MORTALITY OF *Mytella falcata* AND *M. guyanensis* EXPOSED TO DIFFERENT TEMPERATURES

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ABSTRACT

Mytella falcata and *M. guyanensis* are species of potential commercial value for aquaculture. The present study had the objective of estimating the mean tolerance (MT_{50}) of both species of estuarine bivalves exposed to different water temperatures. Significant differences were observed through a variance analysis (p = 0.02) between cumulative mortality from both species. *M. falcata* was more resistant than *M. guyanensis* at a temperature of 33ºC, with a MT_{50} of, respectively, 87.42 and 53.36 hours of exposure. Although there was no mortality of the organisms exposed to the other temperatures, control (24°C), 27°C and 30°C, it was found that there is a decrease in the creation of byssus at temperatures of 30°C and 33°C, indicating a possible physiological stress.

Key words: estuarine bivalves; aquaculture; mean tolerance; mortality curves.

MORTALIDADE DE *Mytella falcata* E *M. guyanensis* SUBMETIDOS A DIFERENTES TEMPERATURAS

RESUMO

Mytella falcata e *M. guyanensis* são espécies de potencial valor comercial para a aquicultura. O presente estudo teve como objetivo estimar a tolerância média (TM_{50}) das duas espécies de bivalves estuarinos, expostas a diferentes temperaturas da água. Observaram-se diferenças significativas, através de análise de variância (p=0,02), entre a mortalidade acumulada de ambas as espécies, sendo *M. falcata* mais resistente que *M. guyanensis*, na temperatura de 33°C, com TM_{50}, respectivamente, de 87,42 e 53,36 horas de exposição. Apesar de não ocorrer mortalidade dos organismos expostos às outras temperaturas, controle (24°C), 27°C e 30°C, constatou-se a diminuição na formação de bisso nas temperaturas de 30°C e 33°C, indicando possível estresse fisiológico.

Palavras-chave: bivalves estuarinos; aquicultura; tolerância média; curvas de mortalidade.

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INTRODUCTION

Temperature and salinity are the abiotic factors that most affect the distribution, abundance, and survival of aquatic species (FREIRE *et al*., 2008; BARBIERI *et al*, 2014; BARBIERI *et al*., 2016). Temperature undergoes great variations in the sea, and is inversely proportional to depth and latitude; it is the most important barrier for the distribution of species due to its direct involvement in basic physiological processes, especially reproduction (HENRIQUES *et al*., 2007). Temperature is one of the main environmental factors that regulate a number of metabolic reactions in ectothermic animals (DAMATO and BARBIERI, 2011).

Conservation and protection of the estuarine environment is extremely hard due to the complex relationship between pollutions, habitat degradation, alien species, current flux modifications, and overexploitation of fishery resources (DUDGEON *et al*., 2006; COLLAÇO *et al*., 2015).

Inserted in this context are the mangroves, which are ecosystems with high diversity of species adapted to these drastic variations, including the temperature, and which provide favorable conditions for feeding, reproduction, and protection, in addition to being considered areas of great importance for nutrient and organic matter cycling (SCHAEFFER NOVELLI, 1995).

Along nearly the entire extension of the Santos estuary there are industrial plants that discard their effluents, which may rise local temperature when they use water to refrigerate boilers (MOURA, 2003). This problem is intensified due to local tide oscillations and depth. Refrigerated systems that use water for thermal exchanges in equipments are called cooling towers (STOECKER and JABARDO, 2002).

In the Bertioga channel, within the estuarine area of the Baixada Santista region, *Mytella falcata* and *M. guyanensis* are estuarine bivalve species of commercial importance (GOMES *et al*., 2010), exploited by some riverside communities as a source of food and income (MARTINS and SOUTO, 2006).

The growth and even survival of organisms in natural environment can be directly influenced by temperature. *M. falcata* and *M. guyanensis* has a potential for aquaculture, not yet explored in Brazil; Therefore, studies to investigate the effect of high temperatures on its survival are required.

This species is important for aquaculture, held in estuarine areas where the temperature is not controlled. Thus, we conducted this study to investigate the mortality at different temperatures (24, 27 and 33°C) as a proxy for metabolic activity (PHAN *et al*., 1993). These three temperatures are the limits recorded in estuarine areas in the coastal region of Santos. We aim to better understand temperature influence on organism's mortality in natural environment.

Despite its social and ecological importance, until the present time, few studies have been performed on the resistance to environmental variations such as temperature. The present study had the objective of evaluating mortality, through Mean Tolerance (MT_{50}), of *Mytella falcata* and *M. guyanensis* subjected to different temperatures.

METHODS

In February 2014, 320 animals of the estuarine bivalve species *Mytella falcata* and *M. guyanensis* were sampled, in the area of the Bertioga channel (23º 56' S ; 46º18'W), state of São Paulo, southeastern Brazil (Figure 1). Juvenile mussels (maximum length of 20 mm) were used in order to avoid gamete emission during the experi-ment (A.P.H.A., 1998). The bivalves were taken to a laboratory where they were individually separated and cleaned from all encrusting organisms on their valves. Next, they were stored for 24 hours in a container with water from where they were sampled, at a temperature of 23°C and salinity of 16, and kept under constant

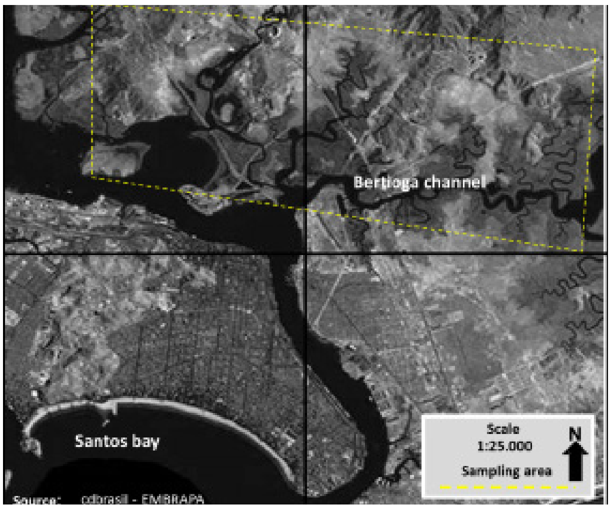

Figure 1. Sampling area of *Mytella falcata* and *M. guyanensis*, Bertioga channel, São Paulo coast, Brazil.

aeration, at a density of 2 animals L^{-1}.

The experiment was installed in an acclimatized room at a temperature of 24°C and kept at a salinity of 16. In the aquariums, organisms of both species were exposed to three different temperatures (27°C, 30°C and 33°C), which were controlled by JALLI automatic thermostats, model JT 500W, and thermometers, with four repetitions. Many organisms was used as control, at room temperature, without thermostats, thus, reaching a total of 16 aquariums. The time required for the mortality of 50% of the animals (MT_{50}) was determined through calculation using the Trimmed Spearman-Karber method (HAMILTON *et al.*, 1977).

Mortality verifications were performed every 6 hours until the end of the 168-hour test. Death was observed through the non-response of the animal to the mechanical stimulus produced by touching the mantle with a small knife. The MT_{50} comparison at different temperatures was performed through ANOVA (completely randomized design).

RESULTS

During the entire experiment the mean temperature of the control treatment was 24°C. This value was close to the one registered in field, at the sampling area (23ºC). After 168 hours of testing, *Mytella falcata* and *M. guyanensis* exposed to temperatures of 27 and 30°C did not reach 50% of mortality. MT_{50} was only observed in organisms exposed to temperatures of 33°C (Figures 2, 3 and 4).

At a temperature of 33°C, there was significant difference (p = 0.02) in the mortality of both species. *Mytella falcata* presented greater resistance than *M. guyanensis*, with MT 50 of 87.42 and 53.36 hours, respectively (Table 1). At temperatures of 27°C and 30°C the behavior of the animals was similar to the animals of the control treatment. Only after 168 hours, at the end of the experiment, total mortality occurred to the *M. falcata* mussels subjected to a temperature of 33°C.

Table 1. Mean tolerance (MT_{50}) of *Mytella falcata* and *M. guyanensis* exposed to a temperature of 33°C, during the 168 hours of the experiment.

MT_{50}	*M. falcata*	*M. guyanensis*	ANOVA (p)
Maximum	102.30 hours	64.71 hours	
Minimum	74.71 hours	44.00 hours	0.02
Mean	**87.42 hours**	**53.36 hours**	

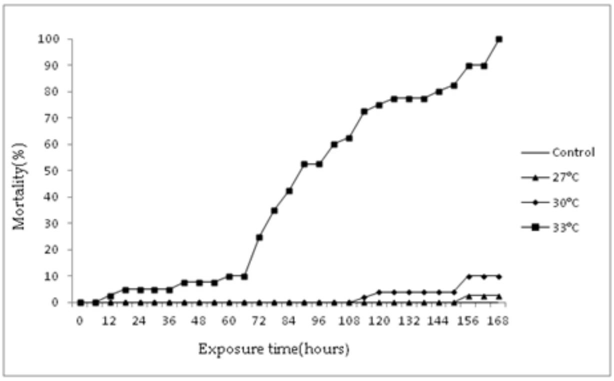

Figure 2. Cumulative mortality curves (%) obtained through the mean of each treatment of *Mytella falcata* exposed to different temperatures (Control = 24ºC).

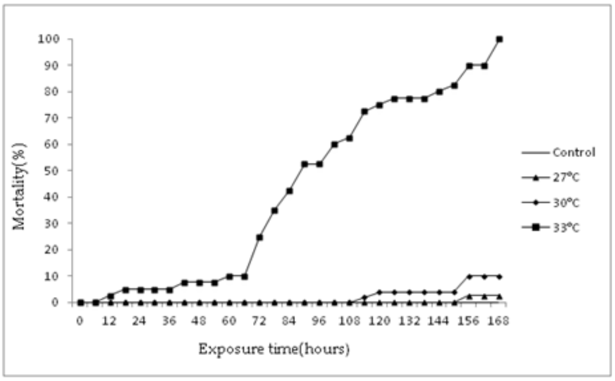

Figure 3. Cumulative mortality curves (%) obtained through the mean of each treatment of *Mytella guyanensis* exposed to different temperatures (Control = 24ºC).

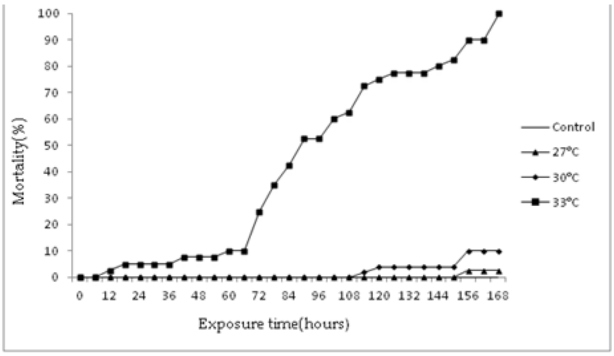

Figure 4. Cumulative mortality curves (%) of *Mytella falcata* and *Mytella guyanensis* exposed to a temperature of 33°C.

DISCUSSION

In the present study, only temperatures above 23°C were chosen to be used since the occurrence of values below this are rare in the studied area (RODRIGUES *et al*. 2003; SUTTI, 2014). BENNETT and JUDD (1992) stated that the temperature that causes mortality in marine organisms depends on exposure time. Corroborating with this information, only after 168 hours of exposure to a temperature of 33°C there was the total mortality of *M. falcata.*

Individuals of the species studied are sessile organisms captured in the region from shallow waters. They are certainly subjected to pronounced vertical temperature gradients that may favor adaptive strategies to optimize energy use at least within a certain variation range. Therefore, to accurately measure temperature effect on *Mytella* spp., the use of experimental ranges relatively narrow is important mainly when studying individuals of species subjected to unstable environments. (PHAN *et al.*, 1993).

The actual tolerance range in nature is often narrower than the potential range in aquaculture. This phenomenon may be evidenced in laboratory through short-term behavioral response, because metabolic cost of physiological regulation under extreme conditions reduces tolerance range in the wild both at the top and lower ends (ODUM, 1988).

Although there are several studies all over the world concerning the resistance of bivalve mollusks at different temperatures, for *Mytella falcata* and *M. guyanensis* studies with such an approach were not found.

GONZÁLES (2003) tested, in Laguna Manuela, Mexico, the resistance of the scallop *Nodipecten subnodosus* through the TLM_{96} method (RAND and PETROCELLI, 1985) for different temperatures and concluded that the species tolerates up to 32°C. This result is similar to the ones obtained in the present study for *M. falcata* and *M. guyanensis*.

On natural banks of Santos bay, São Paulo coastal zone, HENRIQUES *et al.* (2007) diagnosed that the temperature of 34°C, after 24 hours of exposure, is highly lethal for the *Perna perna* mussel. In the Gulf of Mexico, HICKS and MCMAHON (2002) concluded that temperatures above 30°C are also lethal for the same species.

The lower mortality of *Mytella falcata* can be related to the habitat that the bivalve lives in, often encrusted in roots of *Rizhophora mangle*, which remain most of the time submersed at high water, and emerged at low water, depending on the type of tide (spring tide or neap tide) (PEREIRA *et al*. 2003.)

Organisms from the *M. guyanensis* species are found buried in mangroves, in areas away from the water line. As they are always under the mud, they are less exposed to environmental weathering such as sun light, and sudden temperature changes, because the muddy sediment works as a thermal insulating material (PEREIRA *et al.* 2007).

According to data from SUTTI (2014), in the Bertioga channel, water temperature ranges from 22°C to 28°C. These values are close to the ones obtained by PEREIRA (2010), who observed temperatures ranging from 20 to 28°C along the Santos estuary, from November 2004 to March 2005. During the entire experiment, organisms from both species, exposed to 27, 30°C and control, behaved normally, opening and closing their valves when stimulated. On the other hand, organisms that underwent the treatment of 33°C remained with their valves wide open most of the time, presenting late responses, even after physical stimuli.

Although not lethal, temperatures of 27°C and 30°C may have affected important physiological functions such as gametogenesis (VÉLEZ and EPIFANIO, 1981), and life cycle (BUCKLEY *et al.*, 2001). RESGALLA JR. *et al.* (2007), testing the metabolism of *Perna perna* at temperatures of 15°C, 20°C, 25°C, and 30°C concluded that increasing temperature leads to significant increase on oxygen consumption. They also observed lack of byssus formation in organisms subjected to temperature of 30°C, diagnosing this as a symptom of stress (BRODSKY, 2011).

DOWD and SOMERO (2013) observed that mussels tended to spend much less time with the valves in a sealed position following exposure to 33°C body temperature, especially when exposed in air, for three *Mytilus* congeners found on the West Coast of North America: two native species (*Mytilus californianus* and *M. trossulus*) and one invasive species from the Mediterranean (*M. galloprovincialis*). *Mytella falcata* and *M. guyanensis* had the same behavior in the present study.

The organisms studied were sampled at low water, with mangrove substratum nearly or entirely dry. In neap tides, when the tide oscillation is lower than the spring tides, some of the natural banks studied can remain under a water column below 30 cm, and along with thermal effluent from factories can reach temperatures of 33ºC. This fact can still be aggravated with dredging activities performed in specific areas of the Bertioga channel, silting the slopes of the mangrove, making other areas exposed to this condition.

In the present study only cumulative mortality tests were performed, changes in metabolism or physiological stress were not evaluated. Formation of byssus was not visually observed in all tested temperatures. However, they appeared in lower quantities in organisms exposed to temperatures of 30°C and 33°C.

Even if the water temperature of estuaries rarely reaches 33°C, or can maintain this temperature for long periods as in laboratories, other aspects must be taken into account such as human influence, and alterations in physiological functions of these organisms, which may affect their life cycle, even without killing them.

CONCLUSION

Mytella falcata is more resistant than *M. guyanensis* when exposed to high water temperatures. Long exposure periods to temperatures of 33°C were found to be lethal to both species. It was found that there is a decrease in the creation of byssus at temperatures of 30°C and 33°C, indicating a possible physiological stress.

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