



A Non-Invasive Technique for Recording the Cardiac Activity of the Tropical-Subtropical Mangrove Oyster *Crassostrea brasiliana*

IGOR BAKHMET¹, DENIS M. S. ABESSA^{2*}, LUCAS M. BURUAEM², & ESTEFANÍA BONNAIL³

¹ Institute of Biology, Karelian Research Centre of RAS, Laboratory of Ecology of Fish and Water Invertebrates, Petrozavodsk, Russia.

² UNESP, Praça Infante Dom Henrique, s/n, 11330-900, São Vicente, SP, Brazil.

³ UNESCO UNITWIN/WiCop. Department of Physical-Chemistry. Faculty of Marine and Environmental Sciences, University of Cádiz. Campus Río San Pedro, 11510 Puerto Real, Cádiz. Spain.

*Corresponding author e-mail dmabessa@clp.unesp.br

Abstract: In this study, we searched the electric signal of the heart of *Crassostrea brasiliana*; the cardiac activity of 7 individuals was monitored and recorded for a week. The heart beat rates were clearly detected and showed low variation. The technique presented good potential to be used in oysters.

Key Words: mollusk, physiology, heart beat rate, oyster

Resumo: Uma Técnica Não Invasiva para Monitorar a Atividade Cardíaca da Ostra do Mangue *Crassostrea brasiliana*. Foi realizada busca do sinal elétrico do coração de *Crassostrea brasiliana*, e em seguida foi realizado monitoramento cardíaco de 7 animais, mantidos por 7 dias em condição constante. O ritmo cardíaco foi claramente detectado e variou pouco, mostrando que a técnica é promissora para ser aplicada na espécie.

Palavras Chave: moluscos, fisiologia, batimento cardíaco

The mangrove oyster *Crassostrea brasiliana* (Lamarck, 1819; Figure 1) has been used as an appropriate biological model in wide number of investigations of both applied and for fundamental science, in the fields of aquaculture, hydrobiology, ecotoxicology, physiology, and biochemistry (Shumway 1977; Burton 2012; Lannig *et al.* 2006). These oysters represent a dual relevance: economic resource due to the popular high quality of their meat (Pereira *et al.* 2001); and ecological role as intermediate trophic level, contributing significantly to the production of a type of biocenosis associated with estuarine rocky reefs. Oysters act as suitable environmental quality monitors, being able to accumulate anthropogenic pollutants within their

tissues, not only where they are farmed but also where they naturally occur (Doi *et al.* 2014; Reigada *et al.* 2014).

All around the world, investigators have used oysters to monitor the environmental quality by using wide range of methods – hydrochemical, biochemical, hydrobiological, immunological (Cherkasov *et al.* 2007; Lannig *et al.* 2006; Bado-Nilles *et al.* 2008). However, most of these methods have some limitations due to involve large time gaps between the initial exposure to the stressors and the expression of effects. Besides, these methods are destructive and require the organisms to be sacrificed, eliminating thus the possibility of making repeated measures of the same individual along time.

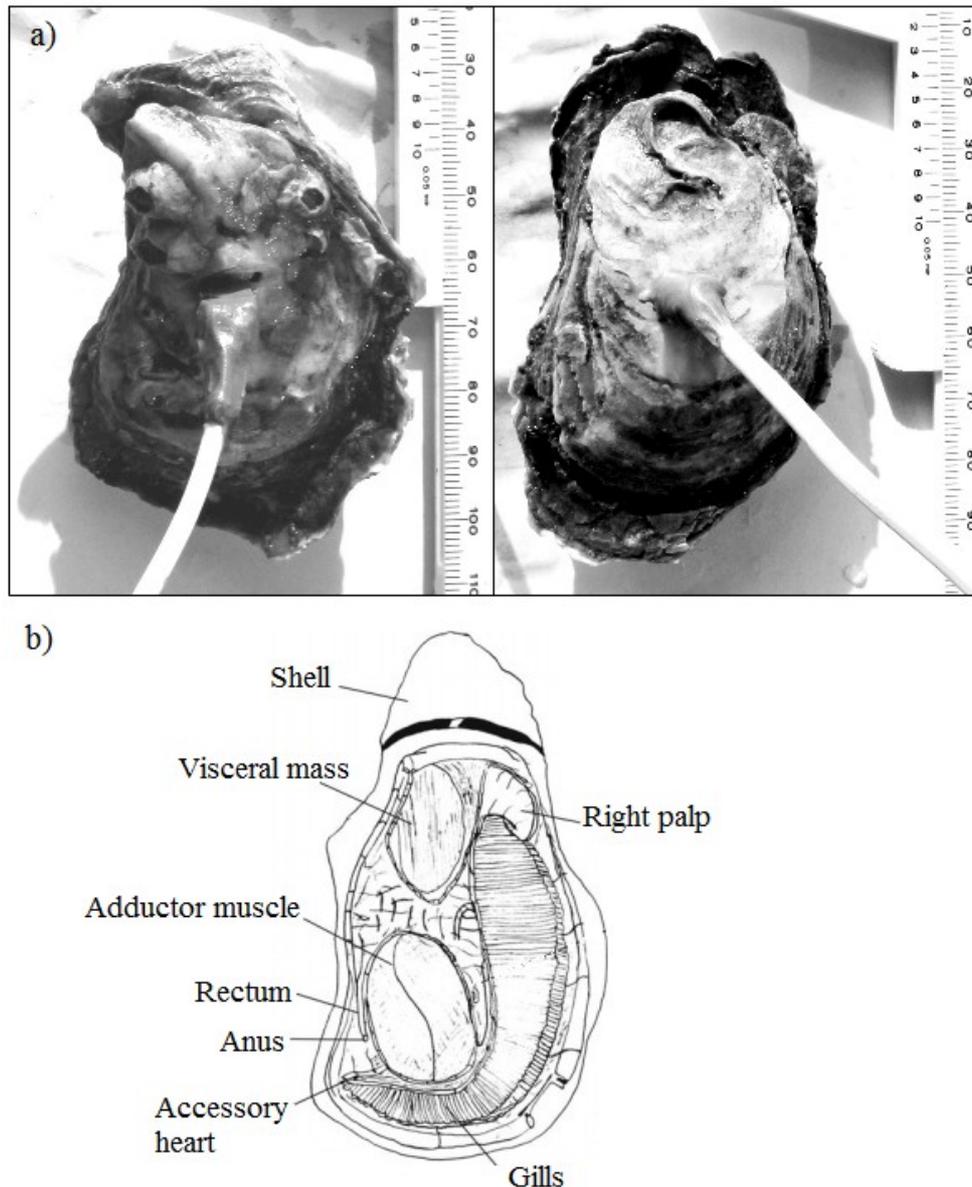


Figure 1. a) Correct location of infra-red sensors on the shell of mangrove oyster (*Crassostrea brasiliiana*) for heart beat rate recording; b) *Crassostrea brasiliiana* anatomy scheme (adapted from Amaral & Simone 2014).

A non-destructive method (CAPMON) was proposed by Depledge & Andersen (1990) to monitor the physiological condition of aquatic bivalves and crustaceans. This method is based on the heartbeat rates (HR) monitoring by means of infra-red sensors installed on the shells of the studied organisms. The cardiac activity may be considered as an appropriate indicative of the organism condition because, as general rule, the HR immediately change as a direct response to alterations of almost all environmental parameters. The monitoring of the heart activity was applied as a method to estimate the physiological conditions of oysters more than 40 years ago (Feng & Van Winkle

1975). However, that method was based on the implantation of electrodes directly in the animals, thus it could be used only in laboratory conditions and, besides that, a wider application of such highly informative parameter was hindered by artifacts evoked by the implantation of electrodes and/or other injuring impacts (Segal 1961). Recently, this drawback was overcome owing to the technique of remote monitoring (Depledge & Andersen 1990). This new approach is based on the attachment of infra-red sensor CNY70 on the mollusks shell at the region of pericardium. The sensor has two illuminators: the first one serves as infra-red source and the second one has the role of receiving the

reflected rays. The movement of heart walls changes the reflected ray, and the received signal is amplified through a special booster and then transformed by an analog-digital converter in order to produce the plethysmogram of heart contraction. Experiments have proved that changes and variations in mussels' heartbeat rates were significantly correlated with various natural factors (Marshall & McQuaid 1993; 1994). Other studies revealed high sensitivity of mussels HR to heavy metals and ammonia (Marchan *et al.* 1999; Curtis *et al.* 2000). In this context, the absence of studies on the cardiac activity of oysters is surprising. The reason of that gap, we suppose, relies in the fact that the shell of oysters is extremely thick and shapeless, thus it makes difficult to set up electrodes to record the HR signals. Despite that, and considering that oysters are a very good biological model, we decided to try to find the way of registering the cardiac activity in this important mollusk.

To achieve that, mangrove oysters of the species *Crassostrea brasiliiana* (= *C. gasar*) were obtained from the commercial oyster farm COPEROSTRA from the region of Cananéia. This is a cooperative maintained by traditional fishers, within the protected area of Cananéia-Iguape-Peruíbe, in the Southern Coast of the São Paulo State (Brazil). The organisms were immediately transported to the laboratory alive, within tanks containing clean seawater and kept aerated. Experiments were performed at the Center of Studies on Aquatic Pollution and Ecotoxicology, which is located in the São Paulo State University (São Vicente, SP, Brazil) during April 2014.

Crassostrea brasiliiana is primarily found in regions significantly influenced by freshwater inflow from the continent and show large variations in salinity. In the estuaries of Cananéia, the Southern coastal region of São Paulo, the distribution of this species is strongly influenced by salinity (Pereira *et al.* 2001). *C. brasiliiana* is cultured in some estuaries of the South and South-East Brazil (Pereira *et al.* 2003), from seeds collected in the natural environment and kept in cropping systems (Gomes *et al.* 2014). Previous studies have shown that the oysters from COPEROSTRA are free from chemical and biological contamination (Doi *et al.* 2014; Reigada *et al.* 2014).

After sorting the mollusks by size (retaining those within 68-75 mm shell length), 7 individuals were held in Plexiglas tanks with aerated seawater

with a salinity of 20 to 25 ‰ under constant light at a temperature of $25 \pm 2^\circ\text{C}$ for at least 7 days prior to use in experiments to ensure recovery from any stress effects due to collection. Water was partially daily replaced. Oysters were daily fed on basis with approximately 1 liter of cell suspension of microalgae *Tetraselmis sp.* cultured in the university facilities.

According Amaral & Simone (2014), pericardium is located between visceral glands and adductor muscle. It wide occupies, anteriorly and 1/2 size of adductor muscle (Figure 1b). Taking into account the expected position of the pericardium, we searched for the right place for attaching the infra-red sensors on the shell of the oysters. In addition, knowing the shell thickness (3-4 mm) we scrapped the shell surface with sandpaper in order to obtain a flat surface for the attachment of the sensor (Figure 1). After several attempts we succeed in finding the best places to attach the sensor and recording heart activity in oyster. For the oysters of sizes which we used, the best place for attaching the sensors is on crosshair two lines: first, from umbo to opposite side of the shell; second, transversely to the first line at the distance of approximately 40 mm (Figure 1a).

The obtained plethysmograms for oysters (Figure 2) allowed a clear discrimination between atrial contractions (short and high peak) and ventricular contractions (a longer and flattened peak). Parts of these recordings are shown in Figure 2 – where the heart-beat signals of the studied individuals are displayed (20 seconds recordings obtained along 7 days of measurements). The signal intensity (as recorded by an oscillograph) could reach 3 V. The HR in the oysters did not varied significantly, as values ranged between 30.2 and 33.5 beats per minute. The average heart rate was 31.1 ± 1.2 beats per min (Figure 2). We would like to underline that during 7 days of observations the relative stable of cardiac activity in every animals was shown (the deviation did not exceed more then 1 beats per min). This value can be used for comparative purposes as HR of *Crassostrea brasiliiana* under non stress situation.

As conclusion it is obviously that the results of our work may serve as a base for future studies in many fields, as general physiology studies (especially with aquatic organisms), biomonitoring, environmental risk assessment, environmental quality assessment, among others.

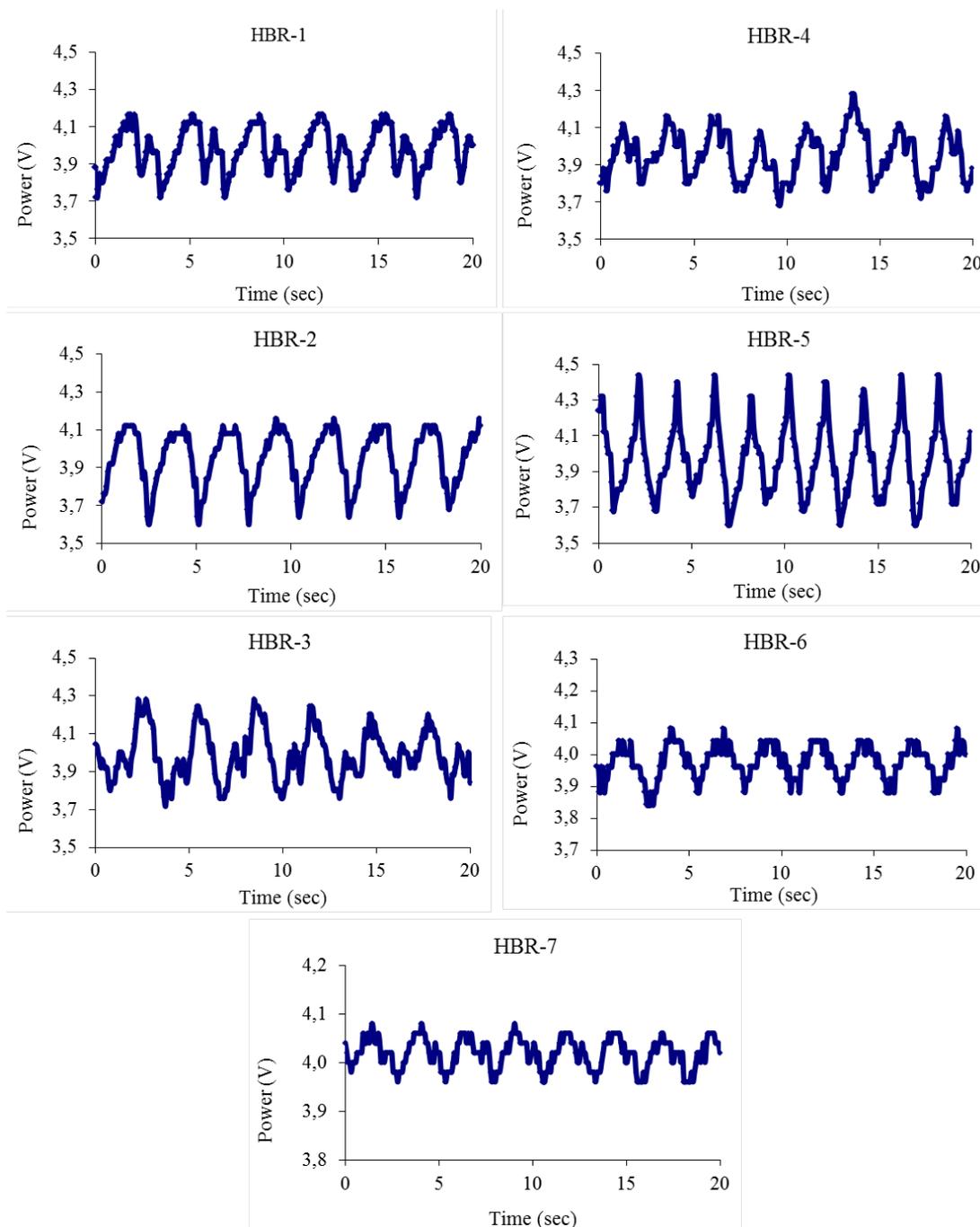


Figure 2. Plethysmograms obtained for 7 individuals of the mangrove oyster (*Crassostrea brasiliiana*) kept under controlled conditions for 7 days (20 seconds of each register).

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