PANAMJAS
Pan-American Journal of Aquatic Sciences

# Influence of physicochemical parameters on *Litopenaeus vannamei* survival

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**Abstract.** Although several studies showed that *Litopenaeus vannamei* is resistance to several stressor conditions, information about survival of shrimps submitted simultaneously to several environment changes are scarce. So, the objective of this study was to evaluate the simultaneous influence of salinity, temperature and oxygen variations on survival of juveniles of *Litopenaeus vannamei*. Each salinity (5, 25, and 35‰) was crossed with temperatures of 20, 25 and 30 ºC and different oxygen levels including 0, 0.5, 1.5, 3 and 6 mg $O_2L^{-1}$. The $LC_{50\text{-}96h}$ values showed a higher survival resistance to oxygen variation at the lowest temperature of 20 °C with higher salinities of 25 and 35‰. In addition, a low survival resistance was observed at the highest temperature of 30 °C in all salinities tested. These results demonstrate that the sensitivity of white shrimp to oxygen variation is directly linked to temperature and salinity. In shrimp, higher temperatures can lead to an increase in metabolism and, consequently, higher oxygen consumption for energy demand. At low salinities, there are compensatory mechanisms to maintain osmotic homeostasis, which increase energy expenditure.

**Key words:** crustacean, physicochemical parameters, aquaculture.

**Resumo: A influência de parâmetros físicoquímicos na sobrevivência do *Litopenaeus vannamei*.** Embora diversos estudos tenham mostrado que *Litopenaeus vannamei* é resistente a diversas condições estressoras, informações sobre a sobrevivência desta espécie de camarão submetida simultaneamente a diferentes condições ambientais ainda são escassas. Portanto, o objetivo deste estudo foi avaliar a influência simultânea da salinidade, temperatura e variações de oxigênio na sobrevivência do camarão. Cada salinidade (5, 25, e 35‰) foi submetida com diferentes temperaturas 20, 25 e 30 ºC e diferentes níveis de oxigênio (0, 0.5, 1.5, 3 e 6 mg $O_2$ $L^{-1}$). Os valores da $CL_{50\text{-}96h}$ mostraram maior sobrevivência às variações de oxigênio quando submetidas à temperatura de 20ºC e salinidades mais altas 25 e 35‰. Enquanto na maior temperatura foi observada menor sobrevivência em todas as salinidades analisadas. Estes resultados demonstram que a sensibilidade do camarão às variações de oxigênio está diretamente relacionada à temperatura e salinidade. Em camarões, altas temperaturas podem levar a um aumento do metabolismo e consequentemente, um maior consumo de oxigênio para suprir a demanda energética enquanto em baixas salinidades, há mecanismos compensatórios para manter a homeostasia osmótica que também representa maior gasto energético.

**Palavras-chave**: crustáceos, parâmetros físico-químicos, aquacultura.

## Introduction

Shrimp farming is one of the most important activities in the global aquaculture industry. The Pacific white shrimp *Litopenaeus vannamei* (Crustacea, Decapoda) is among the most economically important species of cultured animals (FAO, 2015). Originally, *L. vannamei* are native to the eastern Pacific Ocean from Mexico to northern Peru; however, it is now breeding in several countries around the world and currently reaches a larger number of breeding tones than those generated by conventional fisheries (FAO, 2014).

In fact, shrimp farming is a globally exploited activity. In Brazil, shrimp farming began in the 1970s as a source of income for the local population in the northern and northeastern states. Shrimp farming also gained ground in the southern states in the late 1990s. *Litopenaeus vannamei* has since become the most widely cultivated shrimp species in the country, owing to its great adaptability in different ecosystems along the hemispheres (Ostrensky et al., 2000; Bostock et al., 2010).

Animal welfare and farming productivity are closely related to environmental conditions as well as the appropriate management procedures adopted by breeders (Tyler et al., 2017). Physical and chemical factors such as salinity, temperature and dissolved oxygen levels are some of the most important parameters to be established and monitored (Romano and Zeng, 2006). Dissolved oxygen is the most critical variable in shrimp farming, which generally reflects the nursery's environmental conditions. The ideal range of dissolved oxygen concentration suggested for *L. vannamei* ranges from 6 to 10 mg $O_2L^{-1}$ (Brock and Main, 1994). It is known that prolonged exposure to low oxygen concentrations of values below 1.5 mg $O_2L^{-1}$ may be lethal to shrimp. In addition, moderate oxygen concentrations (3 mg $O_2$ $L^{-1}$) are associated with low feed rates, slow growth and low resistance to diseases (Welker et al., 2013). The solubility of oxygen is affected by temperature, atmospheric pressure, salinity and closely influenced by organic matter. Therefore, the solubility of oxygen decreases both with increasing temperature and with increasing salinity (Spanopoulos-Hernández et al., 2005).

One of the problems encountered in the shrimp farming industry is precisely controlling the temperature of the tanks, which is exclusively dependent on the atmospheric temperature and can oscillate throughout the day and the seasons (Barbieri & Ostrensky, 2002). The optimal temperature for *L. vannamei* growth is around 28 °C for adult animals (Wyban et al., 1995); however, the water temperature on farms can vary from 15 to 30 °C in a short time (Chen, 1990), causing thermal stress to the animals, reducing their immunological resistance and increasing the susceptibility to pathogens and infections (Cheng et al., 2005).

In addition, an ionic imbalance in the culture water requires a higher energy expenditure to maintain osmotic homeostasis, thus impairing molting and growth (Brito et al., 2000; Romano & Zeng, 2006). Therefore, salinity is another important factor for the proper development of *L. vannamei*. Even though it is an eurihaline species that tolerates a wide range of salinity from 0.5 to 40‰ (Saoud et al., 2003), an abrupt change can cause harmful effects directly to their metabolism and growth, consequently affecting the survival of this crustacean. In this context, the objective of the present study was to evaluate the mortality of this crustacean under different variables of oxygen, temperature and salinity concentrations.

## Material and Methods

*Shrimp maintenance:* The juvenile Pacific white shrimp *Litopenaeus vannamei* of both sexes (with an average weight of 8 ± 0.8 g) were obtained with the collaboration of the Marine Station of Aquaculture (EMA) of the Federal University of Rio Grande – FURG. The shrimp were transferred to the laboratory and acclimated in tanks under controlled parameters (temperature 20 ± 0.5 ºC, salinity 30 ± 1‰, pH 8.0, 12L/12D, constant aeration, fed commercial feed (GuabiTech active 1.6 mm with 38% brut protein), twice a day), for at least two weeks prior to the experiment. After the acclimation period, the animals were divided into experimental groups as described below.

*Experimental design:* Experiments to evaluate the Mean Lethal Concentration in 96 h ($LC_{50\text{-}96h}$) were performed in three steps (n = 450; n = 10 for each group with one replicate each). The following salinities were defined for each step: 5, 25 and 35. Each salinity was crossed with different temperatures (20, 25 and 30 ºC) and oxygen levels (0, 0.5, 1.5, 3 and 6 mg $O_2$ $L^{-1}$). Mortality was evaluated for 96 hours in all conditions. The experiment was carried out in aquaria containing 15 liters of salt water in the salinities of the study and duly capped during the experiment. Nitrogen gas ($N_2$) was used to achieve the desired oxygen concentration. Oxygen levels and temperature were constantly monitored every 3 hours (Oximeter: DO-5519, Lutran Eletronic Enterprise Co.).

## Results

All values of $LC_{50\text{-}96h}$ in this study are shown in Table I. In the first hours of the experiment, at the lower temperature (20 °C), the occurrence of death in relation to the exposure time increased proportionally with the decrease in oxygen level (Fig. 1a). In the lowest oxygen concentrations (anoxia ± 0.1 mg $O_2$ $L^{-1}$ and 0.5 ± 0.1 mg $O_2$ $L^{-1}$), the occurrence of death was recorded during the first hours of the experiment with mortalities above 50%, rapidly reaching 100% of mortality in all temperatures observed (Figs. 1a, b and c). At 5‰ salinity, deaths were recorded in the control group (6 ± 0.5 mg $O_2$ $L^{-1}$) only at the highest temperature after a 36 hours exposure (Fig. 1c).

At 25‰ salinity and 20ºC was observed a low mortality when animals were exposed to 3 or 1.5 mg $O_2$ $L^{-1}$ (Fig. 2a). However, at 25 ºC, the concentration of 3 ± 0.3 mg $O_2L^{-1}$ showed a greater effect on mortality than 1.5 ± 0.2 mg $O_2$ $L^{-1}$ (Fig. 2b), At 30 °C, except in the normoxic condition (6 mg $O_2$ $L^{-1}$), the mortality reached 100% within the first 18 hours of exposure (Fig. 2c).

At salinity 35‰, no deaths were observed in the control groups (6 mg $O_2L^{-1}$) at any temperature analyzed (Fig. 3a, 3b and 3c). At 20 °C, there were no deaths at the concentration of 3 mg $O_2L^{-1}$ and the percentage of deaths was more pronounced in anoxia and 0.5 mg $O_2L^{-1}$ in the first hours of exposure (Fig. 3a). At 25 °C, a higher mortality rate was observed at 3 mg $O_2L^{-1}$ than at 1.5 mg $O_2L^{-1}$. In addition, concentrations of 0.5 mg $O_2L^{-1}$ and anoxia at 25 °C caused total mortality in the first hours of exposure (Fig. 3b). At 30 °C, all oxygen concentrations (with exception of the control group) were associated with total mortality in the first 30 hours of exposure; total mortality occurred within 12 hours of exposure at lower oxygen concentrations (Fig. 3c).

## Discussion

The demand for products generated by shrimp farming has been steadily increasing in recent years (FAO, 2016), generating interest in farming techniques that minimize production losses. The consequent demand for quality product necessitates approaches incorporating aspects related to sustainability, with particular attention on the quality of the aquatic environment in which the production is practiced. For this reason, the physicochemical parameters discussed in this study are of important relevance for the welfare of the shrimp, as well as for obtaining quality products.

The intermediate temperature and salinity used in this study were 25 °C and 25‰ respectively, which resulted in a $LC_{50\text{-}96h}$ for the dissolved oxygen of 2.59 mg $O_2L^{-1}$. An approximate temperature of 28 ºC may be the most suitable for shrimp rearing (Wyban et al., 1995). A variation in salinity does not seem to be an interference factor at a temperature of 25 ºC, because the $LC_{50\text{-}96h}$ values obtained in the lowest and highest salinity used in this study (5 and 35‰) were 2.79 mg $O_2L^{-1}$ and 2.81 mg $O_2L^{-1}$, respectively. However, these $LC_{50\text{-}96h}$ values are not far from what the literature describes as moderate hypoxia (3 mg $O_2L^{-1}$) (Welker et al., 2013), which may cause mortality over a long period of time. However, in a short period of exposure, hypoxia can leave the animals vulnerable to attack by microorganisms and result in illnesses and an associated low rate of growth and weight gain (Zhou et al., 2009).

**Table I.** Medium lethal concentration ($LC_{50\text{-}96}$) of dissolved oxygen over different salinities and temperatures.

| $LC_{50\text{-}96h}$ | Temperature 20°C (CI) | Temperature 25°C (CI) | Temperature 30°C (CI) |
|---|---|---|---|
| Salinity 5% | 2.67 mg $O_2L^{-1}$ (2.27-3.06) | 2.79 mg $O_2L^{-1}$ (2.50-3.01) | 4.81 mg $O_2L^{-1}$ (4.32-5.11) |
| Salinity 25% | 1.92 mg $O_2L^{-1}$ (0.89-2.99) | 2.59 mg $O_2L^{-1}$ (2.25-2.92) | 4.21 mg $O_2L^{-1}$ (3.00-5.01) |
| Salinity 35% | 1.92 mg $O_2L^{-1}$ (0.89-2.99) | 2.81 mg $O_2L^{-1}$ (2.58-3.01) | 3.62 mg $O_2L^{-1}$ (2.60-4.53) |

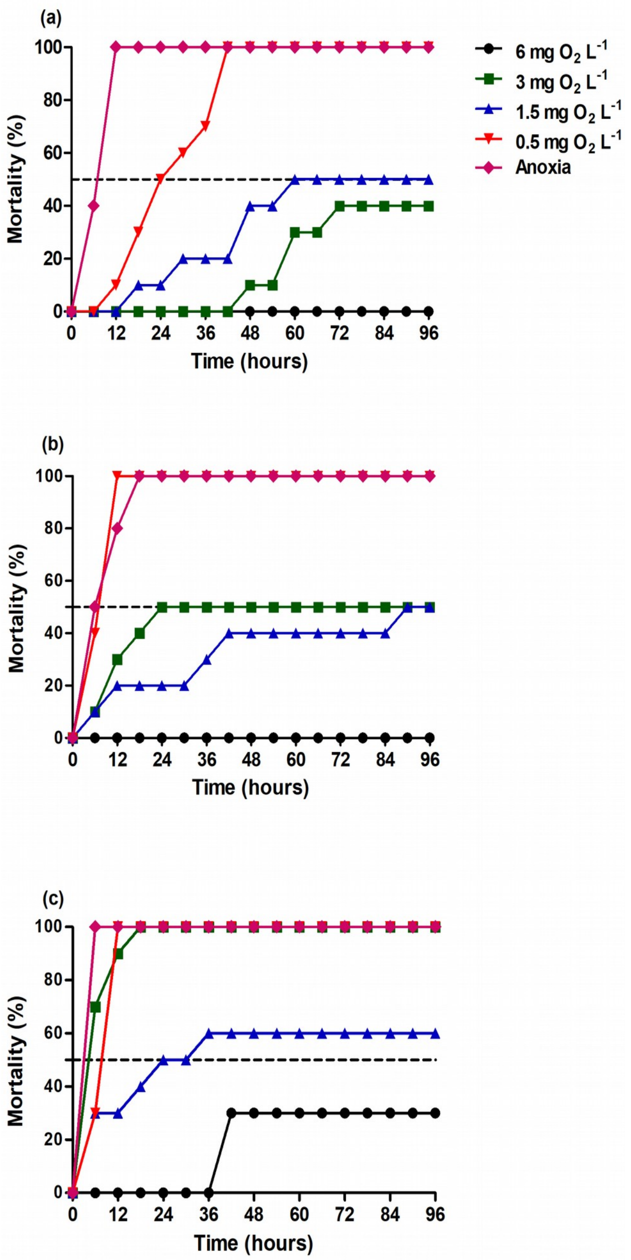


**Figure 1.** *Litopenaeus vannamei* $LC_{50}$ values after 96 hours at 5‰ salinity in different temperatures (a – 20 °C; b – 25 °C and c – 30 °C) and dissolved oxygen concentrations (6, 3, 1.5, 0.5 mg $O_2L^{-1}$ and anoxia). Results are expressed as the percentage of dead animals.

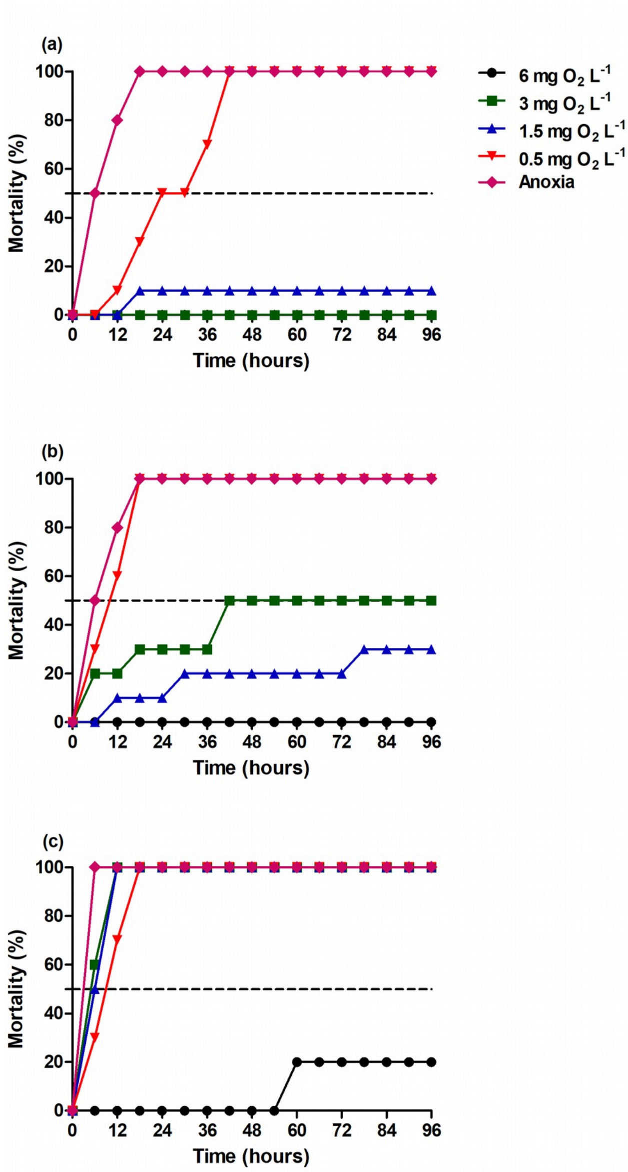


**Figure 2.** *Litopenaeus vannamei* $LC_{50}$ values after 96 hours at 25‰ salinity in different temperatures (a – 20 °C; b – 25 °C and c – 30 °C) and dissolved oxygen concentrations (6, 3, 1.5, 0.5 mg $O_2L^{-1}$ and anoxia). Results are expressed as the percentage of dead animals.

After 96 hours of experimentation, shrimp reared in a lower salinity (5‰) at 20 °C had an $LC_{50\text{-}96h}$ for dissolved oxygen set at 2.67 mg $O_2$ $L^{-1}$, which is higher than the values for other experimental salinities (25 and 35‰) at the same temperature. These data indicate that in high salinities and temperatures of 20 ºC the survival resistance of *L. vannamei* is increased to a condition of almost severe hypoxia. However, it has been reported that temperatures around 19 °C can leave *L. vannamei* in relative immobility, which reduces dietary behavior and results in a consequent weight loss and metabolism (Ponce-Palafox et al., 1997); however, managing the salinity (increasing, in this case) can revert this scenario. It is also known that marine shrimp grown at low salinity obtain the minerals present in the water required for their osmoregulation by active transport, thereby leading to increased energy expenditure (Gong et al., 2004). *Litopenaeus vannamei* in adulthood has a decline in osmoregulatory capacity, which entails greater energy expenditure to maintain its homeostasis, thus

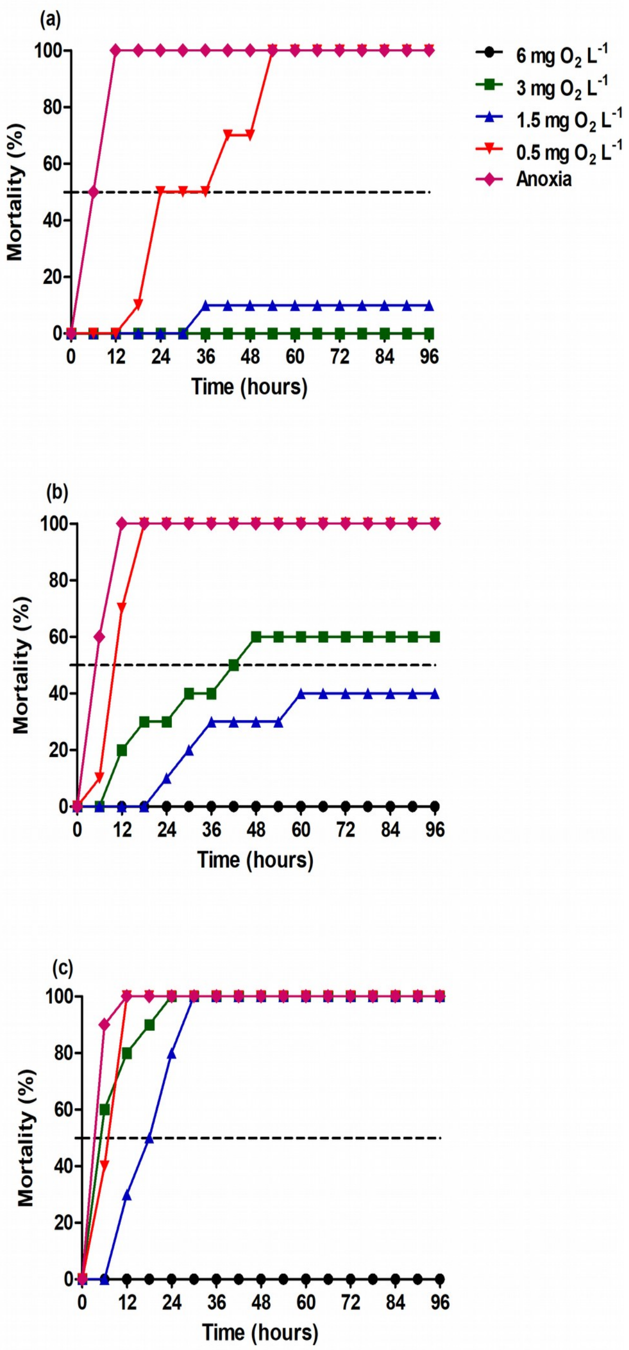


**Figure 3.** *Litopenaeus vannamei* $LC_{50}$ values after 96 hours at 35‰ salinity in different temperatures (a – 20 °C; b – 25 °C and c – 30 °C) and dissolved oxygen concentrations (6, 3, 1.5, 0.5 mg $O_2L^{-1}$ and anoxia). Results are expressed as the percentage of dead animals.

increasing the demand for oxygen (Vargas-Albores and Ochoa, 1992).

In this study, the values of $LC_{50\text{-}96h}$ for the dissolved oxygen in the higher experimental temperature (30 ºC) were evidently higher in all the salinity levels (5, 25 and 35‰, with $LC_{50\text{-}96h}$ of 4.81, 4.21 and 3.62 mg $O_2L^{-1}$, respectively). This finding directly reflects increased metabolism as well as higher oxygen consumption, leading to a lower resistance to restrictions of this gas. In this context, is important to emphasize that the temperature is a factor that directly influences oxygen consumption in aquatic animals. Given that crustaceans do not have the capacity to maintain a constant body temperature, the animal is subject to the thermal variations of its habitat that directly influence its metabolic processes (Martínez-Palacios et al., 1996). Therefore, high temperatures accelerate metabolic processes and use up the available oxygen, which results in a drop in ATP levels and affects physiological and biochemical processes over a long time period (Boyd, 1979; Manushet al. , 2006).

The rate in oxygen consumption generally increases steadily as the temperature rises. For example, a rise in temperature of 10 °C can result in a three-fold oxygen consumption rate. This elevation in the metabolic rate is called the thermal coefficient (Q10), which represents the degree of sensitivity of organisms to temperature (Schmidt-Nielsen, 1999). In fact, Sponopoulos-Hernández et al. (2005) reported an increase in oxygen consumption in *Litopenaeus stylirostris* with an increase in temperature starting from 20 to 30 °C. These results in combination with the data generated in this study demonstrate the greater sensitivity of this organism to variations in oxygen levels at higher temperatures.

In low-salinity conditions, *L. vannamei* has compensatory mechanisms to maintain osmotic homeostasis, such as active $Na^+/K^+$-ATPase ion uptake and the mobilization of amino acids such as arginine, lysine and glycine, leading to energy expenditure (Álvarez et al., 2004). The highest $LC_{50}$ value for dissolved oxygen was obtained at the lowest salinity and highest temperature, being set at 4.21 mg $O_2$ $L^{-1}$. This result can be explained because the osmoregulation processes that consume energy which results in higher oxygen demand even for euryhaline species such as *L. vannamei*. Physiological variations occur at each stage of the animal life cycle and require differentiated care (Vernberg, 1983; Ye et al., 2009). Therefore, values of temperature, salinity and dissolved oxygen are important factors to determine the stocking density and mortality rates in the shrimps farming. In this study, the sensitivity of white shrimp to oxygen fluctuations was directly linked to temperature and salinity. The $LC_{50\text{-}96h}$ analyzes showed higher resistance of shrimp to oxygen variation at a lower temperature (20 °C) with higher salinities (25 to 35‰). In addition, a low resistance was observed at a higher temperature (30 °C) with lower salinities (5 to 25‰). Together, these data contribute significantly to production practices aimed at minimizing animal losses, as well as to the welfare of the shrimp farming industry.

**Acknowledgements**

The authors would like to thank IFS for financial support (Process number A5318/2012 granted to Juliane Ventura-Lima, PhD and Process number A/5352-1 granted to Fábio Everton Maciel, PhD). Also Brazilian agencie CNPq (455818/2014-2) supported this Project. Roberta de Oliveira Lobato was a graduate fellow at Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Litiele Cruz recipient of fellowship CAPES/PNPD Program. Wilson Wasielesky is research fellows at CNPq.

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Received: September 2019
Accepted: April 2020
Published: June 2020