cooling water conduits became a common nuisance in many industrial and power plants that use raw river or lake water for their processes.[5] The fouling effects of the species include clogging, pressure loss and efficiency reduction of water intakes, sieves and filters, pipes, heat exchangers, and condensers. Available information on control measures of *Limnoperna* is chiefly based on the experience gained in Europe and North America from the widespread pest zebra mussel, *Dreissena polymorpha* (Pallas 1771). However, while useful as a general reference, this information cannot be extrapolated to *Limnoperna* directly because species-specific and environmental dissimilarities are associated with very significant differences in physiological tolerances of the two species. As compared with *Dreissena*, *Limnoperna* is significantly more tolerant to both environmental variations (e.g., pH, dissolved oxygen, calcium concentrations, salinity, pollution),[6] and to toxicants used for the mitigation of mussel fouling.[5]

Research on the control of *Limnoperna* in industrial installations has been centered on fouling-resistant materials and coatings,[7−9] toxicants,[5,10] desiccation,[11] oxygen deprivation,[12] and manipulation of water flow.[13] As an alternative to the above, heat treatment has been gaining importance as a nonchemical, comparatively economical, and environmentally innocuous antifouling method which yielded good results with the zebra mussel at several European and U.S. power stations.[14−16] Upper lethal thermal limits have usually been determined as either acute or chronic thermal tolerances. The acute upper lethal temperature is the temperature required to achieve death of the mussels when it is raised at a specific rate. Results of this method are expressed as lethal temperatures (e.g., the temperature required to kill 50% or 100% of the animals; LT50 and SM100, respectively) at a specific heat rate increase and starting (acclimation) temperature.[17−19] On the other hand, chronic upper thermal limits involve

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continuous exposure of animals to a constant temperature for periods long enough to achieve 100% mortality. Also in this case, starting (acclimation) temperature has sometimes been found to affect the final outcome of the exposures.[20] The results of this method are expressed as lethal times, including LT50, mean survival time (MST), and SM100, as a function of a specific temperature (ref 20, this study). For *Limnoperna*, only three surveys on its thermal tolerance have so far been performed. Montalto and Marchese[21] carried out some tests of the combined effects of pH (5 and 10) and temperature (5 and 35 °C) on survival of the mussel. The experimental design of this study was not aimed at the development of control measures, for which reason its usefulness for this purpose is very limited. Rolla and Mota[22] provided some isolated data on survival rates at 10−40 °C, but the lack of methodological details (acclimation temperatures, n, controls, mussel size, etc.) and inadequate observation intervals (24 h) make these data suspect and do not allow comparative analyses with our results. The third survey is that of Perepelizin and Boltovskoy,[17] where only the acute thermal tolerance of the mussel was addressed.

In the present work we investigate the chronic upper lethal temperatures of *Limnoperna.* Our results show that thermal treatment of fouled facilities is a viable alternative for implementing cheaper and environmentally friendlier control strategies.

MATERIALS AND METHODS

Limnoperna fortunei were collected manually from natural populations along the coast of the Río de la Plata estuary off Buenos Aires (34°36′S, 58°20′W) on several occasions between January and October (midsummer to late winter) 2009. Mussels were transported to the laboratory, rinsed and transferred to 20 L aquaria with aerated, dechlorinated (by active air bubbling during 24 h) tap water at river collection temperature.

Mussels were acclimated for the experiments to 12 and 28 °C at a rate of 1 °C day^{-1}. These temperatures are typical for the winter and the summer, respectively, in the lower Paraná River. Experiments at different starting temperatures were scheduled so as to minimize the range between starting and in situ collection temperature. Tests starting at 28 °C were conducted with mussels collected in spring-summer at a water river temperature of 19−27 °C, whereas those starting at 12 °C were carried out during the winter, on animals retrieved from water at 13.5−15.5 °C. During acclimation, animals were fed daily with commercial fish food ("Vitafish baby", 44% proteins, 13% lipids, 14.5% minerals, 5.4% calcium, 2% phosphorus) at a concentration of 0.02 g L^{-1}. The water in acclimation aquaria was renewed every 3 days for mussels acclimated to 28 °C, and every 7 days for those acclimated to 12 °C (this difference did not affect the behavior in the corresponding controls, see below). All experiments were started within ≤40 days of collection.

After acclimation, groups of approximately 20 animals 7 ± 2 mm (small), or 21 ± 2 mm (large) in length were gently isolated, placed in 9 cm Petri dishes covered with a 1 mm mesh nylon cloth to prevent escape, and returned to the aquarium for further work. After 24 h, Petri dishes were examined and loose mussels (i.e., not firmly attached to each other or to the bottom of the dish) were eliminated. We only used bysally attached mussels because loose, unattached organisms can have a reduced tolerance to stress.[23] Subsequently, one Petri dish was placed in each experimental vessel, consisting of a plastic container filled with 3 L of dechlorinated tap water actively aerated and covered with a perforated plastic lid.

Chronic upper thermal tolerance in *Limnoperna fortunei* was evaluated at seven treatment temperatures: 34, 36, 38, 40, 41, 42, and 43 °C, selected on the basis of the highest ambient temperatures where *Limnoperna* is known to thrive,[6] and the operationally feasible conditions currently used in industry.[24] For exposures of up to 24 h (i.e., for temperatures between 38 and 43 °C), plastic containers were immersed in an 8 L controlled temperature (precision: ± 0.1 °C) water bath. Water was gently mixed to ensure homogeneous temperature distribution. For the rest of the experiments (exposures of over 24 h, treatment temperatures of 34 and 36 °C), experimental vessels were placed in a thermoregulated incubator (precision: ± 0.1 °C). All exposures were carried out in triplicate (n = 1700, for a total of 84 experiments; Table 1), plus one control per treatment temperature and size class (14 controls in total). Controls were subjected to the same experimental conditions and exposure times (0.7 to 644.3 h), but without increasing the initial temperature.

For all test groups, temperature was raised from its initial (acclimation) level to each treatment value at a rate of 0.1 °C/min, (which is roughly the rate achievable at most power plants;[14,24]). Once the target temperature was reached, it was maintained constant for the rest of the experiment until 100% mussel mortality was achieved in all replicates. In experiments of less than 24 h mussels were not fed. In all others they were fed daily. Water temperature and dissolved oxygen were permanently monitored with a Hach sensION156 pH-conductivity-dissolved oxygen meter (nominal accuracy, dissolved oxygen: ±1%, temperature: ± 0.3 °C).

From the moment animals began to die (0.2 h to 3 days, depending on temperature), dead mussels were identified and removed at regular intervals (10−30 min for 38−43 °C, 1.5 h to twice a day for 36 °C, and daily for 34 °C). Mussels were considered alive when they closed their valves under a gentle stimulus between the valves in the region of the siphons,[25] or they resisted forcible valve opening.[26] At the end of each run, experimental mussels were allowed to cool to room temperature (12 h) and their vitality was rechecked in order to account for potential recovery events.

A MFANOVA (Multifactorial Analysis of Variance) model was used to contrast mean survival time (MST), and the time required to kill 50% and 100% of the animals (LT50 and SM100, respectively) for each size class and starting temperature. LT50 values were obtained after fitting the Probit Model (BenchMark Dose Software, version 1.4.1c). Data were not transformed and controls were not included in the statistical analyses.

RESULTS AND DISCUSSION

Experimental Results. Postassay recovery of dead mussels was very rare, and mortality in the controls was always below 5%.

Individual mussel mortality times varied between 0.2 and 644.3 h. LT50, MST, and SM100 did not differ significantly between size classes (MFANOVA, $p > 0.290$). Results of exposures at 38−43 °C were independent from acclimation temperature (12 and 28 °C; MFANOVA, $p > 0.234$), but for those at 34 and 36 °C mortalities were significantly associated with acclimation temperature (MFANOVA, $p < 0.001$). Table 1 summarizes the results obtained and the corresponding LT50, MDT and SM100 values for each acclimation and treatment temperature.

Figure 1 shows the time necessary to achieve an effective control of *L. fortunei* (LT50, MST, and SM100), at 38−45 °C.

Table 1. Chronic Upper Lethal Times for Small (7 ± 2 mm) and Large (21 ± 2 mm) *Limnoperna fortunei* Acclimated at Winter (12 °C) and Summer (28 °C) Temperatures and Exposed to Different Treatment Temperatures (Mean ± Standard Error)[a]

acclimation temperature (°C)	teatment temperature (°C)	size (mm)	*N*	LT50 (h)	MST (h)	SM100 (h)
28 °C (summer)	43	7	60	0.6 ± 0.0	0.7 ± 0.1	0.9 ± 0.1
		21	61	0.5 ± 0.0	0.7 ± 0.1	0.9 ± 0.1
	42	7	61	1.2 ± 0.1	1.4 ± 0.0	1.7 ± 0.0
		21	61	0.7 ± 0.0	0.9 ± 0.0	1.2 ± 0.0
	41	7	63	2.5 ± 0.1	2.8 ± 0.1	3.3 ± 0.2
		21	63	1.9 ± 0.2	2.3 ± 0.1	2.9 ± 0.1
	40	7	61	4.4 ± 0.1	4.6 ± 0.1	5.5 ± 0.0
		21	61	3.5 ± 0.2	3.7 ± 0.2	4.8 ± 0.2
	38	7	60	11.8 ± 0.8	13.5 ± 0.8	15.8 ± 0.6
		21	61	12.3 ± 1.1	13.7 ± 1.3	15.7 ± 1.8
	36*	7	59	61.6 ± 7.8	81.7 ± 11.2	106.6 ± 16.3
		21	62	58.2 ± 5.4	84.1 ± 6.5	122.9 ± 8.2
	34*	7	60	372.6 ± 24.1	450.0 ± 22.6	593.5 ± 10.9
		21	60	341.5 ± 12.5	425.8 ± 17.3	580.8 ± 31.8
12 °C (winter)	43	7	61	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
		21	60	0.6 ± 0.0	0.8 ± 0.1	1.1 ± 0.1
	42	7	60	1.0 ± 0.1	1.2 ± 0.0	1.5 ± 0.0
		21	60	1.1 ± 0.1	1.4 ± 0.1	1.8 ± 0.2
	41	7	60	2.0 ± 0.1	2.3 ± 0.1	2.7 ± 0.2
		21	60	1.9 ± 0.2	2.2 ± 0.2	2.6 ± 0.2
	40	7	60	3.4 ± 0.2	3.9 ± 0.2	4.5 ± 0.3
		21	60	3.5 ± 0.2	4.1 ± 0.1	4.8 ± 0.2
	38	7	61	12.5 ± 0.8	13.8 ± 0.7	15.0 ± 0.9
		21	61	12.2 ± 0.8	13.8 ± 0.7	15.2 ± 0.9
	36**	7	60	15.1 ± 0.8	21.1 ± 0.5	26.0 ± 0.5
		21	60	19.2 ± 0.2	22.1 ± 0.3	25.5 ± 0.5
	34**	7	62	54.8 ± 22.7	173.0 ± 39.7	398.9 ± 51.3
		21	62	62.9 ± 23.3	172.7 ± 22.6	374.4 ± 47.8

[a] LT50: time required to kill 50% of the animals; MST: mean survival time; SM100: time required to kill 100% of the animals. Asterisks denote significant differences ($p < 0.001$, MFANOVA) between acclimation temperatures.

Since for this temperature range differences between size classes and acclimation (starting) temperatures were not significant, curves are based on pooled data for all acclimation temperatures and both size classes. The three curves (LT50, MST, and SM100) are very similar. Times required to kill the mussels are higher and more variable at temperatures between 38 and 41 °C; thus, at 38 °C almost 16 h are necessary for SM100, whereas at 41 °C the same result is achieved after only 3 h. When treatment temperature approaches 45 °C, LT50, MST, and SM100 become very similar and drop to almost zero (instantaneous mortality).

Lethal times for exposure temperatures between 33 and 38 °C, based on pooled data for both size classes (which did not differ significantly) are shown in Figure 2. In contrast, the effects of starting (acclimation) temperature (winter, 12 °C; and summer, 28 °C) on mortality rates is evident, especially at exposure temperatures below 36 °C. For example, at 34 °C, mussels acclimated at the winter temperature (12 °C) died several times faster (LT50, MST, and SM100 between 50 and 275 h) than those acclimated at the summer conditions (28 °C, 350 to over 625 h). In other words, at low exposure temperatures time of the year (i.e., acclimation temperature) played a major role in the response of the mussels, whereas at high exposure temperatures (>38 °C) time of the year was not significant, suggesting that the treatment was above the threshold level of the mussels thermal adaptation capabilities (Figure 2).

Comparisons with Other Freshwater Fouling Molluscs. As expected from their original home ranges and current worldwide distribution patterns, our results indicate that *Limnoperna* is much more tolerant to high temperatures than *Dreissena*. Indeed, at 34 °C *Limnoperna* survives between 386.7 and 587.2 h, as opposed to 5.0−23.2 h for *Dreissena*.[20] At higher temperatures the contrast is even higher: at 36 °C *Limnoperna* survives 25.8−114.8 h, while *Dreissena* only 0.5−3.0 h.[20]

*Limnoperna*s native distribution area is tropical and subtropical Southeast Asia, where the temperature of freshwater bodies ranges around 14 °C (winter) to 30 °C (summer).[27] These values are quite similar to those in its invasive area in South America (roughly 14−33 °C, [1,4]). Interestingly, the thermal tolerance of *Corbicula fluminea* (invasive in Europe and North and South America), also tropical-subtropical in its native Asian range, is very similar to that of *Limnoperna*.[28] *Dreissena*, on the other hand, is native from the Ponto-Caspian basin, where the climate is sharply continental with freezing winters and temperatures up to ca. 25 °C in summer.[17] In its invasive area (most of Europe and North America) the climate is mostly cold to temperate

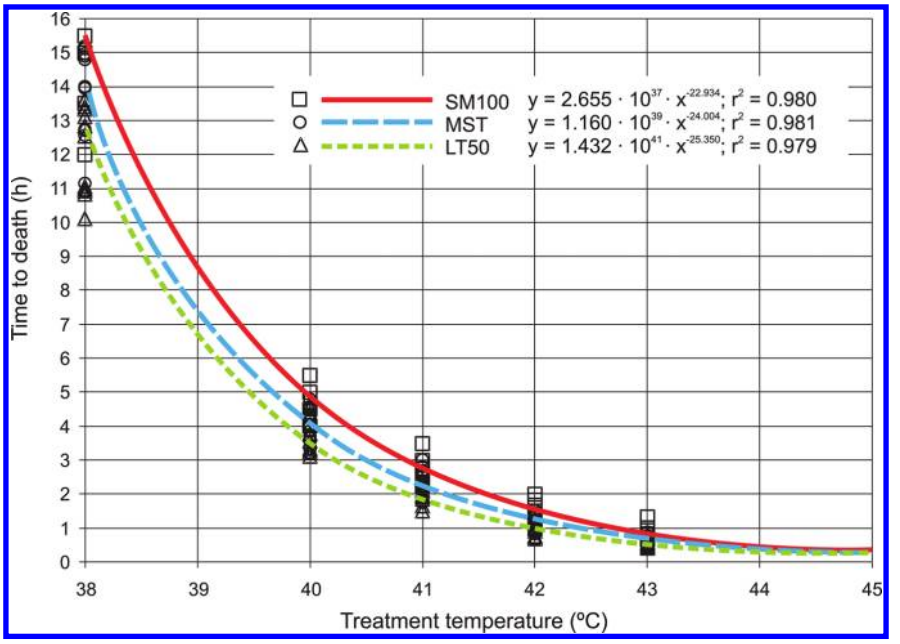

Figure 1. Exposure times necessary to achieve an effective control of *L. fortunei* at temperatures between 38 and 45 °C (actual data points, symbols; and regression, lines). LT50: time required to kill 50% of the animals; MST: mean survival time; SM100: time required to kill 100% of the animals. Based on pooled data for both acclimation temperatures and mussel sizes.

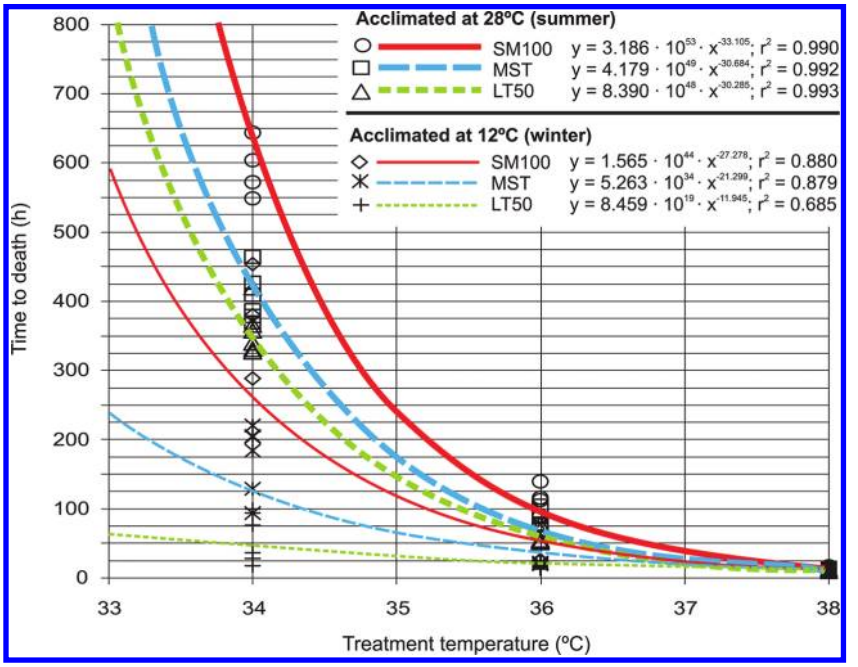

Figure 2. Exposure times necessary to achieve an effective control of *L. fortunei* at temperatures between 33 and 38 °C, as a function of acclimation temperature (winter, 12 °C; and summer, 28 °C) (actual data points, symbols; and regression, lines). LT50: time required to kill 50% of the animals; MST: mean survival time; SM100: time required to kill 100% of the animals. Based on pooled data for small and large mussels.

and winter water temperatures around 0 °C are common. These contrasts in the evolutionary history of the two mussels seem to be reflected in the differences in their tolerance to heat stress in experimental conditions.[17]

It should be pointed out that *Limnoperna* is not only more tolerant to high temperatures than *Dreissena*, but it also is more eurythermic than the zebra mussel. Although *Limnoperna* typically inhabits tropical and subtropical areas, it can also survive in waterbodies where the temperature falls below zero during the winter, like the Paldang reservoir in South Korea.[29]

Implications for *Limnoperna*s Geographic Expansion. These results reinforce the assumption that high temperatures will not represent an obstacle for *Limnoperna*'s expansion throughout northern South America, and eventually reaching Central and North America. Northwards expansion will be facilitated by its tolerance to pollution, low oxygen levels and scarce dissolved

calcium.[1,4,6] In the near future, the freshwaters of North America may be colonized by *L. fortunei*, resulting in strong impacts on invaded ecosystems, especially in the southern and western regions of the United States where dreissenids are hindered by low calcium levels and higher temperatures.[30]

Thermal Treatment for the Control of Fouling by *Limnoperna*. Thermal treatment is economical and environmentally safer than most other control methods, especially chemical treatments.[15] Heat treatment programs aimed at controlling mussel fouling have been successfully implemented for many years in North America,[15,31,32] and in Europe.[14,16] Heat treatment protocols typically use temperatures of up to 43 °C for up to 10 h,[24,33,34] which is well in excess of the thermal tolerance of *Limnoperna* (Figure 1).

Heat treatment is usually implemented by recirculating (rather than discharging) water heated in the condensers back to the precondenser sections of the cooling system. This process is repeated until the water has attained sufficient temperature to kill the fouling mussels. For *Limnoperna*, best results will be achieved by targeting temperatures above 38 °C, which would yield 100% mortalities in 1−16 h year-around (Figure 1). Usually these operational times are short enough to be cost-effective. On the other hand, treatments at temperatures below 38 °C will require much longer periods of exposure (Figure 2), and may render the method economically prohibitive.[15,35,36] It should be borne in mind that, for any given treatment temperature, the total operational time required is higher than that indicated in Figures 1 and 2, because the values illustrated do not include the time necessary to attain the target temperature. This lead time will vary depending on ambient water temperature and operational possibilities, ranging between 1 and over 6 h at a temperature increase rate of about 1 °C/10 min (typical for heat treatments in power plants: [14,24]).

Heat treatments for the mitigation of *Limnoperna* fouling can use either chronic or acute thermal protocols. The use of chronic thermal treatment (this study) involves maintaining operating temperatures at a relatively constant lethal level for a given period of time. In some cases, when operational limitations preclude the use of this method, acute thermal treatments may be applied. This procedure consists in the gradual increase of the water temperature at a given rate until values that induce an acute (instantaneous) mortality of the mussels, followed by a return to normal operating conditions. This alternative allows shorter operational times, but requires higher water temperatures: for *Limnoperna*, temperature increase rates of 1 °C/5−30 min, yield 100% mortalities at 44−50 °C, requiring between 2 and 15 h.[17]

Our data indicate that thermal treatment at 33−38 °C yields considerably faster mortalities at low (12 °C) than at high (28 °C) acclimation temperatures, suggesting that the implementation of this technique would be most effective during the winter. However, the fact that *Limnoperna* has a very extended reproductive period (see below) and fast growth rates[37] may require more than one yearly treatment. In addition, attaining high treatment temperatures is operationally easier at high ambient temperatures (i.e, during the summer). Thus, the best timing for these operations would vary among facilities.

Because the effects of heat are likely to affect byssus attachment strength,[38] alternative protocols combining thermal treatment with manipulations of flow speed may yield interesting results, especially at low temperatures, which require longer exposure times (Figure 2). Although the planktonic larvae of the mussel are probably more sensitive to temperature than sessile adults, the fact that *Limnoperna* reproduces almost continuously during 6−10 months[39] makes the alternative of targeting the larvae unattractive.

Obviously, heat treatments are not without limitations. System shutdowns and flow reversals or redirections may pose major operational challenges. Acute thermal treatments of entire raw water systems may be unfeasible because of high operating water temperatures leading to excessive equipment wear or malfunction.[20] In some cases individual treatment of isolated components (off-line intake embayments, individual heat exchangers, service water systems), and/or a combination of chronic and acute strategies, may offer a viable aternative.[40] Finally, restrictions by state or national regulatory agencies associated with discharge water temperature can also limit the use of thermal treatments.[36]

The results of this study are based on specimens collected along the Río de la Plata estuary, off Buenos Aires, where water temperatures vary annually between 10 and 12 and 28−29 °C. As shown for other organisms,[20] it is conceivable that mussels from areas with a different thermal regime may have slightly different thermal tolerances.

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