ABSTRACT

THE EFFECTS OF INCREASING SALINITY ON THE REPRODUCTION, FEEDING BEHAVIOR, GROWTH RATE, OSMOREGULATION AND SURVIVAL OF THE BARNACLE *BALANUS AMPHITRITE* (CRUSTACEA, THORACICA) FROM THE SALTON SEA, CALIFORNIA.

by

Ana Lilia Escamilla Pérez

Increasing salinities greatly affected feeding behavior, wet weight, reproduction, survival and osmoregulation of the barnacle *Balanus amphitrite* from the Salton Sea. Few adverse effects were apparent at salinities up to 50‰. By 60‰ transient aversive responses were seen and reproductive output was reduced. The 70‰ treatment produced a strong aversive response and long-term reductions in feeding and reproductive activity. LC_{50} after 22 days was 73‰. Eighty parts per thousand was lethal within 15 days upon acute exposure, but the barnacles survived for at least several days at salinities of up 92‰ when the salinity was increased gradually. *B. amphitrite* osmoregulates at lower salinities, and seems to osmoconform at intermediate salinities (40-60‰). It is not clear whether it osmoregulates or osmoconforms at higher salinities. This barnacle species is clearly euryhaline and does not seem to be directly threaten until the Salton Sea reaches a salinity

of approximately 60‰, which at the present rate of increase should occur by the year 2033.

LOMA LINDA UNIVERSITY

Graduate School

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A Thesis in Partial Fulfillment

of the Requirements for the Degree Master of

Science in Biology

June 1994

ACKNOWLEDGMENTS

I am indebted to my advisor, Dr. David L. Cowles for his continuous encouragement, his patience, and for his numerous and helpful revisions. Without his help this thesis would have not been accomplished. Also, I want to thank my Committee members, Dr. H. Paul Buchheim and Dr. H. Thomas Goodwin for their helpful ideas, suggestions and revisions of my manuscript.

I want to thank the Salton Sea State Park Headquarters District Ranger Mr. Dennis E. Imhoff and staff for allowing me to collect samples and for providing me with several references.

I want to acknowledge Sherri Frakes for her valuable help with the blood osmolality assays during the summer months.

I want to thank Paul Simpson from San Diego State University for suggesting several valuable references.

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INTRODUCTION

The Salton Sea, centered at 33° 20' N and 115° 50' W, is a recently formed body of saline water in the California lower desert (Figure 1) (Carpelan, 1958; Arnal, 1961). It is the largest lake in California with average dimensions of 55 by 24 km (Figure 2). Its surface is approximately below sea level (-73 m) and it has no outlet. The temperature of the Salton Sea varies seasonally from 10 to 36°C. In spring the surface may be as much as 5°C warmer than the bottom, but since the maximum depth is only 12 m, winds tend to mix the water rapidly so that normally the vertical temperature difference is less than 1°C (Carpelan, 1958).

Analysis of water from the Salton Sea, conducted from 1905-1955, revealed that the ionic composition is similar to that of the oceans, but it contained relatively less Mg^{2+}, K^{+}, and Cl^{-}; about the same proportion of Na^{+}; and more SO_4^{2-}, Ca^{2+}, HCO_3^{-}, and CO_3^{2-} (Carpelan, 1958). Hely et al. (1966) stated that "sodium and chloride have been the dominant dissolved constituents in the Salton Sea....[and] sulphate (SO_4^{2-}) has been the next most abundant ion. During most of the life of the Sea its content ratio of sulphate to chloride has increased, a trend that is still continuing. Calcium (Ca^{2+}), magnesium (Mg^{2+}), and bicarbonate (HCO_3^{-}) have been relatively minor constituents." Table 1 compares water chemistry of the Salton Sea and the oceans based on a recent study by Schroeder et al. (1993).

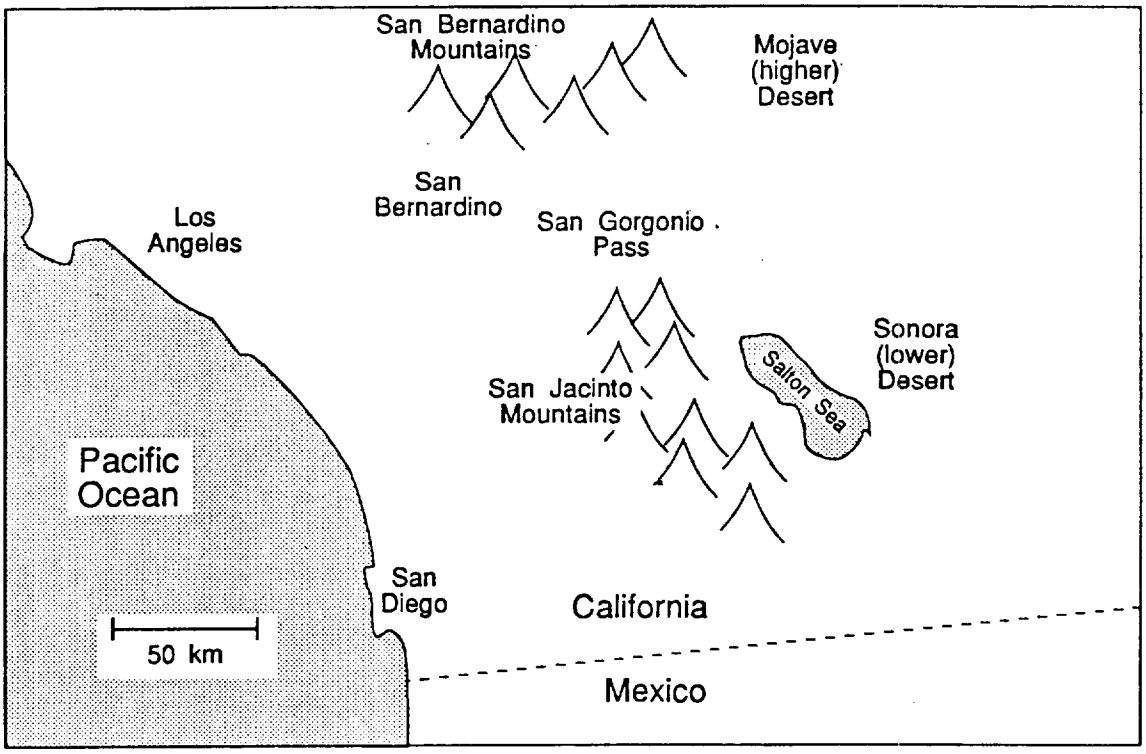


Figure 1. Location of the Salton Sea, California.

Figure 2. Map of the Salton Sea basin (adapted from Hely et al., 1966). Elevations are in feet below sea level.

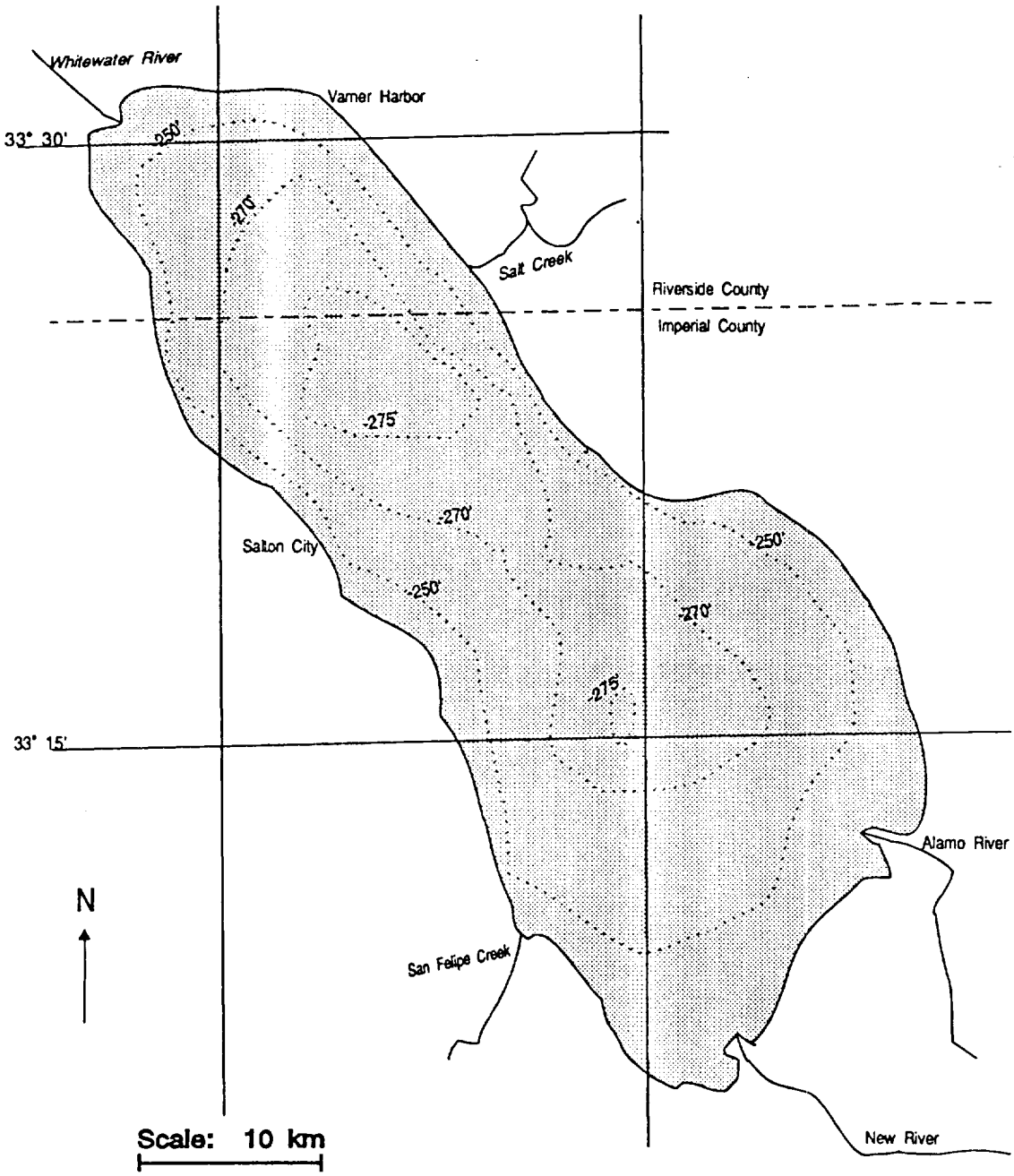

Whitewater River
Varner Harbor
33° 30'
-250'
-270'
Salt Creek
Riverside County
Imperial County
-275'
-270'
Salton City
-250'
-250'
-270'
-275'
33° 15'
Alamo River
N
San Felipe Creek
New River
Scale: 10 km

Table 1. Analyses of undiluted water samples from the Salton Sea and the Pacific Ocean (adapted from Schroeder et al., 1993. Salton Sea data are for 10-11-89)

	Concentration	
Constituent	Salton Sea	Ocean Average
Specific conductivity (μS/cm)	53,700	53,000
pH	8.8	~8
Major constituents (mg/l):		
Ca	950	403
Mg	1,300	1,260
Na	11,000	10,500
K	220	390
Alkalinity (as CaC03)	185	120
S04	10,000	2,650
Cl	17,000	18,900
Br	13	66
Si02	12	4
N as N02 + N03	<0.100	-0.5
N as NH3	0.62	-0.5
N as NH3 + organic	3.7	-0.5
P as hydro + ortho	0.06	0.06
Minor constituents (μg/l):		
Al	50	2
As	9	3.7
B	12,000	4,400
Fe	340	2
Mn	80	0.2
Mo	6	10
Se	2	0.2
Sr	3,400	7,700
U (natural)	5.2	3.2

The Salton Sea formed after continuous floods on the Colorado and Gila rivers ruptured the Rockwood Gate irrigation channel near Yuma, Arizona and flooded the Salton Sink, a basin containing extensive salt deposits, from 1904-1907 (Carpelan, 1958). In 1907, the irrigation channel was repaired, and the water level of the Salton Sea has since been maintained by irrigation waters.

Several authors have debated the origin of the salts present in the Salton Sea. For example, Coleman (1929) thought they came from the volcanigally active mud pots at the south end of the sea. Carpelan (1958) stated that "the salts present in the Salton Sea are the result of evaporation of Colorado River water." However, Arnal (1961) argued that not all the salts present came from the Colorado River. This author made a detailed study of the amount of salts contributed by other sources and by the Colorado River: "of the 254,044,700 tons of salts present in 1955 in the Salton Sea, 140,206,700 tons was contributed by Colorado River water and concentrated by evaporation. The remainder, 113,838,000 tons, was derived from re-solution of salts of the former salina of the Salton Sink" (see Arnal, 1961 for detailed calculations). A common misconception about the Salton Sea is that it was originally connected to the ocean as a northern extension of the Gulf of California. However, Vittor (1968) summarized the evidences that showed that this

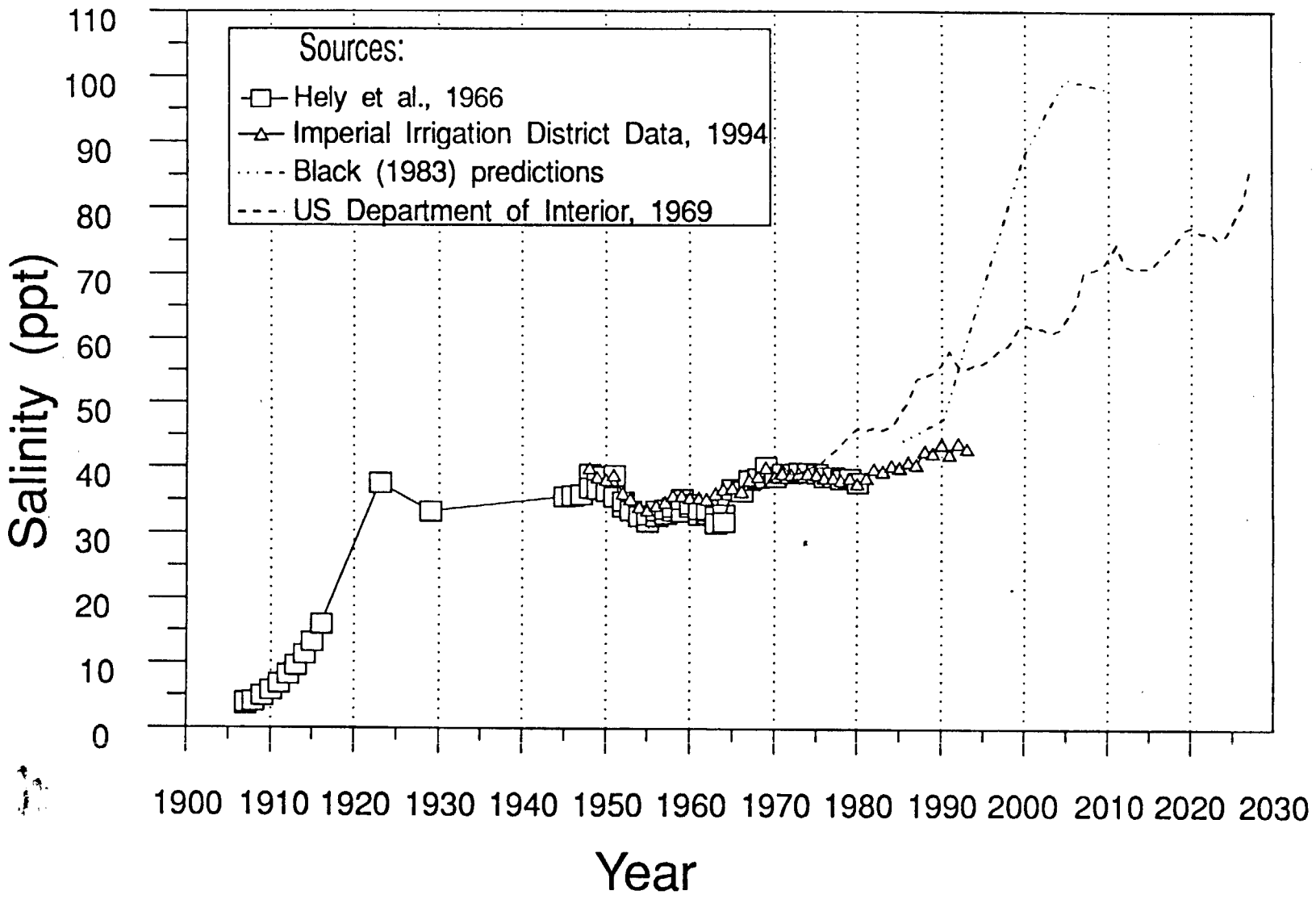
idea is inaccurate. He stated that the vast deposits of freshwater snails and layers of calcium carbonate characteristic of all Quaternary lakes of the Great Basin indicate that Lake Cahuilla, the previous body of water in the Salton Sea basin, was of freshwater origin. In addition, Carpelan (1958) pointed out that the salt deposits in the sink are of an ionic composition most characteristic of the Colorado River, not of seawater from the Gulf of California.

Arnal (1961) stated that the Sea's evaporation rate in this dry, hot desert basin may be as high as two meters per year. Thus it is not surprising that the Sea decreased in volume during its early years. The Salton Sea surface level has varied due to changes in inflows and evaporation rates since the time the lake was formed. Due to the balance between varying inflows and evaporation, the surface elevation has undergone the following changes: In 1907, -59.44 m; 1920, -76.20 m; 1948, -73.15 m; in 1964, -70.53 m; 1968, -70.87 m (Anderson et al., 1986). By 1948, the surface area had decreased from an original 1294.99 km^2 to about 828.80 km^2. Hely et. al. (1966) pointed out that changes of salinity in a body of water that has no outlet are best described in conjunction with changes of water volume and of mineral content "because the salinity at a particular time depends on both the amount of water and the quantity of dissolved minerals." Thus we would expect salinity to

increase as the volume of water decreases. Indeed, Carpelan (1958) estimated that the future salinity increase would be 0.4‰ per year.

This general increase in salinity is well documented. When the sea was first formed, the water was of such low salinity that it contained freshwater species such as mullet, trout, and catfish; and a thriving commercial and sport fishery soon developed. However, as the salinity increased rapidly, from 3.6‰ in 1907 to about 40‰ by 1925, the freshwater species died out, and were replaced later by saltwater species introduced by the California Department of Fish and Game. Some authors seem to imply that the salinity levels have gradually increased since that time. This was clearly true in the early years up to about 1925, but Hely et. al. (1966), Black (1983) and data from the Imperial Irrigation District Data (unpublished data, 1994) show that salinity has actually fluctuated since 1925 (Figure 3) due to variations in freshwater runoff and lake volume accompanied by a steadily increasing total salt load. Salinity fluctuated until the 1980's when it began a steady increase, presumably due to drought. The unusually wet winter of 1992-1993 caused another rise in surface level and a decrease in salinity, but this effect is expected to be only temporary.

Figure 3. Actual and predicted salinity fluctuations in the Salton Sea since 1907.


Sources:
Hely et al., 1966
Imperial Irrigation District Data, 1994
Black (1983) predictions
US Department of Interior, 1969
Salinity (ppt)
110
100
90
80
70
60
50
40
30
20
10
0
1900 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030
Year

Various groups and governmental agencies have attempted to predict what is going to happen to the volume and salinity of the Salton Sea in the future. Past predictions of rapid and steady increases in the salinity levels have so far been inaccurate. For example, The U.S. Department of Interior and the Resources Agency of California (USDI 1969), predicted that in the 1980's salinity would increase from 47 to 57‰ (Figure 3), while in the 1990's it would steadily increase from 57 to 63‰, and that by the year 2010 the salinity would be as high as 72‰. Black (1983) predicted even higher rates of increase such that by the year 2007, the salinity would read 101‰, even with U.S government water conservation measures. Historical values up to 1993 show that those predictions are much too high, but trends of the past 10 years suggest that the predicted steady increase in salinity has at last begun to occur, though at not so rapid a rate as had been projected.

The Salton Sea presents two contrasting pictures--it is a stopping site for about 2 million migratory birds, and at least up until recently it harbored a rich fishery, but it is also California's largest agricultural sump. Surrounded by farms, the Sea receives millions of liters of contaminated water flushed from the surrounding fields and from industrial and residential effluent from México. (Polakovic, 1993). Every year, natural and man-made sources

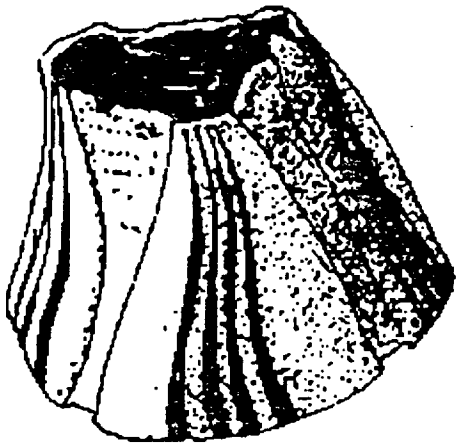
put approximately 4.5 million metric tons of salt into the Sea (Hely et. al., 1966). "Irrigation runoff waters with [salinity] concentrations as high as 3200 mg/l are carried into the Salton Sea by the Alamo and New Rivers" (Hammer, 1986) and by the Whitewater River (Black, 1983). In addition, México continuously pours a toxic mix of pollutants into the New River: "raw and partially treated sewage, slaughter house scraps, industrial toxins, garbage, power plant effluent, agricultural wastewater and detergents" (Polakovic, 1993). He also stated that high levels of DDT, arsenic and selenium have already taken their toll on the eggs of several bird species; other pollutants found are chromium, zinc, lead, and pesticides.

At the present salinity levels, even the dominant fish species--*Tilapia*, Corvina and Sargo (the main sport fish)--which were formerly very abundant, are disappearing. Salinity of 40‰ and above appears to inhibit reproduction of the fish species in the Sea (Black, 1983). Salinities of 50‰ and higher may also threaten the reproduction of pile worms (Black, 1983), amphipods, and maybe even barnacles (Polakovic, 1993).

The subject of my study is the barnacle, *Balanus amphitrite* (Figure 4), which was accidentally introduced to the Salton Sea. According to Newman and Abbott (1980), the preferred habitat of *B. amphitrite* within its natural range

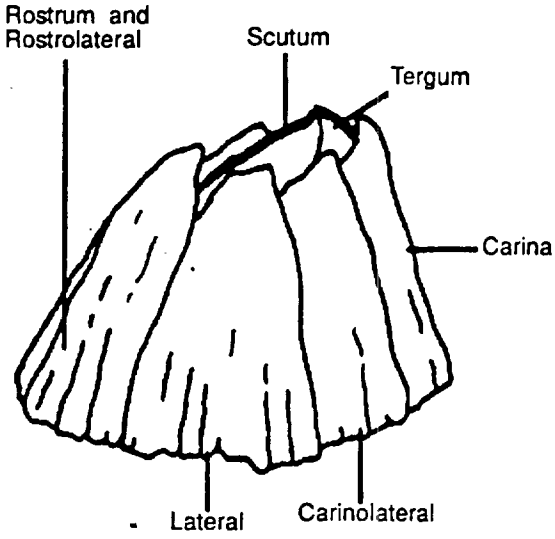
Figure 4. The barnacle *Balanus amphitrite*: a. External view (Hinton, 1987). b. Identification of the external shell plates of a thoracic barnacle (Barnes, 1987). c. Internal barnacle anatomy (Light et al., 1967).

A

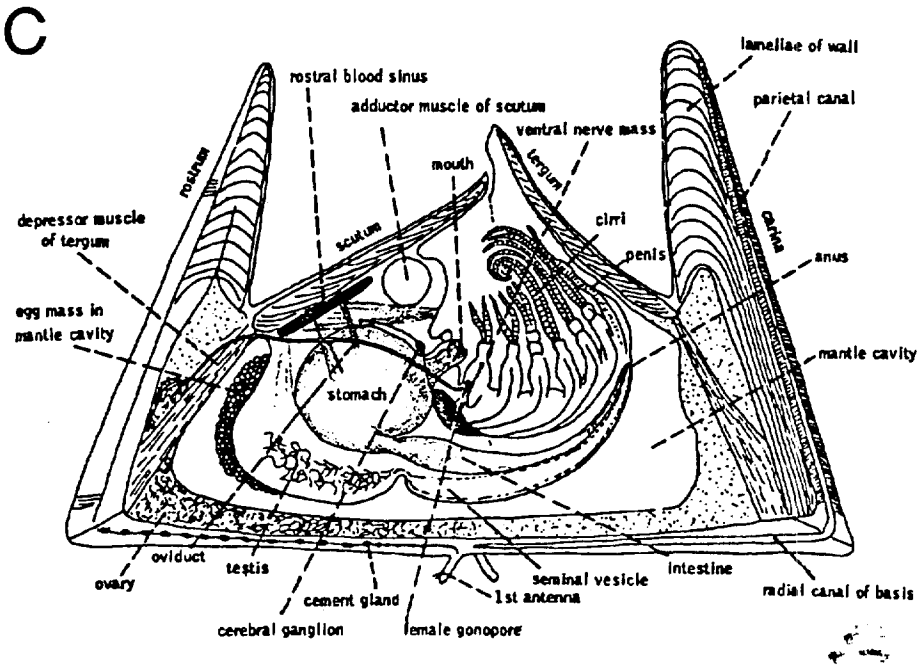


Scale: 1 cm

B



C



is the marine low intertidal zone to 18 m depth in bays and estuaries. It is a fouling barnacle with a worldwide distribution, but cannot survive in very dilute seawater.

It inhabits bays in which the summer temperature is about 20°C, at which temperature reproduction can take place. *B. amphitrite* was first reported to have been observed in the Salton Sea between 1943-1944 (Hilton, 1945). It has been suggested that the barnacle was accidentally transported from the San Diego area by air during Naval seaplane practice flights, as adults on mooring buoys or on ropes (Cockerell, 1945; Newman and Abbott, 1980) or as larvae in the bilge water of Naval flying boats (Hilton, 1945).

The Salton Sea barnacle population was identified as a new subspecies, *Balanus amphitrite saltonensis,* by Rogers (1949) based on morphological differences already apparent between the recently isolated Salton Sea population and that of the open coast. This designation was supported by Henry and McLaughlin (1975) who differentiated between *Balanus amphitrite amphitrite* and *Balanus amphitrite saltonensis* based on a multivariate analysis of 15 morphological characters. However, Newman and Abbott (1980) claimed that the Salton Sea form was also found in Wilmington Harbor (Pacific coast) and that the difference between this putative subspecies and *Balanus amphitrite amphitrite* is

ecotypic. Raimondi (1992) evaluated the hypothesis of Henry and McLaughlin (1975) and also concluded that *Balanus amphitrite saltonensis* is merely an ecotypic variation of *Balanus amphitrite amphitrite*. Though he did find some differences in the larvae of the two populations, Raimondi (1992) stated that the adult differences can be explained by "environmentally induced plasticity whereas the larval differences can be explained by an evolutionary process, probably selection." For the remainder of this paper, I will refer to the barnacle species occurring in the Salton Sea simply as *Balanus amphitrite*.

Balanus amphitrite is now very abundant in the Salton Sea, but many other species in the Sea, such as fish, have suffered sharp declines in recent years which have been attributed mainly to increasing salinity. It was not known what the ecological tolerances of *B. amphitrite* were to increasing salinity, nor what could be expected for the species' continued viability in the Sea if the salinity continues to increase as expected. The purpose of this study was to assess the effect of increasing salinity on reproduction, feeding behavior, growth rate, osmoregulation, and survival of *Balanus amphitrite* and to attempt to predict the level of salinity at which the barnacle population may no longer be viable.

MATERIALS AND METHODS

Collection of physical and chemical data, and collection and maintenance of specimens

Fresh adult barnacles (*Balanus amphitrite*) were collected from the Salton Sea, Coachella Valley, California every fourteen days during the months of June through August and October through December 1993. Barnacles were collected by hand from the docks in Varner Harbor, Salton Sea State Park Headquarters and Visitors Center (Figure 5). "The marina [Varner Harbor] is located on the northeast shore of the Salton Sea. It is nearly round, with a diameter of approximately 100 m (335 feet) and has a maximum water depth of about 5 m (16 feet)" (Vittor, 1968). At the time the specimens were collected [between 7:17 am and 9:43 am], salinity, dissolved oxygen, air and water temperatures were measured at three representative sites within and near Varner Harbor: at the dock (site A), at the open beach, and at a rocky site at the base of the harbor jetty. Dock site B was only used for collection of specimens after August 12, 1993.

Surface salinity was measured on every visit using an Atago 2441 refractometer referenced to deionized water with a precision of +/- 0.5‰. Surface dissolved oxygen was measured with a Yellow Springs Instrument model 57 portable polarographic electrode oxygen meter. In addition, an oxygen and depth profile was taken from the surface to the bottom

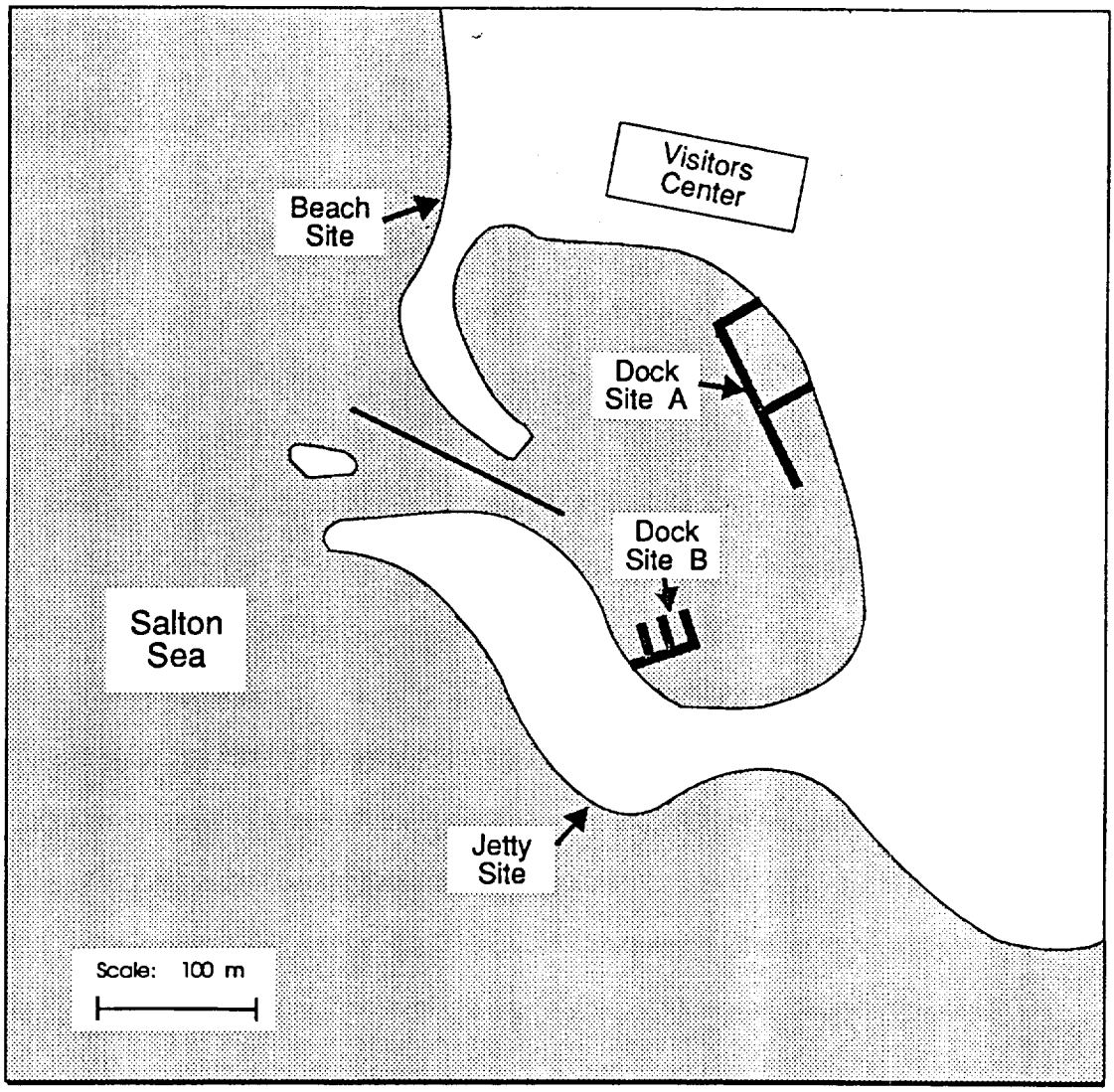
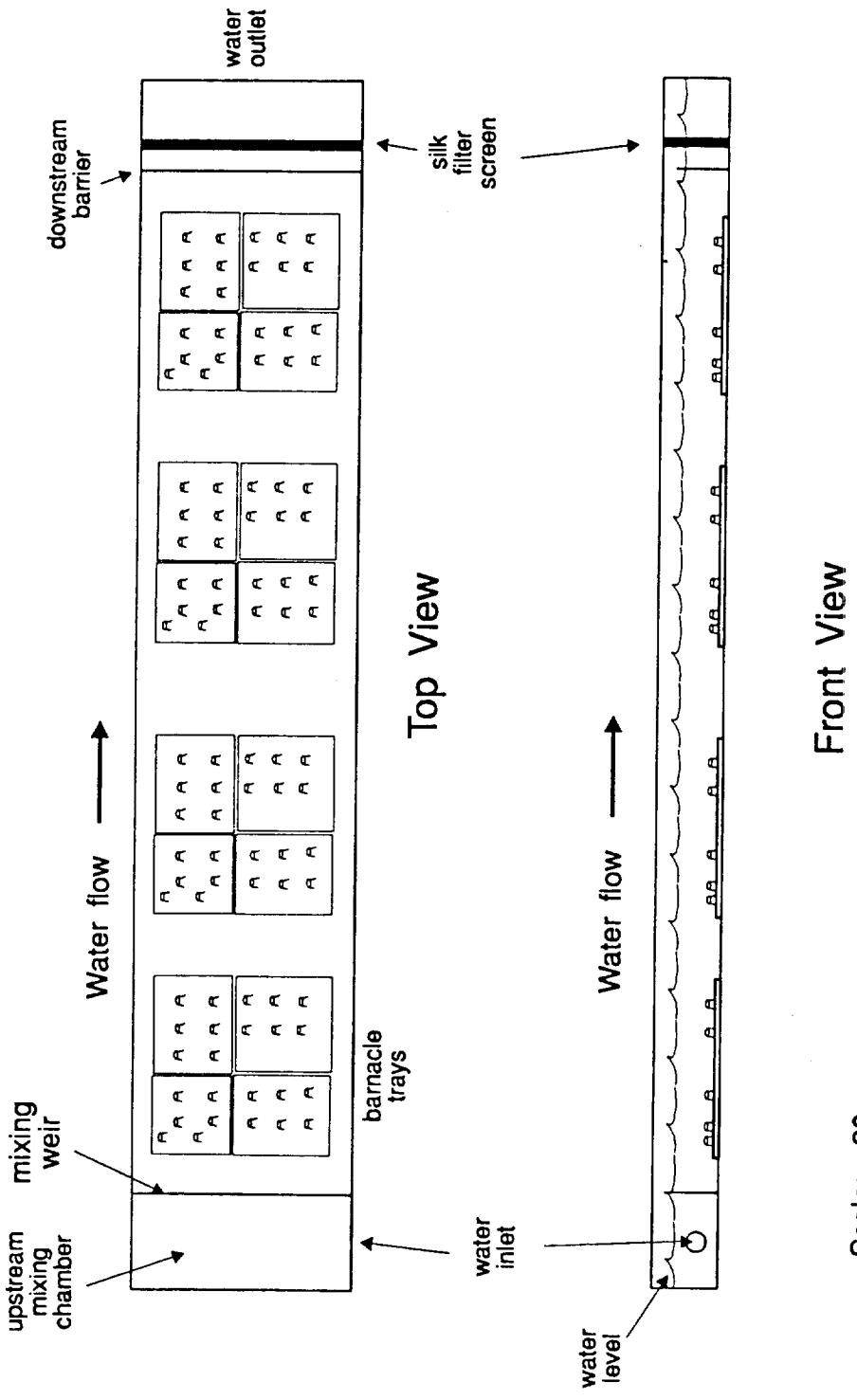


Figure 5. Map of the Salton Sea State Park Marina Headquarters (Varner Harbor) with collection and sampling sites noted.

of the harbor on August 12, 1993 using the same meter and a self-stirring polarographic electrode.

The barnacles were transported to Loma Linda University in continuously aerated insulated containers. Before each experiment, the algae and freshly settled larvae covering the barnacles' shell plates were removed by brushing. The bases of 96 barnacles were then individually glued onto 16 acrylic trays 7 to 9 cm^2 in area, using cyanoacrylate ("super") glue. Six individuals were glued to each tray and were spaced 0.8 to 1.5 cm apart. All the barnacles were carefully oriented in the same direction, perpendicular to current flow, since feeding rate is sensitive to the direction of water flow (Crisp and Bourget, 1985). These trays were placed in an acrylic flume through which 35‰ normal salt water was continuously and slowly circulated at a flow rate of approximately 0.5 cm/s (Figure 6). The flumes were placed inside large sea water tanks (23-28°C) under a 14L:10D light-dark cycle. Continuous aeration was provided by means of air stones. The barnacles were allowed 5 days of acclimation to the laboratory conditions. They were fed 500 ml of freshly hatched *Artemia salina* nauplii every two days. Seawater temperature, dissolved oxygen, and salinity in the flumes were monitored every other day and salinity was adjusted if necessary.

Figure 6. Acrylic flume and trays in which barnacles *Balanus amphitrite* were exposed to different experimental salinities.


water outlet
downstream barrier
silk filter screen
Water flow
Top View
mixing weir
upstream mixing chamber
barnacle trays
water inlet
Water flow
Front View
water level
Scale: 20 cm

Behavior, growth, reproduction, and mortality assays

General procedure--Experimental salinities used were 35, 40, 50, 60, 70 and 80‰, produced by adding a concentrated brine of "Instant Ocean" sea salts to normal seawater until the refractometer indicated that the target salinity was reached. Deionized water was added if salinities became too high. Thirty-five parts per thousand salinity was chosen as the control group since it is the average ocean salinity to which worldwide shore *Balanus amphitrite* barnacles are exposed. Six cohorts were used on the following dates--60‰, June 25-July 23, 1993; 35‰, July 9-August 6, 1993; 80‰, July 23-August 20, 1993; 50‰, August 6-August 25, 1993; 70‰, October 22-November 5, 1993 and 40‰, November 5-December 3, 1993. The barnacles for each experimental group were also checked for baseline rates of feeding behavior two days before the experiment began, and so served as their own control group for this variable. On the sixth day after collection of the barnacles the actual experiment began when the barnacles were transferred to the experimental salinity, as outlined below and in Figure 7. In the rest of this paper this day will be called experimental day zero. It is to be noted that the 50‰ group was accidentally prematurely terminated at day 14.

Figure 9. Oxygen versus depth profile at Varner Harbor on August 12, 1993.

* = Monitor Temperature, Dissolved oxygen and salinity

Experimental Day	Actual Day	Monitored Activity
	1 Friday	Collect barnacles from Salton Sea Begin gluing onto trays Place in normal seawater for acclimation to laboratory conditions
	2 Saturday	Feed, look for larvae, *
	3 Sunday	Finish gluing onto trays
	4 Monday	Feed, measure feeding rate Look for larvae, *
	5 Tuesday	
DAY 0	6 Wednesday	Weigh, look for larvae Place in experimental salinity Wait 30 minutes Feed, measure feeding rate, *
	7 Thursday	
	8 Friday	Feed, look for larvae, *
	9 Saturday	
	10 Sunday	Feed, look for larvae, *
	11 Monday	
	12 Tuesday	Feed, look for larvae, *
	13 Wednesday	
DAY 8	14 Thursday	Feed, look for larvae, Measure feeding rate Check mortality, do "pin test", *
	15 Friday	Weigh
	16 Saturday	Feed, look for larvae , *
	17 Sunday	
	18 Monday	Feed, look for larvae, *
	19 Tuesday	
DAY 14	20 Wednesday	Feed, look for larvae, *
	21 Thursday	
	22 Friday	Feed, look for larvae, *
	23 Saturday	
	24 Sunday	Feed, look for larvae, *
	25 Monday	
	26 Tuesday	Feed, look for larvae, *
	27 Wednesday	
	28 Thursday	Feed, look for larvae Measure feeding rate Check mortality, do "pin test", *
DAY 23	29 Friday	Weigh, end of experiment

Feeding behavior--The feeding behavior of all 96 barnacles was measured in normal seawater (35‰) two days before the experiment began. On day 0, each tray of 6 barnacles was weighed and then transferred to the experimental conditions. The barnacles were left undisturbed for an hour to overcome behavioral effects due to the transfer, then live *Artemia salina* nauplii, which the barnacles readily feed on, were placed in the upstream end of the flume and allowed to drift slowly past the barnacles. A 10 minute waiting period was allowed to elicit a full feeding response in the barnacles. Then the feeding rate of each of the 96 barnacles was measured with a stopwatch by timing the number of cirral beats in 15 seconds. Feeding behavior was measured on day 0, day 8 and day 22 for each experiment. In my experiment, cirri strokes were defined following Crisp and Southward's (1961) definition of normal and fast beat. Normal beat is the most common activity under aquarium conditions and may be defined as "the type of rhythmic behavior in which the cirri are fully expanded and then withdrawn into the mantle cavity again without pause" in rhythm with the opercular movements. In the fast beat the beat movements are faster but with less opercular movement than in normal beat; there is a lack of any noticeable exhalant pulse, but strong and fast rhythmic cirral movements occur. Since the differences between normal and fast beat can only be analyzed fully from

cinematographic records, in my experiment each full cycle was recorded as one stroke or one beat and any beats which did not show this complete cycle were not recorded.

Change in Wet Weight--The barnacles' wet weight was measured at experimental day 0 before being transferred to the experimental salinity, then again at day 8 and at day 24. However, no weight was recorded for salinities 35, 60 and 80‰ on day 8. Weighings were made of each entire tray of 6 barnacles, and mean wet weight was expressed as average grams per individual after subtracting the weight of the tray. Before weighing, water and debris were removed from the tray by thoroughly blotting the tray with a paper towel, but without blotting dry the barnacles' tergum and scutum.

Reproduction--A fine-mesh silk screen was installed in the flume downstream from the barnacles (Figure 6). Every other day before feeding, the screens were checked for the presence of planktonic barnacle (cypris and nauplius) larvae, which would indicate that a reproductive event had taken place. Also, when weighing the trays each barnacle was checked for settled larvae and/or fresh juvenile barnacles growing on the adult shell plates, tergum, or scutum.

Mortality--Every other day, barnacles were assessed for mortality. Prior experience had indicated that there were several reliable signs that the barnacles had died: 1) empty shell plates or brown colored individuals; 2) immobile barnacles hanging outside their shells; 3) no response to gentle prodding of the scutum with a pin (Southward, 1962); or 4) softening of the scutum. Dead barnacles were recorded, and they and their shell plates were removed to prevent the water from fouling and to prevent fungi from infecting healthy individuals.

Blood osmolality assays

The total amount of solutes present in the body can be expressed as osmolality. This variable can be obtained by measuring the freezing point depression of the body fluid because freezing point depression varies in direct proportion with osmolality. Measurements of hemolymph osmolality show the internal physiological changes of an organism as it copes with environmental changes (such as exposure to various salinities) and thus can help determine if an organism is an osmoconformer or an osmoregulator. This involved a parallel set of experiments using a different set of barnacles, which were collected and maintained in the same way as was the first set. The procedure is outlined below and in Figure 8. Algae and larvae were brushed off

Activity

1) Prepare experimental salinity
2) Brush off larvae and algae
3) Randomly select 50+ barnacles
4) Place them in experimental salinity, continuously aerate with an air stone
5) Start timer (30 min, 1 h, or 24 h)
6) Prepare a cooler and place chamber, petri dish and tissue homogenizer on ice
7) Place centrifuge in cold room
8) Place counterbalance test tube on centrifuge
9) Turn on microosmette
10) At the end of assigned time, remove barnacles from experimental salinity
11) Place barnacles on petri dish
12) Remove plates with forceps, place viscera on chamber
13) Discard any dead barnacles
14) Transfer viscera to tissue homogenizer in small amounts and crush until all tissue has been homogenized
15) Scrape off pestle, weigh tissue homogenizer and return to ice
16) Make counterbalance tube 5 g less than tissue homogenizer
17) Place both glass vials in centrifuge at 3rd speed level for 15-90 minutes
18) Pipette three 50 μl aliquots, labeling them in sequence as the liquid is extracted
19) Set micro-osmette at scale III
20) Clean with ethanol and a cotton tip the micro-osmette wire and the vial site
21) Place each vial in micro-osmette, cleaning machine between each sample to avoid sample pollution
22) Make 3 replicates of each sample
23) Take water samples and repeat steps 18-23

Figure 8. Protocol for blood osmolality assays

from clusters of barnacles; then about 40-50 barnacles were randomly selected and placed in experimental salinities (25, 35, 40, 50, 60 and 70‰) for 30 minutes, 1 h, or 24 h. No 80‰ osmolality tests were done because barnacles were already extensively dehydrated by 70‰, and massive numbers of barnacles had to be used to obtain a few drops of hemolymph at this osmolality. At the end of each time period,a subset of approximately 50 barnacles was removed and their shell plates were removed with forceps. Then the viscera were placed in a glass chamber with some moist tissue paper to prevent them from drying, and were placed on ice. The time to remove all the barnacles from the shell was about 15 minutes. Any dead barnacles were discarded. The barnacle viscera were then transferred to a tissue homogenizer which was kept on ice at all times, and the specimens were homogenized thoroughly. They were then centrifuged at 5°C at the third speed level of a desktop centrifuge (IEC Clinical bench model 428) for 15-90 minutes until the hemolymph above the sedimented particulate was clear. Three replicate 50 μl samples of the supernatant hemolymph were removed by a micro-pipette and placed into vials, labeling them in sequence as the liquid was extracted. The samples were not diluted but were placed directly in a micro-osmette model 5004 micro-osmometer and hemolymph osmolality was read using scale III. Sea water osmolality samples were also taken from

the experimental salinities in which the barnacles had been placed.

Statistical analysis

Two types of statistical comparisons were made: among days for each salinity treatment, and among salinity treatments for each day. For both types of comparisons, I used Analysis of Variance and the Scheffé multiple-range tests, using SPSS/PC (SPSS Inc). Comparisons among days were made for feeding rate, wet weight and survival. Comparisons among treatments were made for feeding rate and survival. Because each treatment had a different initial value, these comparisons required that I standardize each day as a percentage of the control (initial) value for each group. Arc Sin transformations were done on these percentage values to normalize them before performing parametric statistical analyses. Groups were compared using ANOVA and the Scheffé multiple-range test, using SPSS/PC (SPSS Inc). This same transformation and analysis was also performed on the feeding rate and survival data. For the mortality assay, the LC_{50} for salinity at 22 days was calculated on a probit-logit scale (Martinez-Palacios, 1990). LC_{50} is the concentration at which 50% of the tested population dies and 50% lives. Reproductive events were simply noted on each date. Changes in blood osmolality in the different salinities were

assessed by Analysis of Variance and the Scheffé multiple-range tests, using SPSS/PC (SPSS Inc). Differences at or below the 0.05% confidence level were deemed significant; those at 0.01% or below were deemed highly significant.

RESULTS

Physical characteristics of the Salton Sea during the period of the experiment

Temperature in the Sea varied considerably on a seasonal basis during the experiment. Dock water surface temperatures were warmer during summer (31-33°C), and considerably cooler in late fall (22-24°C). Surface temperatures at the beach and jetty, representing sites less protected than the dock site, were generally of similar temperature or up to 1.5°C cooler. There was a slight variability in salinity among the sites (up to 1‰), but in general the sites were of similar salinity. Salinity increased, however, from 39‰ in late Spring to 41‰ in late Fall, presumably due to the high evaporation rates the Sea is known for. Oxygen levels were highly variable at the surface throughout the experiment, and were also variable by depth. More often than not, oxygen levels in surface waters were only about 50% saturated or less. On several occasions, however, primary productivity was so high that the surface water was supersaturated with oxygen, especially at the beach site. At the other extreme, oxygen was nearly absent from even the surface water at times in late summer and fall, especially at the dock and jetty sites. This low oxygen appeared to be correlated with a mass die-off of the barnacle population which occurred at this time. Oxygen levels below the surface were even lower than those

at the surface, approaching zero as shown in the oxygen profile in Figure 9, which was taken August 12, 1993 at the dock site A.

Physical and behavioral responses of B. amphitrite to increasing salinity

The measurements of feeding rate, changes in wet weight, reproduction, and survival of *B. amphitrite* at different salinities provided parallel indices of the physiological impact of increasing salinity on the species. These different indices produced generally similar outcomes, while specific differences among the results of the various tests, as outlined below, provided a documentation of the increasingly pervasive physiological effects as salinity increased.

Feeding rates--The barnacles displayed increasingly extreme aversive responses to the increasing salinities, which were clearly detectable in their feeding behavior. Subjects in 35, 40, and 50‰ had little or no immediate change in their feeding rates when transferred to the experimental salinity (Figures 10 and 11). They continued to feed at rates similar to or greater than the control throughout the experiment. Hereafter I will refer in this paper to control rates as normal rates. The groups at higher salinities,

Figure 9. Oxygen versus depth profile at Varner Harbor on August 12, 1993.

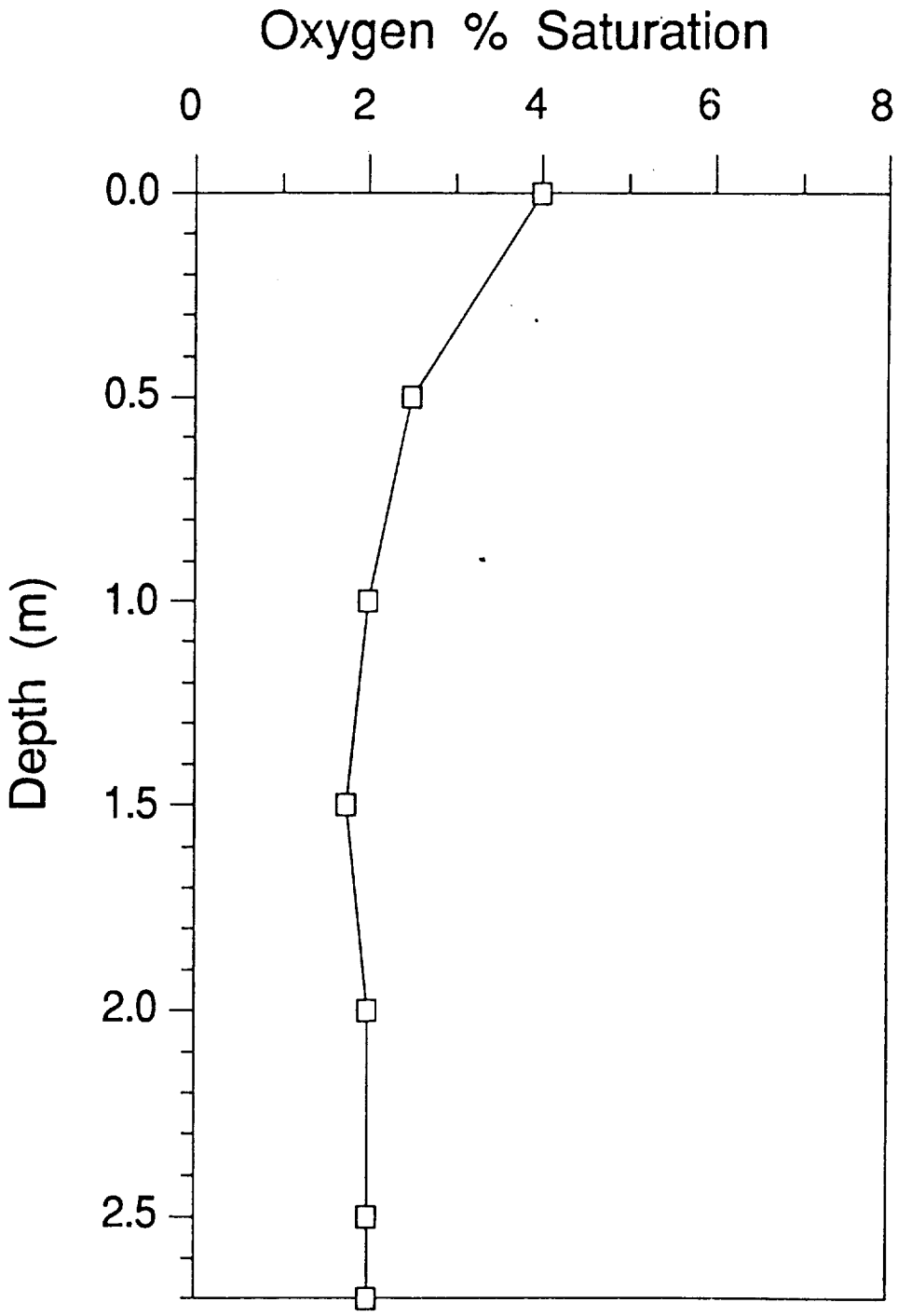

Oxygen % Saturation
0
2
4
6
8
0.0
0.5
1.0
1.5
2.0
2.5
Depth (m)

Figure 10. Feeding rates of *Balanus amphitrite* at different experimental salinities, as a function of elapsed days. Error bars = se.

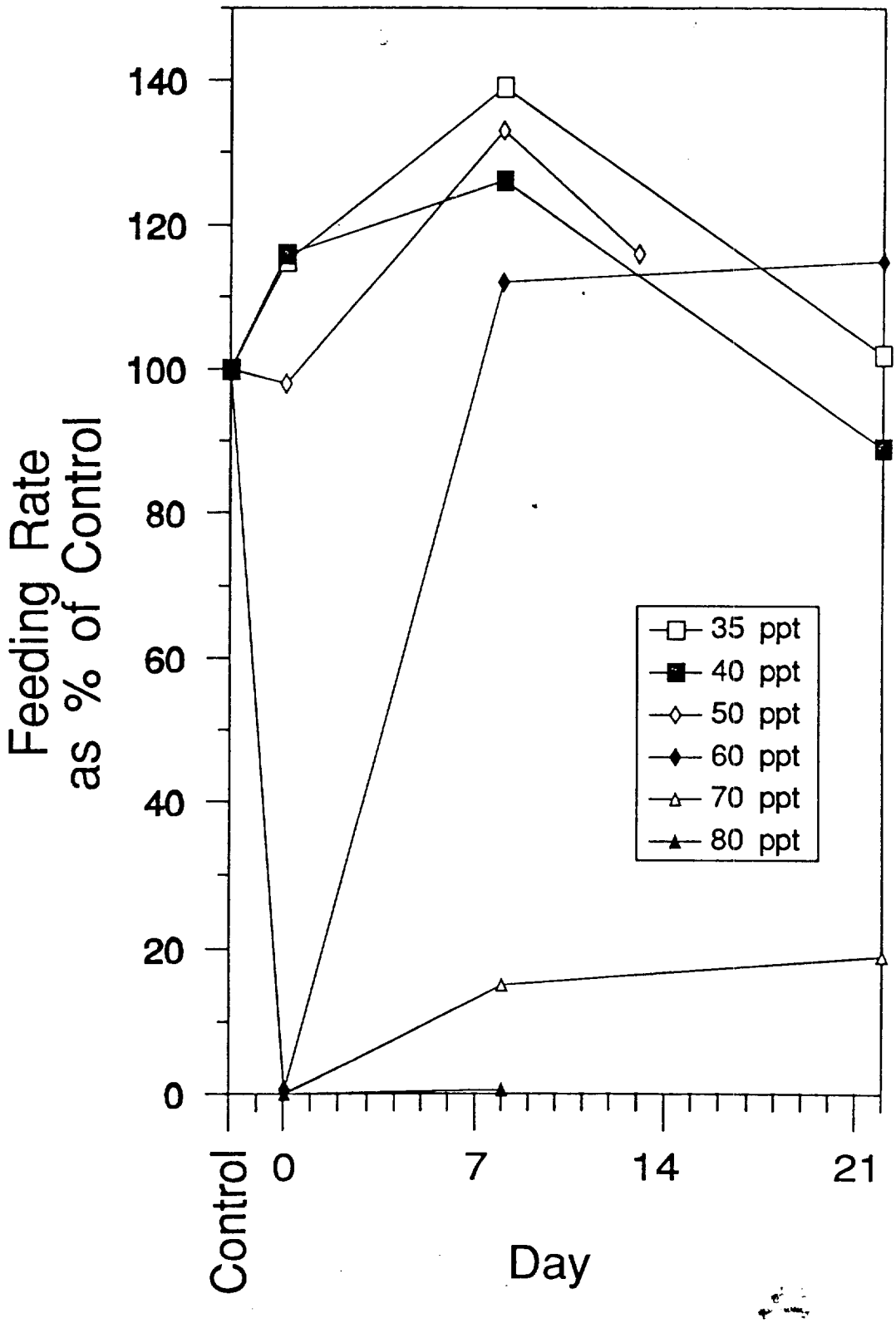
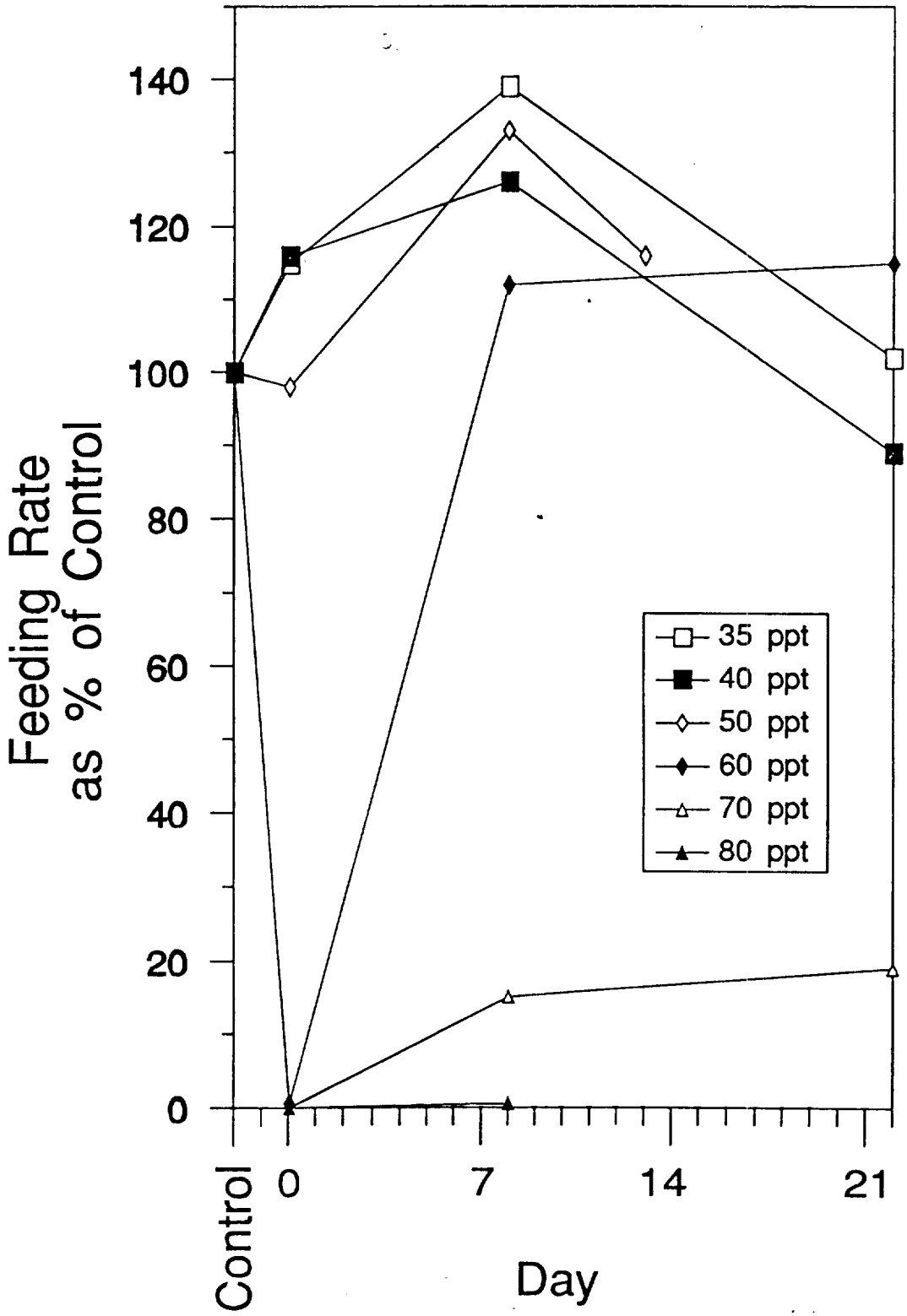

Feeding Rate
as % of Control
140
120
100
80
60
40
20
0
Control
0
7
14
21
Day
35 ppt
40 ppt
50 ppt
60 ppt
70 ppt
80 ppt

Figure 11. Feeding rates of *Balanus amphitrite*, expressed as a percentage of initial (control) feeding rate, at different experimental salinities, as a function of elapsed days. Error bars = se.

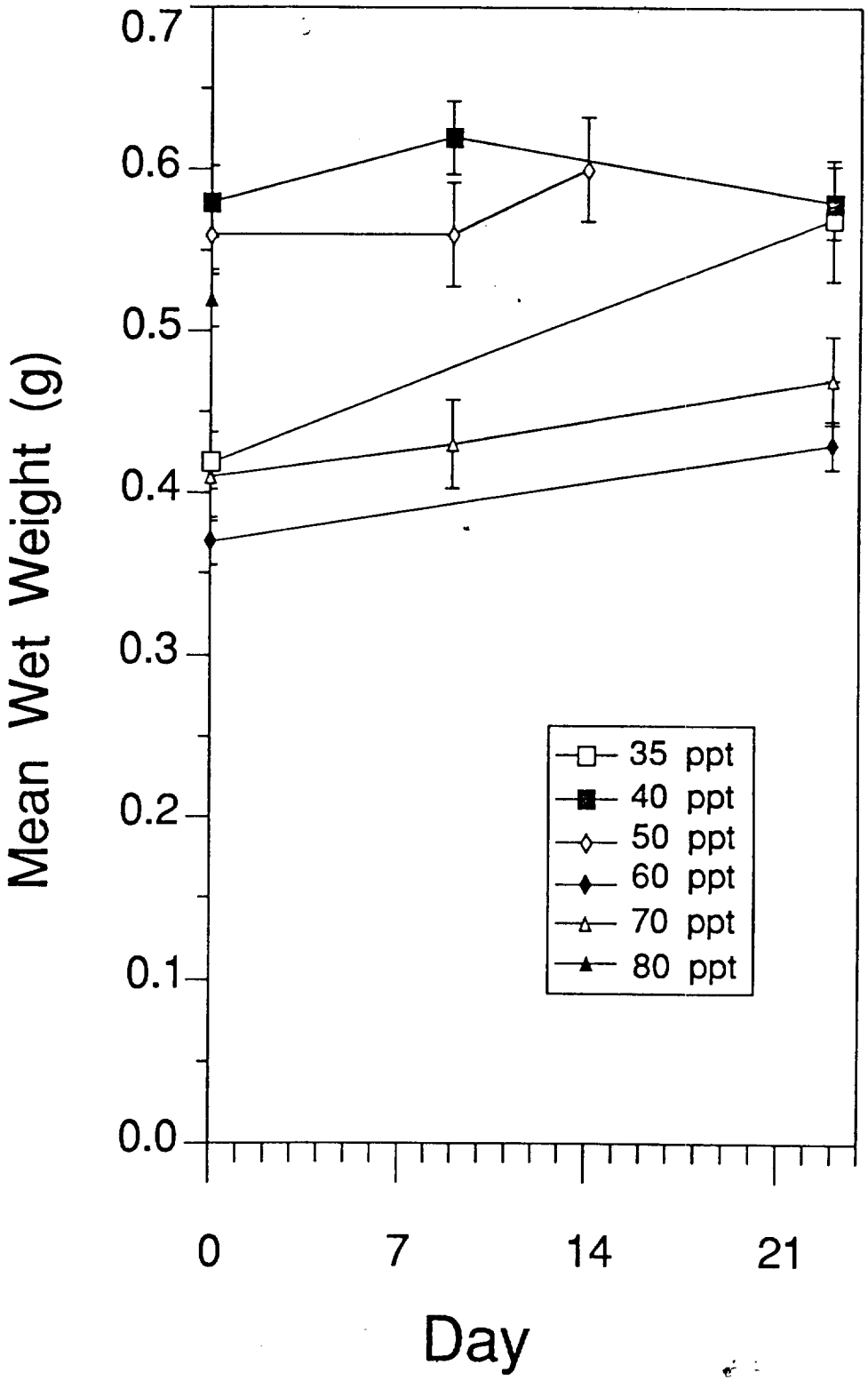

Feeding Rate
as % of Control
140
120
100
80
60
40
20
0
Control
0
7
14
21
Day
35 ppt
40 ppt
50 ppt
60 ppt
70 ppt
80 ppt

(60-80‰), showed a definite acute aversive response to the higher salinity, i.e., they would not feed even 1 h after being immersed in the experimental salinities. The group placed in 60‰ salinity immediately closed up, and had virtually zero feeding response on the first day (Figures 10 and 11). By day 8, however, the barnacles in 60‰ had resumed essentially normal rates, though still lower as a percentage of its control rate than were those in 35, 40, and 50‰. This difference was significant between 60‰ and the 35 and 50‰ groups at day 8. By day 22, the feeding rate of the 60‰ group had recovered further and was no longer lower than that of any of the lower salinity groups. The 70‰ group, in contrast, also closed up on initial transfer to 70‰ salinity, but failed to recover as rapidly or as completely as did the 60‰ group (Figures 10 and 11). By day 8 its rate was still only about 15% that of the groups at lower salinities, a highly significant difference ($P < 0.0001$). Though the 70‰ group exhibited a slowly increasing feeding rate, it never returned to anywhere near normal levels. By day 22, it was still feeding at less than one-third the rate of those at lower salinities, a highly significant difference. The 80‰ group, as the most extreme case, closed immediately and, except for a few feeble strokes recorded on day 8 for a few individuals, did not feed at all until death (Figures 10 and 11). In addition, even when not feeding, a

behavioral difference between barnacles at 70-80‰ and those at lower salinities was observed: most of the barnacles in the high salinities left their valves (scutum and tergum) partly agape, but would not move their cirri. This explains the drastically lower number of recorded strokes. They did show some response when disturbed i.e., they would close their valves. The barnacles at lower salinity, in contrast, frequently closed the scutum and tergum when not feeding, but opened rapidly and fed vigorously when a feeding response was elicited.

Wet Weight--All groups except the 80‰ group gained weight during the experiment (Figure 12). The 35‰ and 60‰ groups gained weight significantly. The 50‰ group gained weight, but since it was terminated prematurely, it is not known whether its weight gain would have been significant by the end of the experiment. The weight gain in the 40‰ group was also not significant, but this group started out with a significantly greater average weight than the 35, 40, and 60‰ groups and it had also reached the largest size by the end of the experiment. It may have been growing more slowly because it may have been approaching the upper size range normally observed for this species. The increase in weight of the 70‰ group was not significant, even though those barnacles started out small as did the faster-growing 60 and

Figure 12. Changes in wet weight of *Balanus amphitrite* at different experimental salinities, as a function of elapsed days. Error bars = se.


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Mean Wet Weight (g)
0
7
14
21
Day
35 ppt
40 ppt
50 ppt
60 ppt
70 ppt
80 ppt

35‰ groups. The 80‰ group died so no final weights could be recorded.

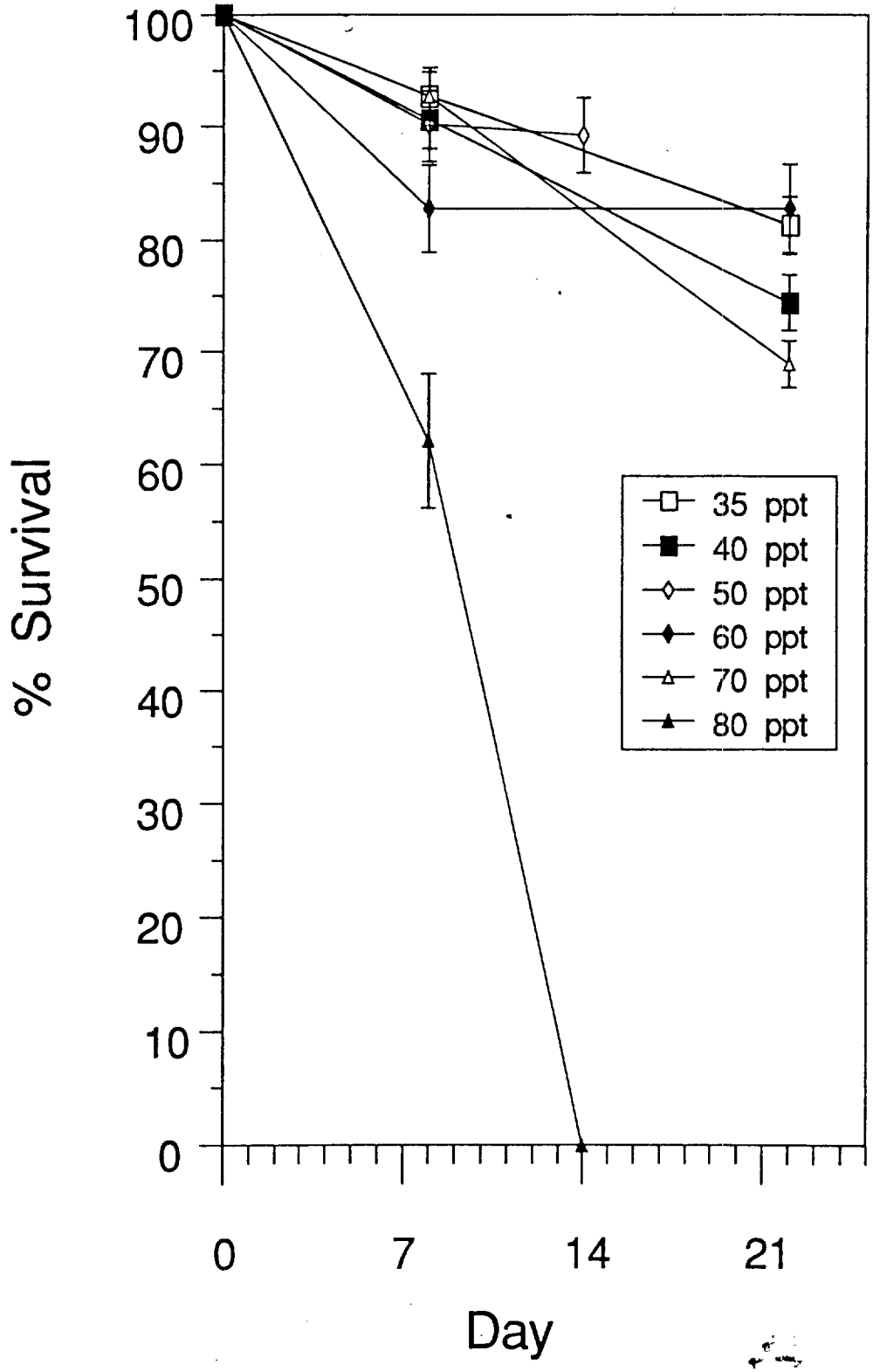
Reproduction--New larvae and settlement of juveniles on the adult barnacles were observed at salinities ranging from 35‰ to 50‰ (Table 3). The 40‰ treatment had the greatest number of larval settlement (34 larvae and 83 juveniles were observed during the experiment). Only two juveniles and no larvae were observed at the 60‰ treatment. It would thus appear that reproduction was quite reduced at this salinity; however, this conclusion must remain tentative since this salinity was one of the earliest tested and identification of barnacle larvae was less certain than it was in later experiments. There was little or no apparent reproduction at either 70 or 80‰.

Mortality--All of the experiments, including the control, experienced some amount of mortality during the experiments probably due to residual handling, transport effects and fungi infection from dead individuals (Figure 13). Survival was high in all salinities for the first week, except for the 80‰ treatment, which had already suffered significantly greater mortality than the rest. High mortality continued in the 80‰ group, and all of them had died by day 14. Mortality continued to be low and not statistically

Table 2. Reproductive success of *Balanus amphitrite* at different salinities. Abbreviations: L = larvae (pelagic nauplii or cyprids) which were caught in the silk screen. J = juveniles (settled individuals which have initiated metamorphosis into the adult form). ppt = salinity in parts per thousand. Cont = control

Salinity	Day															Total	
(ppt)	Cont	0	2	4	6	8	10	12	14	16	18	19	20	22	23	L	J
35								1L				1L	1+L			3+	
				1J									1J		1J		2
40	30L				3L	10L	3L		1L		1L					48	
					2J	2J	50J				1J			2J	26J		83
50		15L	3L	15L	1+L	5L	5L		10L							54+	
															2J		2
60																0	
															2J		2
70																0	
																	0
80																0	
				1J													1

Figure 13. Percent survival of *Balanus amphitrite* at different experimental salinities, as a function of elapsed days. Error bars = se.


100
90
80
70
60
50
40
30
20
10
0
% Survival
0
7
14
21
Day
35 ppt
40 ppt
50 ppt
60 ppt
70 ppt
80 ppt

different among the rest of the groups throughout the rest of the experiment. By the end, the 70‰ group had suffered greater mortality but this difference was not yet significantly different from the other groups. The LC_{50} for salinity was estimated to be approximately 73‰ at 22 days.

Blood osmolality assays

After 24 h, *B. amphitrite* in 35-60‰ appeared to have completely osmoconformed to the salinity of the seawater medium (Figure 14). The blood (hemolymph) of *B. amphitrite* in 25‰ seawater, however, was still substantially higher than that of the seawater after 24 h, a highly significant difference ($P < 0.0001$). Also, the blood of *B. amphitrite* in 70‰ was substantially lower in osmolality than the ambient seawater ($P < 0.0001$) (Figure 14). On the surface, this would seem to indicate that *B. amphitrite* osmoconforms at intermediate salinities of 35-60‰, but osmoregulates at the more extreme high and low salinities of 70 and 25‰. The time series data, however, indicate that the situation may not be as simple as this (Figures 15 and 16). The ready change in blood osmolality of subjects in 35, 40, 50, and 60‰ seawater to the salinity of the ambient medium does appear to be a clear case of osmoconformity within this salinity range. Likewise, the blood osmolality of subjects in 25‰ seawater, which did not approach that of the ambient medium, indicates

Figure 14. Osmolality of *Balanus amphitrite* blood after 24 hours at different experimental salinities.

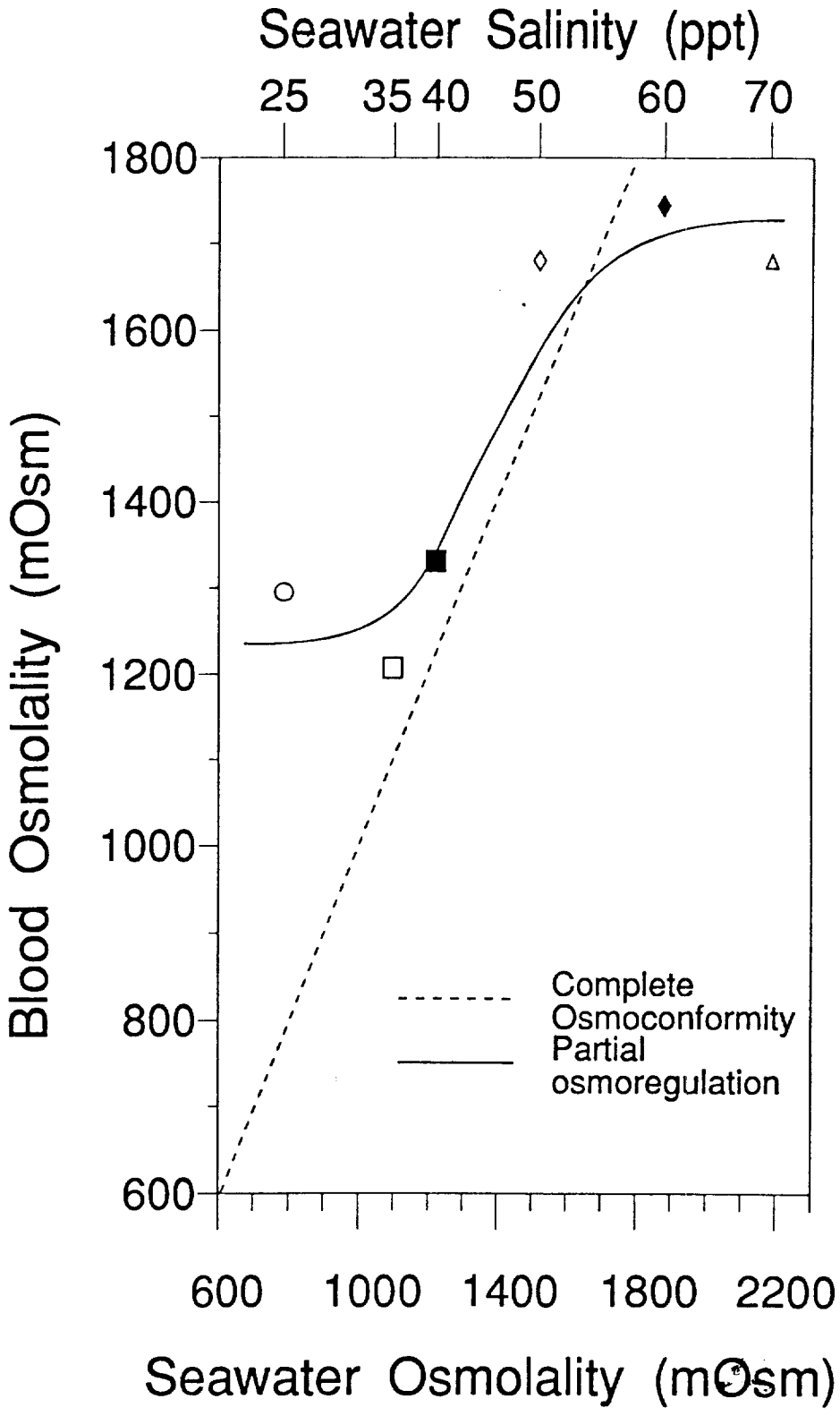

Seawater Salinity (ppt)
25
35
40
50
60
70
1800
1600
1400
1200
1000
800
600
Blood Osmolality (mOsm)
Complete Osmoconformity
Partial osmoregulation
600
1000
1400
1800
2200
Seawater Osmolality (mOsm)

Figure 15. Time series of changes in blood osmolality of *Balanus amphitrite*, placed in different salinities. Error bars = se.

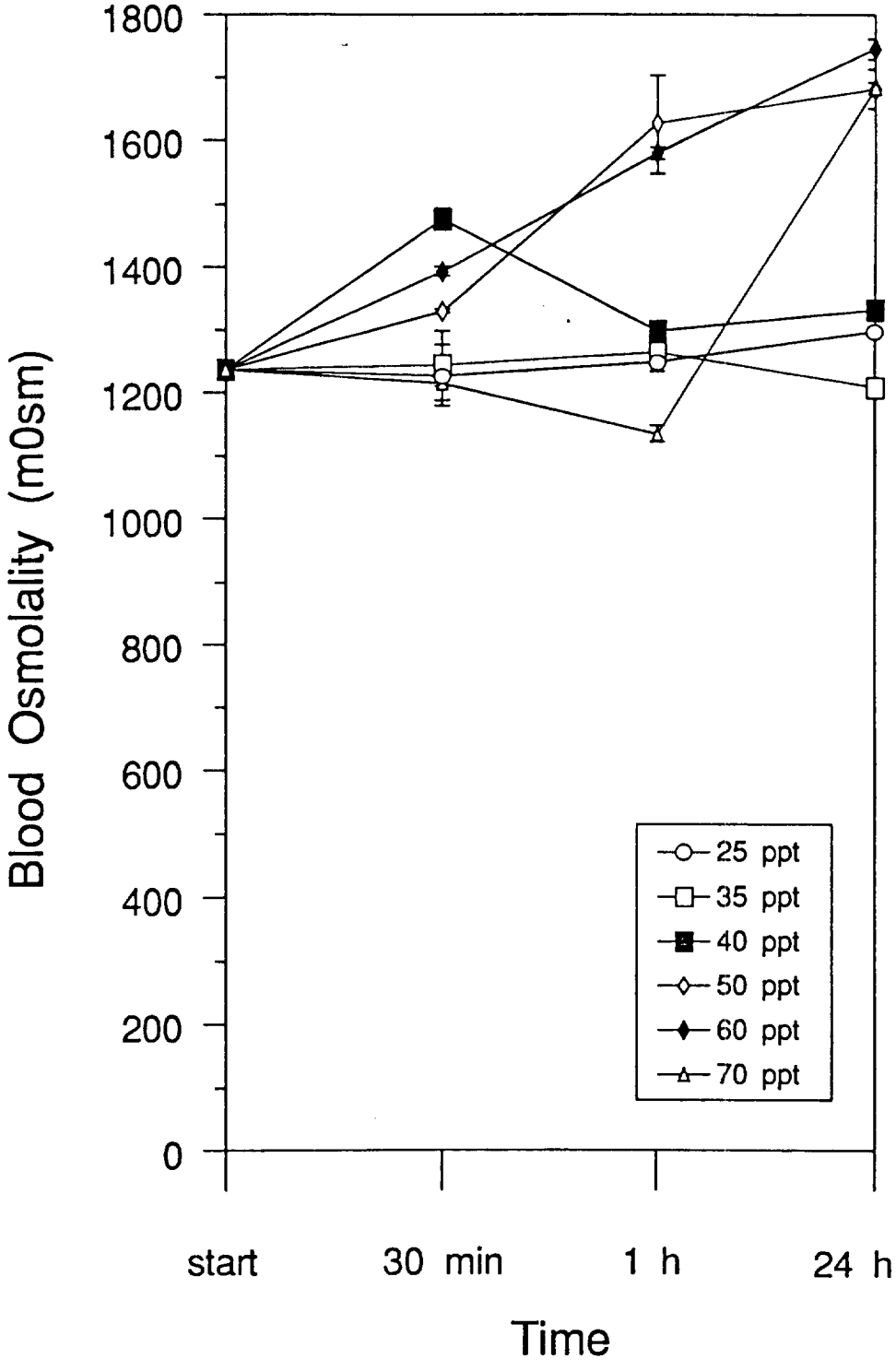
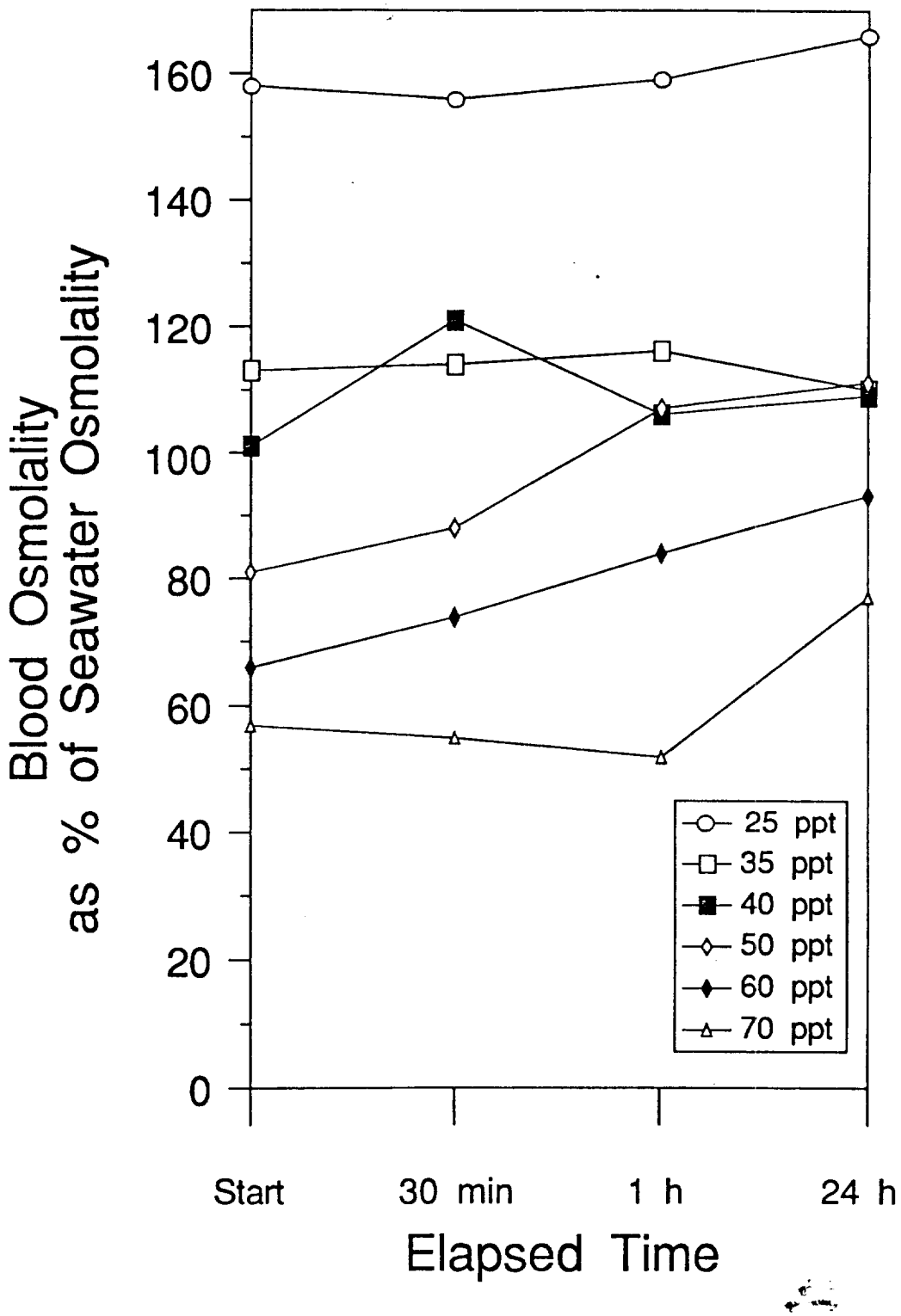

1800
1600
1400
1200
1000
800
600
400
200
0
Blood Osmolality (m0sm)
25 ppt
35 ppt
40 ppt
50 ppt
60 ppt
70 ppt
start
30 min
1 h
24 h
Time

Figure 16. Time series of changes in blood osmolality of *Balanus amphitrite* placed in different salinities, normalized to percentage of ambient seawater osmolality.


Blood Osmolality
as % of Seawater Osmolality
160
140
120
100
80
60
40
20
0
25 ppt
35 ppt
40 ppt
50 ppt
60 ppt
70 ppt
Start
30 min
1 h
24 h
Elapsed Time

osmoregulation at this lower salinity. It cannot be determined from the data, however, whether *B. amphitrite* acts as an osmoregulator or as an osmoconformer at higher salinities such as 70‰. The time-series data for 70‰ show a pattern that is inconsistent with pure physiological osmoregulation, and may or may not be consistent with osmoconformity. On placement into the 70‰ seawater, the subjects' blood initially remained at constant osmolality, characteristic of the 35‰ water they had been transferred from (Figure 15). After 1 h, however, the blood osmolality began to increase. If the subjects were physiologically regulating or partially regulating their blood osmolality, one would expect to see no change, or an initial partial change followed by stabilization at a new level, neither of which were seen. Though blood osmolality of the 70‰ group was still significantly lower than that of the seawater after 24 h, it cannot be determined from the dataset whether the blood osmolality had stabilized at this value, indicating osmoregulation, or was continuing to increase, indicating osmoconformity. The best interpretation of the 70‰ data is that the subjects initially used behavioral osmoregulation, closing up tightly when exposed to the 70‰ water. This allowed them to keep their blood out of contact with the seawater for at least an hour, maintaining the initial low osmolality. However, after a time blood-

osmolality began to climb in spite of the subjects' aversive response. It is not clear from the data whether the subjects could stabilize blood osmolality at a higher level by osmoregulation, or would inevitably conform to the higher seawater osmolality. *B. amphitrite*, then, appears to be a clear osmoregulator at 25‰ and an osmoconformer between 35 and 60‰, but it may or may not osmoregulate at higher salinities.

DISCUSSION

Physical and chemical characteristics of the Salton Sea during the period of the experiment

The wide ranges of temperatures and oxygen levels observed in the Sea during this study have been reported by others as well. Carpelan (1958) stated that the organisms present in the Salton Sea have to cope with great temperature ranges and a high summer temperature; furthermore, they must cope with large diurnal fluctuations in pH and in the dissolved oxygen concentration resulting from photosynthesis and respiration. In addition, the most severe stress they have to deal with is the stress "occurring annually when decomposition of settled zoo- and phyto-plankton brings about periods of oxygen depletion at the bottom". Furthermore, there are also high concentrations of sulfide and ammonia that make the summer season the most challenging for many organisms to survive. Carpelan (1961) also observed some occasional anoxic periods at the Salton Sea during summer and fall along with a fish "kill" and high concentrations of sulfide. He suggested that the depletion of oxygen was due to dinoflagellate blooms and bacterial decomposition. Carpelan (1958) stated that "concentrations of dissolved oxygen ranged up to 11.8 mg/L at the surface 60 9.3 mg/L at the bottom during winter. During calm periods in summer, however, the water at depths below 8-9 meters became anoxic for periods as long as 1-3 days; the surface waters,

too, became depleted of oxygen, but only for an hour or so at about dawn. Concentrations of sulfide as high as 85 μg-at./L and of ammonia as high as 50 μg-at./L were found during the anoxic periods in summer...Periods when oxygen is absent at the bottom are periods without wind." These anoxia periods may be directly or indirectly responsible for the mass die-off of barnacles that occurred in late summer. Vittor (1968) noted the anoxic conditions and also the growth of mats of blue-green algae which grew over the barnacles, preventing cirral activity and resulting in suffocation or starvation. Presumably this extensive mortality of benthic barnacles during summer anoxia and algal overgrowth forms the source of the vast windrows of dead barnacles washed up on the beaches.

Physical and behavioral responses of B. amphitrite to increasing salinity

Feeding rates--In the Balanidae, a feeding barnacle may use two patterns of active cirral basket movement: normal beat and fast beat (Crisp and Southward, 1961). According to Hunt and Alexander (1991), adult barnacles use two main feeding techniques: they filter-feed by beating their cirri rhythmically or they hold their cirri extended in the circulating water so that food is either trapped passively or actively, depending on the speed of the water current. At

water flow speeds of <2-3 cm/s, barnacles tend to be active feeders (Crisp and Southward, 1961; Trager et al., 1990). The rate of cirral activity observed in the *B. amphitrite* in this experiment at salinities of 35-60‰ was in the range of rates observed for normal beat, or of "fast beat" (Crisp and Southward, 1961) used for active feeding.

The transient closing response of the barnacles placed in moderately high salinities (60‰), on the other hand, most likely corresponds to the "testing" behavior described by Crisp and Southward (1961). In this behavior, which "most often occurs under seemingly unfavorable conditions," the valves "hardly open and the cirri are not protruded," at least at first, but this behavior is frequently a precursor to full beating. Presumably the strength of the unfavorable stimulus is correlated with the tightness with which the opercular valves are closed and with the length of time that passes before the barnacle relaxes the valves, tests the water, and feeds. For example, barnacles regularly close up briefly after a mild stimulus such as being touched, or even when a shadow passes over them. Stronger or more prolonged unfavorable conditions, such as exposure to low tide, elicit tighter closing for a longer period of time. The evasive behavior of closing the opercular valves for long periods of time, in fact, is typical of acorn barnacles such as *Balanus* when encountering adverse environmental conditions (Barnes

et al., 1963). The length of time the barnacle remains closed may thus serve as a gauge of the strength of the negative stimulus being avoided, and perhaps also of the magnitude of the internal physiological adjustments necessary to adapt to the new conditions. In this regard the 60‰ seawater would rank as a fairly strong negative stimulus because the barnacles remained tightly closed for at least an hour after initial exposure to it, but it could eventually be adjusted to at least as far as normal feeding was concerned because the subjects had resumed nearly normal feeding rates by 8 days.

Even higher salinities (70 and 80‰) in the same line constitute a correspondingly stronger negative stimulus and a greater physiological challenge, one which, in the most extreme case (80‰), cannot be adequately adjusted to on acute exposure and leads to the death of the barnacles. The barnacles cannot remain closed indefinitely because their lack of exposure to oxygenated water while closed leads to anaerobic metabolism. Crustaceans in general have low tolerance to anoxia due to the buildup of toxic byproducts of metabolism, leading to harmful acidification in cellular pH and to metabolic derangements through end-product inhibitory effects (Hochachka and Somero, 1984). Barnacles are frequently seen to allow their opercular valves to gape slightly under these conditions, letting air in and

providing some access to oxygen (Barnes et al., 1963), so they cannot be considered to be operating anaerobically. When they are fully prevented access to oxygen, however, such as they would be when fully closed underwater, survival time under these truly anaerobic conditions is on the order of not much more than 3 or 4 days (Barnes et al., 1963). They must eventually open or die, even in the face of a strong negative external stimulus. The eventual opening of the *B. amphitrite* after several days in the 70 and 80‰ experiments does not necessarily mean that they had been able to physiologically adjust to these salinities, but may have simply been an indication that they had reached their limits to tolerance to the anaerobic conditions prevalent when closed. The fact that *B. amphitrite* had not, in fact, been able to fully adjust to the higher salinities was shown by the fact that feeding in the 70‰ group never rebounded to normal levels, and by the lack of active feeding and rapid death of the 80‰ group. The atypical gaping of those in the higher experimental salinities should not be interpreted as "testing" behavior typical of barnacles faced with a mild aversive stimulus, but rather as a signal of the breakdown of normal function and metabolic control due to strongly adverse physiological conditions brought about by exposure to the highly saline medium and by prolonged anaerobiosis.

Such a posture is often observed in barnacles after death or when death is imminent.

Wet Weight--Carpelan (1961) noted that barnacle growth was greatest in summer, when a basal diameter of 8-9 mm was reached within 30 days. During the coldest part of the year the growth rate was less than one-tenth the summer rate. Vittor (1968) reported that the highest growth rates in *B. amphitrite* occur during February and March; this species can reach a maximum diameter of 1.5 cm. Bourget (1987) stated that "growth is greater in spring and summer and considerably reduced in winter, while steadily decreasing after the first growth season." In addition, Crisp (1960) stated that "the rate of growth....appears to accelerate with increasing size, though it is probably not constant but falls, since the beating rate usually decreases with growth. After maturity, growth slows down." Those observations may help explain some of the variability in my dataset. Specifically, the 40‰ group was tested in the fall (November 5-December 3); and had a greater mean size, lower growth rate, and slower feeding rate than did the groups tested during the summer. These differences can most clearly be interpreted as effects of the season of testing and of the barnacles' larger size rather than as an adverse effect of the experimental salinity, especially since 40‰ is the

closest salinity of any of those tested to the present salinity in which the population is living and thriving.

Up until the study of Darling and Wilbur (1993), the literature did not show a consensus on non-destructive estimation of barnacles' fresh weight. Most authors' techniques require the destruction of the organism in order to find out its growth rate (Costlow and Bookhout, 1957; Clare et al., 1994). In this experiment I chose to measure change in wet weight because from all the proposed methods of measuring growth, it seemed to be the least disturbing for the barnacles and would not involve the destruction of organisms which would be needed for further tests.

Reproduction--Physiological stresses due to high salinity may be expected to result in reduction or cessation of reproduction. In this species, however, reproduction continued at salinities ranging from 35 up to 50‰, suggesting that physiological stress in this range of salinities is insufficient to deter reproduction. *B. amphitrite* can thus be identified as a euryhaline species. These observations agree with those of Crisp and Costlow (1963) in which *Balanus amphitrite* and *Balanus eburneus* nauplii were found to successfully develop in a salinity range of from 20 to 50‰. Vittor (1968) stated that "the ability of eggs and larvae to withstand extremes in

salinity and temperature may be essential to the success of barnacles in areas where wide fluctuations occur." This author mentioned that barnacle colonies had a high population density in salinities ranging from 19 to 38‰ in the Salton Sea State Park Marina. It is interesting to note that the species is still found there in very high densities, even though the salinity now usually exceeds 40‰. Crisp and Costlow (1963) suggested that larvae developing within reproductive females 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." Though both the larvae and the adults are affected by extremely high salinities, the former seem to be the most affected (Vittor, 1968).

Crisp and Stubbings (1957) and Rittschof et al. (1984) summarized the factors influencing larval selection of a particular substratum: depth, illumination, surface contour and texture, the presence of previously settled individuals of the same species or of related species, larval competence, local abundance, the velocity gradient of currents at the surface of the substratum, molecular and bacterial films, and chemical cues. Rittschof (1993) pointed out that live barnacle body odors act as pheromones for settlement and metamorphosis of larvae.

In our experiment, larvae generally settled directly on the adults, as early as 4 days after the experiment began. This observation differs from that of Thorson (1957) who stated that in estuaries, *B. amphitrite* "began to settle after about one to two and a half weeks."

Mortality--Schmidt-Nielsen (1983) stated that a euryhaline animal is one "which can tolerate wide variations in the salt concentration of the water in which they live." During this experiment the salinity at the Salton Sea (June-November 1993) ranged from 39-41‰ which is distinctly more saline than the ocean where the species is normally found. *B. amphitrite*'s thriving population in the Salton Sea and its survival in a wide range of laboratory salinities clearly identify it as a euryhaline species. Also, after the 70‰ experiment was finished, barnacles were left in the acrylic flume and the salinity was allowed to slowly increase for 28 days due to evaporation. The barnacles were monitored for survival and continued to be fed. At the end of this time, only 10 individuals (15.9%) had died, even though the salinity by then had reached 92‰. Adults of this species can thus tolerate exposure to salinities of at least 92‰ for quite some time if the high salinities are approached gradually. Simpson (personal comm.) stated that *B. amphitrite*'s ultimate salinity tolerance is 100‰ for both

short and long-term exposure. Hedgpeth (1967) reported the upper salinity tolerance of *B. eburneus* and *B. amphitrite* to be 76‰. This agrees well with my LC_{50} estimates of 73‰, but the long-term experiment described above shows that this number applies only to acute exposure, and maximum tolerable salinity can be much higher if the salinity is approached gradually.

Blood osmolality assays

Most previous studies of osmoregulation in crustaceans have addressed the more common situation of dilution of the seawater medium to brackish water, such as that encountered in estuaries. Kirschner (1991) stated that in this situation most euryhaline crustaceans are osmoregulators, while others are at least partial osmoregulators. This pattern is clearly seen in *B. amphitrite* in the hyposaline conditions, though the range of diluted salinities I tested was not great enough to determine how strong the osmoregulation pattern is. As Fyhn (1976) pointed out, previous osmoregulation data about *B. amphitrite* and *B. glandula* may not be representative because it was not always clear that the barnacles were measured in a steady state. This author stated that "above a sea water osmolality of about 500 mOsm [corresponding to about 17‰ salinity] the hemolymph of *B. improvisus* conforms osmotically with the sea water but is

slightly hyperosmotic to it. This....seems to be a general feature of cirripeds." In this regard, *B. amphitrite* is a better osmoregulator than is *B. improvisus* because *B. amphitrite* was strongly osmoregulating even at 25‰. The fact that the *B. amphitrite* were at a steady state in their osmoregulation is clearly illustrated by the lack of any significant change in internal osmotic potential for 24 h in these individuals (Figure 16).

The hypersaline (>35‰) experiments extend these observations in the other direction, and in this range quite different results are observed than found in the hyposaline experiment. *B. amphitrite* appears to osmoregulate little if at all in moderate hypersalinity (40-60‰), and it is unclear whether it osmoregulates or not at 70‰ or higher. Determination of whether this species osmoregulates at very high salinities will require longer-term tests than were conducted in this experiment. This conclusion is in agreement with that of Fyhn (1976) and Foster (1970) who found that barnacles needed a substantial period such as 5 to 7 days to fully acclimate from 100 to 50% seawater.

The closing response observed in the barnacles upon being immersed in the highest salinities is a commonly observed aversive response which serves to "retard the osmotic equilibration between hemolymph and sea water" (Fyhn, 1976). The effects of this aversive behavior were

clearly seen in the time-dependent change in blood osmolality in the 70‰ individuals, with the initial lack of change in blood osmolality due to the closing response and the subsequent climb in osmolality due either to later partial opening of the opercular valves or to partial penetration of hyperosmotic seawater into the mantle cavity even with the valves closed. In either case, there is no clear evidence for physiological osmoregulation in these hypersaline conditions.

Several authors have obtained hemolymph from intact barnacles either by syringe (Waite and Walker, 1992) or by a glass micro-pipette (Fyhn, 1976) and report obtaining substantial amounts of hemolymph from just a few individuals (10 or less). This method appears to have some serious drawbacks when applied to *Balanus amphitrite*, however. The barnacles, when closed, retain a definite residual supply of seawater within the mantle cavity (Figure 4c). The volume of this seawater generally exceeds that of the hemolymph. This fact, along with the configuration of the body and mantle cavity within the plates, makes it very difficult to sample only hemolymph and not also residual seawater when inserting a syringe from outside the shell plates. For this reason I chose to dissect away the shell plates, in the process disposing of the residual water inside. Then, I homogenized the living tissue and centrifuged out the cellular portion,

leaving the liquid hemolymph supernate undiluted for testing. This process recovered most of the hemolymph that was present in the animal tissues, and yet I still had to process 30-50 individuals in order to yield 150-200 μl of hemolymph, especially at higher salinities. These results show that the actual hemolymph volume, especially in high salinities, is low and any method producing large volumes of hemolymph under these conditions should be examined very carefully for possible dilution of the hemolymph by seawater from the mantle cavity.

Several of my hemolymph samples, especially those from individuals collected during the summer months, had a yellow or bright orange color, whereas those individuals collected in October-November had a pale colored hemolymph. The differences in color can most likely be attributed to the reproductive potential of the individual. Phospholipid production and content in the hemolymph increases during the months of high reproduction and drops during the rest of the year. Yellow and orange pigments (chromolipid, astaxanthin, and various carotenoids) are also normally present in some barnacles' hemolymph in varying amounts (Waite and Walker, 1992).

When counteracting adverse environments, aquatic organisms may use the following regulatory strategies: ion regulation which takes place at the gills and regulates the

passage of specific ions into and out of the body, volume regulation which controls the amount of fluid present in cells, and osmoregulation which regulates the total content of osmotically active particles within the cell (Kinne, 1967). The barnacle *B. amphitrite* seems to use all three of these mechanisms. For ion regulation, barnacles seem to be an effective chloride ion regulator in 20‰ and 40‰ seawater solutions (Vittor, 1968); for cell volume regulation--also known as isosmotic intracellular regulation, barnacles greatly rely on adjustments of intracellular concentration of α-amino compounds (Jeuniaux et al., 1961). Fyhn (1976) found out that when *B. improvisus* is exposed to hypersaline sea water, proline dominates the intracellular amino acid pool and constitutes 86% of the total concentration of amino acids. When *B. improvisus* is acclimated from low to high sea water osmolality, the proline concentration levels can vary by as much as 2000 times. Thus, regulation of intracellular proline level seems to be a main factor responsible for the euryhalinity of this barnacle species, but how this high proline accumulation occurs is unknown (Fyhn, 1976). Kinne (1967) concluded that "the main osmoregulatory organs are gills (often responsible for salt balance), gut (often responsible for water balance), antennal glands (in some crustaceans), nephridial canals, and body surfaces." Forster and Goldstein (1979) summarized how cell volume regulation

takes place--"regulation of intracellular amino acid concentration has been shown in Crustacea in response to osmotic stress. This regulation is achieved via control of transport of amino acids across the cellular membrane and also by control of the cellular level of amino acid metabolism." The amino acid taurine is considered to play a key role in intracellular osmoregulation in invertebrates (Simpson et al., 1959). Fang et al. (1992) made a detailed study on the osmoregulation of several marine species including crustaceans, and found out that the following amino acids are involved in the cell volume regulation process: glycine, proline, arginine, glutamate, and alanine.

SUMMARY AND CONCLUSIONS

Balanus amphitrite from the Salton Sea is clearly a euryhaline species. Although adults can survive acute exposure of up to 70‰ salinity (LC_{50} is approximately 73‰ after 22 days), and perhaps up to 92‰ or higher if the salinity increases gradually, the species displays an increasingly pervasive suite of behavioral, physiological, reproductive, and survival effects as a consequence of the increasing salinity. Few adverse effects are apparent at salinities up to 50‰. By 60‰, reproductive potential appears to be sharply decreased and the barnacles exhibit clear short-term aversive responses to the hypersaline water. By 70‰ protracted changes in normal behavior are seen and by 73‰, 50% of the population will die within 3 weeks on acute exposure. Response to sudden exposure to 80‰ is rapid and profound and death ensues within 15 days. The population of *B. amphitrite* in the Salton Sea does not appear to be directly threatened by the rising salinity at the present time. The threat is likely to become more imminent when salinities begin to exceed 60‰. At the rate of increase in salinity seen for the Sea over the past 10 years, 60‰ salinity should be reached by about the year 2033. This analysis does not take into account indirect effects, such as threats to the species' food supply. Any effects of increasing salinity to *B. amphitrites'* prey species such as small plankton (Barnes, 1987) and copepods may bring about reduction in the population well before any

direct salinity effects become apparent. For at least the case of the copepods, this food supply does not appear to be in any more imminent danger than is the barnacle itself, however, because Dexter (1993) found that *Apocyclops dengizicus*, the most abundant copepod in the Salton Sea, can continue to grow and reproduce at salinities up to 68‰, a level comparable to that found for *Balanus amphitrite* in this study.

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