

Salinity and temperature tolerance of the caridean
shrimp, *Palaemon peringueyi* (Caridea: Palaemonidae)

By

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degree of
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Abstract

Temperature and salinity are two of the most important environmental factors that influence the physiology of aquatic organisms, especially in the estuarine and coastal ecosystems. The interaction of salinity and temperature can be complex, with one variable acting as a modulating factor on the effects of the other and both factors have a huge impact on organisms as they can influence, modify and control an organism's physiological rates. Studies on the effects of salinity and temperature on the survival of the caridean shrimp, *Palaemon peringueyi* were conducted for this study. Density experiments of *P. peringueyi* were first conducted in order to determine shrimp densities to be used in tests. An influence of appropriate densities (5, 10 and 15 individuals per litre) to the survival of adult shrimps (i.e. carapace length ≥ 9 mm) after 96-hour exposure was investigated. Highest survival (80%) was recorded for the density of five individuals per litre, with survival varying between 60 and 80%. The lowest survival was recorded for the highest density (i.e. 15 individuals). Therefore, individual instead of group exposures were used for salinity tolerance tests. *P. peringueyi* were able to tolerate test salinities (range: 5 to 55 in increments of 5) and highest survival ($\geq 80\%$) was recorded at salinities of 15–45. Difference in survival between salinities was significant ($p = 0.000541$) and these were driven mainly by differences between 55 vs all salinities. Temperature tolerance was highest at 15°C, 20°C and 25°C where survival ranged between 73% and 100%. Temperatures of 5°C and 35°C resulted in mortalities. Temperature was thus found to have a more pronounced effect on survival ($p = 0.000$) while salinity had a less significant effect. The interactive effects of temperature and salinity were found to have a significant influence on the survival of *P. peringueyi* ($p = 0.002$).

Keywords: *Palaemon peringueyi*, density, salinity tolerance, temperature tolerance

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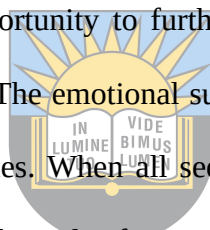
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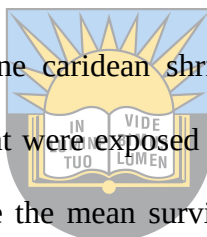


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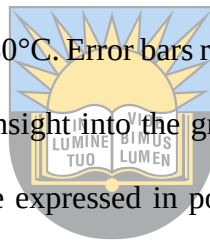
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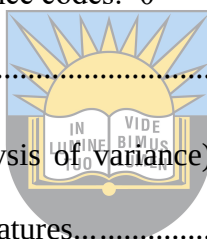
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Chapter 1

General Introduction



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Introduction

An estuary is defined as “a semi-enclosed coastal body of water which has a free connection with the open sea and within which seawater is measurably diluted with freshwater derived from land” (Cameron and Pritchard, 1963; Pritchard, 1967; Elliott and McLusky, 2002). This definition is, however, based on estuaries located in the northern hemisphere whereas southern hemisphere estuaries such as South African and Australian estuaries with varied characteristics were ignored (Day, 1980; Hodgkin and Lenanton 1981; Roy *et al.*, 2001). This, therefore, led to a redefinition of an estuary in order to put more emphasis on the fact that freshwater input into an estuary must be acquired from rivers and that on occasion salinities can surpass that of full-strength sea water. Subsequently, Potter *et al.*, (2010) defined an estuary as “a partially enclosed coastal body of water that is either permanently or periodically open to the sea and which receives at least periodic discharge from a river(s), and thus, while its salinity is typically less than that of natural sea water and varies temporally and along its length, it can become hypersaline in regions when evaporative water loss is high and freshwater and tidal inputs are negligible.” This definition takes into account the characteristics of South African estuaries and recognised that these estuaries may not necessarily be freely connected with the sea but are either periodically or permanently open to the sea (Day, 1980; Whitfield and Elliot, 2011).

The ecological integrity of estuaries worldwide is increasingly compromised by a range of human impacts such as rapid population growth and uncontrolled industrial development in industrial regions (Chapman, 2007; Rainbow, 2007). Water quality in these estuaries is often compromised by excessive nutrient input, chemical contaminant accumulation and habitat degradation (Rainbow, 2002). Much like other estuaries in the world, South African estuaries are no exception, and the integrity of local estuaries is also under great threat (Begg, 1978; Heydorn, 1986; Ramm, 1990; Whitfield, 2000; Turpie, 2004). The major factors affecting local

estuaries are mostly man-made and comprise of agricultural and industrial pollution, catchment land-usage, coastal development, freshwater abstraction and over-exploitation of biotic resources (Lubke *et al.*, 1997; Marrant and Quin, 1999; Whitfield *et al.*, 2012; van Niekerk *et al.*, 2013).

South African estuaries are classified into nine categories based on geomorphological and/or physical characteristics. These include estuarine lakes, estuarine bays, estuarine lagoons, predominantly open estuaries, large temporary closed estuaries, small temporary closed estuaries, large fluvially dominated estuaries, small fluvially dominated estuaries and arid predominantly closed estuaries (van Niekerk *et al.*, 2020). South Africa has approximately 290 estuaries across four biogeographical regions (i.e. cool temperate, warm temperate, subtropical and tropical) (Harris *et al.*, 2019; van Niekerk *et al.*, 2020) and more than 90% have restricted inlets (or mouths) due to strong wave action and high sediment availability, while more than 75% of these systems close off for varying phases due to the formation of sand bars across the mouth (Whitfield, 1992; Cooper, 2001; Taljaard *et al.*, 2009; Whitfield and Elliott, 2011). Predominantly Open Estuaries make up about 45 of the 290 South African estuaries (van Niekerk *et al.*, 2020). Predominantly Open Estuaries (also known as tidal river lagoons) are open to the sea for more than 90% of the time (similar to the Permanently Open Estuary type of Whitfield 1992). Some are permanently open due to recurrent river flow or the existence of a large tidal prism (van Niekerk *et al.*, 2020). These systems have a moderate tidal prism, and parameters including bathymetry, river inflow and tidal cycle determine the horizontal salinity gradient and vertical salinity stratification (Mackay and Schumann, 1990). Salinities in these systems generally vary between 0 and 35 (van Niekerk *et al.*, 2020) and in the upper reaches of some systems (e.g. Kariega Estuary) salinities greater than 40 have been recorded during severe droughts because of extremely reduced or non-existent river inflow and elevated summer evaporation rates (Whitfield and Bruton, 1989).

Salinity is a vital factor in species distribution within estuaries (Wooldridge, 1999; Rowe, 2002). For example, *P. peringueyi* (sand shrimp) is more abundant in the middle and lower reaches of predominantly open estuaries that are characterised by high salinities (de Villiers *et al.*, 1999; Nyalungu *et al.*, 2014). The distribution of fauna (e.g. *P. peringueyi*) in an estuary can be influenced by physiological responses of dealing with varying physical and chemical parameters in estuaries, including salinity, pH, temperature and dissolved oxygen (Gonzalez-Oreja and Saiz-Salinas, 1998; de Villiers *et al.*, 1999; Teske and Wooldridge 2003).

Emmerson (1985) concluded that built-in aspects such as body size and the stage at which the organisms moult play an essential role in the biology of many crustaceans. Hagerman and Weber (1981) and Schmidt-Nielsen (1983) also highlighted that parameters such as temperature, dissolved oxygen and salinity also play crucial roles in the biology of crustaceans.

According to Kinne (1963), the tolerance of an organism to salinity describes its potential for recruitment into the estuarine environment whilst the salinity tolerance range is thermally influenced. *P. peringueyi*, for example, is euryhaline and has been recorded at salinities within the range of 10 to 50 (Wooldridge, 1999) but is generally distributed from the middle to lower reaches where salinities generally range between 20 and 35 (Nyalungu *et al.*, 2014). However, a number of crustaceans occupy restricted areas within estuaries, at which salinity variation is generally within the range tolerated in the laboratory (Teske and Wooldridge, 2003; Masikane *et al.*, 2014). Therefore, in order for an organism to maintain an internal environment composition within certain limits, the acclimation to a particular salinity may be necessary and would involve the modification of exchange surfaces such as the gut epithelium and the gills (Blust, *et al.*, 1992). Some crustaceans (e.g. *Palaemon longirostris*) can change their integument permeability in response to low salinity (Rainbow, 1997).

According to Brett (1987) temperature and salinity are the most prominent abiotic variables that affect the physiology of aquatic organisms. Temperature has a direct effect on the rate of all biological processes while salinity places an osmoregulatory demand on organisms. In addition to the effects of the individual variables, the interaction of salinity and temperature can be complex, where one factor exacerbates the effects of the other (Vernberg, 1983). Thermal and saline effects on any given species vary among life stages and changes in physiology are age-dependent, therefore, resulting in a variation in metabolic responses (Vernberg, 1983).

Distribution and biology of *P. peringueyi*

P. peringueyi (McPherson, 1990) is a hyperbenthic estuarine sand shrimp species that is distributed throughout the length of the Southern African coastline from Kosi Bay on the east coast (Broekhuysen and Taylor, 1959; Wood *et al.*, 2017) to Walvis Bay on the west coast (Barnard, 1950) but is more prevalent towards the southeast and south coast (de Villiers *et al.*, 1999). *P. peringueyi* forms part of the four species of the genus *Palaemon* (i.e. *capensis*, *concinus*, *debilis* and *peringueyi*) that have been recorded in southern Africa which are characterised by freshwater, marine and estuarine species (Kensley, 1972). *P. peringueyi* (Stebbing, 1915) from Southern African waters was previously thought to be the same species as *Palaemon pacificus* (Stimpson, 1860), a species documented from the Red Sea to Japan and Polynesia. It was determined that *P. peringueyi* is a distinct species after a number of specimens from various zones within the species' distribution range were evaluated. The rostrum length and the pleural shape of the fifth abdominal segment allow for easy differentiation between the two species (Macpherson, 1990).

P. peringueyi has an adult marine phase and a juvenile phase which can be completed either in the marine environment, in rock pools, or within estuaries (Emmerson, 1985; de Villiers *et al.*, 1999; Bernard and Froneman, 2005). Reproduction and early larval development take place within marine coastal waters and there are no records of the species ever reproducing in estuaries (Emmerson 1985, Bernard and Froneman 2005, Froneman 2006). The last zoeal stage and the postlarvae subsequently migrate to protected nursery habitats (Emmerson, 1986). Juveniles spend between nine and twelve months in these habitats, where upon they return to the near shore habitat (Wooldridge, 1999).

P. peringueyi is considered a dominant part of the hyperbenthos within the littoral zone of Predominantly Open and Large Temporarily Closed Estuaries along the south-eastern coastline of southern Africa (Emmerson, 1986; Bernard and Froneman, 2005; Richoux and Froneman, 2008; Whitfield *et al.*, 2008). However, the species was recorded to have a higher abundance in Predominantly Open Estuaries compared to Large Temporarily Closed Estuaries (Whitfield and Bate, 2007). The reduction in shrimp abundances in Large Temporarily Closed Estuaries is caused by the existence of a sand bar across the estuarine mouth which prevents shrimp recruitment into the estuaries (de Villiers *et al.*, 1999; Bernard and Froneman 2005; Whitfield and Bate 2007). *P. peringueyi* recruitment into large temporarily closed estuarine systems occurs during breaching and storm surge overtopping events (Froneman 2006; Whitfield *et al.* 2008), which may result in the recruitment of large volumes of juveniles into estuaries (Whitfield and Bate 2007). Highest *P. peringueyi* recruitments into Predominantly Open Estuaries take place in summer, followed by winter (Emmerson, 1986).

P. peringueyi can be collected from the field all year round and high densities can be collected in the lower reaches, particularly in the winter season in the Kariega Estuary (see Nyalungu *et al.*, 2014). Within estuarine systems, the shrimps are commonly found in the middle to lower reaches where salinity ranges between 25 and 35 and in association with seagrass (*Zostera*

capensis) where shrimp densities may reach 400 individuals per square meter (Wooldridge, 1999; Nyalungu *et al.*, 2014). The reduction in shrimp abundance in the upper reaches may be caused by the shrimp's physiological tolerances (Allan *et al.*, 2006) and the restricted cover of seagrass with which they are associated (Bernard and Froneman, 2005). Sea grass beds act as a sanctuary for *P. peringueyi* as it limits predation by restricting predator access (Walsh and Mitchell, 1998) and function as zones of increased food availability (Kneib and Wagner, 1994; Adams *et al.*, 1999). Stable isotope and lipid analysis studies indicated that the species can be considered a detrital feeder which mainly consumes plant material mostly obtained from the sea grass with which they are associated (Froneman, 2002; Richoux and Froneman, 2007; 2008).

This shrimp species is of great ecological importance as it forms an essential link in the estuarine food chain (Froneman and Henninger, 2010), since it likely plays an essential part in nutrient and energy dynamics of Southern African estuaries (Froneman and Henninger, 2010). It has been identified as a significant constituent in the diet of a number of commercially important fishes, which include: *Argyrosomus japonicus* (the kob), *Lithognathus lithognathus* (white steenbras), *Alosa sapidissima* (shad), *Lichia amia* (Garrick), *Epinephelus marginatus* (Yellowbelly rockcod) and *Pomadasys commersonnii* (Spotted grunter) (de Villiers *et al.*, 1999).

In South Africa, studies on *P. peringueyi* have mainly focussed on the shrimp's population dynamics (Emerson, 1983; 1985; 1986; 1987; Robertson, 1984; Bernard and Froneman, 2005; Froneman, 2006), the aspects of the shrimp's feeding ecology (Froneman, 2001, 2002; Richoux and Froneman, 2007; 2008; Froneman and Henninger, 2010) and on thermal and saline effects on the shrimp's biology (Robertson, 1984; Achituv and Cook, 1984; Emmerson, 1985; Allan *et al.*, 2006). Although studies of *P. peringueyi* from temperate South African waters have been conducted and considering the fact that one parameter (i.e. temperature) exacerbates the

effects of the other (i.e. salinity), there is little information that considers the effects that global climate change may have on the survival of the shrimp species.

In South Africa, the warm-temperate coast is a region of increasing thermal variability, characterized by a large annual thermal range when compared with the cool-temperate and subtropical coasts. The South African coastal environment is mainly dominated by the western boundary Agulhas current on the south-eastern seaboard and the cooler eastern boundary Benguela current on the West coast. The characteristics of both currents have been shown to demonstrate effects of global warming (Whitfield., 1998; James., *et al*, 2007; Rouault., *et al*, 2009; Moloney., *et al*, 2013; Mead., *et al*, 2013). According to Rouault, *et al*. (2009) a 1.5°C increase in sea surface temperatures had been shown within the Agulhas current over the past 4 decades as a result of the current's intensification, which is brought on by a rise in trade and westerly winds in the southern Indian Ocean.

Therefore, with these and many other marine ecosystems around the world exhibiting anthropogenically caused variations in temperature patterns, changes in organism phenologies, characteristics, distribution, productivity and the interaction of species (e.g. Hoegh-Guldberg and Bruno, 2010; Somero, 2010; Doney *et al.*, 2012), a study on the interactive effects of salinity and increasing water temperatures on the survival of *P. peringueyi* is necessary in order to determine the overall impact global climate change may have on coastal and estuarine environments. Results from such studies will contribute to the already existing body of work on the effects of salinity and temperature (i.e. taking global climate change into account) and how they may alter the survival and/or distribution of *P. peringueyi* in warm temperate and subtropical regions.

Collection site

P. peringueyi were collected at a seagrass area in the lower reaches of the predominantly open freshwater deprived Kariega Estuary (33° 41' S; 26° 44'E), Kenton-on-Sea in the Eastern Cape during high tide (Figure 1.1). The estuary was selected because of its seagrass cover, easy accessibility, and its good ecological condition (Harrison *et al.*, 2000). Most of the seagrass beds (i.e. *Z. capensis*) from the Kariega Estuary were removed by a large episodic flood in 2012, which significantly altered the physico-chemical characteristics of the estuary (Nodo *et al.*, 2018). However, the seagrass beds have since recovered subsequently, in terms of cover, over the course of a few years (Wasserman, *et al.*, 2020). An assessment of the Kariega Estuary had shown the estuary to be in a good ecological state with regards to its ichthyofaunal communities, and the water quality of the system was considered to be in an adequate state (Harrison *et al.*, 2000). Due to the massive dams in the basin, the estuary consistently receives minimal freshwater supplies, yet it is nevertheless vulnerable to significant, sporadic river flooding (Wasserman, *et al.*, 2020). In comparison to other Eastern Cape estuaries, the Kariega Estuary has a superior abundance of macrobenthic organisms, more in particular, crustaceans (e.g. *Cleistostoma edwardsii*, *Cymodocella algoense*, *Sesarma catenata*, *Uca urvillei* and *Upogebia africana*) mainly because of the low freshwater input of the estuary (Hodgson, 1987).

The estuary's marine dominance has led to the wide distribution of macrophytes (i.e. *Z. capensis*) along its whole length and in a littoral belt surrounding the spring low tide level (Ter Morshuizen and Whitfield, 1994; Wasserman, *et al.*, 2020). *Z. capensis* can extend its range upstream during extended periods of low freshwater input due to the presence of uniform euhaline conditions and weakly formed longitudinal salinity gradients. (Wasserman, *et al.*, 2020). The system's eelgrass beds range in width from roughly 5.2m in the lower reaches to 3.3m in the upper reaches (Ter Morshuizen and Whitfield, 1994). Comparatively, shrimps

could have been collected from the Swartkops Estuary, which has a more extensive seagrass cover, however, the Swartkops Estuary is known for its poor contamination state (Binning and Baird, 2001).



Figure 1.1: Map illustrating the location of the Kariega estuary at Kenton-on-Sea in the Eastern Cape Province, South Africa. The yellow circle represents the area of shrimp collection whilst the yellow dot represents the approximate location of Kariega Estuary in the Eastern Cape Province (Image credit: Google Earth®)

Aim

The study was aimed at investigating the impacts of salinity and temperature on the survival of the caridean shrimp *P. peringueyi* from the predominantly open Kariega Estuary in the Eastern Cape, South Africa.

Specific objectives:

- Determine shrimp density to be used in experiments.
- Determine the effects of salinity on the survival of *P. peringueyi*.

- Determine the effects of temperature on the survival of *P. peringueyi*.
- Determine the combined effects of salinity and temperature on the survival of *P. peringueyi*.

Chapter outline

Chapter 2: Salinity tolerance of the caridean shrimp *P. peringueyi* (Caridea: Palaemonidae)

- A density experiment was conducted to determine appropriate shrimp densities to be used in salinity and temperature experiments. Based on the results from the density experiment, adult shrimps (CL $\geq$ 9 mm) were individually exposed to triplicate containers of six salinity treatments (5, 15, 25, 35, 45 and 55). Experiments were conducted over 96 hours and survival was monitored at 24-hour intervals.

Chapter 3: Temperature tolerance of the caridean shrimp *P. peringueyi* (Caridea: Palaemonidae)

- The effect temperature on the survival of the shrimp were investigated where adult shrimps (CL $\geq$ 9 mm) were individually exposed to triplicate containers of seven temperatures treatments (5, 10, 15, 20, 25, 30 and 35 °C). Experiments were conducted over 96 hours and survival was monitored at 24-hour intervals.

Chapter 4: Combined effect of salinity and temperature on the survival of the estuarine caridean shrimp *P. peringueyi* (Caridea: Palaemonidae).

- The combined effects of salinity and temperature on the survival of the shrimp were investigated over a range of salinities (15, 20 and 25) and temperatures (10, 15, 20, 25 and 30 °C) using the method described earlier.

Chapter 5: Synthesis

NB: Each chapter is followed by its own cited references.



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Chapter 2

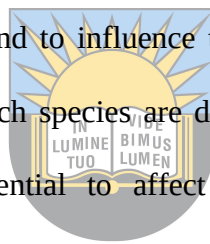
Salinity tolerance of the caridean shrimp *P. peringueyi* (Caridea: Palaemonidae)



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Introduction

One of the most prevalent factors in an aquatic ecosystem that directly affects the growth, distribution, metabolism, and oxygen consumption of estuarine organisms is salinity. Salinity is regarded as a major influence on marine organisms' behaviour, geographic distribution, larval recruitment and dispersal (Anger, 1991; 1996; Spivak and Cuesta, 2009; Smyth *et al.*, 2014). Salinity plays a crucial role in species distribution within estuaries (Wooldridge, 1999; Rowe, 2002). It has a vital function in the structuring of macrobenthic communities as the salinity gradient in estuarine systems functions as an environmental stressor for euryhaline marine species and as a physiological barrier for stenohaline marine and freshwater species (Teske and Wooldridge, 2002). Salinity places an osmoregulatory demand on aquatic organisms and changes in salinity tend to influence the manner in which communities are structured and the boundaries in which species are distributed. Therefore, any alteration to environmental salinity has the potential to affect the animals' capability to perform fundamental biological processes and thus their survival and thriving ability.



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Aquatic organisms can be defined as either stenohaline or euryhaline depending on their ability to tolerate salinity. Stenohaline organisms have a tolerance for only a small range of environmental salinity whilst euryhaline organisms tolerate environmental salinities and salinity fluctuations within a specified range (Smyth and Elliot, 2016). The survival of estuarine populations within estuaries is partially reliant on their ability to tolerate salinity variations that are characteristic of their surrounding environment (Masikane *et al.*, 2014). For example, the euryhaline sand shrimp *P. peringueyi* can tolerate wide variations in salinities (i.e. ranging between 10 and 50) and is commonly more abundant in the middle and lower reaches of predominantly open estuaries which are characterised by high salinities (de Villiers *et al.*, 1999; Nyalungu *et al.*, 2014). The distribution of estuarine organisms such as *P. peringueyi* in

estuaries is largely attributed to by their ability to respond to the constantly changing physico-chemical parameters of their surrounding medium (i.e. dissolved oxygen, salinity, temperature and pH) (Gonzalez-Oreja and Saiz-Salinas, 1998; de Villiers *et al.*, 1999; Teske and Wooldridge, 2003).

Studies on the effects of water temperature and salinity on larval shrimp (i.e. penaeid shrimps) have demonstrated that salinity has a greater effect on survival than temperature (Kumlu, 2000, 2001; Zacharia and Kakati, 2004; Ch and Shailender, 2013). During most of their early life cycles, penaeid shrimps are subjected to harsh climatic conditions and rapid variations in estuarine environments (Raj and Raj, 1982). Therefore, it is anticipated that postlarvae and juveniles of most species will be highly adaptive and osmoregulate well at varying salinities, in contrast to adults (Kumlu, 2001).



The ability of an estuarine organism to tolerate salinity variations contributes highly to its potential of recruitment or rather range expansion within an estuary (Kinne, 1963). *P. peringueyi*, for example, has a tolerance for a wide range of salinities (Wooldridge, 1999) and has been recorded from the middle to lower reaches where salinities generally range between 20 and 35 (Nyalungu *et al.*, 2014). Crustaceans, however, can inhabit all parts of an estuary, from freshwater heads to saline mouths, and they are also said to possess the ability to inhabit restricted areas of estuaries within which salinity generally varies within the range tolerated in the laboratory (Teske and Wooldridge 2003; Masikane *et al.*, 2014). Some crustaceans (e.g. *Palaemon longirostris*) can change their integument permeability in response to low salinity (Rainbow, 1997).

Materials and Methods

Collection of shrimps

P. peringueyi were collected at a seagrass area in the lower reaches of the Kariega Estuary (33° 41'S; 26° 44'E) during high tide. The shrimps were collected at a salinity of 35 (i.e. salinity was measured on-site using a handheld refractometer) using a small seine net and a dip net (2mm mesh). The number of collected shrimps did not differ significantly between the two methods. Shrimps were then transported to the laboratory in sealed, aerated tanks filled with water collected from the sampling site (transportation time: ≤ 2 hours). In the laboratory, shrimps were acclimated for at least 24 hours at a salinity of 35 before the commencement of the experiments.

Experimental procedure

Density

This study was designed to determine the number of shrimps that should be exposed per replicate container without negatively affecting survival. The behaviour of individuals of *P. peringueyi* when confined in small spaces (e.g. 1L container) is not known, therefore, it was necessary to perform this pilot study in order to determine the effects of density on survival. Three shrimp densities were considered in this study, and these included 5, 10 and 15 individuals per 1L glass container. Each density treatment was replicated three times. The shrimp densities were then exposed to a salinity of 35 for a 96-hour period (following Anderson *et al.*, 2004). Test containers were sealed with perforated parafilm to limit evaporation that may affect the test salinity whilst simultaneously providing an aeration source for the test media. The test media were not aerated but the dissolved oxygen levels in test media were regularly monitored to ensure that they remained at acceptable levels, and the test media were renewed after 48 hours to ensure that depleting oxygen levels had no confounding effect. A photoperiod of 12 hours light: 12 hours dark was maintained in all experiments and survival was monitored at 24-hour intervals. This process followed a study by Masikane *et al.*, (2014). Dead shrimps

were defined as those that did not respond even to mechanical stimuli. Dead shrimps were removed from test containers to prevent water fouling and test media were renewed after 48 hours to ensure the reduction of potential confounding factors (e.g. pH, oxygen and ammonia) that may negatively alter the test results.

Salinity tolerance

The highest survival in shrimp density (survival $\geq 80\%$) (following Anderson *et al.*, 2004) that was obtained from the density experiment was exposed to six salinities (5, 15, 25, 35, 45 and 55) to determine the salinities at which tests should be evaluated. The acclimation salinity of 35 was used as the control. The effect of salinity (range: 5 - 45) has been studied on the standard metabolic rate of this shrimp (see Allan *et al.*, 2006). However, with *P. peringueyi* having a wide tolerance to salinity (range: 10 – 50) it was decided to include salinity extremes (i.e. 5 and 55) in order to determine the shrimp's response when exposed to salinities beyond its range of tolerance. The desired salinities were obtained by either diluting natural seawater (salinity 35) with distilled water for salinity decreases or by dissolving commercial sea salt (Red Sea Salt) in natural seawater for salinity increases. Natural seawater was collected at the mouth of the Kariega Estuary. Before commencing with the experiment, shrimps were measured with a Vernier caliper 24 hours prior experiments to ensure that the shrimps had sufficient time to recover from handling stress. Adult shrimps with carapace length >9 mm (CL being the region from the anterior tip of the rostrum to the dorsal midline of the posterior margin of the carapace) were then selected and acclimated to the experimental salinity by either increasing or decreasing salinity by ≤ 5 units every 2 hours. Once the desired salinity had been reached, healthy shrimps (i.e. actively swimming and undamaged) of nearly the same size class were selected and used for the experiment. Shrimps were fed on crushed fish flakes (following Allan, *et. al.*, 2006) a day prior to the experiment to ensure that starvation was not a confounding

factor of salinity. The experiment was conducted over a 96-hour period and survival was monitored at 24-hour intervals. Test containers were sealed with perforated parafilm to avoid evaporation that may influence test salinities and provide aeration to the test media (following Masikane *et al.*, 2014). The test salinities and temperature were monitored three times a day (in the morning, noon and in the afternoon). Where salinities had increased or decreased, these were adjusted accordingly by adding appropriate amounts of distilled water or natural seawater. A photoperiod of 12 hours light: 12 hours dark was maintained in all experiments and the test media were renewed after 48 hours to reduce water fouling and ensure the minimization of confounding variables. The salinity media were not aerated (i.e. following Masikane *et al.*, (2014) on the salinity tolerance study of the amphipod *Grandidierella lignorum*), however, the dissolved oxygen levels in the test media were regularly monitored, and the test media were renewed after 48 hours to ensure that depleting oxygen levels had no confounding effect.

Statistical analysis

The density and salinity tolerance experiments were analysed graphically. A survival of $\geq 80\%$ was used as a reference in order to accept density and salinity results. The study followed protocol for short term test conditions (i.e., 24-, 48- or 96-hours duration) from Anderson *et al.*, 2004 which stipulates that the test acceptability criteria control survival in toxicity testing is $\geq 80\%$. This is part of the quality assurance and quality control procedures. All analyses were performed in RStudio employing a one-way ANOVA to determine significant differences in survival between experimental salinities. This was followed by a postHoc (TukeyHSD) test to indicate where significant differences occur between groups.

Results

The mean survival of shrimps decreased with increasing density and increasing time (Figure 2.1). Highest survival was recorded at density of 5 individuals per litre, where average survival was 80% after 96 hours of exposure. On the other hand, no shrimps survived beyond 24 hours of exposure in the density treatment of 15 shrimps per litre (Figure 2.1). The mass mortality at 15 shrimps per litre may have been a result of a confounding factor (i.e. oxygen depletion) since the test media were not aerated.

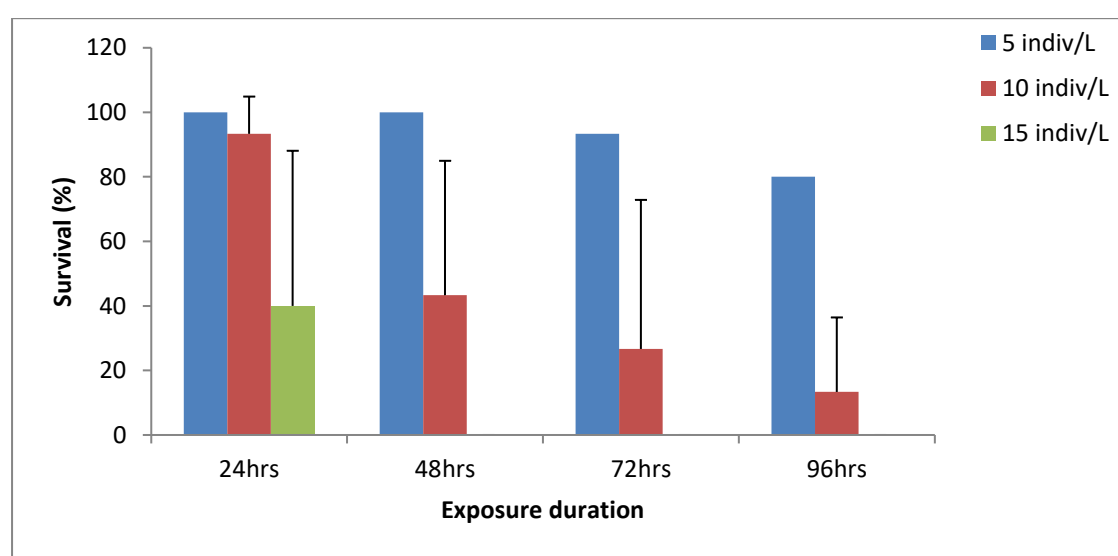


Figure 2.1: *P. peringueyi* mean survival at different densities over a 96-hour period. Error bars represent one standard deviation (+1SD) above the mean survival, providing insight into the greater variability in survival within the dataset. Standard deviation values are expressed in points on the test scale. Error bars were calculated using the standard deviation of the dataset.

For toxicity testing, standard protocol dictates that five individuals must be used per container when using shrimp species (e.g. Anderson *et al.*, 2004). Therefore, since the current study was conducted using *P. peringueyi* as the test organism, the same protocol was adopted. However, based on the density data, it was decided that the salinity tolerance test will be conducted using a density of four shrimps per 1L container. The main aim behind reduced density (from five

to four individuals per litre) was to reduce variability on survival. The experiment, with a reduced density, was then conducted in triplicate trials over a 96-hour period and average survival was analysed graphically. Mean survival decreased with increasing time and varied greatly between salinities at 72-hour and 96-hour exposure. Survival dropped below 80% after 72hrs of exposure in all salinities and further dropped to <25% after 96-hour exposure in all salinities (Figure 2.2).

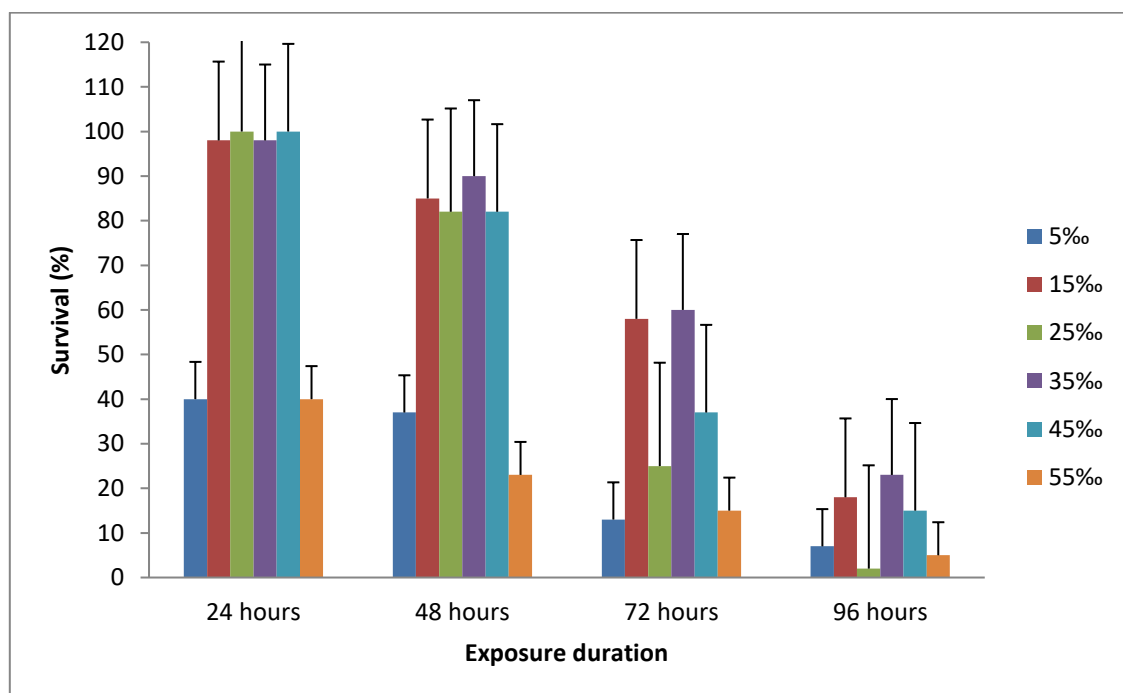


Figure 2.2: Tolerance of the estuarine caridean shrimp *P. peringueyi* to various salinity treatments. Shrimps in this experiment were exposed in groups of four. Error bars represent one standard deviation (+1SD) above the mean survival, providing insight into the greater variability in survival within the dataset. Standard deviation values are expressed in points on the test scale. Error bars were calculated using the standard deviation of the dataset.

Test salinities during the salinity tolerance test deviated by as little as 0.49 units (Salinity treatment of 45) and as much as 1.50 units (salinity treatment of 35) while temperatures deviated by as little as 0.49°C and as much as 1.14°C (Table 1).

Table 2.1. Salinity and temperature conditions that were recorded for the salinity tolerance test. In this experiment, shrimps were exposed in a group of 4 in each test container.

Salinity Treatment	Salinity			Temperature (°C)		
	N	Mean	SD	N	Mean	SD
5	12	4,92	0,90	12	10,33	0,49
15	12	16,33	1,97	11	10,91	1,14
25	12	25,83	0,58	11	10,18	0,60
35	12	29,67	3,50	11	9,91	0,94
45	12	45,67	0,49	12	10,42	0,67

Due to the high mortality rate and low mean survival rate that may have been caused by potential confounding factors (i.e. ammonia build-up, oxygen depletion, etc.) as a result of high shrimp densities in test containers, the experiment had to be modified. Observations conducted during the experiment indicated that the shrimps may have also been exhibiting cannibalistic behaviour due to the small space confinement as limbs were often observed in the test containers. Therefore, due to these factors the salinity tolerance test was repeated with a slight modification. In this modified experiment, shrimps (i.e. 3 replicates) were individually exposed to test salinities. In all salinities, shrimps were exposed to media of approximately 150ml to reduce movement thus ensuring that exhaustion had no confounding effect. Survival was $\geq 80\%$ in all salinities except 5 and 55 where survival dropped to 60% and 33% respectively (Figure 2.3).

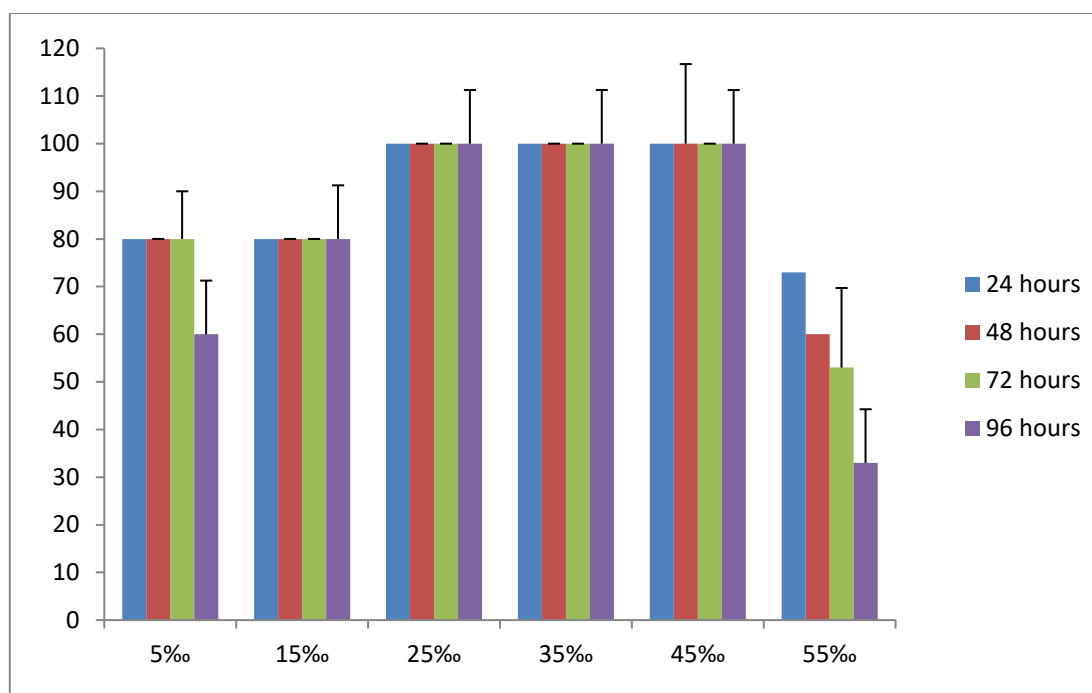


Figure 2.3: Tolerance of the estuarine caridean shrimp *P. peringueyi* to various salinity treatments when shrimps are exposed individually in test containers. Error bars represent one standard deviation (+1SD) above the mean survival, providing insight into the greater variability in survival within the dataset. Standard deviation values are expressed in points on the test scale. Error bars were calculated using the standard deviation of the dataset.

In this salinity tolerance test, salinities did not vary, whilst temperatures varied by as little as 0.87°C and as much as 1.16°C (Table 2.2).

Table 2.2. Salinity and temperature conditions that were recorded for the salinity tolerance test. In this experiment, shrimps were exposed individually in test containers.

Salinity Treatment	Salinity			Temperature (°C)		
	N	Mean	SD	N	Mean	SD
5	12	5,00	0,00	12	11,58	1,16
15	12	15,00	0,00	12	11,75	0,87
25	12	25,00	0,00	12	11,75	0,97
35	12	35,00	0,00	12	11,83	0,83
45	12	45,00	0,00	12	11,75	0,97

A one-way ANOVA indicated a significant difference ($p = 0.000541$) in mean survival between experimental salinities when the shrimps were individually exposed (Table 2.3).

Table 2.3: One-way ANOVA (analysis of variance) indicating a significant difference in survival between experimental salinities when shrimps were exposed individually.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11311.111	5	2262.222	10.180	0.000***
Within Groups	2666.667	12	222.222		

A postHoc (TukeyHSD) test was run to check where significant differences between groups occur. The test showed that significant differences only occurred between 55 vs all salinities (i.e. 55 vs. 15, 55 vs. 25, 55 vs. 35 and 55 vs. 45). No significant differences occurred between the other groups (Table 2.4).

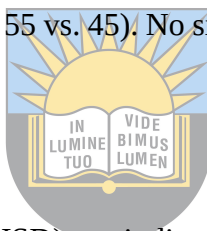


Table 2.4: Results of postHoc (TukeyHSD) test indicating where significant differences occur between groups after 96 hours of exposure. Significant differences occurred between 55-15, 55-25, 55-35 and 55-45 (i.e. Significance codes: 0 '***'; 0.001 '**'; 0.01 '*'; 0.05 '.'; 0.1 ' '); 1)

Salinity	diff	Lwr	upr	p adj
25-15	2.00e+01	-20.88	60.88	0.59
35-15	2.00e+01	-20.88	60.88	0.59
45-15	2.00e+01	-20.88	60.88	0.59
5-15	-2.00e+01	-60.88	20.88	0.59
55-15	-4.67e+01	-87.55	-5.78	0.02*
35-25	-1.42e-14	-40.88	40.88	1.00
45-25	0.00e+00	-40.88	40.88	1.00

5-25	-4.00e+01	-80.88	0.88	0.06
55-25	-6.67e+01	-107.55	-25.78	0.00**
45-35	1.42e-14	-40.88	40.88	1.00
5-35	-4.00e+01	-80.88	0.88	0.06
55-35	-6.67e+01	-107.55	-25.78	0.001**
5-45	-4.00e+01	-80.88	0.88	0.06
55-45	-6.67e+01	-107.55	-25.78	0.001**
55-5	-2.67e+01	-67.55	14.22	0.31



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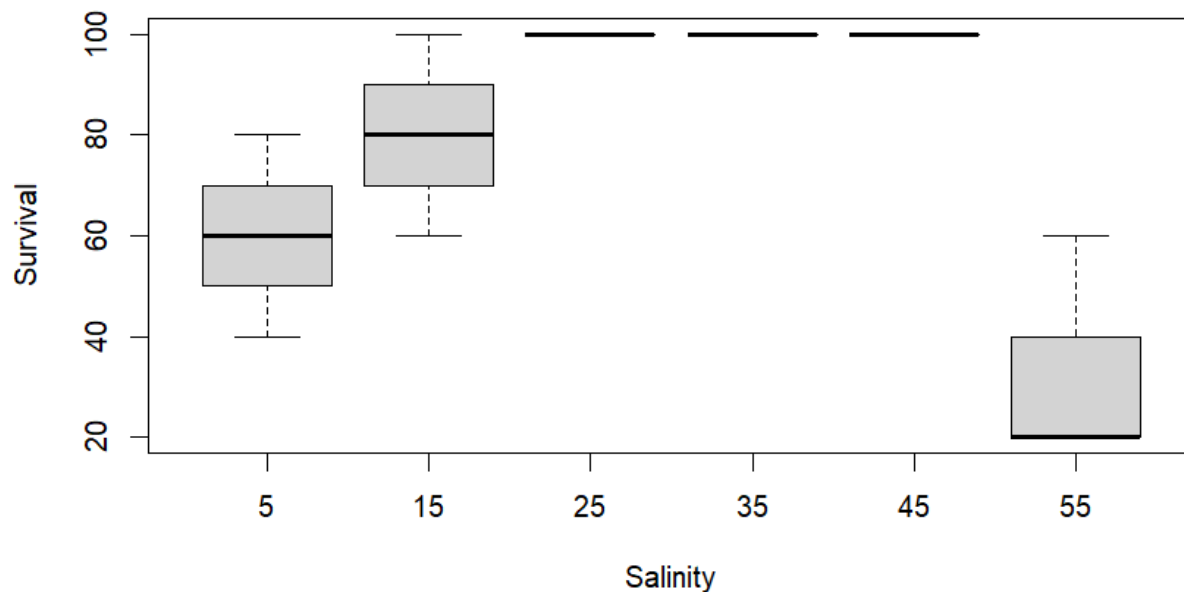


Figure 2.4: Salinity tolerance of the estuarine caridean shrimp *P. peringueyi*. The box and whiskers plot indicates the differences in mean survival at the various salinity treatments after 96 hours of exposure. The box (i.e. interquartile range) represents the middle 50% of the data and the lines inside the boxes represent the median. The "whiskers" represent the min and max values within a 1.5 range times the interquartile range.

Discussion

Results from the current study indicate that group exposure has a negative impact on the survival of the shrimps, *P. peringueyi*. This may be due to space competition, cannibalistic behaviour, oxygen depletion, pH or ammonia build-up and it requires further investigation. The cannibalistic nature of this shrimp is also not well understood. Survival of *P. peringueyi* at the lowest density (i.e. 5 individuals per litre) varied between 60% and 100%. If tests using *P. peringueyi* will conform to batch exposure, then density will confound test results. This study, therefore, recommends that salinity tests for *P. peringueyi* employ the individual exposure

method whilst employing all quality control protocols (i.e. media aeration, measurement of oxygen saturation and ammonia build-up levels, etc) to eliminate any confounding effect on the test results (following Anderson *et al.*, 2004).

Mean survival of *P. peringueyi* over 96 hours varied between salinities. Highest survival was recorded at salinities 25, 35 and 45 where mean survival was 100% but was not significantly different to mean survival at 15 which was at 80%, on average. These salinities correspond to salinities that have been measured in the middle and lower reaches of the Kariega Estuary where high shrimp abundances occur (Nyalungu *et al.*, 2014). Allan *et al.* (2006) reported a lack of a significant difference in respiration rate at these four salinities (i.e. 15, 25, 35 and 45) and suggested that this was due to the fact that *P. peringueyi* is well adapted to inhabiting environments characterised by variations in salinity and temperature. Adaptation to wide salinity variations is not uncommon in crustaceans, however, and studies on different shrimps (e.g. *Penaues monodon*, *Palaemon serratus*, *Litopenaues setiferus* and *Farfantepenaeus paulensis*) have shown salinity to have little or no effect on oxygen consumption (Rosas *et al.*, 1999; Lemos *et al.*, 2001; Salvato *et al.*, 2001).

Survival at 5 dropped to 80% after 24 hours then dropped to a further 60% after 96 hours of exposure. Lowest survival was recorded at 55 where survival was reduced to 73% within the first 24 hours and dropped to a further 60% after 48 hours, 53% after 72 hours and ultimately 33% after 96 hours.

P. peringueyi exhibited a wide salinity tolerance where highest peak in survival (i.e. under laboratory conditions) was recorded at salinities generally recorded in the middle and lower reaches of predominantly open estuaries (e.g. Kariega Estuary). A significant decrease in survival was only observed at the salinity extremes when the shrimps were exposed to hypo and hypersaline conditions. *P. peringueyi* exhibited a wide tolerance to salinity variations,

however, salinities beyond the shrimps' tolerance range had a negative impact on survival. These results are comparable with previous studies on the effects of salinity tolerance on the shrimp. Kirkpatrick and Jones (1985) found that *P. peringueyi* suffer mortalities at salinities <5 and Allan *et al.* (2006) reported that salinity increases resulted in an increase in the shrimp's respiration rate. The mortalities experienced by *P. peringueyi* at the salinity of 5 (under laboratory conditions) coincide with findings from Kirkpatrick and Jones (1985). The latter authors' comparison of *Palaemon affinis* to other *Palaemon* species showed that *Palaemon* species of marine and brackish water inhabitancy lacked tolerance for low salinities. Mortalities at low salinity levels, therefore, indicate that the shrimp's upper distribution in predominantly open estuaries is reduced due to physiological tolerances (Emmerson, 1986).

Modifications to environmental salinity have a potential impact on the animals' ability to perform important biological processes and thus their survival and thriving ability (Smyth and Elliott, 2016). Even though *P. peringueyi* may be euryhaline, salinities beyond the shrimp's tolerance range may have negative impacts on its physiology, therefore, limiting the shrimp's distribution within the estuarine environment.

Estuaries serve as important nursery sites for numerous aquatic inhabitants including fishes and shrimps (e.g. *P. peringueyi*) (McHugh, 1967; Brito and Pena, 2007). The occurrence of caridean shrimps in high densities suggests that they serve a vital function in the structural determination and dynamics of seagrass communities (Kikuchi, 1974; Hooks *et al.*, 1976). Emmerson (1983) showed that the percentage cover and presence of submerged macrophytes were positively correlated with shrimp abundance in estuaries. Additionally, a period of decreased *P. peringueyi* numbers followed the mass reduction of *Zostera* beds in the Kromme Estuary due to siltation during flooding.

The life cycle of *P. peringueyi* is inclusive of a marine and an estuarine phase (Emmerson 1983) and is therefore, highly dependent on salinity. The study used adults (>9mm CL), not juveniles, while the critical salinity tolerance range for inhabiting estuaries is in juveniles, thus further research is required to test for the salinity tolerance of juveniles. The presence and/or quality of a salinity gradient connected to the ocean attracts larvae to nursery regions, suggesting that postlarvae possess a sensitivity to salinity fluctuations (Williams and Debler, 1968; Hughes, 1969 and Young and Carpenter, 1977). This, therefore, implies that changes in ambient salinity would negatively affect shrimp recruitment into estuaries which would result in a reduction of *P. peringueyi* in estuaries thus changing the structure and dynamics of these estuarine communities.



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Chapter 3

Temperature tolerance of the caridean shrimp *P. peringueyi* (Caridea: Palaemonidae)



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Introduction

Temperature is regarded as an important environmental factor for shrimps and other invertebrates (Rahman, *et al.*, 2007). It is regarded as one of the most significant aspects influencing aquatic organisms since it affects the kinetic energy of molecules and biochemical reactions (Somero, 1969; Fry, 1971; Mora and Ospina, 2001). Temperature directly affects other environmental variables such as increasing salinity and decreasing dissolved oxygen (Vargas-Albores *et al.*, 1997). Temperature also influences the rate of all biological processes (Allan *et al.*, 2006) more especially in organisms with a life cycle that involves an estuarine phase (Newell 1979). Temperature and salinity are two of the most vital natural variables that have a direct effect on marine organisms, specifically in the estuarine and coastal ecosystems (Shi *et al.*, 2011) where thermal and saline conditions vary diurnally and seasonally.

Individual parameters (i.e. rate of growth, longevity, rate of excretion, food intake and basic metabolism and population parameters (i.e. mortality, reproduction rate, recruitment and population size/distribution) are all temperature dependent (Shaw and Bercaw, 1962; Brey, 1995; Southward *et al.*, 1995; Kröncke *et al.*, 1998; Perry *et al.*, 2005; Pörtner *et al.*, 2008). Aquatic ectotherms, for example, are greatly affected by temperature variations because they are unable to physiologically regulate their body temperature but rather their body temperature adapts to that of their surrounding environment (Madeira *et al.*, 2012) and are, therefore, required to inhabit zones within their thermal preference (Portner and Peck, 2010).

It is predicted that, as temperature rises (i.e. due to global climate change), species distribution and abundance will be altered based on their temperature tolerance and adaptability (Harley *et al.*, 2006). If, however, rising aquatic temperatures do not physiologically favour species, alterations in ranges of distribution (i.e. individually or by populations) (Perry *et al.*, 2005;

Cheung *et al.*, 2009; Sunday *et al.*, 2011, 2012), shifts in phenology (i.e. timing of annual events) (Edwards and Richardson, 2004), mortalities, local extinctions and the eventual loss of species may occur (Roessig *et al.*, 2004; Parmesan, 2006; Hoegh-Guldberg and Bruno, 2010; Portner and Peck, 2010). In contrast, aquatic ectotherms that occupy temperate regions, characterized by seasonal variability (Cheung *et al.*, 2009; Sunday *et al.*, 2011, 2012), generally have broader temperature tolerance ranges and have a lower sensitivity to changes in temperature.

The sand shrimp *P. peringueyi* can grow and remain active at temperatures between 10°C to 30°C (Wooldridge, 1999), but postlarvae and sub-adults are intolerant of temperatures greater than 32°C (Robertson, 1984). The shrimp may tolerate low temperatures but only for limited periods (de Villiers *et al.*, 1999) and thermal conditions below 4.3°C tend to be lethal (Robertson, 1984).



Literature has emphasized how crucial it is to investigate a species' physiological limits at levels comparable to those found within their natural habitat, otherwise, there may be a discrepancy concerning a species' realized niche (or range) and the range predicted by physiological tolerance. (Helmuth *et al.*, 2002; Sánchez-Fernández *et al.*, 2012).

The rising of aquatic temperatures and increased thermal variability has been increasingly evident in South Africa (Rouault *et al.*, 2010; Lima and Wetthey, 2012; Schlegel *et al.*, 2017). Therefore, since temperature is one of the most imperative aspects directly influencing the physiology of aquatic organisms, obtaining knowledge on thermal limits and requirements of aquatic organisms such as *P. peringueyi* is very vital. This knowledge helps identify key components of biodiversity that are susceptible to changes in temperature in order to properly prioritize conservation efforts to minimize biodiversity loss (Mutia, 2011).

Materials and Methods

Shrimps were individually exposed to seven different temperatures ranging between 5 and 35°C under controlled conditions (i.e. controlled salinity) in a controlled temperature room. The experimental procedure was similar to that of salinity tolerance for individual exposure described in chapter 2. Adult shrimps (CL > 9 mm) were acclimated to the experimental temperatures overnight (following Allan *et al.*, 2006). The desired temperatures were obtained by either increasing or decreasing temperature by ≤ 2 degrees every 2 hours, at a constant salinity of 35. Allan *et al.*, (2006) adjusted temperature by 0.5°C/h, however, they were working on the shrimp's respiration rate and could, therefore, not allow the shrimp to overcome the effects of salinity before measuring its respiration. This study, therefore, opted for 2°C every 2 hours to ensure that the shrimp had ample time to acclimatize to the test conditions. Once the desired temperature had been reached, healthy shrimps of nearly the same size class were selected and used for the experiment. The shrimps were individually exposed to the experimental temperatures in 150ml glass containers to reduce movement, thus ensuring that exhaustion had no confounding effect. The test media were not aerated but the dissolved oxygen levels in test media were regularly monitored to ensure that dissolved oxygen remained at accepted levels and the test media were renewed after 48 hours to ensure that depleting oxygen levels had no confounding effect. The experiment was conducted over a 96-hour period and survival was monitored at 24-hour intervals. A photoperiod of 12 hours light: 12 hours dark was maintained in all experiments.

Statistical analysis

All analyses were performed in RStudio employing a one-way ANOVA to determine significant differences in survival between experimental temperatures. This was followed by a postHoc (TukeyHSD) test to indicate where significant differences occur between groups.

Results

Highest mean survival after 96 hours was observed at 20°C where a 100% survival was recorded. This was followed by 15°C and 25°C where survival after 96 hours was recorded at 87% and 73% respectively. Survival decreased drastically, however, when temperature was shifted towards the shrimp's thermal tolerance limits (i.e. 10 and 30°C) and no shrimps survived beyond 24 hours in temperatures 5°C and 35°C (Figure 3.1).

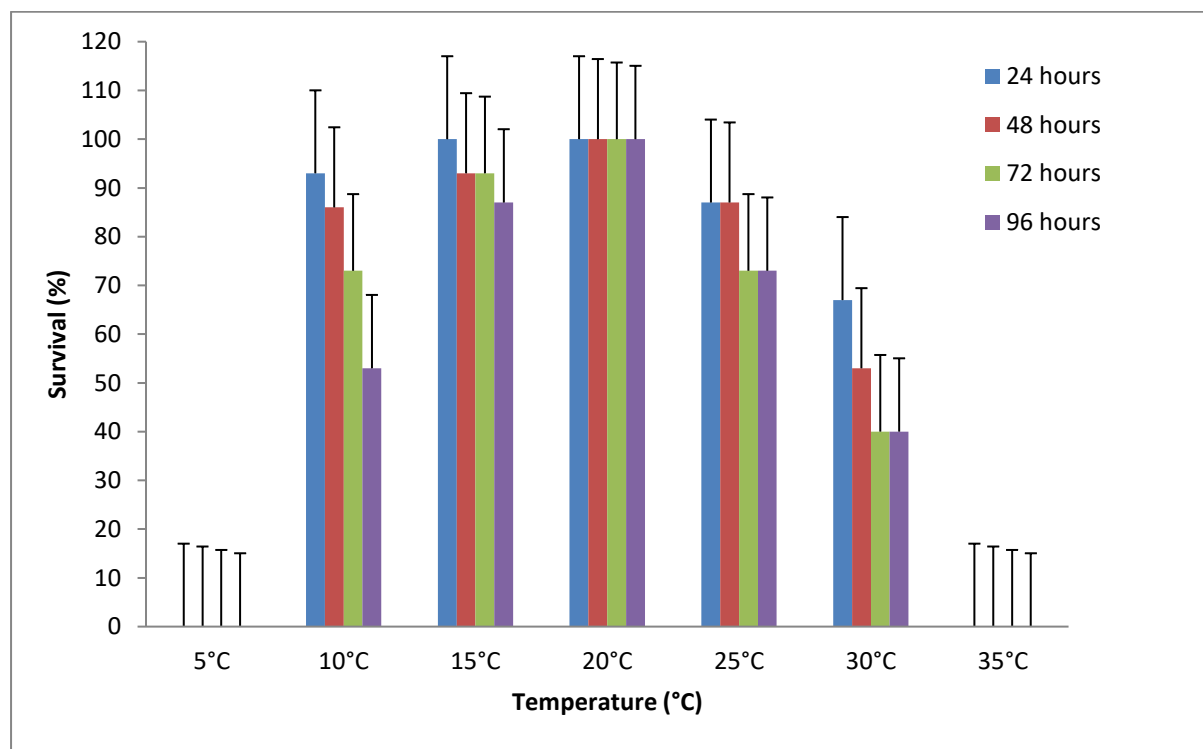


Figure 3.1: Mean survival in tolerance of the estuarine caridean shrimp *P. peringueyi* to various temperature treatments at individual exposure. Error bars represent one standard deviation (+1SD) above the mean survival, providing insight into the greater variability in survival within the dataset. Standard deviation values are expressed in points on the test scale. Error bars were calculated using the standard deviation of the dataset.

An analysis of variance (one-way ANOVA) indicated a significant difference ($p = 0.000$) in survival after 96 hours between temperatures (Table 3.1).

Table 3.1: One-way ANOVA (analysis of variance) indicating a significant difference in survival between experimental temperatures.

	Sum of Squares	df	Mean Square	F	Pr (>F)
Temperature	28495.238	6	4749.206	41.556	.000
Residuals	1600.000	14	114.286		

A postHoc (TukeyHSD) test was run to check where significant differences between groups occur. The test revealed that significant differences occurred between 5-10, 5-15, 5-20, 5-25, 5-30, 15-10, 20-10, 35-10, 30-15, 35-15, 30-20, 35-20, 30-25, 35-25 and 30-35 (Table 3.2).

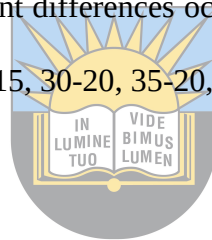


Table 3.2: The postHoc (TukeyHSD) test results indicating where significant differences occur between groups after 96 hours. Significant differences occurred between 5-10, 5-15, 5-20, 5-25, 5-30, 15-10, 20-10, 35-10, 30-15, 35-15, 30-20, 35-20, 30-25, 35-25 and 30-35 (i.e. Significance codes: 0 '***'; 0.001 '**'; 0.01 '*'; 0.05 '.'; 0.1 ' '; 1).

Temperatures	Diff	lwr	upr	p adj
10-5	5.333e+01	23.528	83.138	0.000***
15-5	8.667e+01	8.667e+01	116.472	0.000***
20-5	1.000e+02	70.195	129.805	0.000***
25-5	7.333e+01	43.528	103.138	0.000***
30-5	4.000e+01	10.195	69.805	0.006**
15-10	3.333e+01	3.528	63.138	0.024*
20-10	4.667e+01	16.862	76.472	0.002**

35-10	-5.333e+01	-83.138	-23.528	0.000***
30-15	-4.667e+01	-76.472	-16.862	0.002**
35-15	-8.667e+01	-116.472	-56.862	0.000***
30-20	-6.000e+01	-89.805	-30.195	0.000***
35-20	-1.000e+02	-129.805	-70.195	0.000***
30-25	-3.333e+01	-103.138	-43.528	0.000***
35-25	-7.333e+01	-103.138	-43.528	0.000***
35-30	-4.000e+01	-69.805	-10.195	0.006

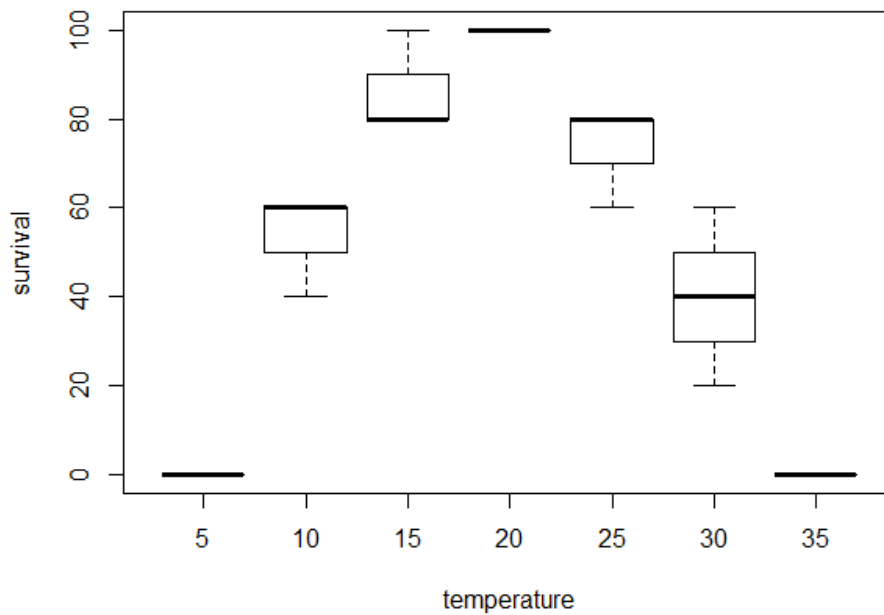
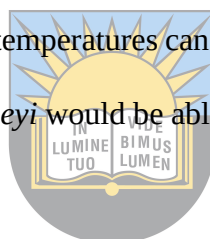


Figure 3.2: Temperature tolerance of the estuarine caridean shrimp *P. peringueyi*. The plot indicates differences in mean survival at the various temperature treatments after 96 hours of exposure. The box (i.e. interquartile range) represents the middle 50% of the data and the lines inside the boxes represent the median. The "whiskers" represent the min and max values within a 1.5 range times the interquartile range.

Discussion

P. peringueyi was shown to have a tolerance for temperatures ranging between 10 and 30. Highest survival after 96 hours was recorded at 15, 20 and 25°C where survival was $\geq 73\%$ and no significant difference was observed between the three temperatures. However, survival below or beyond these temperatures decreased drastically and was recorded below 60% after 96 hours. A 100% mortality was recorded at the temperature extremes (i.e. 5°C and 35°C) where no survival was recorded beyond 24 hours of exposure. This, therefore, suggests that *P. peringueyi*, prefers temperatures between 15°C to 25°C which are temperatures generally recorded in the Kariega Estuary (Heyns and Froneman, 2010). However, the low survival at 30°C was unusual as the species is widely distributed along the Southern African coastline and can be found in estuaries where water temperatures can rise above 30°C in the shallows. It was, therefore, expected that the *P. peringueyi* would be able to tolerate at least 30°C with ease and this requires further investigation.



Results from the present study are comparable with reports from previous studies on *P. peringueyi* (e.g. Achituv and Cook, 1984; Robertson, 1984; de Villiers *et al.*, 1999). Achituv and Cook (1984) reported the most conducive temperature for the growth of *P. peringueyi* to be 25°C and proposed that *P. peringueyi* may favour warmer waters over the cold waters. De Villiers *et al.* (1999) found that *P. peringueyi* tolerates wide temperature variations (range: 10 to 30 °C) and that lower thermal conditions can be tolerated but only for restricted periods while temperatures greater than 30 °C result in mortalities (Robertson, 1984; de Villiers *et al.*, 1999; Allan *et al.*, 2006). Robertson (1984) reported that postlarvae and sub-adults of *P. peringueyi* do not tolerate temperatures $> 32^{\circ}\text{C}$ and temperatures below 4.3°C were lethal to the shrimp.

The present study found that increases in water temperature beyond the shrimp's upper tolerance point have a negative impact on the livelihood of the shrimp, *P. peringueyi*. This is in correspondence with previous studies on temperature effects on the physiology of the shrimp (e.g. Allan *et al.*, (2006) who reported increases in *P. peringueyi* respiration rates when subjected to temperature increases towards the shrimp's upper tolerance). These results on temperature effects are not uncommon within the crustacean community. Studies on crustaceans (e.g. *Penaeus chinensis*, *Penaeus japonicas*, *Penaeus californiensis*, *Litopenaeus vannemai*, *Litopenaeus stylirostris*, *Macrobrachium nipponense*, *Macrobrachium americanum*, *Palaemon peringueyi*, *Palaemon longirostris*, and *Palaemon varians*) have all shown a similar trend where increasing temperatures have an effect on the shrimp's physiology. Studies on the effects of temperature have also shown temperature increases to have resulted in increased metabolic rates (Chen and Lai, 1993; Chen and Nan, 1993; Villarreal and Ocampo, 1993; Dong *et al.*, 1994; Zhang *et al.*, 1998; Jiang *et al.*, 2000; Allan *et al.*, 2006; Spanopoulos-Hernández *et al.*, 2005; García-Guerrero *et al.*, 2011; Valenzuela-Quinónez *et al.*, 2011; González-Ortegón *et al.*, 2013; Ponce-Palafox *et al.*, 2013).



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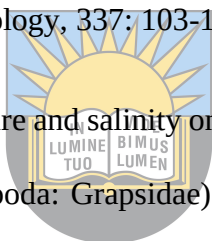
Temperature, being the prominent factor affecting biological processes is regarded as the most important environmental factor that often has a direct impact on all biological processes. Therefore, *P. peringueyi* thermal studies are a necessity in order to meet the need of understanding temperature effects on coastal and estuarine environments. IPCC, (2014) predicted an increase of 2.0°C - 4.0°C in mean ocean temperatures by the end of the century which, therefore, further emphasizes the importance of understanding and/or predicting the potential impacts of rising water temperatures as a very vital research focus. Knowledge on the effects of temperature will help determine the potential impacts of rising water temperatures (i.e. due to global warming) on coastal and estuarine communities.

Increasing marine temperatures and the associated rise in anomalous thermal events (e.g. marine heatwaves) have the potential to surpass the physiological thresholds of marine organism (IPCC, 2014). This could compromise the energetic process (e.g. growth, reproduction, and behaviour) and ultimately impact the fitness and survival of marine organisms (Doney *et al.*, 2012; Huey *et al.*, 2012; Lefevre, 2016; Abram *et al.*, 2017). Marine ectotherms (e.g. *P. peringueyi*) are more at risk to the dangers of thermal climatic changes, specifically warming (Pinsky *et al.*, 2019), due to the fact that they lack the ability to physiologically adjust their body temperature (Pörtner, 2002) and inhabit zones within their range of tolerance (Pörtner and Peck, 2010). Therefore, results from the present study will assist in determining the thermal tolerance of *P. peringueyi* which will ultimately help in determining the vulnerability of the shrimp to temperature changes and whether the species can regulate such limits under increasing climatic stress (Stillman, 2003; Somero, 2010, 2012).

According to Schwenk *et al.*, (2009) an organism's vulnerability towards a rise in temperature depends mainly on its thermal tolerance, so knowledge on a species' tolerance to temperature is the initial step towards understanding how vulnerable these species are. Understanding the thermal tolerance of species such as *P. peringueyi* will add to the existing body of knowledge on aquatic organisms' thermal tolerances and requirements so as to properly prioritize conservation efforts to combat effects changes in temperature may have on biodiversity, population stability and the livelihood estuarine and coastal structures.

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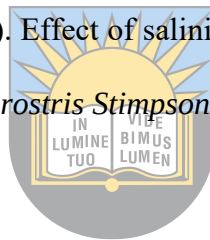
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Chapter 4

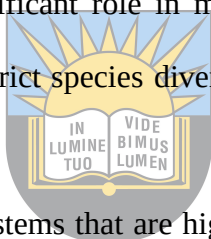
Combined effect of salinity and temperature on the survival of the estuarine caridean shrimp *P. peringueyi* (Caridea: Palaemonidae).



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Introduction

Global aquatic habitats are being affected by climate change, which is connected to human-caused increases in greenhouse gases (IPCC—Intergovernmental Panel on Climate Change 2013), at a rate that is unprecedented (Hoegh-Guldberg and Bruno 2010; Firth and Fisher 2012). Throughout the century, warming will continue, causing long-term, widespread changes in several climate drivers, which include atmospheric and oceanic surface temperatures, precipitation patterns, sea level rise and extreme weather events. (IPCC—Intergovernmental Panel on Climate Change 2013). Salinity, sea surface temperature and the rise in sea levels are strongly correlated with water balance elements. As a direct or indirect result of global climate warming, all these factors are shifting and will continue shifting in the future. Sea surface temperature and salinity have a significant role in marine creatures, have the potential to directly impact ecosystem health, restrict species diversity and are crucial for the incubation period of juveniles.



Estuaries are important aquatic ecosystems that are highly productive and offer an extensive range of ecosystem services (Barbier *et al.*, 2011; Costanza *et al.*, 1997, 2014). Estuarine ecosystems are more susceptible to climatic change effects than other ecosystems because they serve as transitional areas between marine, freshwater and terrestrial environments (Poloczanska *et al.*, 2007). Increased seawater intrusion into transitional water bodies (e.g. estuaries) is anticipated due to sea level rise (Liu and Liu, 2014; Chen *et al.*, 2016; Vargas *et al.*, 2017). Due to a decrease in river input, reduced freshwater levels may subsequently reach these aquatic bodies (Dailidienė and Davulienė, 2008; Jakimavičius and Kovalenkovienė, 2010; Vargas *et al.*, 2017) thus resulting in elevated salinity levels. At the same time, lower freshwater inflow increases mouth closure duration in many estuaries, which reduces marine-estuarine connectivity, affects salinity regimes and recruitment.

It is well understood that physiological responses to salinity and temperature effects vary according to species and the two factors may combine to affect shrimp growth and survival (Staples and Heales, 1991; O'Brien, 1994). However, the effects of temperature on the growth and survival of penaeid shrimps is more significant (Parado-Esteva, 1998). The biological effects of salinity and temperature variations on marine organisms are considered to be among the most important drivers of the marine environment (Ponce-Palafox *et al.*, 1997). The physiological response of an organism to physical changes in the surrounding environment (e.g. salinity and temperature) affects its ability to carry out biochemical reactions, which in turn affects the organism's metabolism, distribution and ultimately, its survival and growth at the different stages of their life cycle (Magnuson *et al.*, 1979; Reynolds and Casterlin, 1979). In crustaceans, stress that is due to the effects of salinity typically results in late growth, survival reduction, feeding and growth rates, as well as in prolonged or suspended moulting cycles, metabolic changes in the partitioning of energy and in changed behaviour (Anger, 2003). Besides individual salinity variation effects, interactive effects in conjunction with another environmental factor such as temperature could further limit species distribution on an ecological and geographical scale and thus, select for the evolution of special adaptations (Anger, 2003).

Salinity and temperature have a huge impact on aquatic organisms as they can influence, modify and control an organism's physiological rates (Hernández, *et al.*, 2006). The synergistic effects of salinity and temperature can be complex, with one factor influencing the effects of the other (Vernberg, 1983). Therefore, knowledge of the physiological processes and limitations of marine species is necessary to forecast how they will respond to temperature variability in future climate change scenarios (Sinclair *et al.*, 2016). Despite the importance of global projections of mean climate conditions, the ecological effects of climate change are more likely to result from responses to extremes than from changes in average circumstances

(Coumou and Rahmstorf 2012) and will exhibit significant spatial variability as, for example, some regions become wetter and others drier (Durack *et al.*, 2012; Hobday and Lough 2011).

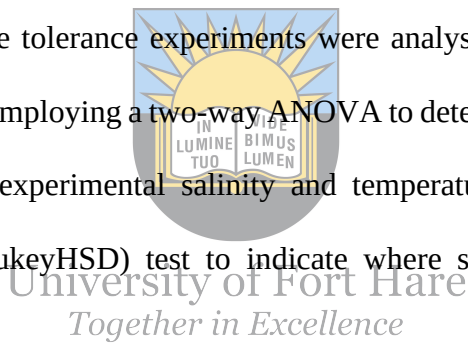
Materials and Methods

The interactive effects of salinity and temperature on the survival of *P. peringueyi* were investigated over a range of salinities and temperatures using the methods described in chapters 2 and 3. *P. peringueyi* were individually exposed to combinations of five different temperatures (i.e. 10°C, 15°C, 20°C, 25°C and 30°C) and three salinities (i.e. 25, 35 and 45) under controlled conditions (i.e. controlled salinity and temperature) in a controlled temperature room to determine the combined effects of both physico-chemical parameters on the survival of *P. peringueyi*. Both salinity and temperature ranges were selected based on the results obtained from chapters 2 and 3. The selected salinity range included only optimum salinities where survival was highest and omitted those which would induce stress, whereas for temperature, the range also included temperatures with low survival rates (e.g. 30°C) which were expected to induce stress. The aim behind this was to test the effects potential global climatic change scenarios (i.e. warming) might have on shrimp survival at the areas where they are most abundant. Adult shrimps (CL > 9 mm) were acclimated to the experimental salinities and temperatures overnight (following Allan *et al.*, 2006). Temperature was controlled using a water bath and salinity was adjusted by either diluting natural seawater (i.e. salinity 35) with distilled water for salinity decreases or by adding commercial sea salt for salinity increases. Temperature and salinity were adjusted daily to keep the variations within $\pm 0.5^{\circ}\text{C}$ and $\pm 0.5\text{ppt}$, respectively, using a mercury thermometer and a portable refractometer. Once the desired salinity and temperature had been reached, healthy shrimps of nearly the same size class were selected and used for the experiment. The shrimps were individually exposed to the

experimental temperatures in 150ml glass containers to reduce movement, thus ensuring that exhaustion had no confounding effect. Test containers were sealed with perforated parafilm to limit evaporation that may affect the test salinity whilst simultaneously providing an aeration source for the test media. The test media were not aerated but dissolved oxygen levels in test media were regularly monitored to ensure that they remained at acceptable levels. The test media were renewed after 48 hours to ensure that decreasing dissolved oxygen levels had no confounding effect. The experiment was conducted over a 96-hour period and survival was monitored at 24-hour intervals. A photoperiod of 12 hours light: 12 hours dark was maintained in all experiments and salinity and temperature were monitored on a regular basis.

Statistical analysis

The salinity and temperature tolerance experiments were analysed graphically. All analyses were performed in RStudio employing a two-way ANOVA to determine significant differences in mean survival between experimental salinity and temperature combinations. This was followed by a postHoc (TukeyHSD) test to indicate where significant differences occur between groups.



Results

Mean survival at 10°C varied between salinities, where highest survival (i.e. 100%) after the first 24 hours was observed at 35 and 45. A drastic decline in mean survival after 48 hours was then observed at all test salinities and no shrimp survival was observed after 96 hours (Figure 4.1). Highest mean survival at 15°C was recorded at 35 where mean survival was 80% after 96 hours, on average. Mean survival dropped to 67% when salinity was decreased to 25 and to a further 60% when salinity was increased to 45 (Figure 4.2). Mean survival at 20°C was relatively much higher across all salinities. Mean survival was recorded at 100% between salinities 25 and 35 but dropped to 93% at 45 after 96 hours (Figure 4.3). Mean survival at

25°C was very high and was >90% across all salinities. Mean survival was recorded at 100% at salinities 35 and 45 after 96 hours. Mean survival was reduced to 93% after the first 24 hours at 25°C and no other mortalities were recorded after (Figure 4.4). Mean survival at 30°C varied with time across all test salinities and highest survival of 33% after 96 hours was observed at 35 (Figure 4.5).

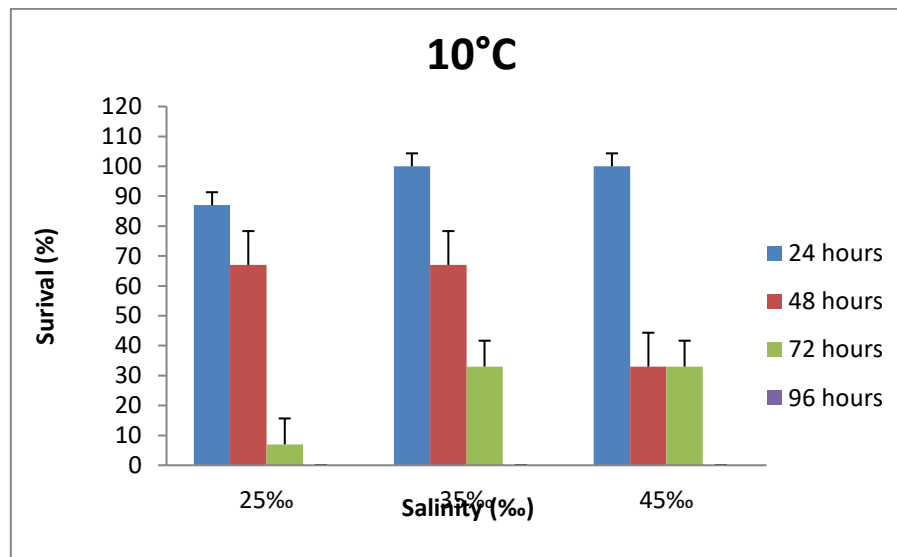


Figure 4.1: Mean survival of the estuarine caridean shrimp *P. peringueyi* exposed to various salinities at a constant temperature of 10°C. Error bars represent one standard deviation (+1SD) above the mean survival, providing insight into the greater variability in survival within the dataset. Standard deviation values are expressed in points on the test scale. Error bars were calculated using the standard deviation of the dataset.

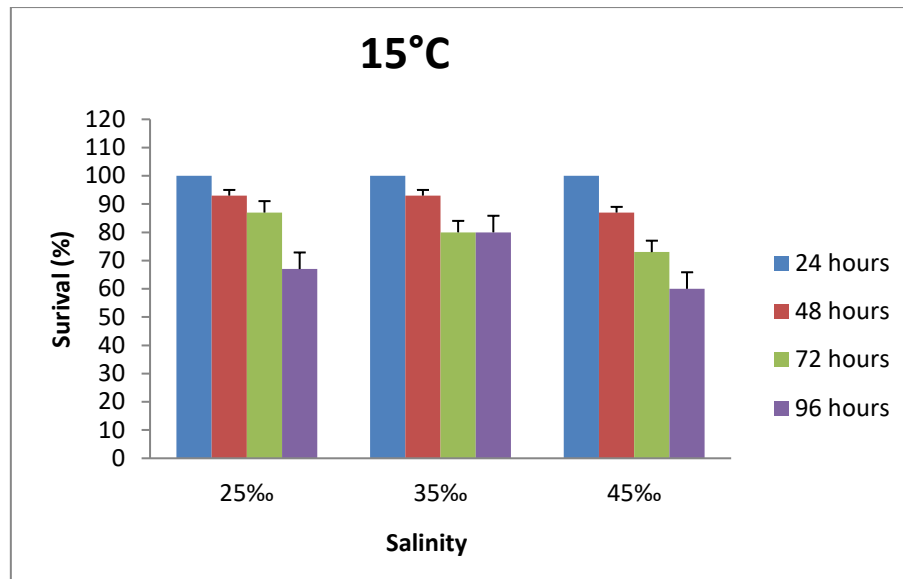


Figure 4.2: Mean survival of the estuarine caridean shrimp *P. peringueyi* exposed to various salinities at a constant temperature of 15°C. Error bars represent one standard deviation (+1SD) above the mean survival, providing insight into the greater variability in survival within the dataset. Standard deviation values are expressed in points on the test scale. Error bars were calculated using the standard deviation of the dataset.

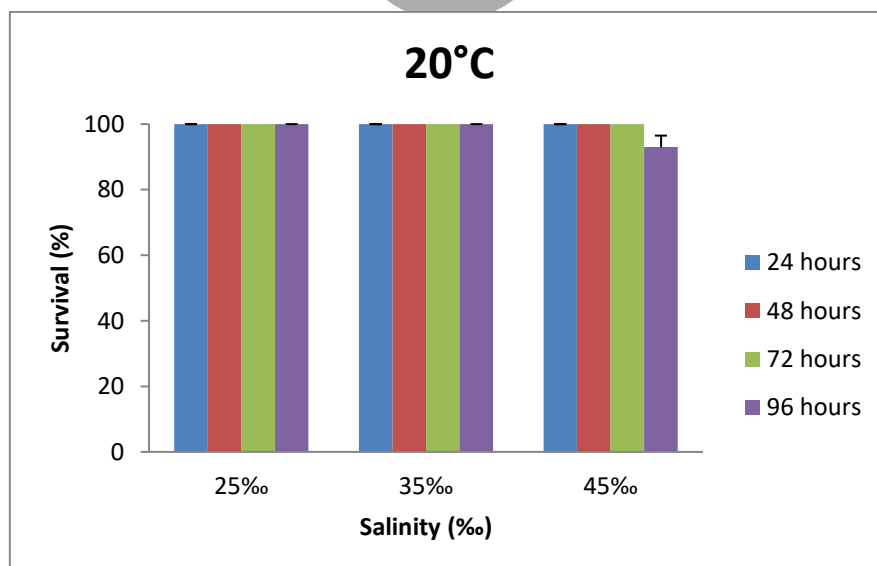


Figure 4.3: Mean survival of the estuarine caridean shrimp *P. peringueyi* exposed to various salinities at a constant temperature of 20°C. Error bars represent one standard deviation (+1SD) above the mean survival, providing insight into the greater variability in survival within the

dataset. Standard deviation values are expressed in points on the test scale. Error bars were calculated using the standard deviation of the dataset.

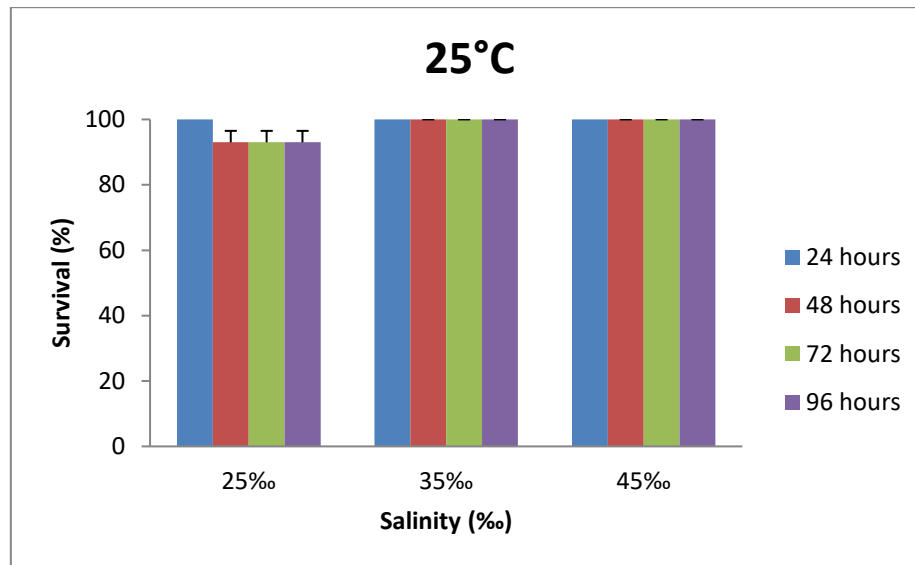


Figure 4.4: Mean survival of the estuarine caridean shrimp *P. peringueyi* exposed to various salinities at a constant temperature of 25°C. Error bars represent one standard deviation (+1SD) above the mean survival, providing insight into the greater variability in survival within the dataset. Standard deviation values are expressed in points on the test scale. Error bars were calculated using the standard deviation of the dataset.

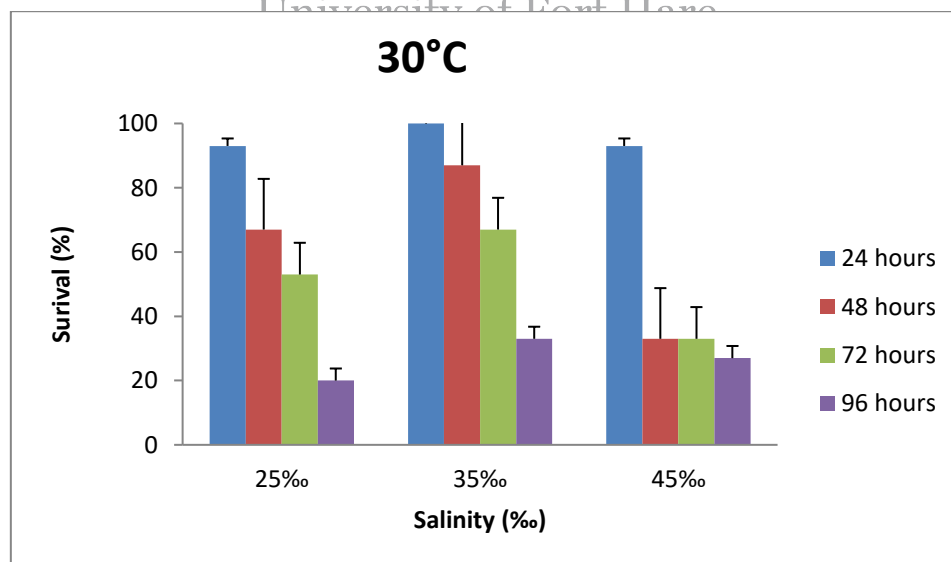


Figure 4.5: Mean survival of the estuarine caridean shrimp *P. peringueyi* exposed to various salinities at a constant temperature of 30°C. Error bars represent one standard deviation (+1SD) above the mean survival, providing insight into the greater variability in survival within the

dataset. Standard deviation values are expressed in points on the test scale. Error bars were calculated using the standard deviation of the dataset.

Significant differences in mean survival were observed between 10, 15 and 30°C with all test temperatures. No difference in survival was observed between temperatures 20 and 25°C (Figure 4.6).

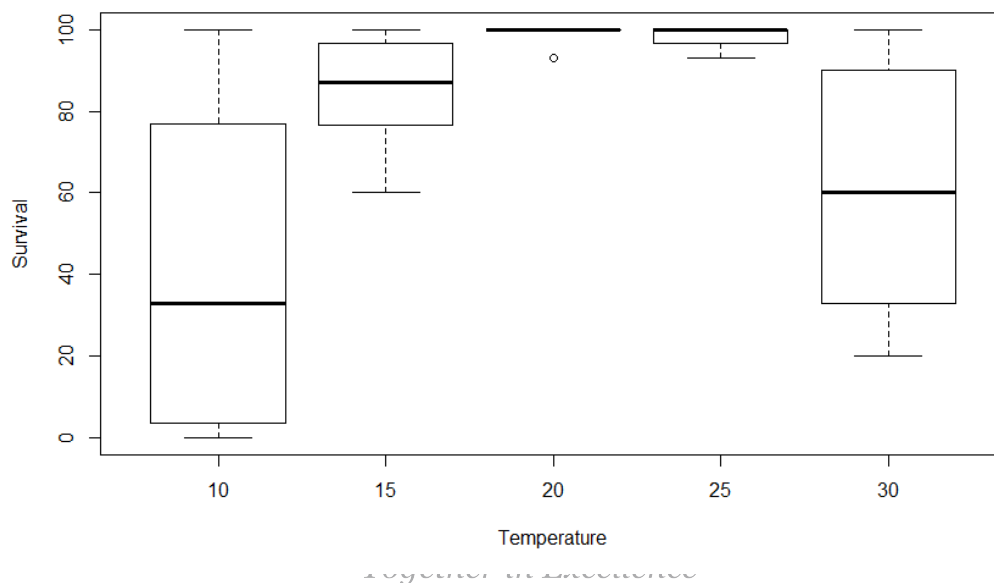


Figure 4.6: Combined effect of salinity and temperature on the survival of *P. peringueyi*. The plot indicates difference in mean survival between the five test temperatures (i.e. 10, 15, 20, 25 and 30°C) at all test salinities. The box (i.e. interquartile range) represents the middle 50% of the data and the lines inside the boxes represent the median. The "whiskers" extending from the box represent the min and max values within a 1.5 range times the interquartile range.

A factorial ANOVA revealed that the difference in survival was more significantly driven by temperature and salinity had little effect (Table 4.1). The interaction between salinity and temperature were also shown to have a significant influence on the survival of *P. peringueyi* ($p = 0.002$).

Table 4.1: Factorial ANOVA indicating differences in survival between salinity and temperature combinations.

	Sum of Squares	df	Mean Square	F	Pr (>F)
Salinity	385	2	192.6	1.444	0.262
Temperature	29311	4	7328	14.22	4.95e-08
Salinity: Temperature	415	4	103.7	0.778	0.554
Residuals	28342	55	515		

A postHoc (TukeyHSD) test was conducted to identify differences among the three groups. Statistical differences were found to be driven by differences between 10°C, 15°C and 30°C and all test temperatures. No significant statistical differences were recorded between temperatures 20-25°C (Table 4.2).

Table 4.2: The postHoc (TukeyHSD) test results indicating where significant differences occur between groups. Significant differences occurred between 15-10, 20-10, 25-10, 30-15, 30-20 and 30-25 (i.e. Significance codes: 0 ‘***’; 0.001 ‘**’; 0.01 ‘*’; 0.05 ‘.’; 0.1 ‘ ’; 1).

Temperatures	Diff	lwr	upr	p adj
15-10	41.083	14.946	67.220	0.000***
20-10	55.500	29.363	81.367	0.000***
25-10	54.333	28.196	80.470	0.000***
30-10	14.917	-11.220	41.054	0.498
20-15	14.417	-11.720	40.554	0.532
25-15	13.250	-12.887	39.387	0.611
30-15	-26.167	-58.304	-0.030	0.050.
25-20	-1.167	-27.304	24.970	0.999
30-20	-40.583	-66.720	-14.446	0.001**
30-25	-39.417	-65.553	-13.280	0.001**

Discussion

Lowest shrimp mean survival after 96 hours of exposure was observed at 10°C where mean survival was 0% in all test salinities. This, however, increased with increasing temperature and highest shrimp mean survival was obtained at the combination of 20°C and 25°C with all test salinities, where mean survival after 96 hours ranged between 93% to 100%. Mean survival of *P. peringueyi* was highest at salinities and temperatures similar to those found in their natural habitat as observed by Heyns and Froneman (2010) and Nyalungu (2014). These results are comparable with previous studies on the effects of salinity and temperature on other crustaceans, including penaeids, (e.g. Lester and Pante, 1991) that indicate that survival is highest at temperatures and salinities similar to those recorded at their natural habitat.

Studies of prawns in brackish waters have revealed that salinities beyond their osmotic points have a higher impact on survival compared to temperature (Ch and Shailender, 2013), although extreme temperatures are associated with high mortality (Aktas and Cavdar, 2012; Crisp *et al.*, 2017). This corresponds with the results from the present study which found temperature to be the more influential factor behind the shrimp's mean survival when shifted towards the shrimp's upper and lower limits. Although test salinities outside the shrimp's osmotic point were omitted from the study, a steady decline in shrimp survival was observed when salinity was lowered to 25 and even more so when in combination with temperatures towards the shrimp's thermal limits, thus suggesting an exacerbating effect of the one physico-chemical parameter on the other. The findings of this study are, therefore, comparable with previous studies on the effects of salinity and temperature on crustaceans, which follow the same trend when it comes to salinity and temperature tolerance (e.g. CTMax studies on *Farfantepenaeus aztecus* (Re *et al.*, 2005), *Litopenaeus stylirostris* (Re *et al.*, 2006) and *Penaeus semisulcatus* (Kir and Kumlu, 2008)).

Salinity and temperature influence the physiology and, partly, the survival and manner in which organisms are distributed within the aquatic environment (Re *et al.*, 2005). Temperature and salinity have a direct and indirect influence on aquatic organisms. Temperature directly influences organismal activity, and salinity has an indirect influence on physiological responses, such as growth, life cycle, metabolism, nutrition and intra- and inter-specific relationships (Kinne, 1971). Robertson (1984) found that combinations of elevated temperatures and low salinities result in mortalities of *P. peringueyi*. The present study is in correspondence with this result and has concluded that the combined effects of temperature and salinity have a significant influence on the survival of *P. peringueyi* ($p = 0.002$).

These findings on the interactive effects of salinity and temperature on the survival of *P. peringueyi* are critical from a South African perspective as we can now add to the existing body of work and make educated conclusions on the impacts global climate change may have on the livelihood of the shrimp species. Though the test species may have not been exposed to stressors beyond its tolerance range, it is quite evident that thermal and saline conditions beyond the shrimp's tolerance points result in mass mortalities. It can thus be concluded that the predicted shifts in both parameters that may be brought about by global climate change will have detrimental effects on the species' livelihood and survival.

Species distribution and abundance is predicted to shift according to the species' temperature tolerance and its adaptability, due to the rise in temperature (Harley *et al.*, 2006). In order to deal with the predicted shifts in thermal stressors associated with climate change, certain species will physiologically rely on mechanisms to tolerate extreme body temperatures, and many will behaviourally regulate their body temperatures (Kearney *et al.*, 2009). In comparison to terrestrial endotherms, aquatic ectotherms lack the ability to control their body temperature physiologically (Portner, 2002). *P. peringueyi*, for example, is an obligate poikilotherm and like other ectotherms, lacks the ability to physiologically regulate its body temperature

(Madeira *et al.*, 2012), therefore, its distribution within estuaries is limited by its thermal preference (Portner and Peck, 2010). As such, the shrimp species is increasingly exposed to the threat of thermal climatic changes, especially warming (Pinsky *et al.*, 2019). It can thus be concluded that exposure to rising water temperatures and increased thermal variability, coupled with the changes in salinity brought about by global climatic change will not only be physiologically unfavourable to *P. peringueyi* but will also result in shifts in its distributional ranges and abundance of the shrimp species. Furthermore, exposure to such harsh environmental conditions and with one factor exacerbating the effects of the other, it can only be concluded that future global climate change scenarios will have a detrimental effect on the survival of the species.

Though this study focused on the salinity and temperature tolerance of adult *P. peringueyi*, it should be noted that salinity and temperature tolerances are more relevant to juveniles than adults (i.e. juveniles are the main occupants of estuaries as they recruit into estuaries at around 6mm) and tolerances for juveniles are typically very different. Therefore, since juveniles are the ones that spend the most time in estuaries and are thus most affected by salinity changes, the current study recommends that further research be conducted to further test the ecological relevance of its data by looking at juvenile tolerances. This will result in the understanding of the physiological responses of *P. peringueyi* to multiple environmental drivers (i.e. salinity and temperature) which will be the starting point toward better models of biotic responses to climate change.

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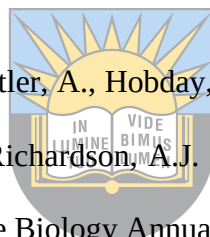
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Chapter 5

Synthesis



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This study examined salinity tolerance, temperature tolerance and their combined effect on the survival of the caridean shrimp, *P. peringueyi*. This study was conducted in order to understand how the shrimp species is physiologically adapted to the changing estuarine environment, especially with the environmental changes that would be brought about by global climate change. *P. peringueyi* is said to be euryhaline and tolerant to a wide range of aquatic temperatures (Wooldridge, 1999). These are rather important adaptations within the estuarine environment, where the fluctuations in both salinity and temperature occur frequently and widely.

The study was aimed at investigating the saline and thermal effects on the survival of the shrimp species. To achieve this, it was necessary to first determine the appropriate shrimp density to be used so that density did not have a sub-lethal effect during salinity, temperature and interactive effects testing. Group exposure was found to have a negative impact (i.e. which might not be a density induced effect but might be due to a confounding factor such oxygen depletion and requires further investigation) on the survival of *P. peringueyi*. This may be due to space competition and/or cannibalistic behaviour and it requires further investigation. Therefore, this study recommends that salinity and temperature tests using *P. peringueyi* as a test organism, employs the individual exposure method.

The salinity tolerance of *P. peringueyi* investigated appears to correspond to the areas that the shrimp inhabits within the estuarine environment. Its wide tolerance is mostly attributed to the fact that the species is widely distributed within estuaries and is, therefore, subjected to wide variations in salinity from its surrounding environment. Throughout this study the shrimp displayed a wide salinity tolerance with highest peak in survival recorded at salinities generally recorded in the middle and lower reaches of predominantly open estuaries (e.g. Kariega Estuary). *P. peringueyi* is more abundant in the middle and lower reaches of predominantly open estuaries that are characterised by high salinities (de Villiers *et al.*, 1999; Nyalungu *et al.*,

2014). The decline in shrimp survival when exposed to lower salinities further emphasized that the shrimps, being occupants of the lower and middle reaches of the estuary, are exposed to hyposaline conditions for shorter periods. This corresponds with previous studies on *P. peringueyi* which state that the shrimp's upper distribution in predominantly open estuaries is reduced due to physiological tolerances (Emmerson, 1986).

Results on the effects of temperature on *P. peringueyi* are comparable with previous studies. *P. peringueyi*, being a species that has a high abundance in the warm-temperate waters of the Eastern Cape in comparison to the cooler waters of the Western Cape (Hanekon, 1982; Achituv and Cook, 1984), was found to have a preference for cool to warm water temperatures between 15 and 25°C. This was not surprising as these are the temperatures that are generally recorded in the shrimp's natural habitat. Extreme temperatures beyond the shrimps' tolerance range, however, had a negative effect on survival with temperatures 5 and 35 resulting in 100% shrimp mortalities within 24 hours of exposure.

Temperature was found to be the more influential factor driving shrimp mortalities when compared to salinity. The interactive effect of temperature and salinity on survival was also found to be significant. The interactive effect of the two factors is usually very complex, with one variable exacerbating the effects of the other. Though the shrimp species is adapted to wide salinity and temperature ranges, as is evident in the high survival rates at the different temperatures, an increase in shrimp mortalities was, however, observed when salinity was shifted below 35. This is unusual as these are salinities that are recorded at the shrimps' natural habitat. This was observed as an effect of a potential confounding factor and the study refers that it be further investigated. Previous studies on crustaceans have also shown that an interaction between the two variables has significant effects on the physiology of organisms with one variable exacerbating the effects of the other. *P. peringueyi*, however, was not exposed to combinations of extreme stressors beyond its preference range thus the potential of

the interactive effects of salinity and temperature on its survival was not fully tested. However, the study has provided sufficient data that corresponds with previous studies on the combined effects of salinity and temperatures on the survival of *P. peringueyi*. This study does, however, suggest that the combined effects of salinity and temperature be further investigated with the inclusion of extreme salinity conditions beyond the shrimp's osmotic point. This study also notes that saline and thermal tolerances of *P. peringueyi* are more relevant to juveniles than adults and recommends that further research be conducted to further test the ecological relevance of its data by looking at juvenile tolerances, since they are more affected by the ever-changing estuarine conditions (i.e. salinity and temperature).



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