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The School of Science and Technology  
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Comparison of the Barnacle,  
*Balanus amphitrite*,  
in Different Environments

by

Katsura Matsuda

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A Thesis submitted in partial satisfaction  
of the requirements for the degree of  
Master of Science in Biology

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Each person whose signature appears below certifies that this thesis in their opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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## ABSTRACT OF THE THESIS

Comparisons of the Barnacle, *Balanus amphitrite*, in Different Environments

by

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The Salton Sea is a saline lake located centrally in the Colorado Desert, California. Salinity as well as nutrients of the Sea have been increasing since 1907, thus it is currently facing ecological collapse. The barnacle, *Balanus amphitrite saltonensis* at the Salton Sea was once thought to be a subspecies of *B. amphitrite* due to distinctive morphological differences between populations at the Sea and San Diego Bay. However, only one subspecies, *Balanus amphitrite amphitrite*, currently exists based on genetic studies. My study investigates physiological, survival, and morphological differences of *B. amphitrite* populations from the Salton Sea and San Diego Bay.

Weight-specific oxygen consumption rate was measured for 3 hours to compare respiratory responses of both populations at 18 experimental combinations of salinity (0, 15, 30, 36, 45, and 60 ‰) and temperature (15, 25, and 35 °C). Although no overall significant difference in respiration occurred, significant differences between populations were found in 45 ‰ at 15 °C and in 0 ‰ at 35 °C. Salton Sea barnacles consumed more oxygen in higher salinities and at higher temperatures than San Diego Bay barnacles.

Results of survival experiments ( $T_{50}$ ) in the same 18 conditions for 48 hours indicated that no animals died at 15 ° and 25 °C while animals tested in low salinities (0 and 15 ‰) at 35 °C for both populations resulted in mortality of greater than 50 % of the

treatment populations within 48 hours. Mortality rate showed that Salton Sea barnacles survived longer than San Diego Bay barnacles in low salinities at high temperature and that temperature had a greater influence on survival than salinity.

Morphological differences were examined by measuring animal diameter, height, and test thickness for both populations. Statistical analyses indicated that there were significant differences in those measurements between populations. Possibly as a result of phenotypic plasticity induced by different environmental conditions, external morphology is distinct between the populations.

Although *B. amphitrite* at the Salton Sea and San Diego Bay are the same species, Salton Sea barnacles may be adapted to tolerate the elevated conditions of salinity and temperature that occur in that environment.

## CHAPTER ONE

### INTRODUCTION

#### **Objective of the Study**

The objective of this study was to compare the physiological, survival, and morphological differences of the barnacle, *B. amphitrite*, between the Salton Sea and San Diego Bay in California. Since the Salton Sea barnacles thrive in the consistent conditions of high salinity and high temperatures throughout the year in the Salton Sea, individuals from the Sea were compared with the same species from San Diego Bay with more variable conditions of salinity and temperature. In order to compare these populations, oxygen consumption, survival rate, and morphology were measured and analyzed.

#### Hypothesis for Each Comparison

##### ***Physiological Comparison***

Oxygen consumption is an indirect measure of metabolisms commonly used in the study of crustaceans (McMahon and Wilkens, 1983). Unlike other invertebrates, barnacles consume more oxygen when cirral movement of the animals is active (Newell and Northcroft, 1965) and this occurs when animals are in optimal conditions. Therefore, I test the hypothesis that the Salton Sea barnacles will consume more oxygen at their optimal range of high salinity and high temperature, which resemble the environmental conditions of the Salton Sea. In contrast, the San Diego Bay barnacles will consume more oxygen at lower salinity and lower temperature, which resemble the marine environment, compared with the Salton Sea barnacles. In order to address this hypothesis, weight-specific oxygen consumption rate was measured in 18 combinations of salinity

and temperature in order to compare the physiological differences between the Salton Sea and San Diego Bay populations.

### ***Survival Comparison***

There is a need for sessile organisms such as barnacles at the Salton Sea to survive more extreme conditions when compared with marine conditions. Since the Salton Sea barnacles are thriving in high salinity and high temperature of the Sea, the hypothesis tested is that the survival rate of the Salton Sea barnacles will be greater than that of San Diego Bay barnacles in the experimental conditions of high salinity and high temperature. In order to determine the differences in tolerance to the 18 combinations of salinity and temperature used in the oxygen consumption experiment, survival rates of the animals was measured.

Death of the animals is often hard to determine in survival experiment. In this experiment, tension of the scutum was measured in order to determine whether the animal was dead or alive. The hypothesis is that live animals will have greater scutum tension than dead animals for both the Salton Sea and San Diego Bay populations. To address this hypothesis, death of the animals was determined by measuring the tension required to remove one side of the scutum from live as well as dead barnacles during survival experiments.

### ***Morphological Comparison***

Distinctive morphological differences between the Salton Sea and San Diego Bay barnacles were reported by Rogers (1949). Casual observation of both populations also suggests that the morphology of the animals differ between the Salton Sea and San Diego Bay. The hypothesis is that there will be significant differences in the diameter and

height of the barnacles from the Salton Sea and San Diego Bay. The Salton Sea barnacles will be taller and narrower due to lack of predation, space for settlement, and tidal action while the San Diego Bay barnacles will be shorter and wider due to predation, enough space for settlement, and wave exposure. In order to address this hypothesis, the differences in morphology, diameter and height of the animals were measured.

Casual observation also suggested that there is a difference in animal test thickness. The hypothesis is that the test thickness of the Salton Sea population will be thinner compared to the San Diego Bay population, which will have thicker tests. In order to address this hypothesis, animal test thickness was measured to clarify the difference in their morphology.

### **Controversy Over *Balanus amphitrite saltonensis***

The barnacle *Balanus amphitrite* Darwin (1854), also called the striped barnacle, is a very abundant cirriped crustacean of the Order Thoracica (Suborder Balanomorpha) in the Salton Sea. *Balanus amphitrite* is a very common member of the intertidal fouling communities of harbors and protected embayments. The native range of *B. amphitrite* is the Southwestern Pacific and Indian Ocean, while present distribution is in tropical and warm temperate waters throughout the world (Edmondson, 1933). *Balanus amphitrite* can be described as a small, conical, sessile barnacle which can grow up to about 1.5 cm in diameter (Edmondson, 1933). According to Edmondson (1993), the test color is whitish with purple or brown longitudinal stripes, and the surface of the test plates is longitudinally ribbed. The animal's body is covered and protected by the paired structures of interlocking tergum and scutum.

According to Linsley and Carpelan (1961), *B. amphitrite* was introduced into the Salton Sea by U. S. Navy seaplanes from San Diego Bay early in the 1940s. Since the Salton Sea was a closed lake, the population has been isolated, and morphological differences between the Salton Sea and San Diego Bay populations have become distinctive (Rogers, 1949). Rogers (1949) argued that the barnacles from the Salton Sea were a subspecies of *B. amphitrite* based on their morphological differences and subscribed to them the name *B. amphitrite saltonensis*. However, after Henry and McLaughlin (1975), who also recognized *B. amphitrite saltonensis* as a subspecies, no publications have further indicated that the barnacles from the Salton Sea are a subspecies of *B. amphitrite* found in San Diego Bay. In 1985, Flowerdew suggested to synonymize the *amphitrite* variety *saltonensis* with the variety *amphitrite* based on genetic analysis. Therefore, *B. amphitrite amphitrite* is the only subspecies of *B. amphitrite* that exists around the world today. Since controversy over *B. amphitrite saltonensis* as a subspecies needs to consider the issues of genetic analysis as well as phylogeny of the animals, investigating whether *B. amphitrite* at the Sea is a subspecies or not is beyond the scope of this study.

### **History of the Salton Sea**

The Salton Sea, the largest inland body of water in the state of California, is located in a closed desert basin in Riverside and Imperial Counties in southern California. According to the report from the Salton Sea Restoration Project (2005 online source), the first flood occurred in 700 AD when the Colorado River silted up its normal outlet to the Gulf of California and swung northward through two overflow channels. Since 700 AD, at least 11 floods have been recorded over hundreds of years in the Salton Sea.

Fialkowski and Newman (1998) stated that the current Salton Sea was formed when a man-made irrigation canal from the Colorado River broke and flooded the depression that extended 82 meters below sea level in 1907. Since then, salinity, nutrients, and heavy metals have been increasing annually (Fialkowski and Newman, 1998).

### **How the Salton Sea Has Changed Over Time**

#### Salinity

The Salton Sea has been heading toward ecological collapse over the last few decades (Salton Sea Restoration Project, 2005 online source). Initially, the Sea maintained a salinity level of 3.65 ‰ in 1907. However, by 1929 the salinity level had already increased to 32.06 ‰ (Arnal, 1961). Carpelan (1958) also mentioned that the salinity of the Sea was 33 ‰, which was still slightly lower than ocean salinity.

According to the report from the Salton Sea Ecosystem Restoration Program (Department of Water Resources, online source), the current salinity level of the Salton Sea is approximately 48 ‰, calculated to be 30 % greater than ocean salinity. Because of the continued agricultural wastewater inflows and lack of an outlet, the Salton Sea is increasing in salinity and may soon be too saline to support fish and other elements of the present food chain as a result of high evaporation rates (Hart *et al.*, 1998). Ross (1915) came to the conclusion that leaching of salts from the lake bottom had been completed by 1911. Since then, the changing salinity of the Sea has been dictated by fluctuations in water level as well as continued concentration of salts by evaporation. Tostrud (1997) also estimated that 80 million tons of salt had dissolved from flooded salt deposits and were added to the Sea water by 1914.

A report by the Salton Sea Ecosystem Restoration Program (Department of Water Resources, online source) suggested that the Sea's surface area is about 365 square miles today although the size of the Sea will be significantly reduced due to the recent approval for the quantification settlement agreement inflow that reduces inflow to the Sea (Department of Water Resources, online source). The proposed reduction in inflow will additionally cause salinity to rise faster than it would without a reduction in inflow. Along with significant environmental changes, such as a rapid increase in salinity of the Salton Sea, changes in vertebrate as well as invertebrate populations will be observed (Hart, *et al.*, 1998).

#### Chemical Component of the Sea

The earliest water quality data on the Salton Sea were presented by MacDougal (1907) who compared the major ion composition of the Sea to normal seawater in 1907 and Ross (1915) who also collected data on cations and anions as well as total-dissolved solids during every May or June from 1907 to 1914. According to their studies, the composition of the Salton Sea underwent dynamic changes during the period of the Sea's formation in 1905 to 1907 as a result of dissolution of flooded salt deposits. Therefore, physical and chemical characteristics of the Sea were already different than the ocean in 1958 when Carpelan studied salinity, ionic composition, dissolved oxygen, and pH at the Sea. Carpelan's study (1958) indicated that Salton Sea water contained less Magnesium ( $Mg^{2+}$ ), Potassium ( $K^+$ ), and Chloride ( $Cl^-$ ) than ocean water, while Sodium ( $Na^+$ ) was about the same proportion of the ocean. Calcium ( $Ca^{2+}$ ), sulfates ( $SO_4^{2-}$ ), bicarbonate ( $HCO_3^-$ ), and carbonate ( $CO_3^{2-}$ ) were greater than that of ocean water. The calcium ion content of the Sea, which is greatly related to the formation of barnacle tests, has also

changed over time since the Sea was formed. Carpelan (1958) measured the amount of  $\text{Ca}^{2+}$  at the Sea (0.850 ‰), and it was already higher than the ocean (0.413 ‰). In 1945, just a few years after *B. amphitrite* was introduced to the Sea. Calcium reached saturation levels around 1950, while  $\text{SO}_4^{2-}$  reached its saturation levels around 1980 at the Sea. Based on published as well as unpublished data from the Imperial Irrigation District (IID) and the Coachella Valley Water District (CVWD) (Tostrud, 1997),  $\text{Ca}^{2+}$ ,  $\text{SO}_4^{2-}$ , and  $\text{HCO}_3^-$  have precipitated out of the Sea as calcite and gypsum. Holdren and Montaño (2002) also indicated that the Salton Sea is supersaturated with respect to calcite, gypsum, and other minerals. Holdren and Montaño (2002) suggested that  $\text{HCO}_3^-$  has undergone the greatest relative change of the major ions, decreasing from 1.6 % of the total major ion concentration in 1916 to 0.6 % in 1999.

#### pH

Carpelan (1985) concluded that the pH of the Salton Sea ranged from 8.3 to 8.6 in winter and from 8.5 to 8.8 in summer. The Salton Sea's water contained a different ratio of  $\text{CO}_3^{2-}$  to  $\text{HCO}_3^-$  than the ocean, and the pH was higher (Carpelan, 1985). The surface pH of the Sea measured by Holdren and Montaño in 2002 averaged approximately 8.2 and showed little differences between river water and the Salton Sea samples. The city of El Cajon, California, collected data on the pH levels of San Diego Bay from September 1994 to January 2001. Since pH range of the Bay was from a minimum of 6.5 to a maximum of 8.5 (California Water Quality Control Boards Region 9, 2005), pH of both locations, the Salton Sea and San Diego Bay overlapped in their respective ranges.

## Temperature

Mean water column temperature of the Salton Sea ranged from a minimum of 13° to 14 °C in early January to a maximum of 30° to 34 °C in July to September (Watts *et al.*, 2001). Maximum mean water column temperatures were 30.8 °C on September 9<sup>th</sup>, 1997, 32.8 °C on July 3<sup>rd</sup>, 1998, and 30.1 °C on August 28<sup>th</sup>, 1999 (Holdren and Montaña, 2002). Maximum surface temperature observed at the southern part of the Sea on August 24<sup>th</sup>, 1999, indicated the highest record of 36.5 °C while a minimum surface temperature of 14.2 °C, was observed at the same location on January 22<sup>nd</sup>, 1999 (Holdren and Montaña, 2002). Seasonal minimum temperature for the mean water column temperature always occurred in January and was 13.9 °C on January 27<sup>th</sup>, 1997, 13.2 °C on January 6<sup>th</sup>, 1998, and 13.8 °C on January 25<sup>th</sup>, 1999. Temperature differences between the top and bottom of the water column in the Sea showed a range of 1 ° to 3 °C during the cooler months and from 3 ° to 9 °C during the summer. Watts *et al.* (2001) suggested that higher wind speeds in 1998 relative to 1997 or 1999 probably were responsible for the accelerated heating during May to June in 1998, although the coolest Spring was observed in the same year. Downward mixing by wind-generated turbulence of heat taken up by surface waters can diminish heat loss via backradiation and increase heat gain via conduction (Watts *et al.*, 2001). The Sea cooled from September to January except for daily surface (0 to 2 m) warming although the midlake water column tended to be isothermal during this period. By conductive and evaporative cooling of surface waters and periodic windy conditions, the Salton Sea water was presumably circulated and mixed daily or almost daily.

## Dissolved Oxygen

The Federal Water Quality Administration (FWQA, 1970) presented information studied from 1963 to 1969 on nutrients in the Sea and described the Sea as “objectionably eutrophic.” The high algal growth was leading to dissolved oxygen super-saturation in surface waters as well as depletion at the bottom of the water column. According to Watts (2001), periods of stratification were sporadically interrupted by mixing that increased bottom water temperature throughout the warming period as the Salton Sea started to warm from January to August and also distributed oxygen downward to bottom waters. Surface oxygen levels in the Sea during several months were often greater than 200 % saturation, while some oxygen depletion was observed in the bottom waters throughout the year (Holdren and Montaño, 2002). According to Watts *et al.* (2001), dissolved oxygen concentrations ranged from 0 to > 20 mg/l with surface waters typically containing > 4 mg/l during the day. When the Sea was stratified during the spring and summer seasons, an oxygen gradient existed between surface (6 to 20 mg/l) and bottom waters (0 to 3 mg/l). Anoxic conditions are developed rapidly in bottom waters during stratification periods (Watts *et al.*, 2001). Dissolved oxygen concentrations were often severely depleted especially at the Sea bottom where oxygen was less than 2 mg/l from April through November. The maximum observed surface dissolved oxygen concentration at the northern part of the Sea was less than 1 mg/l on both September sampling dates (Holdren and Montaño, 2002). The August and September surface oxygen minima has the effect of causing extensive fish kills during the season (Holdren and Montaño, 2002).

From 1954 to 1956, when Carpelan (1958) studied the oxygen regime of the Sea, salinity increased from 33 to 42 ‰ with lowered oxygen solubility. According to Carpelan (1958), one of the reasons why oxygen solubility was reduced was because of increased introduction of fish biomass such as tilapia, which increased the respiration at the Sea. Decomposition rates of settled zoo- and phyto-plankton, which were also inducing oxygen depletion at the bottom of the Sea were observed. Although temperature generally has a large effect on dissolved oxygen, the thermal regime did not change notably from 1954 to 1956 (Watts *et al.*, 2001). However, in surface waters, mid-day dissolved oxygen concentrations were more variable from 1997 to 1999 when compared with those from 1954 to 1956. Large variations were observed among years as well as seasons from 1997 to 1999, while little inter-annual variation and small seasonal differences were recorded from 1954 to 1956. Surface waters from 1997 to 1999 were supersaturated at times between January and July (10 to 20 mg O<sub>2</sub>/l) and then hypoxic or anoxic at times between August and September (Watts *et al.*, 2001).

### **Destruction or Preservation of the Salton Sea**

#### **Importance of the Salton Sea and Significance of the Project**

The Salton Sea is recognized by the Salton Sea State Park as Southern California's largest inland water recreation area and the second largest state park in California since 1955. A gradual increase in salinity and its consequences were already recognized soon after the Sea was formed. Therefore, various salinity control measures have been studied and implemented as early as the mid-1950s in order to save this unique environment, which support the lives that rely on the Sea (Department of Water Resources, online source). According to the report from the Salton Sea Restoration

Project (Online source), over 400 species, including endangered species, have been recorded in the area with 100 breeding species of many vertebrate and invertebrate animals. While the Salton Sea is best known for its fish and bird populations, 24 reptiles such as snakes and lizards and over 20 mammals such as bats, mice, coyotes, deer, bobcat and bighorn sheep have been recorded in the desert and wetland areas. Therefore, the Salton Sea provides many recreational opportunities such as fishing and bird watching because of the wide ecological diversity. The Salton Sea Authority has also emphasized that the Sea is perhaps most important to a large segment of the migratory birds of the Pacific flyway since about 95% of wetlands in California have been lost. Therefore, the Salton Sea has an important role, not only as habitat for over 400 species of animals, but also for millions of migratory birds for food resources and stopover points.

However, according to the report from the Salton Sea Restoration Project (Online source), many species in the Sea are in a state of crisis due to the increased salinity and nutrient flushes from agricultural run-off which have steadily increased over the years. The Salton Sea is a uniquely troubled, yet valuable natural resource to humans, to wildlife, and to the agriculture industry. In the opinion of Verdin (2000), “The popularly conceived notions that the Salton Sea is dead or is dying are totally out of whack.” The findings on the reality of the Sea show an ecosystem which is worth keeping. Even though Verdin (2000) believes that the Sea is not dead or dying, the reality is that the Sea is heading toward ecological collapse (Holdren and Montaña, 2002). Loss of the Sea will also lead to the loss of huge economic income from agricultural crops as well as jobs that people in the associated valleys rely on. Mike Chrisman, California Resources Agency Secretary, announced in January, 2005 in Sacramento, California

(Business Wire) that the Salton Sea is receiving \$750,000 for a pilot study of removing selenium from the Sea as a part of the Resources Agency's Salton Sea ecosystem restoration study. Chrisman said, "The state is strongly committed to restoring the Salton Sea ecosystem and the permanent protection of the fish and wildlife dependent on that ecosystem." Besides ecological importance, the Salton Sea also plays an important role for agriculture, which is a billion dollar mainstay of two valley's economies around the Sea. The Sea has recreational and economical values for humans as being the place for sport fishing and a repository for agricultural drainage. The newsletter on the Salton Sea Restoration Project published in January, 2005, stated, "Any future state actions to restore the Salton Sea will be the sole responsibility of the State of California."

Since the barnacle, *Balanus amphitrite*, including its planktonic larvae, are a food supply for fish and birds at the Sea, decreased numbers of barnacles may result in loss of those organisms that feed on the barnacle, even leading to loss of revenue for the communities around the Sea. Therefore, learning how *B. amphitrite* physiologically responds and survives environmental changes in relation to extreme conditions of salinity and temperature will provide us with a better understanding of how lower trophic invertebrates respond and, potentially adapt, to such harsh environments as the Salton Sea.

### **Background and Significance of Specific Experiments**

#### Oxygen Consumption Experiment

Oxygen consumption is the most frequently used parameter in crustacean respiration physiology (McMahon and Wilkens, 1983). Wolvekamp and Waterman (1960) state that the metabolic rate of crustaceans is generally related to temperature as it is in other poikilotherms. The stress effect of temperature on crustacean metabolism

often decreases with the duration of exposure to the altered temperature as homeostatic metabolic adjustments are made in individuals and species (Wolvekamp and Waterman, 1960). The rate of oxygen uptake of intertidal invertebrates was studied by Newell and Northcroft (1967) who demonstrated that metabolism, measured by the rate of oxygen uptake at rest, is maintained at a relatively constant value over a wide temperature range between 6.5 °C and 22.5 °C. The species they used were a wide variety of common intertidal invertebrates including the anemone *Actinia equina* L., the polychaete *Nephtys hombergi* L., the winkle *Littorina littorea* L., and the cockle *Cardium edule* L. In each of the species examined, there was a rate of oxygen uptake at minimal activity, which did not vary with temperature over the approximate range 6.5 to 22.5 °C (Newell and Northcroft, 1967).

A previous study of Newell and Northcroft (1965) also showed that the cirriped *Balanus balanoides* has a level of maintenance metabolism which is independent of temperature over much of the normal environmental temperature range. They also pointed out that the lower rate of oxygen uptake of *Balanus balanoides* represents respiration at minimal activity, so that the contribution made by muscular or ciliary activity is probably small. Therefore, the level of the rate of oxygen uptake with temperature changes is likely to reflect changes in the “maintenance metabolism” of the organism, or oxygen used for purposes other than activity (Newell and Northcroft, 1965). Thiyagarajan, *et al.* (2003) stated that stress factors such as temperature and salinity may have a significant effect on the amount of stored energy reserves in *Balanus trigonus* larvae due to an increased metabolic activity. According to their study, the effect of temperature on larval development and attachment of *Balanus trigonus* was

comparatively stronger than that of salinity. Therefore, the effect of dynamic changes in temperature rather than the differences in salinity may also have a greater influence on the rate of oxygen consumption of *Balanus amphitrite*, although their study was on larvae. Sixtus (1978) concluded that the Salton Sea population of *B. amphitrite* has shown a change in adaptive capacity indicating a developing difference between it and the San Diego Bay population since the Salton Sea population is able to maintain metabolic stability under short-term temperature changes.

#### Life Cycle of *Balanus amphitrite*

Calcagno *et al.* (1998) indicated that *B. amphitrite* from the Mediterranean coast can survive a mean of 77 days and a maximum of 1.26 to 1.40 years. Calcagno *et al.* (1998) also found the barnacle to have a mean life span of 22 months and a maximum life span of 5 to 6 years in South Africa. These barnacles are hermaphrodites, but cross-fertilization occurs in dense populations (Edmondson, 1933). In such cases, males deposit sperm directly into the mantle cavity of adjacent functional females via a long tube. Fertilized eggs are brooded in the mantle cavity, and it may be several months before the free-swimming planktonic larvae are released (Edmondson, 1933). The immature state of *Balanus* can be found from January to February and even some through July, while peaks of spawning occur from March to June and from August to September (Carpelan 1958).

In tropical waters, breeding of barnacles is continuous throughout the year, although the intensity may vary within local environmental conditions. The reproductive biology of barnacles may depend upon a variety of factors including temperature, photoperiod, salinity, and food supply. In the Salton Sea, the extremely high productivity

of *B. amphitrite* resulted in the covering of the entire beach around the Sea with their tests in 1961 (Linsley and Carpelan, 1961). Some beaches at the Sea are still covered by the barnacle tests today.

### Survival Experiments

Survival experiments on *B. amphitrite* for the effect of salinity and temperature have been studied for decades. According to Nilsson-Cantell (1948) and Shatoury (1958), *B. amphitrite* is apparently able to survive and sometimes breed in abnormally high and low salinities. They also demonstrated that sudden changes in their experimental conditions of salinity, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, and 85 ‰, did not appear to be harmful to *B. amphitrite*. Anil *et al.* (1995) showed that the salinity tolerance of *B. amphitrite* larvae depended largely on water temperature. In their study, barnacles experienced a 21 % mortality rate in 10 ‰ and a 13 % mortality rate in 20 ‰ at an optimum temperature of 23 °C. However, 58 to 99 % mortality was observed at higher and lower temperatures than the optimum temperature. In 30 ‰, the mortality rates were only 10 to 19 % at temperatures of 23 to 27 °C, and 35 to 71 % at temperatures of 15, 20, and 30 °C (Anil *et al.*, 1995). Other experiments on larvae of *B. amphitrite* developed at different salinities showed that some degree of adaptation to salinity change might be possible during development and might help to account for the remarkably wide range of habitat successfully colonized by this species. In salinities between 25 ‰ and 40 ‰, development took place at the normal rate although only a proportion of the eggs hatched, and development was delayed when the larvae were exposed to salinities between 15 ‰ and 25 ‰ or between 40 ‰ and 60 ‰. Salinity tolerance was not influenced appreciably by temperature within the range normally encountered by the animals. Even though *B.*

*amphitrite* generally inhabit warm and temperate seas, the lowest experimental temperatures that the animals are able to tolerate may be close to 0 °C since the *B. amphitrite* complex was found in 3 °C water in Shinji lake, Japan (Iga, 1973). Anil and Kurian (1996) observed that lower mortality rates of *B. amphitrite* occurred at 20 °C than at 30 °C in different salinity levels of 15, 25, and 35 ‰ in general. Another salinity tolerance study of adult *B. amphitrite* was carried out by Simpson and Hurlbert (1998) determined the effects of 7 salinity levels (43, 60, 70, 75, 80, 90, and 100 ‰) reached by salt addition and evaporative concentration on *B. amphitrite* mortality. Mortality was usually greater than 50 % at 80 ‰ or greater salinities after 12 days of experiment. The 12 day lethal concentration (LC<sub>50</sub>) in evaporated water was 89 ‰ and in salt-added water was 83 ‰. Simpson and Hurlbert (1998) concluded that there was no significant difference between the two salinity manipulation methods ( $p > 0.3$ ).

#### Scutum Resistance Experiment

The test of *Balanus amphitrite* consists of a circular wall composed of a number of more or less rigidly articulated compartmental plates with or without a calcarous basal disc (Brooks *et al.*, 1969). The orifice of the barnacle is occupied by four opercular valves (or plates) called the paired “terga” and “scuta.” In this experiment, to determine whether the animal was dead or alive, resistance force (g) of scutum (pl) was measured after survival experiments. Simpson and Hurlbert (1998) also performed survival experiments on *B. amphitrite* from the Salton Sea and used the criteria of death by touching its operculum to see if it would give way or not. While this method could underestimate the number of dead barnacles, tissue decay was very rapid so that a barnacle was unequivocally recognized as dead within 24 hours of death (Simpson and Hurlbert, 1998).

## Morphological Experiment

Morphological appearance of *B. amphitrite* was described in detail by Brooks *et al.* in 1969. Brooks *et al.* (1969) indicated that the test wall of Suborder Balanomorph includes eight primary plates at most: a rostrum, paired rostrolaterals, paired laterals, paired carinolaterals, and a carina. In *Balanus*, the rostralateral plate is considered to be fused into the rostrum (See Figure 2.6.A). Therefore, the true rostrum has been eliminated, and a total of six compartmental plates makes *B. amphitrite* take on a different appearance from the eight compartmental plates of *Chthamalus*.

The sessile barnacle, *B. amphitrite*, has a rigid, symmetrical test with articulated opercular valves (Newman, 1996). According to Newman (1996), the higher balanoids develop their structures within the plates in order to have greater strength as well as utilize less building material, facilitate rapid growth, and to defend against erosion and predation. All thoracican barnacles have solid wall plates covered by a thin, more or less persistent chitinous cuticle. In general, the plates grow primarily by adding their margins basally and laterally (diametrically), and demarcated layers frequently show faint lines of organic matrix in their test corresponding to growth lines on their exterior test (Ushirokawa and Yamaguchi, 1989).

According to Brooks *et al.* (1969), both determinate and indeterminate growth occurs in the *Balanomorpha*. It was also demonstrated that, in many species of *Balanus*, the full size of the animal may be reached within the first year after settlement with only low growth rate occurring after the second year. However, growth of the test wall and base of *Balanus* appears to continue throughout its life. Linsley and Carpelan (1961) even found that the basal diameter of *B. amphitrite* in the Salton Sea during summer was

9 mm after 30 days of settlement, which was also consistent with their growth rate observed in laboratory conditions.

#### Animal Test Thickness Experiment

Morphological differences between the Salton Sea and San Diego Bay barnacles were apparent in their test thickness during animal collections for this study, as Rogers (1949) and Henry and McLaughlin (1975) recognized decades ago. When Simpson and Hurlbert (1998) studied the effects of salinity on the growth and strength of *B. amphitrite* from the Salton Sea with 5 different salinity levels (30, 39, 48, 57, and 65 ‰), *B. amphitrite* wall thickness on the settling plates was greatest at 39 and 48 ‰ while the diameter of the animals was also greatest at 39 and 48 ‰. Relative to their diameter, *B. amphitrite* grown at salinities above 39 ‰ were shorter and had thicker walls than those that grow in seawater below 39 ‰. The force required to break the test was greatest for animals grown in 48 ‰, although strength of the wall declined steadily as the salinity increased. Simpson and Hurlbert (1998) also showed that *B. amphitrite* from the Salton Sea grown at 48 ‰ seemed to be able to withstand physical stress. At the higher salinities, *B. amphitrite* were shorter and had thicker walls relative to their diameters, which may strengthen their structural stability. Although results of Simpson and Hurlbert's (1998) experiment indicated that there is an effect of salinity on the growth and strength of *B. amphitrite* from the Salton Sea, they concluded that observed effects of salinity on the growth rate and size of these barnacles may not reflect an evolutionary change within the population.

## CHAPTER TWO

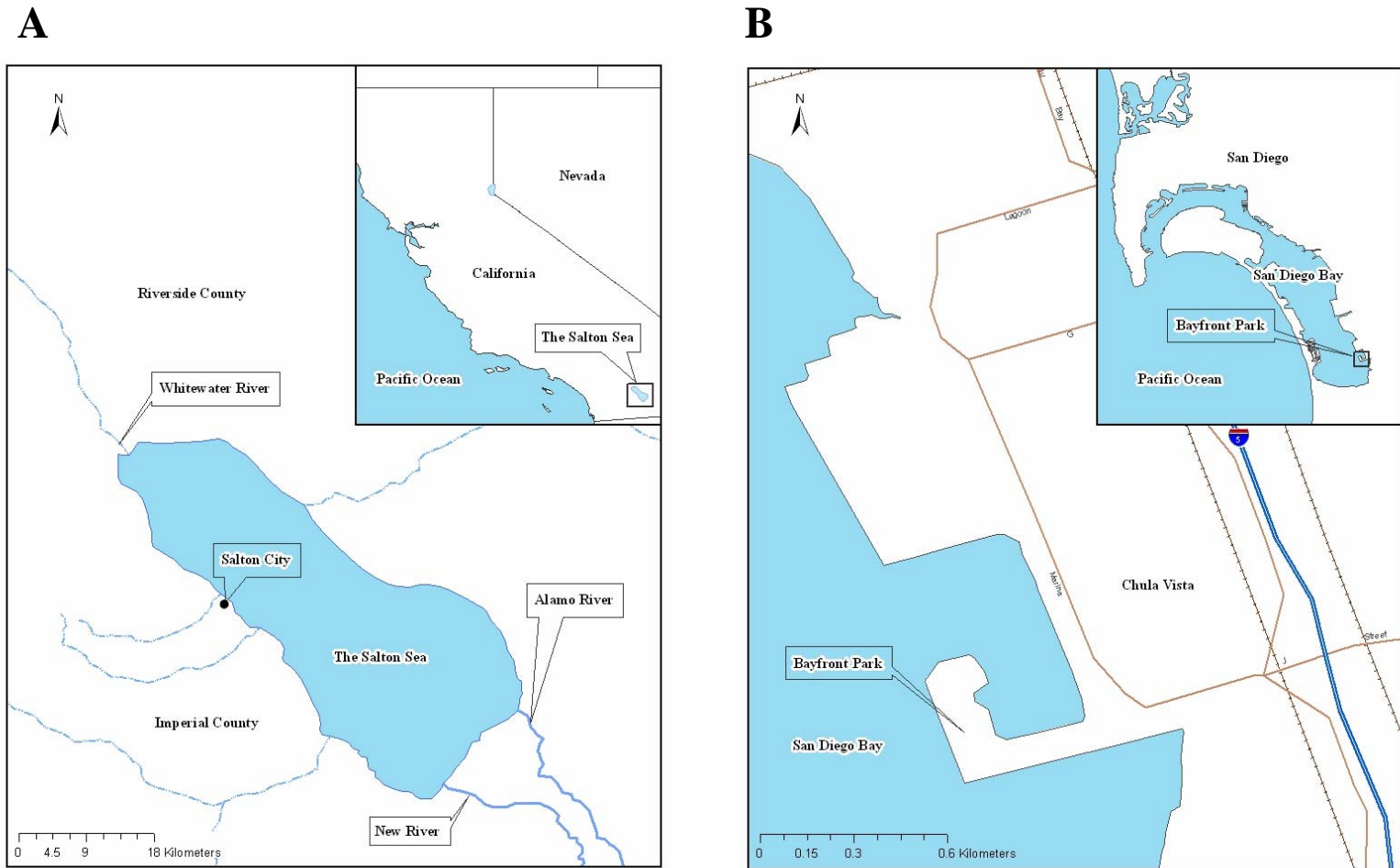
### MATERIALS AND METHODS

#### **Project Location**

Animal collection sites were located at the embankments of Salton City at the Salton Sea (33° 32' N, 115° 94' W) (Figure 2.1.A) and Bayfront Park at the southern end of San Diego Bay (32° 37' N, 117° 06' W) (Figure 2.1.B) in California. Laboratory work was done at the Marine Research Group Laboratory and room A540 of the Islet Transplant Laboratory Research wing of Loma Linda University Medical Center, on the campus of Loma Linda University, California.

#### **Collection and Care of Animals Before Experiments**

In order to study the influence of extreme conditions on physiological responses, individuals from two populations of *B. amphitrite* from the Salton Sea and San Diego Bay were compared. According to Yamaguchi (Pers. Comm.), this species can attain a diameter of up to 1 cm, and the animals become sexually mature after the first year. Therefore, adult barnacles with diameter approximately 1 cm were collected from both sites and brought back to the Marine Research Group Laboratory at Loma Linda University in order to measure respiratory responses and survival rates in 18 combinations of salinity and temperature (Table 2.1) as well as measurements for tension of tergum/ scutum and animal test thickness. Collection of the animals was done at several times throughout the experiments as Figure 2.2 and 2.3 indicate. During the collection of animals, barnacles were carefully scraped off the substrate with a 1/2" flat edged Cold Chisel and a hammer to avoid breaking the base of the test from the substrate.

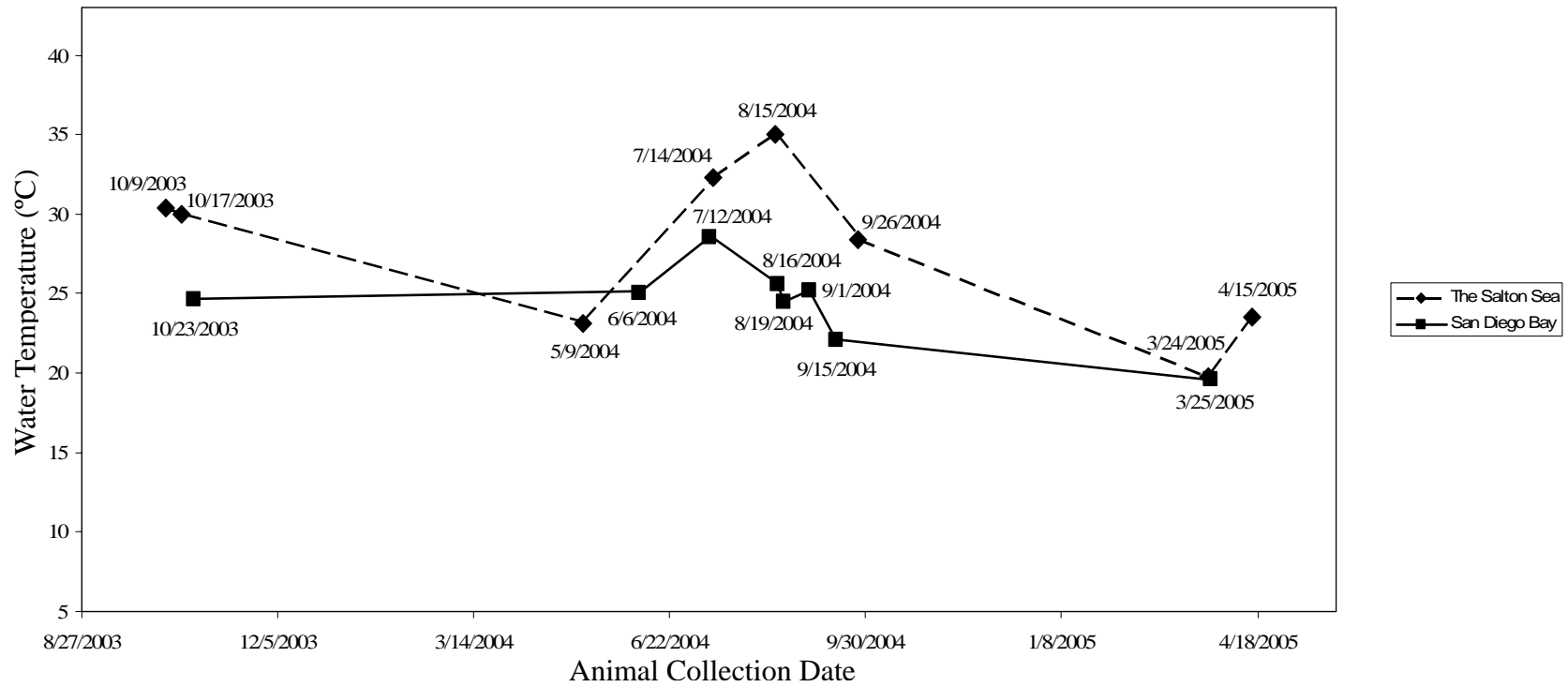


Map source: Street map of U.S.A., ESRI

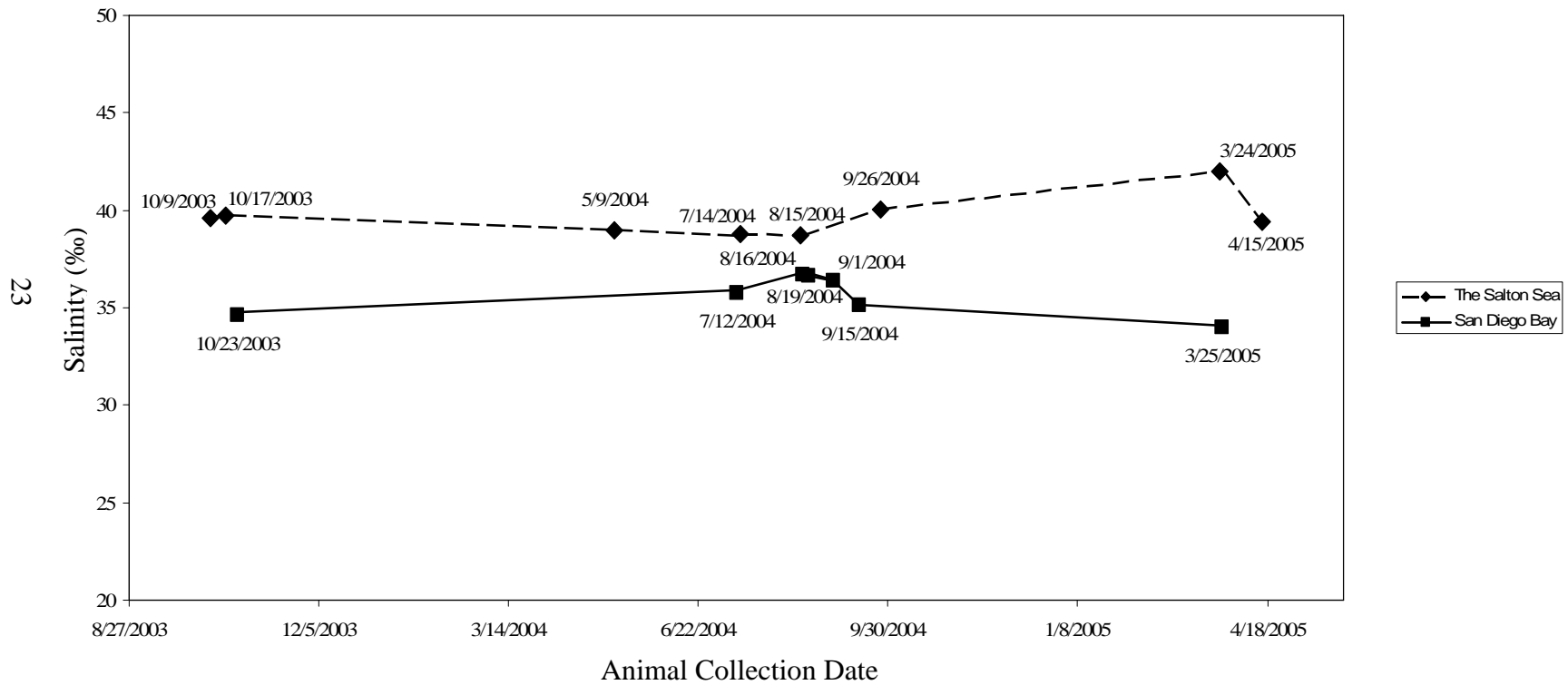
**Figure 2.1.** Map of animal collection sites at **A:** Salton City at the Salton Sea, CA. **B:** Bayfront Park at San Diego Bay, CA.

**Table 2.1.** Eighteen combinations of experimental conditions for the Salton Sea and San Diego Bay barnacles.

Salinity (‰) Temperature(°C)	0	15	30	36	45	60
15						
25						
35						



**Figure 2.2.** Water temperature (°C) of animal collection sites for Salton City at the Salton Sea and Bayfront Park at San Diego Bay. Dates are the day and year of animal collections.



**Figure 2.3.** Salinity (‰) of animal collection sites for Salton City at the Salton Sea and Bayfront Park at San Diego Bay. Dates are the day and year of animal collections.

The Salton Sea population tended to grow on top of each other more densely than the San Diego Bay population. Therefore, barnacles from the Salton Sea were much easier to collect than San Diego Bay barnacles. At each collection, measurements of salinity and water temperature were recorded with a Model WP-84 Conductivity-Salinity-Temperature meter while dissolved oxygen and air temperature were also recorded simultaneously with a TPS90D-DO<sub>2</sub> Meter. Although salinity of the Salton Sea and San Diego Bay was recorded, constituents of the salt for both locations were not measured or calculated.

Animal tests were brushed clean to rid them of any algae or dirt, and each population was placed separately in a 10 gallon aquarium tank, with conditions set to represent the environment where each group was collected. Water temperature of the Salton Sea tank was set to  $30 \pm 2$  °C, which fell within the range of water temperatures of the collection site of the Salton Sea (Figure 2.2) throughout the entire study. For the San Diego Bay tank, water temperature was set to  $25 \pm 2$  °C, which also fell within the range of the collection site of San Diego Bay (Figure 2.2) throughout the whole study. In order to control the water temperature, 50 Watt Proquatics Hydromatic Aquarium Heaters for 10 gallons (range 20 ° to 33 °C) were used. The salinity level of the Salton Sea group was kept at  $39 \pm 2$  ‰, which was the mean salinity level of the collection site (Figure 2.3), while the San Diego Bay group was kept at  $35 \pm 2$  ‰ sea water, which also represented the mean salinity level of the collection site of the animals (Figure 2.3). For the experiments, hypertonic salinities were made by adding Kiln Dried Sea Salt (Bolekscrafts) to sea water collected from the Pacific Ocean. Salton Sea water was not used for either experiments or laboratory conditions throughout this study, and it is

recognized that this should be done in a further study. In order to be consistent with the acclimation of the animals, the same temperature and salinity levels were used for all acclimation tanks throughout the investigations. Water circulation within the tank was done with a Rio Aqua Pump/ Powerhead 90, and aquarium tanks were kept aerated in the Marine Research Group Laboratory of Loma Linda University. Animals were fed with freshly hatched Brine Shrimp, *Artemia salina* nauplii, every other day. Aquarium water was changed every other week to clean all leftover Brine Shrimp and dead barnacles.

Prior to each experiment, animals were acclimated to laboratory conditions for at least 5 days. Before each experiment, animals were brushed clean and wiped with paper towel except for the tergum and scutum in order to avoid absorbing excess seawater inside the test. Brushing the animals' test was important for avoiding any possible confounding affects of algal respiration with barnacle respiration. Morphological measurements described in Morphological Experiment of Materials and Methods section were recorded prior to each experiment.

### **Oxygen Consumption Experiment**

In order to measure the rate of oxygen consumption of the barnacles, a closed cell Strathkelvin 928, 6-Channel Dissolved Oxygen System respirometer was used in room A540 of the Islet Transplant Laboratory located in Research wing of Loma Linda University Hospital on the campus of Loma Linda University. The apparatus consisted of six chambers and allowed the six individual oxygen electrodes to be sealed into each of the chambers while recording the rate in  $\mu\text{l/h}$  every 20 s for 3 hours (Figure 2.4). Three hour intervals were chosen for the experiment since preliminary data showed that the animals used up available oxygen in the 3 ml sealed chambers after 4 hours at each



experimental condition. After 4 hours of treatment, the rate of oxygen consumption leveled off.

One of the six chambers contained only sea water without an animal and was used as a control while the other five chambers were used as treatments. Consumption rates measured by the probes were transferred to a computer through the Strathkelvin 928 interface and plotted as lines. Final rates of oxygen consumption over 3 hours for each individual barnacle were calculated by the computer and recorded at the end of each treatment. Furthermore, final oxygen consumption rate was divided by the body weight of the animal in order to calculate the weight-specific oxygen consumption rate. Probe membranes were changed every three to four weeks to ensure optimal operation of the system recorder. The water bath of the system was connected to a recirculating chiller (range from - 5 ° to 40 °C), which maintained treatment temperatures at 15°, 25°, and 35 °C.

The highest experimental temperature, 35 °C, was chosen based on the highest Salton Sea water temperature, 33 °C, in 2003 (Figure 2.5). Creating extreme, but non-lethal conditions for the barnacles was important for choosing experimental conditions. The other two temperatures were chosen at successive 10 °C intervals below 35 °C giving 25° and 15 °C.

Oxygen consumption rates were recorded in 18 combinations of salinity and temperature. Six salinity levels (0, 15, 30, 36, 45, and 60 ‰) were chosen to represent the extreme range of water in which *B. amphitrite* can be found. According to a study by Pérez (1994), *B. amphitrite* from the Salton Sea appeared to be threatened the most when the salinity level exceeded 60 ‰. By 60 ‰, reproductive rates sharply decreased and the



animals clearly showed short-term aversive responses to the hypersaline water. Therefore, as the highest-nonlethal salinity level, 60 ‰, was chosen to represent the extreme hypersaline condition. Between 0 and 60 ‰, three other salinity levels with intervals of 15 ‰ were chosen, while 36 ‰ was also included in order to represent the approximate salinity level of normal seawater.

Oxygen consumption experiments began by selecting 5 individual barnacles within a 2.69 to 10.54 mm size range of test diameter from either the Salton Sea or San Diego Bay tank. The Salton Sea and San Diego Bay populations were alternately exposed to the treatment. Animals were unfed at least 12 hours prior to the start of each experiment. Other measurements on morphology of the animals were done at this time as explained in Morphological Experiment of Materials and Methods section. Each chamber in the experimental set-up held 3.3 ml of salinity-controlled sea water, and the temperature inside each chamber was checked with a thermometer to ensure the specified temperature was reached. Before every experiment, probes were calibrated to zero with an oxygen-free solution of Sodium sulphite in distilled water, as well as to 100 % air saturated water by bubbling air through the sea water at the same temperature as the treatment. After calibration of the probes, animals were sealed inside the chambers with the probes. Measurements of oxygen consumption rate were done without stirring fleas since each chamber size was small (3 ml). In order to acclimate the animals to the experimental conditions, recording was initiated after the animal had been exposed to the chamber conditions for 5 minutes. The whole apparatus was covered with Styrofoam to help keep the temperature constant. After the experiment, animal weight, including the test, was measured again as well as animal body weight only (without the test). If the

animal was dead at this point of dissection, the measurements were discarded to exclude the data of dead barnacles in order to make sure that the experiment was performed on only live animals. All treatment and control experiments were replicated three times, thus 540 barnacles were used for both the Salton Sea and San Diego Bay populations, resulting in a total of 1080 animals.

After all measurements were completed, oxygen consumption rate was analyzed by a Model I, three-way ANOVA for “Group,” “Temperature,” and “Salinity” followed by post-hoc Tukey pairwise analyses. For further analysis of significant results on the three-way ANOVA of oxygen consumption data, a Simple Main Effects (SME) was performed by singling out each group for analysis. Comparing one group (the Salton Sea or San Diego Bay population) on two variables (salinity and temperature) that showed significant interactions at a time helped to see where the significance existed. Along with the SME, a Post Hoc Tukey test was performed. The data were also analyzed by Pearson correlation coefficients for salinity, temperature, animal whole weight before experiment, diameter, height, and oxygen consumption rate for each group.

### **Survival Experiment**

In order to determine the tolerance of *B. amphitrite* to the 18 combinations of salinity and temperature used in oxygen consumption experiments, survival rates of animals to each condition were measured. Each survival test was run for 48 hours, and animals were checked every 8 hours to see whether they were dead or alive. In order to determine the death of the animal, the scutum of the barnacle (Figure 2.6.A) was pulled with forceps attached to a spring scale to measure the resistance. If the scutum of the animal showed strong resistance (191 to 202 g) and closed up tightly, the barnacle was



considered alive. On the other hand, dead barnacles had much less resistance (133 to 156 g) with their scutum, which sometimes caused their body, especially cirri, to come out of their tests completely. An OHAUS spring scale (range from 0 to 2000 g) with forceps attached with 1 cm wide, 3M Micropore tape at the end was used to measure how much tension it took to pull the scutum from the tests. Only one side of the scutum was pinched with the forceps attached to the spring scale, and the point of the forceps holding the scutum was wrapped with tape to hold the shell strong enough to pull out from the animal test. The spring scale was carefully pulled until one side of the scutum came off, and the measurement was recorded. In order to be consistent with measuring resistance, the spring scale was pulled at the same rate, as much as possible. Measured tension range was from 0 to 500 g with intervals of 25 g. Each measurement was recorded along with the other morphological measurements described in Morphological Experiment of Materials and Methods section.

The total number of animals used in this experiment was 468 for both populations, Salton Sea and San Diego Bay, thus 234 animals for each group. A 27.5 w × 59 l × 36 h cm water bath was connected to the same chillers used in the oxygen consumption experiment to keep the temperatures stable at 15°, 25°, and 35 °C. In the water bath, 40, 180 ml cups were placed side by side, floating in 20 cm of water. Each cup contained one of 18 different experimental conditions of sea water with an individual barnacle brushed-clean from either the Salton Sea or San Diego Bay. A total of 12, two-day experiments were done in the Marine Research Group Laboratory with 13 replications for each condition. Controls of the experiment were set as 36 ‰ at 25 °C, as representative of ocean salinity and temperature. Preliminary data on oxygen saturation of sea water in

each container were collected with a TPS90D-DO<sub>2</sub> meter to make sure that low oxygen saturation was not a confounding factor in the survival rate of the animals. Each container had 150 ml of water, and oxygen saturation was measured every 8 hours for 2 days. Results of the preliminary data showed that each experimental condition, even at the condition with highest temperature (35 °C) and salinity (60 ‰) had an oxygen saturation of over 59 %. As a result, oxygen availability was not considered a factor in survival.

After all experiments and measurements were completed, survival rates for each experimental condition were calculated and graphed. Survival rate was analyzed by T<sub>50</sub>, which shows when 50 % of a population succumbs to the treatment condition.

#### Scutum Resistance Experiment

In order to test the hypothesis that there is a significant difference in the resistance force of the scutum between live versus dead barnacles from the Salton Sea and San Diego Bay, tension to remove one side of the scutum from a series of live barnacles (not used in any experiments) was measured. One hundred barnacles were freshly collected from each location and acclimated to laboratory conditions used for previous experiments for at least 5 days. Measurements of tension were recorded by using an OHAUS spring scale (see description in Survival Experiment of Materials and Methods section).

A one-way ANOVA was conducted in order to evaluate the effects of the “state” (dead or alive) of the animals, on tension of the scutum.

#### **Morphological Experiment**

Although *B. amphitrite* from the Salton Sea and San Diego Bay appeared different during the collection of the animals (Figure 2.7.A and B), barnacles from those





two locations were recognized as the same species by Flowerdew (1985) based on genetic analysis. Newman of Scripps Institution, University of California in San Diego (Pers. Comm.) stated that *B. amphitrite* with uneroded tests (Figure 2.7.A and 2.8.A) could be distinguished externally by the presence of disparietal rather than transparietal radii (summits of radii oblique rather than parallel to the base) (Pers. Comm.). If the animal tests were eroded (Figure 2.7.B and 2.8.B), internal investigations were performed by dissecting the animals to see whether the labrum, an unpaired outgrowth arising just in front of the mouth and more or less covering it, was multidenticulate or not (Newman, Pers. Comm.). Yamaguchi (1997a) described the base color of the test of *B. amphitrite* as “dirty white.” He also described the radii found on the entire external surface except the central trapezoidal areas of the rostrum and lateral compartments as grayish blue to purplish blue or reddish purple. Although the patterns of stripes on external tests in the current study varied between individuals, both color and general pattern of *B. amphitrite* from the Salton Sea appeared consistent with those of San Diego Bay barnacles. However, the reddish-purple stripes of some animals from San Diego Bay faded and became a dirty whitish color (Figure 2.8.B).

For morphological comparisons between populations, the following measurements were recorded for each experimental treatment: (1) diameter; (2) height; (3) animal whole weight (including test) before the experiment; (4) animal whole weight (including test) after the experiment; (5) animal body (only) weight; (6) shell weight. A SPI 2000 Dial Plastic Caliper accurate to .001 inch was used for measuring the length of the animals in inches. Measurements were then converted into millimeters using the conversion factor: (X inch • 2.54 cm • 10 mm). A Mettler Toledo PG-S balance

measuring to 0.001 g was used for weighing the animals in grams. After the experiment, each animal was removed from the test by cutting the scuta depressor muscle connected to the scutum (Figure 2.6.B) and extracting the animal body attached to the test out with forceps. The removed tergum and scutum were placed with the empty test inside a drawer to dry in ambient air. The weight of the whole shell was measured the next day.

In order to determine if there was a statistical difference in the morphology of the two populations used in all experiments, a one-way Multivariate Analysis of Variance (MANOVA) was used on a total of 1288 animals from both locations. Variables used for determining morphological differences were diameter and height of the animals. An ANOVA on each dependent variable was conducted as a follow-up test to the MANOVA. Using the Bonferroni method, each ANOVA was tested at the 0.05 level. Since some animals used in experiments contained other substrates such as rocks with the base of their tests, total weigh including tests did not represent an accurate measure. In addition, animal body weight was only measurable after experiments, thus body only weights of animals after treatments were not used for statistical analysis on morphological comparisons between Salton Sea and San Diego Bay individuals.

#### Animal Test Thickness Experiment

Animal test thickness was measured with an SPI 2000 Dial Plastic Caliper ranging from .001" to 6" by pinching the top end of the carina (external test compartment) (Figure 2.6.A) attached to the tergum of the barnacle. By measuring test thickness, the relation to morphological differences in strength of the tests between the Salton Sea and San Diego Bay barnacles could be inferred. Measuring the top end of the carina gave a more accurate thickness measure than the compartment of the rostrum

attached to scutum (Figure 2.6.A) since the shape of the carina was straighter than the curved shape of the rostrum. Measuring test thickness for both groups could provide evidence of differences in test formation. One hundred animals from each location were collected for this test thickness experiment.

A one-way ANOVA was conducted to evaluate the relationship between test thickness and “group.” The independent variable, “group”, included two levels: Salton Sea and San Diego Bay. The dependent variable was test thickness. Pearson correlation coefficients were computed for the variables animal diameter, animal height, and test thickness.

## CHAPTER THREE

### RESULTS

#### Oxygen Consumption Experiment

In order to evaluate the effects of different combinations of temperature and salinity on weight-specific oxygen consumption rate ( $\mu\text{l/h/g}$ ) of *B. amphitrite* from the Salton Sea and San Diego Bay, a three-way ANOVA was conducted. The means (with standard errors) of weight-specific oxygen consumption rate as a function of the three factors for the Salton Sea and San Diego Bay groups are presented in Table 3.1.

ANOVA results showed that there was no significant difference ( $F_{1, 503} = 0.65, p > 0.05$ ) (Table 3.2) in weight specific oxygen consumption rate at 18 different combinations of experimental conditions between groups. However, there was a significant interaction between group and salinity ( $F_{5, 503} = 3.33, p < 0.01$ ) (Table 3.2). There was also a significant interaction between salinity and temperature on oxygen consumption ( $F_{10, 503} = 2.39, p < 0.01$ ) (Table 3.2). Following the three-way ANOVA, the results of a two-way ANOVA on salinity and temperature for the Salton Sea group still resulted in a significant interaction ( $F_{10, 252} = 2.23, p < 0.05$ ) (Table 3.3.A) while for the San Diego Bay group there were no significant interactions, ( $F_{10, 252} = 1.05, p = 0.40$ ) (Table 3.3.B).

Effects of experimental temperatures on oxygen consumption rate for both the Salton Sea and San Diego Bay groups are shown in Figures 3.1 to 3.3. Comparisons of mean weight-specific oxygen consumption rate between the Salton Sea and San Diego Bay at 15 °C (Figure 3.1) indicated that the Salton Sea barnacles consumed significantly more oxygen in water of 45 ‰ than did the San Diego Bay barnacles (SS =  $249.03 \pm 39.44$ ; SDB =  $130.27 \pm 16.13$ ;  $F_{1, 28} = 7.77, p < 0.01$ ). Comparisons of













consumption rates in other salinities at 15 °C did not show any significant differences between groups. At 25 °C with all experimental salinity levels (Figure 3.2), there was no significant difference in weight-specific oxygen consumption rates observed between groups. At 35 °C (Figure 3.3), consumption rates of the San Diego Bay group were generally lower than the Salton Sea group, except at 0 ‰ (SS =  $165.75 \pm 19.12$ ; SDB =  $247.62 \pm 31.85$ ;  $F_{1,28} = 4.86$ ,  $p < 0.05$ ).

Effects of salinity on weight-specific oxygen consumption rate for both the Salton Sea and San Diego Bay groups are compared in Figures 3.4 to 3.9. These graphs indicated that there was a significant difference in consumption rate at only the 15 °C in 45 ‰ and 35 °C in 0 ‰ conditions when species were compared over all combinations. From the observation of Figures 3.4 to 3.9, the San Diego Bay group consumed significantly more oxygen compared than the Salton Sea group in the lower salinity (0 ‰) at 35 °C, while the Salton Sea group consumed significantly more oxygen than the San Diego Bay group in higher salinities with low temperature, such as 45 ‰ at 15 °C.

The highest weight-specific oxygen consumption rate over all experimental conditions within the Salton Sea group was observed in 36 ‰ at 25 °C (Figure 3.10), while the highest rate for the San Diego Bay group was seen in 30 ‰ at 25 °C. There were no significant differences between the three temperatures at 36 ‰ for either the Salton Sea group (Figure 3.10) or the San Diego Bay group (Figure 3.11). On the other hand, there were significant differences ( $p < 0.05$ ) between 15 ° and 25 °C and between 15 ° and 35 °C in 30 ‰ for the San Diego Bay group (Figure 3.11). However, there was no significant difference in consumption rates between 25 ° and 35 °C in 30 ‰ for the San Diego Bay group. It can be seen in Figure 3.10 and 3.11 that both groups have lower

















respiratory activities at 15 °C compared within the other temperatures over the range of salinities, except in 0 ‰, for the Salton Sea group. Significant differences between 15 °C and higher temperatures, 25 ° and 35 °C, occurred in 15 ‰ ( $p < 0.05$ ) and 30 ‰ ( $p < 0.01$ ) for the Salton Sea group (Figure 3.10). In 0 ‰ (Figure 3.10), significant differences in consumption rates between 15 ° and 25 ° and between 15 ° and 35 °C were not seen, although consumption rates were significantly different ( $p < 0.01$ ) between 25 ° and 35 °C. All other experimental conditions at 36, 45, and 60 ‰ in Figure 3.10 did not indicate any significant differences. The San Diego Bay group indicated another significant difference between 15 ° and 25 ° and between 15 ° and 35 °C in 30 ‰ ( $p < 0.05$ ) as well as in 45 ‰ ( $p < 0.01$ ) (Figure 3.11). At the other experimental salinities of 0, 15, 36, and 60 ‰ seen in Figure 3.11, no significant differences in oxygen between temperatures occurred.

Correlation coefficients ( $r$ ) were computed among the following seven variables for each group: salinity, temperature, animal diameter, animal height, animal whole weight before experiment, oxygen consumption rate, and weight-specific oxygen consumption rate. The results of the analyses for the Salton Sea group presented in Table 3.4.A show that 8 out of the 20 correlations were statistically significant ( $p < 0.05$ ), while results for the San Diego Bay group presented in Table 3.4.B indicated that 9 out of the 20 correlations were statistically significant ( $p < 0.05$ ).

Table 3.4.A and 3.4.B indicate small, but significant correlations ( $r = +/- 0.10$  to  $+/- 0.29$ ) between temperature and weight-specific oxygen consumption rate for both the Salton Sea ( $r = 0.215$ ) and San Diego Bay ( $r = 0.188$ ), animal diameter and height for both the Salton Sea ( $r = - 0.127$ ) and San Diego Bay group ( $r = - 0.262$ ), as well as whole





weight before experiment and weight-specific oxygen consumption rate for both the Salton Sea (- 0.152) and San Diego Bay (- 0.168). Diameter and animal whole weight before experiment showed medium correlations ( $r = +/- 0.30$  to  $+/- 0.49$ ) for both the Salton Sea ( $r = 0.483$ ) and San Diego Bay ( $r = 0.319$ ). Animal height was strongly correlated ( $r = +/- 0.50$  to  $+/- 1.0$ ) with animal whole weight before experiment for both groups, (SS:  $r = 0.558$ , SDB:  $r = 0.510$ ). For the San Diego Bay group, a small, significant correlation existed between salinity and weight-specific oxygen consumption rate ( $r = - 0.124$ ) while for the Salton Sea group no such correlation was found. The correlation of diameter with weight-specific oxygen consumption rate for San Diego Bay group was significant ( $r = - 0.014$ ) while for the Salton Sea group it was not. The Salton Sea group showed a small, significant correlation of height with weight-specific oxygen consumption rate ( $r = - 0.168$ ) while there was no relationship of height with weight-specific oxygen consumption for the San Diego Bay group.

For non weight-specific oxygen consumption rate, correlation analyses were done with animal diameter, height, and whole weight before experiment for both populations (Table 3.4.A and 3.4.B). Only the Salton Sea group (Table 3.4.A) showed a small, but positive-significant correlation ( $r = 0.122$ ) between animal diameter and non weight-specific oxygen consumption rate. The San Diego Bay group (Table 3.4.B) still showed a negative correlation ( $r = - 0.116$ ) between animal diameter and non weight-specific oxygen consumption rate. The Salton Sea group showed a positive correlation ( $r = 0.117$ ) between animal whole weight before experiment and non weight-specific oxygen consumption rate (Table 3.4.A). However, the San Diego Bay group (Table 3.4.B) still

showed a negative correlation ( $r = -0.034$ ) between animal whole weight before experiment and non weight-specific oxygen consumption rate.

### **Survival Experiment**

Analysis of the times in which 50 % of the treatment group died ( $T_{50}$ ) was conducted in order to evaluate the effects of 18 experimental combinations of salinity and temperature on the survival of barnacles from both the Salton Sea and San Diego Bay over 48 hours. Comparisons of percent survival between the groups at three different temperatures are shown in Figures 3.12 to 3.14. The only temperature that reduced the treatment groups to 50 % or less was 35 °C (Figure 3.14). However, the graph indicates that only in 0 and 15 ‰ was 35 °C lethal beyond 50 % for both groups. The San Diego Bay group exposed to 0 ‰ was reduced to 50 % within approximately 18 hours, while the Salton Sea group in 0 ‰ took approximately 21 hours to be reduced to 50 %. In 15 ‰ the San Diego Bay group took approximately 29 hours to fall to 50 %, whereas the Salton Sea group withstood approximately 44 hours of the experiment before falling to 50 %. All barnacles from San Diego Bay in 0 ‰ were dead within 40 hours, while barnacles from the Salton Sea were able to tolerate this condition for an additional 8 hours. At 15 ° and 25 °C, no group was reduced to 50 % over the 48 hours of the experiment (Figures 3.12 and 3.13). For 35 °C in 60 ‰ (Figure 3.14), the slopes for percent survival of both the Salton Sea and the San Diego Bay populations were calculated. For both populations,  $T_{50}$  values were estimated at 35 °C in 60 ‰ using the linear equations  $y = -0.6319x + 106.5$  for the Salton Sea and  $y = -1.1813x + 113.85$  for San Diego Bay. The estimated  $T_{50}$  for the Salton Sea population was 89 hours, while the  $T_{50}$  for the San Diego Bay population was 54 hours. The results indicated that the San







Diego Bay population is more susceptible to the high temperature of 35 °C at the outer edges of salinities, 0, 15, and possibly 60 ‰ compared to the Salton Sea population.

Comparisons of percent survival between groups at 6 different salinities are shown in Figures 3.15 to 3.20. In 0 ‰ (Figure 3.15), the San Diego Bay group at 35 °C was reduced to 50 % within approximately 18 hours while the Salton Sea group at the same temperature took approximately 21 hours to fall to 50 %. Both the Salton Sea and San Diego Bay group at 35 °C died out at 40 and 48 hours, respectively. Similarly, in 15 ‰ (Figure 3.16), the San Diego Bay barnacles at 35 °C took approximately 27 hours to be reduced to 50 % while the Salton Sea barnacles at 35 °C were reduced to 50 % within approximately 44 hours. However, both groups took a longer time to be reduced to 50 % compared to the groups in 0 ‰ and did not die out completely by the end of experiment at 48 hours. Neither group was reduced to 50 % during the experiments at the other experimental salinities of 30 ‰ (Figure 3.17), 36 ‰ (Figure 3.18), 40 ‰ (Figure 3.19) and 60 ‰ (Figure 3.20). Other than all temperatures in 0 and 15 ‰ (Figures 3.15 and 3.16), the only group which was reduced to almost half its population was from San Diego Bay in 60 ‰ at 35 °C (Figure 3.20).

#### Scutum Resistance Experiment

The primary purpose of this study was to determine if there was a significant difference in the tension measured in grams of the scutum for animals that were dead versus those that were alive. A one-way ANOVA was conducted in order to evaluate the effects of “state” (alive or dead) and tension of the scutum. Means and standard errors on scutum resistance measurements are presented in Table 3.5. According to Table 3.6, there was a significant difference in the tension of the scutum between live versus dead















barnacles. The barnacles that were alive had significantly stronger resistance of the scutum to an outside stimulus compared to the dead barnacles, which showed significantly weaker or no resistance to pulling of the scutum with forceps.

### **Morphological Experiment**

A one-way MANOVA was conducted to determine if animal diameter (mm) and height (mm) were different for the two populations. The results indicated a significant difference between groups for both animal diameter ( $F_{1, 1286} = 101.14, p < 0.001$ ) and height ( $F_{1, 1286} = 111.98, p < 0.001$ ). Means and standard errors measured for diameter (mm) and height (mm) were the following: Salton Sea (n = 644, Diameter:  $7.53 \pm 0.06$  mm, Height:  $8.23 \pm 0.06$ ) and San Diego Bay (n = 644, Diameter:  $8.32 \pm 0.06$  mm, Height:  $7.29 \pm 0.07$  mm). There is a significant difference between the populations for the morphological variables, animal diameter (mm) and height (mm) ( $F_{2, 2570} = 99.64, p < 0.01$ ). Barnacle tests of the Salton Sea population are significantly taller and thinner than those in San Diego Bay (Figure 3.21).

### **Animal Test Thickness Experiment**

A one-way ANOVA was conducted to evaluate if there was a significant difference in animal test thickness (mm) between groups. The independent variable was group (Salton Sea and San Diego Bay populations), while the dependent variable was animal test thickness measured for both populations (the Salton Sea, n = 100; San Diego Bay, n = 100). Mean test thicknesses for both groups are listed in Table 3.8, and the results of the ANOVA are shown in Table 3.9. Taken together, these results show that the San Diego Bay population has significantly thicker tests than the Salton Sea population ( $F_{1, 198} = 11.11, p < 0.01$ ) (Table 3.9). Since there is a significant difference in animal test







thickness between the Salton Sea and San Diego Bay populations, differences in *B. amphitrite* appearance between those locations may be related to test thickness.

Comparisons of mean animal diameter, height, and test thickness between the groups are shown in Figure 3.21. Pearson correlation coefficients are computed among the three variables: animal diameter, animal height, and test thickness. According to Table 3.10a and 3.10b, there is a small correlation between animal diameter and height ( $r = -0.208$ ) for the Salton Sea group while for the San Diego Bay group there is no significant relationship for these variables. A small significant correlation ( $r = .239$ ) between animal height and test thickness for the Salton Sea group was also found, while for the San Diego Bay group there was no significant correlation for these measures. Interestingly, the San Diego Bay group shows a small correlation between animal diameter and shell thickness ( $r = .290$ ) whereas the Salton Sea group does not show this relationship. Since some tests contained substrate debris, test weight was not incorporated into the correlation analysis.



## CHAPTER FOUR

### DISCUSSION

#### **Oxygen Consumption Experiment**

Although there was no overall significant difference in weight-specific oxygen consumption rates in 18 combinations of salinity and temperature between *B. amphitrite* from the Salton Sea and San Diego Bay, both salinity and temperature had a significant effect on the amount of oxygen consumed by the animals during the experiment. Significant differences in oxygen consumption were seen under analysis of specific combinations of salinity and temperature. Further statistical analysis indicated that there was a significant interaction of salinity and temperature on oxygen consumption for the Salton Sea group, while for the San Diego Bay group there was no such interaction.

#### Cirral Activity with Respiration and Related Issues

After analyzing the results of the oxygen consumption experiment and in consultation with the primary literature, the crucial relationship between respiration and cirral activity of the barnacles became more distinct. Southward (1955a, 1955b, and 1957) was the first person to study the effects of temperature on cirral activity in barnacles. Southward and Crisp (1965) suggested that the beating of barnacle cirri function as a means of respiratory exchange and food capture. Newell and Northcroft (1965) also related cirral activity to oxygen uptake. Cirral activity in barnacles has been used as an indicator of active metabolic rates (Crisp and Southward, 1961). A constant relationship between normal cirral activity and the active metabolic rate at any given temperature has been demonstrated (Crisp and Southward, 1961), with the relationship based on the log of the cirral beat rate being inversely proportional to the log of the body

weight (Newell and Northcroft, 1965). Cirral activity is believed to have a respiratory function since the beat starts spontaneously in still water in the absence of any apparent external stimulus (Southward and Crisp, 1965). Newell and Northcroft (1965) established that the lower level of oxygen uptake is associated with a lack of cirral activity and that the rate of respiration approximates the basal metabolic rate of the animal. Respiration rate has been shown to increase when cirral activity is resumed (Newell and Northcroft, 1965).

According to Newell and Northcroft (1965), temperature and size of the animals correspond well with observed variations in cirral activity. They also indicated that the cirri of small barnacles beat faster than those of large ones at a particular temperature. Although size has been shown to have a larger influence than temperature on the oxygen consumption per cirral beat, as Newell and Northcroft (1965) showed, large animals have a lower oxygen requirement than small ones. Therefore, collecting and using a standardized size range of animals in the current study was an important issue in measuring oxygen consumption. In addition the size of the animal, body weight was also important for measuring oxygen consumption rate.

The relationship between the values of  $\log Q_{10}$  standard metabolic rates based on oxygen consumption, versus body weight has been demonstrated in many animals (Prosser, 1973) in order to reduce the inherent inaccuracies of cirral beat measurements. Newell and Northcroft (1965) studied the relationship between the rate of cirral beat of *B. balanoides* and the body weight as an exponential function. They calculated the amount of oxygen consumed per cirral beat by dividing oxygen uptake ( $\mu\text{l}/\text{mg}/\text{h}$ ) by the number of cirral beats per hour. They indicated that the temperature has little effect on the

amount of oxygen consumed per cirral beat in any one size of animal. The amount of oxygen required per cirral beat was markedly size-dependent and varied with the logarithm of the body weight as an exponential function. Since my study calculated weight specific oxygen consumption rates, animal body weight should not be a confounding factor.

Although Newell and Northcroft (1965) found that temperature has little effect on the amount of oxygen consumed per cirral beat, Southward (1957) stated that the cirral activity of barnacles is influenced largely by the range of temperature. My study supports what Southward (1957) suggested regarding temperature range as a factor influencing the amount of oxygen consumed by the animals. Southward (1957) also suggested that the cirri activity and temperature at which this activity was greatest could be related to the geographical distribution of the species and to the temperature experienced in the normal habitat. Since *B. amphitrite* from the Salton Sea and San Diego Bay are acclimated to different temperature ranges at each location throughout the year (Figure 2.2), different cirral activities at the same experimental temperature would be expected for both populations during temperature treatments.

Southward (1955b) suggested that the fast type of beat is not related to respiratory need, but may be related to utilizing more oxygen than the normal type of beat and may also be an adaptation to assist in feeding or respiration in still water. Since Salton Sea barnacles do not experience daily tidal variations, but instead are influenced by wave actions occurring mostly by wind, cirri movement of the Salton Sea barnacles may be faster compared to San Diego Bay barnacles. Although specific experimentation into

cirral speed was beyond the scope of the current work, casual observations in the laboratory suggested that this was the case.

Cirral activities are also related to the age of the barnacles (Southward, 1955b). Southward (1955b) found that the cirral beat of older barnacles was slower and indicated lesser activity levels compared with younger barnacles. Southward (1955b) made it clear that determining the exact age of the barnacles was not possible to do, thus the critical effect of age on cirral activity was not possible to determine. However, animal size at settlement as well as growth rate can suggest an estimated age of the animals. In this study, similarly sized animals, with diameter of approximately 1 cm, were collected although the size cannot be related to the age of the animals.

In the Balanidae, a feeding barnacle may use two patterns of cirral movement; normal and fast (Crisp and Southward, 1961). Crisp and Southward (1961) described active cirral movement as rhythmical, while non-active cirral movement was described as maintaining the extended cirri motionless in circulating water. Crisp and Southward (1961) also emphasized that, depending on the speed of the water current, the animal uses active (flow speed < 2 to 3 cm/s) or passive cirri movement in order to capture food. Since there is no tidal action at the Salton Sea, barnacles from the Salton Sea may demonstrate more active cirral movement compared to San Diego Bay barnacles, which are exposed to daily tidal action and use passive cirral movement. Since cirral movement is also related to respiration of the animal, more frequent cirral activity may relate to the higher oxygen consumption rates of the Salton Sea barnacles. The results of the current study showed the greatest and least cirral activities based on the animal's highest and lowest oxygen consumption rates at their most optimal and least favorable combinations

of salinity and temperature. The greatest cirral activity for both the Salton Sea and San Diego Bay groups was observed at 25 °C in different salinity levels. For the Salton Sea group, the highest cirral activity occurred in 36 ‰, while for the San Diego Bay group it occurred in 30 ‰. The least cirral activity for both groups was observed at 15 °C in different salinity levels. For the Salton Sea group, the least cirral activity occurred in 15 ‰, while for the San Diego Bay group it occurred in 60 ‰. Therefore, the oxygen consumption rates resulted from the cirral movements can imply that the Salton Sea barnacles are most active in higher salinity at normal temperature of 25 °C as well as least active in lower salinity at low temperature of 15 °C when compared with the San Diego Bay barnacles, which are most active in lower salinity at normal temperature of 25 °C as well as least active in higher salinity at low temperature of 15 °C.

#### Effects of Groups on Oxygen Consumption

As was stated previously, there was no overall significant difference in the amount of oxygen consumed over combinations of experimental conditions between the Salton Sea and San Diego Bay populations. Therefore, although *B. amphitrite* is recognized as a tropical species (Darwin, 1854), it can be concluded that *B. amphitrite* has a wide range of tolerance to environmental changes, including salinity ranging from 0 to 60 ‰ and temperature ranging from 15 to 35 °C, as this experiments have demonstrated. Because *B. amphitrite* found in the Salton Sea and San Diego Bay are the same species, and also much of the conditions are the same in both environments, animal responses should naturally overlap except at the outer edges of those conditions. Evidence from the data of the current study suggests this to be the case.

Although groups did not show significant differences on weight-specific oxygen consumption rate, differences in animal diameter and height between the populations showed correlations to oxygen consumption rate (Table 3.4.A and 3.4.B). The Salton Sea group showed a positive, significant correlation between animal diameter and oxygen consumption rate (Table 3.4.A) while the San Diego Bay group showed a negative correlation (Table 3.4.B). For animal height and oxygen consumption rate, the San Diego Bay group showed a positive correlation (Table 3.4.B) while the Salton Sea group showed a negative correlation (Table 3.4.A). The correlation analysis on oxygen consumption rate indicates that, for the Salton Sea group, the amount of oxygen consumed by the animal increases as animal diameter increases. Since the Salton Sea does not have enough space for the barnacles to settle and grow in diameter, animal diameter may be more related to the actual size of the animal body. In contrast, it appears as though height may be more related to body size in San Diego Bay population since there is ample room for settlement and test diameter is less important.

The reasons why correlations between animal whole weight before experiment and oxygen consumption rate were negative for the San Diego Bay group (Table 3.4.B) may be related to the relationship between surface area and volume of the animal body. From spherical equations for surface area ( $SA = 4 \cdot \pi \cdot r^2$ ) and volume ( $V = 4/3 \cdot \pi \cdot r^3$ ), the ratio of those two values decreases as volume increases at a greater rate than area (Willmer *et. al*, 2000). Therefore, as SA/ V ratio decreases, the animals are less efficient at gas exchange.

## Effects of Salinity on Oxygen Consumption

As the analysis on oxygen consumption experiment indicates, there was a significant effect of salinity on oxygen consumption rate for the barnacles used in the experiment. A significant interaction was also seen for the combination of group and salinity on oxygen consumption rate. Since the correlation analysis for the San Diego Bay group indicated a small, significant interaction between salinity and oxygen consumption rate while the Salton Sea group did not show any correlation between salinity and oxygen consumption, the data suggests that salinity was not the major factor that influenced oxygen consumption rate for the Salton Sea barnacles. Pérez (1994) described the rate of cirral activity of *B. amphitrite* at salinities of 35 to 60 ‰ as the “normal” or even “fast” beat used for active feeding. Since the speed of cirral activity has been related to the amount of oxygen consumption, it is clear that faster beating implies higher consumption rate, although fast cirral activity may not be in response to respiratory need (Southward, 1955b). In agreement with this, this current study found that the Salton Sea barnacles, which normally inhabit the high salinity of 40 ‰ (Figure 2.3) compared to ocean barnacles, consumed more oxygen than San Diego Bay barnacles between the normal and high salinities of 36 and 45 ‰ at all temperatures (Figure 3.7 and Figure 3.8). On the other hand, San Diego Bay barnacles consumed more oxygen than the Salton Sea barnacles in the lower salinities of 0, 15, and 30 ‰ at the normal temperature of 25 °C (Figure 3.4, Figure 3.5, and Figure 3.6). At 60 ‰, animals seemed to be stressed and less active at all temperatures due to the fact that oxygen consumption rates were low (Figure 3.10 and Figure 3.11). The amount of oxygen consumption in different salinities implies optimal ranges of salinity for both the Salton Sea and San

Diego Bay barnacles. Since the Salton Sea group is facing higher salinity at the Sea compared to ocean salinity, the Salton Sea group is more active and consumes more oxygen at higher salinity than the San Diego Bay group.

#### Effects of Temperature on Oxygen Consumption

According to Newell (1975), temperature is one of the major physical factors that influences the metabolic rates of intertidal invertebrates. The metabolism of a wide variety of common intertidal invertebrates is usually measured by the rate of oxygen uptake at rest (Newell and Northcroft, 1967), and Newell and Northcroft (1967) suggested that intertidal invertebrates are able to maintain their metabolism at a relatively constant value over a wide temperature range between 6.5 ° and 22.5 °C. Therefore, invertebrates have a mechanism for regulating the level of maintenance metabolism in order to minimize the effects of temperature variation (Newell and Northcroft, 1967).

A significant effect of temperature on oxygen consumption rate was observed in this current study, although the combination of group and temperature did not indicate a significant interaction on the amount of oxygen consumed. A small correlation between temperature and oxygen consumption rate was observed for both the Salton Sea and San Diego Bay groups.

Animals from both the Salton Sea and San Diego Bay examined in these experiments generally showed the lowest oxygen consumption rate at 15 °C compared to 25 and 35 °C (Figure 3.10 and Figure 3.11). Since Southward and Crisp (1965) observed very short pauses interrupting the normal cirral rhythm when an animal was about to enter the inactive phase at the lower temperature, lower consumption rate could result from the inactivity of the barnacles at low temperature. Casual observation of this study

also indicated low cirral activities at 15 °C. By lowering their cirral activities at a low temperature, the animals may be able to put their energy into responding to unfavorable conditions. At 25 °C, both animals were active and generally consumed the most oxygen compared to the other experimental temperatures (Figure 3.10 and Figure 3.11), although the Salton Sea group consumed more oxygen in salinities above 36 ‰, while the San Diego Bay group consumed more oxygen in salinities below 30 ‰ (Figure 3.2). The Salton Sea barnacles consumed more oxygen than San Diego Bay barnacles at the highest temperature of 35 °C except in 0 and 60 ‰ (Figure 3.3). Both animals showed that the oxygen consumption levels at 35 °C were lower than at 25 °C (Figure 3.10 and Figure 3.11). However, the Salton Sea barnacles consumed more oxygen at 35 °C than at 25 °C in salinities of 15 and 30 ‰ (Figure 3.10). The data of current study suggest that the Salton Sea group is able to compensate for extreme changes in environmental conditions by adjusting their metabolism to combinations of high salinity and low temperature as well as low salinity and high temperature. Because water temperature throughout the year at the Sea is generally higher than at San Diego Bay (Figure 2.2), current study suggests that the optimal range of water temperature for the barnacles at the Sea has shifted towards a higher temperature than for San Diego Bay barnacles.

Southward (1955a) suggested that the ability to adapt to colder or warmer temperatures than the animal experiences elsewhere in its distribution determines the differences in the frequency of beating of their cirri. Southward (1957) also indicated that the rate of cirral beat appeared different depending on different species and the location (tidal level) where they are from. According to Southward (1957), the tropical species *B. amphitrite* was active at higher temperatures and showed a greater frequency

of beating than the northern species *B. balanus*, which was active at much lower temperatures than *B. amphitrite*. In Southward's (1957) study, twelve specimens of the tropical species, *B. amphitrite*, were used for the experiment. Below 10 °C, only 5 individuals were active, and beating was not observed below 6 °C. Between 6.8 ° and 24.9 °C, the mean frequency of beating increased linearly from 1.5 to 9.3 beats/ 10 sec. The maximum frequency was reached at 29.9 °C with a mean of 13.5 beats/ 10 sec. Beating slowed down slightly between 30 ° and 36 °C and ceased completely at 38.4 °C. Previous findings by Southward (1957) suggest this studies results for the Salton Sea barnacles being higher consumers than San Diego Bay barnacles at 35 °C, since *B. amphitrite* from the Sea inhabits an environment in which the average temperature is 30 °C (Figure 2.2). Southward (1955a) also observed a more rapid cirral beat at high temperatures, which were within the animal's exposure ranges for tropical species such as *B. perforatus*, compared with the temperate species, *B. balanoides*. For the temperate species, the opposite was true since at much lower temperatures, which were within their normal exposure range, cirral activities were high.

When Sixtus (1978) studied aspects of the physiology and biochemistry of thermal adaptation in two populations of barnacles (*Balanus amphitrite* Darwin), his studies did not address issues of salinity, but instead focused on temperature, since temperature appeared to be the most important environmental factor influencing the barnacles in the Salton Sea at the time. Even though both salinity and temperature are important environmental factors, Gunter (1957) indicated that temperature was the most important environmental factor influencing the existence of life in an ecosystem, since temperature limits the distribution of the animals as well as their activity. Because

temperature is a measure of molecular agitation, it controls the rate of chemical reactions, and also has an effect on growth and metabolism (Gunter, 1957). Sixtus (1978) studied the physiology and biochemistry of thermal adaptation of *B. amphitrite* from the Salton Sea and San Diego Bay and concluded that the San Diego Bay population was temperature-independent between 15 ° and 25 °C, and relatively temperature-dependent between 25 ° and 30 °C. As well, the Salton Sea population was temperature-independent between 20 ° and 25 °C while temperature-dependent between 25 ° and 30 °C, and relatively temperature-dependent between 30 ° and 35 °C. Newell and Northcroft (1967) suggested that the standard metabolic rate of a marine intertidal ectotherm is relatively temperature-independent within its normal range of environmental temperatures. Therefore, it is concluded from the present study that normal metabolic responses to the higher ecological temperature range of the Salton Sea barnacle is higher than for that of the San Diego Bay barnacle (Figure 3.10 and 3.11).

Newell (1966) found that the rapid increase and equivalent decrease in oxygen consumption rates with increase in temperature, demonstrated by his study of the  $Q_{10}$  values (the factor by which a reaction velocity is increased for a temperature rise of 10 °C) of his San Diego Bay population, occurred when the temperature reached the upper limits of the organism's thermal tolerance. However, the Salton Sea population does not show this pattern. Sixtus (1978) concluded that the difference in respiratory response to various temperature ranges between the two populations was due to the difference in tidal exposure that the animal faced on a daily basis at each location. The San Diego Bay barnacles are intertidal and face regular tidal fluctuations, while most of the Salton Sea barnacles live beneath the water surface continuously or the embankments of the ports

where boats are parked. Therefore, Sixtus (1978) stated that the reason why the Salton Sea population does not follow the pattern of the San Diego Bay population at high temperatures is because of a change in upper lethal temperatures of the population since the Salton Sea has a wider environmental temperature range than any of the other locations.

Morris (1991) demonstrated that the Crustacea show sophisticated respiratory gas exchange and transport since their adaptations occur at the cellular as well as enzyme level with respect to temperature sensitivity in order to regulate O<sub>2</sub> demand and CO<sub>2</sub> production. Morris (1991) also showed that both aquatic and terrestrial species from more stable temperate regions show fewer adaptations with respect to temperature.

Since barnacles respond to day-to-day fluctuations in air temperature during periods of exposure, and since acclimation is essentially an adjustment to long-term fluctuations in temperature (Newell, 1969), exposure to different levels of environmental factors, such as temperature, may result in differing effects on physiological responses of animals in different conditions at the Salton Sea than at San Diego Bay. Therefore, it can be concluded that the fluctuations of water temperature at the Sea have the effect of shifting the metabolism of the animals up to respond to such an extreme environment.

#### Combined Effects of Salinity and Temperature on Oxygen Consumption

As the analysis of the oxygen consumption experiment indicates, the combination of salinity and temperature has a significant effect on the oxygen consumption rates of *B. amphitrite*. The results showed that the highest weight-specific oxygen consumption rate for the Salton Sea group was observed in 36 ‰ at 25 °C while the San Diego Bay group consumed the most in 30 ‰ at 25 °C over all combinations of salinity and temperature.

These results may indicate the optimal range of conditions for both populations since barnacles consume the most oxygen when they are active at their optimal ranges. Since salinity at the Sea is higher than at San Diego Bay (Figure 2.3), such an environmental difference between the two locations may have influenced the animals' optimal salinity range at the normal temperature of 25 °C.

Significant differences in oxygen consumption rate between 15 ° and higher temperatures (25 ° and 35 °C) for the Salton Sea as well as the San Diego Bay groups occurred at different salinity levels (Figure 3.10 and 3.11). The Salton Sea group showed a significant difference between 15 ° and the higher temperatures of 25 ° and 35 °C in salinities of 15 and 30 ‰ (Figure 3.10), while the San Diego Bay group showed a significant difference between 15 ° and the higher temperatures of 25 ° and 35 °C in the higher salinity levels of 30 and 36 ‰ (Figure 3.11). The data suggest that the Salton Sea group was stressed in lower salinities at low temperature since the Salton Sea group inhabits an environment, which is typically high in salinity with high temperatures. On the other hand, the San Diego Bay group was less stressed in lower salinities at low temperature since this population inhabits an environment in which they face lower salinity and temperature on a regular basis than at the Salton Sea.

Although there was no overall significant difference in oxygen consumption rate between groups, further analysis indicated that there was a significant difference in oxygen consumption in 45 ‰ at 15 °C as well as in 0 ‰ at 35 °C between the groups (Table 3.1). In 45 ‰ at 15 °C, the Salton Sea population consumed more oxygen (249.03 µl/h/g) than San Diego Bay population (130.27 µl/h/g) while, in 0 ‰ at 35 °C, San Diego Bay population consumed more oxygen (247.62 µl/h/g) than the Salton Sea population

(165.75  $\mu\text{l/h/g}$ ). The significance of this is that the Salton Sea group may have been able to consume more oxygen at the low temperature of 15 °C than the San Diego Bay group since the Salton Sea group was within the tolerable range of salinity in 45 ‰. On the other hand, the Salton Sea group was more stressed and showed a lower metabolic rate in 0 ‰, even at the optimal temperature of 35 °C. Since the Salton Sea barnacles are rarely, if ever, exposed to such highly diluted water at high temperature, low salinities constitute a real stress to the barnacles from the Sea.

### **Survival Experiment**

As the results of the survival experiments indicated, all animals from the Salton Sea and San Diego Bay survived at 15 ° and 25 °C in all salinity levels (Figure 3.12 and Figure 3.13). However, at 35 °C, neither the Salton Sea nor San Diego Bay populations could tolerate and survive the conditions of low salinity of 0 ‰. All animals from the Salton Sea and San Diego Bay exposed to 0 ‰ died within 48 hours, while 50 % of both groups died in 15 ‰ within 45 hours (Figure 3.14). The current study suggests that since the optimal temperature range for the Salton Sea population has shifted to a higher temperature which includes exposure to 35 °C compared with the San Diego Bay population, the Salton Sea barnacles are able to tolerate and live slightly longer than the San Diego Bay barnacles at the severe salinity levels of 0 and 15 ‰ at 35 °C. Since the Salton Sea population is regularly exposed to higher water temperatures than the San Diego Bay population, the combination of low salinity and high temperature affected the San Diego Bay population more severely than it did the Salton Sea population. Since the combinations of only high temperature and the normal to high salinity were not fatal to both populations, high temperatures appear to have a large effect on the survival of the

barnacles. Schmidt-Nielsen (1983) found that euryhaline animals can tolerate wide variations in the salt concentration of the water in which they live, and their finding supports what this study concluded. That is that the Salton Sea barnacles have experienced a shift in their physiological tolerance up to a higher temperature and salinity range than that of the San Diego Bay barnacles. Pérez (1994) has also indicated from her study that the thriving species of *B. amphitrite* in the Salton Sea is clearly a euryhaline species. Although *B. amphitrite* is an intertidal barnacle widely distributed in temperate and subtropical coasts (Darwin, 1854) and may already have the adaptability to survive higher temperatures than occur on coastal shores, the combination of high temperature and low salinity may be a fatal condition for the barnacles even from the Salton Sea.

The ability of the barnacles to survive extreme environments such as the Salton Sea, is likely correlated to the ability of the animals to withstand daily periods of desiccation. Desiccation has been reported as a very important factor determining the upper limits of distribution in barnacles (Connell, 1974). The stressing effect of desiccation is often enhanced by the occurrence of very low tides during the high temperature hours in later Spring (López Gappa *et al.*, 1993). Desiccation tolerance of *B. amphitrite* was studied by Calcagno and Luquet (1997) in order to characterize the response to air exposure in relation to vertical distribution by means of laboratory and field experiments. After 48 hours of exposure to 0 % relative humidity, only 20 % of the animals died while the remaining died at the end of 120 hours. With 75 % relative humidity, survival rate was 60 % after 120 hours. Their study indicated that mortality rates in both conditions of humidity were greatly increased after 48 hours of exposure. Jones and Greenwood (1982) demonstrated that *B. amphitrite* can tolerate a water loss

above 40 %, resulting in a high desiccation tolerance among crustaceans, and that this species has the capability of maintaining ventilation in air. Crisp and Costlow (1963) showed that *B. amphitrite* has increased tolerance to extreme environments since the species occupies many saline outer banks and occurs at higher levels of the intertidal zone. In the case of the Salton Sea, some *B. amphitrite* at the Sea are likely to be exposed to severe conditions of desiccation and become more tolerant of extreme conditions than coastal barnacles. However, measurements of desiccation were not within the scope of this research.

Barnes and Barnes (1957) suggested that *B. amphitrite* is able to continue interacting with the aerial environment by expelling the water contained within the mantle and adjusting the operculum plates to allow air access to the tissues. By expelling the water contained within the mantle cavity when air-exposed, the animal may be able to maintain a moderate degree of aerobic metabolism during emersion, thus avoiding the accumulation of toxic end products of anaerobic metabolism.

Although *B. amphitrite* shows a wide range of tolerance to the extreme environment of the Salton Sea, Tiffany *et al.* (2002) indicated that the abundance of the barnacles may be threatened when the salinity reaches 50 ‰ because of the weakening of their tests or decreased growth rates. Vittor (1986) suggested that the collapse of aggregations of *B. amphitrite* due to the weakening of the tests seems to be a major cause of adult mortality. Hedgpeth (1967) also reported the upper salinity tolerance of *B. eburneus* and *B. amphitrite* to be 76 ‰, although this number applies only to acute exposure, and maximum tolerable salinity can be much higher if exposure to the salinity is gradual (Pérez, 1994). Pérez (1994) also stated that adult barnacles are able to survive

up to 92 ‰ if the salinity increases gradually. In the current study, it was found that less than half of the barnacles from the Salton Sea died in acute exposure of 60 ‰ with a high temperature of 35 °C for up to 48 hours. If the Salton Sea barnacles are exposed to gradual increase in salinity, the animals at the Sea may be able to survive high salinity levels at the Sea in the future as Pérez (1994) has previously suggested.

#### Scutum Resistance Experiment

Statistical analysis indicated that there was a significant difference in the tension of the scutum between dead and live animals for *B. amphitrite*. Therefore, by measuring the scutum tension of the animals, the state of the animals (whether the animal is alive or dead) can be determined. Tension that is greater than 144.93 g, the tension of the dead barnacle, can indicate live animals. Mean tension of the live animals was 196.50 g. At the end of Southward's work (1955b), practically all specimens had the tergum and scutum slightly open, often with the cirri protruding. This reaction can be termed the gaping response, and probably indicates acute respiratory difficulties. Having strong scutum resistance is important for sessile organisms, such as barnacles, to survive their intertidal habitat, since closing their opercular valves tightly will protect them from desiccation, predation, and wave action.

#### Morphological Experiment

Casual observations of current study suggested that the populations exhibit different morphologies. From statistical analyses on morphology of the animals used in all the experiments, it was concluded that the morphology of the Salton Sea barnacle test is significantly narrower and taller than that of the San Diego Bay barnacle. The cause of the differences in morphology of the barnacles may involve phenotypic plasticity.

Phenotypic plasticity, the degree to which different phenotypes are produced by the same genotype in different environments, may play an important role in the morphological differences between the two populations. Since morphological differences between the Salton Sea and San Diego Bay barnacles are due to different ecophenotypes (Van Syoc, 1992), it is reasonable to assume the differences reflect environmental factors rather than genetic differences. Phenotypic variations can be induced by different ecological conditions in which the organisms are found. The difference in the levels of environmental factors such as salinity and temperature between the Salton Sea and San Diego Bay may induce the phenotypic plasticity of *B. amphitrite*.

Beadman *et al.* (2003) reported that shell characteristics such as strength and thickness of seed mussels were different when grown at different shore heights. Mussels grown at higher shore levels attained characteristics that are beneficial to avoiding predators such as crabs, whelks, and sea stars. Steffani and Branch (2003) also studied mussels for phenotypic plasticity and found that differences in environmental conditions impacting the mussels account for most of the variability, including differences in spawning periods, reproduction, and growth. According to Trussell and Smith (2000), the expression of defensive morphologies in prey is often correlated with predator abundance or diversity over different environmental scales. Further, Trussell and Smith (2000) suggested that phenotypic variation could also reflect within-generation developmental responses to environmental cues. Phenotypic plasticity induced by different environmental causes may be related to the formation of test thickness differences between the Salton Sea and San Diego Bay populations. Since the Salton Sea is a unique environment for this species to grow and reproduce in compared with their usual

intertidal or estuarine habitats, changes in morphology may be a response to reduced predatory or wave exposure pressures. In addition, changes in morphology may be responses to changes in the chemical nature of the Sea.

Carpelan (1961) noted that the growth rates of barnacles were greatest in summer, when a basal diameter of 8 to 9 mm was reached within 30 days, while the coldest season of the year showed growth rates of less than one-tenth the summer rates. Experiments on the effects of salinity on *B. amphitrite* growth and test strength in the Salton Sea by Simpson and Hurlbert (1998) showed that changes in the wall morphology can compensate for the reduced material strength as the wall becomes weaker with increases in salinity at the Sea. Clare *et al.* (1994) also noted the inherently easy-breakage and weak tests of the Salton Sea barnacles compared to coastal barnacles and indicated the difference in how the Salton Sea barnacles settle in larger aggregations than the ocean barnacles. Since settlement substrates, such as rocks and embankments, are limited at the Salton Sea barnacles when compared with the coast of San Diego Bay, the Salton Sea group tends to settle in aggregations and grow narrower and taller than the San Diego Bay group.

#### Animal Test Thickness Experiment

When test thickness was examined, it was found that the Salton Sea population tended to grow on top of each other and was attached to their substrates much less than San Diego Bay individuals. This made barnacles from the Salton Sea easier to collect than those from San Diego Bay. Also, as is mentioned earlier, tests of the Salton Sea population were generally taller and thinner in diameter than the San Diego Bay population. San Diego Bay barnacles had thick, hard tests while the Salton Sea barnacles

tended to be thinner and more fragile. Linsley and Carpelan (1961) suggested that *B. amphitrite* from the Salton Sea were broken easily because of their thinner shells, which resulted from faster growth rates at the Sea. Simpson and Hurlbert (1998) pointed out the fact that aggregation collapse appears to be a main factor of barnacle death at the Sea, and the cause may be the fact that the tests of *B. amphitrite* at the Sea appear to be much weaker than that of *B. amphitrite* in the ocean. Since the present study found that the tests of barnacle from the Salton Sea was significantly thinner than that of San Diego Bay barnacles, these findings are in agreement with the findings of Simpson and Hurlbert (1998).

Below, three possible factors which may cause differences in test thickness between barnacles from the Salton Sea and San Diego Bay are submitted.

### ***Predation and Competition***

One of the possible reasons why there is a difference in test thickness may be the lack of predation and competition with other species at the Sea. Ortega (1981) showed that *B. amphitrite*, a fast growing barnacle, is capable of withstanding a wide range of environmental conditions although its intertidal distribution is considered to be limited by predation and competition. However, in the Salton Sea, interspecific competition and predation are virtually nonexistent, thus Linsley and Carpelan (1961) recorded daily settlement rates greater than 30 individuals/ cm<sup>2</sup>.

Brookes (Pers. Comm.) studied the difference in test thickness of *Littorina obtusata* snails in predation and predation-free treatments and found that predation cues caused the snails to have increased calcification rates, while increased shell thickness was positively related to increased body growth. The shore mussel, *Mytilus edulis*, produces

thicker shell lips in response to predators compared to control mussels raised in predation-free environments (Smith and Jennings, 2000). In the same study, significantly greater shell thickness was observed in mussels raised with Whelks than with crabs. Therefore, attack mode of predators also has an impact on the thickness of animal shells and tests. Since the barnacles at the Salton Sea have almost no predators, there is no need to infuse their test with much  $\text{Ca}^{2+}$  to protect themselves from predation. Therefore, barnacles at the Sea are able to put the energy usually used to reinforce the test with  $\text{Ca}^{2+}$  into growth as well as reproduction.

### ***Food Supply and Wave Exposure***

Since the Salton Sea provides a richer food supply than is available in inshore ocean waters (Hart *et al.*, 1998), it may be that the animal's soft tissues out-grow test deposition (Hurlbert, personal comm.). From casual observations in the current study, numbers of animals in a single aggregation at the Sea were much greater than aggregations at San Diego Bay.

Effects of different wave intensity on shell thickness were observed in the mussel, *Mytilus galloprovincialis*, by Steffani and Branch (2003). Growth rate, condition, and shell shapes of mussel were measured at two regions 500 km apart where different wave exposure exists. *Mytilus galloprovincialis* grew faster and had better shell conditions at the site with extreme wave action than sheltered shores, probably due to greater water flow. Interestingly, Steffani and Branch (2003) demonstrated that mussel shells were thickest on sheltered and extremely exposed shores while thinner (lighter) shells were found at moderately exposed sites. Therefore, the degree of wave exposure is an important physical factor influencing the characteristics of mussel populations. An

increase in shell thickness can protect mussels, and in fact any sessile intertidal inhabitant, from the destructive effects of wave action, although it is metabolically costly to use the energy for forming the shell. Therefore, lack of wave action at the Sea may have the larger impact on the formation of *B. amphitrite* tests than food availability for Salton Sea barnacles.

### ***Salinity***

Simpson and Hurlbert (1998) indicated that the lateral test walls of *B. amphitrite* weakened as salinity increased, although faster growth at salinities below 57 ‰ were observed. They suggested that the weakened tests may have resulted from either structural or chemical changes within the test wall. Barnes *et al.* (1970) demonstrated that *B. amphitrite* has relatively large parietal pores compared to other marine barnacles. Therefore, if the pores become larger with salinity, the integrity of the test may be compromised (Simpson and Hurlbert, 1998). Since the salinity effect on test growth and strength of *B. amphitrite* from the Salton Sea with 5 different salinity levels (30, 39, 48, 57, and 65 ‰) did not seem to have a lack of CaCO<sub>3</sub> (Simpson and Hurlbert, 1998), the necessary ions were probably available during the animals growth at all salinities. However, trace elements incorporated into the walls may have affected the wall strength. According to Lowenstam and Weiner (1989), the substitution of Ca<sup>2+</sup> with Mg<sup>2+</sup> during the mineralization process tends to increase as salinity increases. They also showed that the prevalence in the test matrix of other trace elements, which do not substitute for Ca<sup>2+</sup>, such as Boron, also tend to rise with increased salinity. Therefore, if salinity in the Salton Sea increases, the Salton Sea barnacles may have larger pores and have increased elemental deposition of ions other than CaCO<sub>3</sub>, which may result in weaker tests. Further

examination of tests by electron microscopy and chemical analysis may help to elucidate the situation.

### **Ecological Application**

The Salton Sea is a unique ecosystem, into which many of its marine fish and invertebrates were purposely introduced by humans in order to establish a sport fishery. In addition to this anthropogenically-influenced situation, unusual conditions of temperature, nutrient loading, and elevated salinity are creating an extreme environment for the organisms that inhabit the Sea, including the barnacle, *B. amphitrite*. Carpelan (1958) stated the importance of this organisms' ability to tolerate large ranges of temperature and fluctuations in pH, as well as concentration changes in dissolved oxygen resulting from photosynthesis and respiration in the Sea when the salinity was not yet an issue. Sixtus (1978) suggested that adaptation to a changing environment is a prime necessity for the survival of sessile organisms such as barnacles in the Salton Sea. Inflow of freshwater into the Salton Sea may be reduced in the near future as a result of water conservation and water transfers to coastal California (Watts *et al.*, 2001). The estimated reduction ranges from 25 to 43 %, depending on legislative and other decisions (Tetra tech, 2000). Therefore, Tetra tech (2000) has suggested that the water surface level of the Sea may drop from between 2 to 6 m, and the area of the Sea may be reduced from 980 km<sup>2</sup> to between 675 and 834 km<sup>2</sup>, and the salinity may increase and then drop to as low as 38 ‰. In any case, numerous political and engineering decisions will play a huge role in those changes. Pérez (1994) also indicated that a salinity of 60 ‰ could be reached by about the year of 2033 if the increased rate of salinity observed for the Sea over the past 10 years continues. Current engineering projects propose to stop the increase of salinity

at the Sea by creating evaporation ponds that would utilize diked-off sections of the existing Sea. Those decisions should take into account how modifications of the Sea will involve complex mixing, thermal and dissolved oxygen regimes, and how they could affect the future value of the Sea for people as well as wildlife.

Pérez (1994) stated that *B. amphitrite* from the Salton Sea is clearly a euryhaline species. Fialkowski and Newman (1998) also stated that *B. amphitrite* was an excellent tool for monitoring heavy metals in aquatic biota. The findings of their research suggested that the Salton Sea was not yet greatly polluted by heavy metals derived from industrial, agricultural, and domestic waste water. Although *B. amphitrite* from the Salton Sea was shown to survive in the gradual increase of salinity up to 92 ‰ (Pérez, 1994), this species is expected to become extinct within the Salton Sea when the salinity reaches 70 to 80 ‰, and may show remarkable declines in abundance at salinities as low as 50 ‰ (Simpson and Hurlbert, 1998). Since *B. amphitrite* in the Sea make up about 5 % of the diet of the longjaw mudsucker, *Gillichthys mirabilis* Cooper (Gobiidae: Pisces) (Linsley and Carpelan, 1961) and some of the birds at the Sea such as diving ducks use barnacles as a food source, losing *B. amphitrite* from the Sea may affect the wider ecosystem to some, as yet, unknown extent. Simpson and Hurlbert (1998) also mentioned that the loss of *B. amphitrite* from the Salton Sea could result in habitat loss for the organisms that are associated with them and the eventual loss of the *B. amphitrite* test beaches, as well as any faunal assemblage especially associated with the barnacle. Therefore, maintaining current conditions or even improving the salinity levels at the Salton Sea for the organisms that inhabit the Sea, including *B. amphitrite*, is important for the future ecological relationships of the Sea.

Animals differ in their ability to tolerate environmental factors such as temperature (Schmidt-Nielsen, 1990). Schmidt-Nielsen (1990) also indicated that some animals have a very narrow tolerance range while others have a wide range of tolerance. In the current study, *B. amphitrite* from the Salton Sea was able to tolerate and survived a wide range of environmental factors of salinity and temperature longer than barnacles of the same species from San Diego Bay. Southward (1995b) suggested that the difference in activity of animals from high and low tide levels may be related as much to the difference in rate of growth, and possibly of general metabolism, as to any adaptation to temperature differences between the two levels. Since most of the Salton Sea barnacles are under water experiencing submersion at all times due to lack of tidal action and settlement substrates, differences in physiological responses to extreme environmental conditions may include a change in their upper limits of salinity and temperature tolerance.

Southward (1957) suggested that there is a strong possibility that *B. amphitrite* contains physiological varieties adapted to different temperature regimes. The range of cirral activity of *B. amphitrite* as well as *B. improvisus* and *B. balanus* is closely related to the geographical distribution (Southward, 1957). The tropical and warm temperature species *B. amphitrite* was active at higher temperatures and showed a greater frequency of beating than the northern species *B. Balanus*, which was active at much lower temperatures than *B. amphitrite*. Therefore, even though *B. amphitrite* from the Salton Sea and San Diego Bay are the same species, animals from the Salton Sea are acclimated to different environmental conditions of salinity and temperature throughout the year and may be adapted to survive the differences in such conditions. Though the current study,

there is evidence that morphological and physiological upper limits of the Salton Sea barnacles have changed in response to changes in the conditions of the Sea when compared with barnacles from the intertidal environment of San Diego Bay.

### **Future Study**

Suggested future studies based on the results of this study are the following: (1) genetic analysis comparing populations; (2) affects of differences in chemical components of the Salton Sea and San Diego Bay water on physiology (metabolism), survival, and morphology of both populations; (3) growth rate of both populations at their optimal conditions based on this study; (4) investigation of biomineralization by the Salton Sea barnacles in the Salton Sea water verses coastal seawater with XRD and/ or electron microscopy.

### Genetic Analysis

Genetic analysis of the two populations between the Salton Sea and San Diego Bay needs to be done again in order to confirm the study done by Flowerdew (1985). Since there may be a change at the genetic level for the barnacles from the Sea in order to adapt to the high salinity and high temperature of the Salton Sea environment, more recent genetic analyze may help clarify the controversy of whether *B. amphitrite saltonensis* is a subspecies of *B. amphitrite* or not.

### Chemical Components of the Salton Sea and San Diego Bay

Chemical components of the Salton Sea water may be significantly different than San Diego Bay water due to the fact that the Sea is a closed lake and also an agricultural drainage for Imperial and Riverside counties around the Sea. Therefore, investigating the ratio of chemical components such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  of the Sea as well as San Diego Bay

and the link of water composition to animal physiology, survival, and morphology between the Salton Sea and San Diego Bay, may further elucidate why differences between these populations exist.

#### Comparisons of Growth Rate

Since this study suggests that the optimal conditions of the barnacles from the Salton Sea and San Diego Bay differ, observing the growth rate of the animals at their optimal ranges for both populations may indicate the same growth rate for both populations. San Diego Bay barnacles may grow as fast as the Salton Sea barnacles at their optimal conditions and possibly lead to their morphological changes.

#### Lateral Pore Formation

Integrity of animal tests is related to the pores of the test (Simpson and Hurlbert, 1998). Therefore, investigating the pores of the Salton Sea barnacles with increases in salinity with XRD or microscopy to see increased elemental deposition of ions other than  $\text{CaCO}_3$  may result in weaker tests. By introducing larvae of the barnacles to changes in salinity and temperature, changes in formation of their test including the pores may be analyzed.

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