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https://dx.doi.org/doi:10.21220/s2-kw6p-nk21

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THE INFLUENCE OF ENVIRONMENTAL SALINITY ON HEMOCYANIN FUNCTION IN THE BLUE CRAB, *CALLINECTES SAPIDUS*

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

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by

Alan Lawson Weiland

1976

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

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Approved, April 1976

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ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. Charlotte P. Mangum, under whose guidance this investigation was conducted, for her patient guidance and criticism throughout the investigation. Thanks are due to Dr. Robert Black and Dr. Eric Bradley for their careful reading and criticism of the manuscript. Special thanks go to Mr. Glen Bean for his generous help in constructing apparatus and keeping it functioning during the experiments.

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ABSTRACT

The blood PH of blue crabs is significantly lower when acclimated at 35 °/oo salinity than at 25 °/oo, 15 °/oo, or 5 °/oo. The pattern of PH change closely resembles that of blood osmolality and chloride. There is a large increase in PH and a concomitant decrease in blood osmolality and chloride between 35 °/oo and 25 °/oo, followed by smaller changes at lower acclimation salinities that were generally not significant.

The blood oxygen parameters indicate little significant change in oxygen affinity over the salinity range. The PH increase at the lower salinities apparently counters the effect of the lower ionic concentration on hemocyanin affinity. The compensation is not perfect, but the two factors seem to balance each other well enough so that the respiratory parameters remain within a relatively narrow range.

The increase in blood PH at lower salinities may be the result of the input of ammonia produced in deamination of the intracellular pool of free amino acids as the cells conform to osmotic changes in body fluids.

INTRODUCTION

The effects of various ions on *in vitro* oxygen equilibrium properties of invertebrate respiratory pigments have been intensively studied in recent years. The responses of different oxygen carrying pigments to salt vary in magnitude and direction. In the case of crustacean hemocyanin the oxygen affinity increases as the concentration of inorganic salts increases. (Spock, 1967; Larimer and Riggs, 1964; Pickett et. al., 1966).

Recent work indicates that differences in ionic concentration found *in vivo* in the hemolymph of the crab *Carcinus meanas* acclimated to different salinities are large enough to significantly alter oxygen affinity of buffered blood samples (Truchot, 1973).

Environmental salinity has also been found to affect blood PH in two crustaceans. As salinity is lowered the PH of the blood increases in *Carcinus meanas*. The PH change is independent of Pco_2 and the mechanism of the change is unknown (Truchot, 1973). Interestingly, the opposite trend was found for the barnacle *Pollicipes polymerus* (Fyhn et. al., 1972). In this species PH decreases with decreasing salinity as a result of the input of CO_2 produced in aerobic metabolism, which increases in dilute media. An increase in aerobic metabolism in dilute media is the most common response among crustaceans although some species exhibit the opposite response or none at all (Dehnel, 1961; Lance, 1964; Hagerman, 1970).

It is clear that environmental salinity can affect oxygen binding properties of crustacean hemocyanin *in vitro*. However, the effect of envirommental salinity on *in vivo* respiratory function where different factors are changing and interacting simultaneously is unknown.

Callinectes sapidus was chosen for this *in vivo* study because it is a remarkably euryhaline crustacean that lives in unstable estuarine habitats. The species is found in waters ranging from fresh to hypersaline (Hedgpeth, 1967; Mangum and Amende, 1972). The species is capable of maintaining a hyperosmotic blood but the mechanism is not powerful enough to insure true homeosmoticity resulting in significant changes in dilute media (Ballard and Abbot, 1969; Lynch et al., 1973). The consequences of these ionic changes for *in vivo* oxygen transport by hemocyanin were investigated in the present study.

MATERIALS AND METHODS

Collection and acclimation of experimental animals

Freshly caught crabs from the Chesapeake Bay were purchased from commercial sources during January, February, June and July. Only undamaged animals with no missing appendages were used. They were maintained in large volumes (75 - 250 l) of aerated recirculating water and fed beef liver every two days until three days before the experiments. The animals were all adult males in intermolt stage C_4, ranging in carapace length from 6.3 to 7.5 cm (mean = 6.88 cm $\pm$ 0.05 S.E.; n=82). With one exception, the body weights of each group of experimental animals are homogeneous ($P > .05$ according to Student's t). The summer animals acclimated to 5 °/oo salinity were somewhat lighter (171 gm wet weight) than the winter group acclimated to the same salinity (206 gm) despite a similar carapace length (weights differ significantly; $P < .05$).

The crabs were allowed to acclimate for 7 - 12 days to water of either 5, 10, 25 or 35 °/oo salinity at 21 - 23 ° C. Water (19-34 °/oo salinity) was collected from the York River estuary and Wachapreague Inlet in Virginia. Minor increases in salinity were made by dissolving artificial sea salt (Dayno Sales Co.); dilutions were made with distilled water. Salinity was determined with glass hydrometers calibrated with Copenhagen Normal Water; chlorinity with a Buchler-Cotlove Chloridometer.

Sampling

A single three ml sample of prebranchial blood was taken from each crab at the base of the fourth leg (infrabranchial sinus) within 30 sec of removing the animal from water. The sample was injected under mineral oil and centrifuged at high speed (Sorvall SS - 1) for 10 minutes to express the fluid from the clot. This procedure was necessary for measurement of the PH of a concentrated protein solution, which requires a liquid junction capillary electrode, but it introduced the possibility that any one of the respiratory parameters might be influenced by the diffusion of gases into the sample at the blood-oil interface. To test this possibility, a set of samples was halved and aliquots injected under (1) air equilibrated oil, and (2) oil bubbled for 15 minutes with N_2 gas. As concluded previously (Mangum and Shick, 1972) there is no perceptible effect on PH by using an oil seal. In an earlier test at low temperature, (2 ^{o}C), there was no change in PO_2 of samples injected directly into the electrode chamber or first under oil (Fyhn and Mangum, unpublished). In two of three tests at 22 ^{o}C, PO_2 was decreased by 2.5 - 3.5 mm Hg (14-20%), but in a third it was increased by 1.0 mm Hg (7%). Members of the paired PO_2 measurements obtained at 22 ^{o} C without and with the use of deoxygenated oil are homogeneous (Mann-Whitney U test) with one another, and with the results for samples injected directly into the electrode chamber without exposure to oil ($P > .05$, according to Student's t).

The fluid was then taken back into a hypodermic syringe, and 50 ul injected into the thermostated cell of a Radiometer Blood Gas Analyzer PO_2 electrode (E5046). Aliquots of the same sample were

taken anaerobically into the capillary of the liquid junction PH electrode, and PH measured on at least three successive subsamples. 0.2 ml aliquots were taken into the extractor syringe of a Scholander micro-gasometric analyzer which contained potassium ferricyanide to elicit the oxygen bound to hemocyanin, and the gas analyzed volumetrically (Hoffman and Mangum, 1970). Osmotic concentration of two 0.2 ml aliquots was determined with a Precision Instrument Co. Osmette freezing point osmometer. Chloride ion concentration was determined on at least two 0.1 ml aliquots with a Buchler-Cotlove chloridometer.

Data analysis

Bartlett's test for homogeneity of variance was performed to determine if parametric statistical procedures could be used. In most cases the result was not significant and a normal distribution was assumed. These data were tested by analysis of variance with one-way classification (ANOVA), with winter and summer data treated separately and acclimation salinities considered as treatments. If a significant difference within the four treatments was indicated by ANOVA, the salinity pairs that differed significantly were identified by the Student-Newman-Keuls test (SNK).

In the few cases when the distribution was not normal, non parametric tests were utilized. The Kruskal-Wallis H test was performed and the heterogeneous variables were identified by the Mann-Whitney U test.

Correlations between homogeneous parameters were described by I, and those between heterogeneous parameters by Spearman Rank. The criterion of significance is $P = .05$.

RESULTS

Osmotic Response to Low Salinity

As salinity is lowered the pattern of changes in osmolality and chloride ion concentration is similar to that previously reported (Lynch et al., 1973). Blood chloride is hypoionic to the medium at 35 °/oo, slightly hypoionic at 25 °/oo and then hyperionic and strongly but imperfectly regulated down to 5 °/oo (Figure 1). Blood osmolality is hyperosmotic at 35 °/oo and approximately paralleled the medium down to 25 °/oo. From 25 °/oo to 5 °/oo osmolality is also strongly but imperfectly regulated (Figure 1).

The osmotic and ionic parameters are consistently higher in winter than in summer animals, in spite of acclimation to the same laboratory temperature. The difference is not always significant, however (Table I).

PH (Prebranchial)

The PH of both winter and summer animals at 35 °/oo is significantly lower than the PH at the other salinities (Table II). Summer animals show no significant difference between PH values at 5 °/oo, 15 °/oo or 25 °/oo. Winter animals at 5 °/oo have significantly higher PH values than animals at 15 °/oo. There are no other significant differences.

The pattern of PH change closely resembles that of blood osmolality and chloride (Figure 1). There is a large increase in PH and a

Fig 1. The relationship of environmental salinity to osmolality and chlorosity of crab hemolymph.

The relationship of environmental salinity to osmolality and chlorosity of crab hemolymph

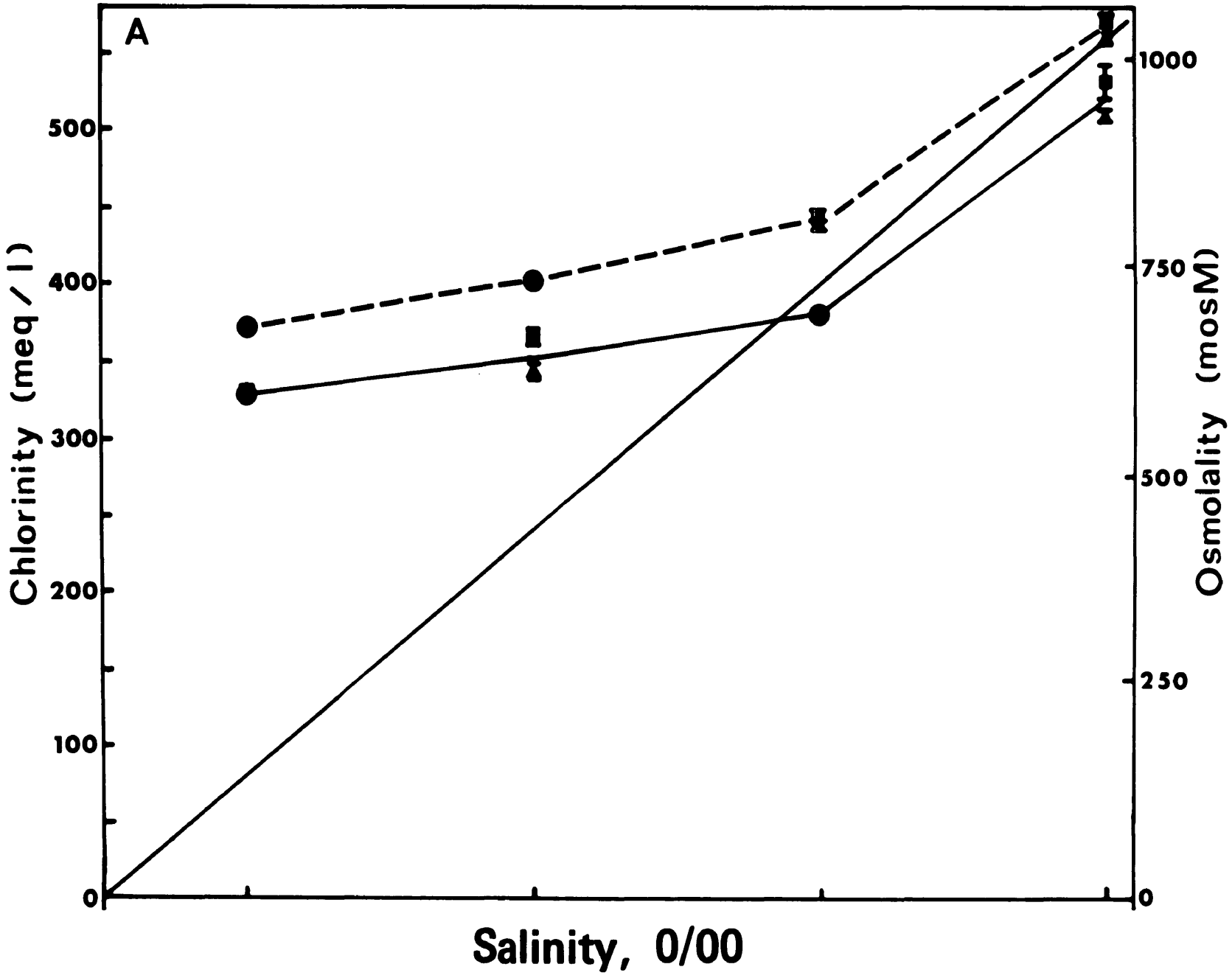

Table I

A. PH

Summer Runs

1. Bartlett's test for homogeneity of variance = 3.675 < 7.815, N.S.

2. Analysis of variance (Anova), one - way classification.

   F = 8.94 > 4.50, Significant at 0.01 level.

3. Student - Newman - Keuls test

Salinity Pairs	PH	
35°/oo - 5°/oo	0.0627 > 0.0481	Significant at 0.01
35°/oo - 25°/oo	0.0685 > 0.0550	Significant at 0.01
35°/oo - 15°/oo	0.0832 > 0.0593	Significant at 0.01
25°/oo - 5°/oo	0.0058 < 0.0366	N.S. at 0.05
15°/oo - 5°/oo	0.0205 < 0.0441	N.S. at 0.05
15°/oo - 25°/oo	0.0147 < 0.0366	N.S. at 0.05

	35°/oo	5°/oo	25°/oo	15°/oo
PH	7.590	7.653	7.658	7.673

A line under the means indicates these do not deviate significantly at 0.05.

Winter Runs

1. Bartlett's test = 0.494 < 7.815 N.S.

2. Anova F = 14.05 > 4.30 Significant at 0.01

3. SNK Test

Table I (con't)

Salinity Pairs	PH		
35°/oo - 15°/oo	0.0770 >	0.0612	Significant at 0.01
35°/oo - 25°/oo	0.1040 >	0.0728	Significant at 0.01
35°/oo - 5°/oo	0.1410 >	0.0762	Significant at 0.01
15°/oo - 25°/oo	0.0270 <	0.0470	N.S. at 0.05
15°/oo - 5°/oo	0.0640 >	0.0550	Significant at 0.05
5°/oo - 25°/oo	0.0370 <	0.0472	N.S. at 0.05

	35°/oo	15°/oo	25°/oo	5°/oo
PH	7.489	7.566	7.593	7.630

A line under the means indicates these do not deviate significantly at 0.05 level.

A comparison of winter and summer PH values with Student's test.

Salinity	Winter PH		Summer PH	Significance	t value
5°/oo	7.630	<	7.653	N.S. at 0.05	1.135 < 2.110
15°/oo	7.566	<	7.673	Significant at 0.01	5.340 > 2.878
25°/oo	7.593	<	7.658	Significant at 0.01	4.617 > 2.898
35°/oo	7.488	<	7.590	Significant at 0.01	5.913 > 2.845

Table I (con't)

B. P_vO_2

Summer Runs

1. Bartlett's test = 1.390 < 7.815 N.S.

2. Anova F = 3.47 > 2.92 Significant at 0.05

3. SNK test

Salinity Pairs	P_vO_2	
35°/oo - 25°/oo	1.310 < 2.276	N.S. at 0.05
35°/oo - 5°/oo	2.430 < 2.749	N.S.
35°/oo - 15°/oo	3.410 > 3.064	Significant at 0.05
5°/oo - 25°/oo	1.120 < 2.330	N.S.
15°/oo - 25°/oo	2.100 < 2.814	N.S.
15°/oo - 5°/oo	0.980 < 2.331	N.S.

35°/oo	25°/oo	5°/oo	15°/oo
9.0mm Hg	10.3	11.4	12.4

A line under the means indicates these do not deviate significantly at 0.05.

Winter Runs

1. Bartlett's test = 11.725 > 7.815 Significant 0.05

2. Non Parametric Kruskal - Wallis H test.
   H = 2.348 N.S.

25°/oo	15°/oo	35°/oo	5°/oo
11.3	13.2	13.4	13.7

A line under the means indicates these do not deviate significantly.

Table I (con't)

A comparison of winter and summer P_vO_2 values with Student's test.

Salinity	Winter P_vO_2		Summer P_vO_2	Significance	t value
5°/oo	13.7mm Hg	>	11.4	N.S. at 0.05	1.594 < 2.110
15°/oo	13.2	>	12.4	N.S. at 0.05	0.590 < 2.110
25°/oo	11.3	>	10.3	N.S. at 0.05	1.156 < 2.110
35°/oo	13.4	>	9.0	Sign. at 0.05	2.752 > 2.101

Table I (con't)

C. C_vO_2

Summer Runs

1. Bartlett's test 1.261 < 7.815 N.S.

2. Anova F = 4.231 > 2.922 Significant at 0.05.

3. SNK test

Salinity Pairs			C_vO_2
25°/oo - 5°/oo	0.1512	> 0.1290	Significant at 0.05
15°/oo - 5°/oo	0.1684	> 0.1556	Significant at 0.05
35°/oo - 5°/oo	0.2070	> 0.1674	Significant at 0.05
15°/oo - 25°/oo	0.0172	< 0.1290	N.S.
35°/oo - 25°/oo	0.0558	< 0.1520	N.S.
15°/oo - 35°/co	0.0386	< 0.1260	N.S.

	5°/oo	25°/oo	15°/oo	35°/oo
0.708 Vol %		0.557	0.539	0.501

A line under the means indicates these do not deviate significantly.

Winter Runs

1. Bartlett's test 0.459 < 7.815 N.S.

2. Anova F = 1.269 < 2.900 N.S. at 0.05

15°/oo	35°/oo	5°/oo	25°/oo
0.584	0.585	0.687	0.698

A line under the means indicates these do not differ significantly.

Table I (con't)

Comparison of Winter and Summer values t test.

Salinity	Winter C_vO_2		Summer C_vO_2	Significance	t test
5°/oo	0.687 Vol%	<	0.708	N.S.	0.277 < 2.110
15°/oo	0.584	>	0.539	N.S.	0.024 < 2.110
25°/oo	0.698	>	0.557	Sig. at 0.05	2.208 > 2.110
35°/oo	0.585	>	0.501	N.S.	1.280 < 2.10

Table I (con't)

D. Chloride

Summer Runs

1. Bartlett's test = 3.511 < 7.815 N.S.

2. Anova F = 120.10 > 4.50 Significant at 0.01

3. SNK test

Salinity Pairs

25°/oo - 35°/oo	107.6 > 22.3	Significant at 0.01
15°/oo - 35°/oo	129.6 > 25.6	Significant at 0.01
5°/oo - 35°/oo	167.3 > 27.6	Significant at 0.01
15°/oo - 25°/oo	22.9 > 22.8	Significant at 0.01
5°/oo - 25°/oo	59.8 > 26.2	Significant at 0.01
5°/oo - 15°/oo	37.7 > 22.9	Significant at 0.01

Winter Runs

1. Bartlett's test = 2.887 < 7.815 N.S.

2. Anova F = 85.84 > 4.50 Significant at 0.01

3. SNK Test

Salinity Pairs

35°/oo - 25°/oo	156.1 > 37.1	Significant at 0.01
35°/oo - 15°/oo	165.9 > 41.4	Significant at 0.01
35°/oo - 5°/oo	193.3 > 44.7	Significant at 0.01
15°/oo - 25°/oo	9.8 < 26.9	N.S.
5°/oo - 25°/oo	37.2 > 32.4	Significant at 0.05
5°/00 - 15°/oo	27.4 > 26.1	Significant at 0.05

Table I (con't)

Comparison of Winter and Summer Values

Salinity	Winter Chloride		Summer Chloride	Significance	t test
5°/oo	336.7	>	322.4	N.S.	1.340 < 2.101
15°/oo	364.1	>	343.9	Sig. at 0.05	2.102 > 2.101
25°/oo	373.9	<	382.1	N.S.	0.846 < 2.101
35°/oo	530.0	>	489.8	Sig. at 0.01	3.170 > 2.878

Table I (con't)

E. Weights

Summer Runs

1. Bartlett's test = 0.411 < 7.815 N.S.

2. Anova F = 0.600 < 2.92 N.S.

$5^{o}/oo$	$25^{o}/oo$	$15^{o}/oo$	$35^{o}/oo$
171.0	174.0	178.1	185.5

A line under the means indicates these do not deviate significantly at 0.05.

Winter Runs

1. Bartlett's Test = 7.383 < 7.815 N.S.

2. Anova F = 1.25 < 2.92 N.S.

$15^{o}/oo$	$25^{o}/oo$	$35^{o}/oo$	$5^{o}/oo$
183.0	190.4	202.8	206.3

A line under the means indicates these do not deviate significantly.

Comparison of Winter and Summer Weights; t test.

Salinity	Winter		Summer	Significance	t test	
$5^{o}/oo$	206.3	>	171.0	Sign. at 0.01	3.36 >	2.88
$15^{o}/oo$	183.0	>	178.1	N.S.	0.39 <	2.11
$25^{o}/oo$	190.4	>	174.0	N.S.	1.36 <	2.11
$35^{o}/oo$	202.8	>	185.5	N.S.	1.19 <	2.08

concomitant decrease in blood osmolality and chloride between 35 °/oo and 25 °/oo, followed by smaller changes at lower acclimation salinities that were generally not significant. The inverse relationship of blood PH and the osmotic parameters extends to the seasonal difference; winter animals, which have a more concentrated blood, also have a lower blood PH.

P_vO_2 (Prebranchial partial pressure of oxygen)

There is no significant change in P_vO_2 when winter animals are acclimated to the four salinities. Summer animals at 15 °/oo have a significantly higher P_vO_2 than those at 35 °/oo (see Table I). There are no other significant differences. Salinity appears to have little effect on P_vO_2.

C_vO_2 (Prebranchial total oxygen content in volume percent)

For the winter animals the total oxygen concentration of prebranchial blood including both dissolved and hemocyanin bound oxygen, does not change significantly at any acclimation salinity. The C_vO_2 value of summer animals at 5 °/oo is significantly higher than the values at the other salinities. There are no other differences (see Table I). Comparing winter and summer values, the only significant difference is at 25 °/oo.

DISCUSSION

A Possible Explanation of PH Change with Acclimation Salinity

Fyhn et al ('72) suggested that intracellular osmolality in the gooseneck barnacle Pollicipes polymerus is not maintained by changes in free amino acids. Moreover, body fluid PH varies directly with acclimation salinity. This response results from the input of CO_2 produced in aerobic metabolism, which increases in dilute media.

VO_2 of adult Callinectes sapidus does not change when acclimation salinity is reduced from 30 to 10 °/oo (Laird '73), and therefore it is unlikely that the accompanying increase in blood PH is due to a reduction in Pco_2. It is more likely that the PH increase at low salinity results from an excess of base, an hypothesis suggested earlier for Carcinus meanas by Truchot ('73) although the effective base was not identified.

Both Callinectes sapidus and Carcinus meanas are capable of extracellular osmotic regulation. The regulatory mechanisms in these species can dampen the change in osmotic concentration of the blood when salinity is altered but neither regulates perfectly enough to achieve homeosmoticity in dilute media. Extracellular regulation is accompanied by intracellular regulation. In both species a pool of free amino acids participates in this intracellular regulation

(Schoffeniels and Gilles, 1970; Gerard and Gilles, 1972; Siebers et al., 1972).

As salinity decreases the amount of free amino acids in the tissues decreases and at the same time the ammonia concentration in the hemolymph increases in both species (Gerard and Gilles, 1972; Siebers et al., 1972). These events are thought to result from increased deamination of the free amino acids (Gerard and Giles, 1972).

Blue crabs in 50% seawater have considerably higher (10x) ammonia levels than those in undiluted seawater. Specifically, ammonia increases by $1.2 - 1.3 \times 10^{-4}$ M/l, a quantity which could cause the observed PH increase (see Table II). In Carcinus meanas transferred from 38 to 11°/oo salinity the ammonia increase is even greater (7×10^{-4} M/l; Siebers et al., 1972), which could also cause the greater blood PH change. In this species the ammonia concentration does not decrease significantly from the elevated value for at least 10 days following the exposure to a dilute salinity. The actual magnitude of the PH change induced by the addition of that quantity of base to the blood depends on its buffering capacity, which is not known.

By far the greatest change in osmolality and chloride ion concentration in the blood is between 35°/oo and 25°/oo. Further reductions in salinity cause decreases in osmolality and chloride ion concentration, but the amount of decrease is much less as shown in Figure 1. Assuming that the free amino acid pool is controlled, catabolically, the greatest increase in deamination and hence ammonia

Table II

Values Determined and Animals Acclimated

at 22.0°c ± 1°c

Abbreviations

P_eO_2 = Partial pressure of oxygen in external environment in millimeters of mercury.

Wt. = Wet weight of animals in grams.

Cm. = Carapace length in centimeters.

P_vO_2 = Prebranchial partial pressure of oxygen.

C_vO_2 = Prebranchial oxygen content in Volume percent.

OSMO = Osmolality in Milliosmoles.

CL- = Chloride ion concentration in milli-equivalents per liter.

C_cO_2 = Oxygen carrying capacity in volume percent.

S_v = Venous percent oxygen saturation.

Table II (con't)

Summer 5°/oo Salinity

mm Hg P_eO_2	Wt.	cm.	PH	P_vO_2	C_vO_2	OSMO	CL-	C_cO_2	S_v
148.0	163.7g	—	7.675	7.0	0.704	723.3	353.3	—	—
148.0	164.4	—	7.594	12.0	0.884	705.5	365.2	—	—
148.0	182.9	—	7.728	8.0	0.648	683.9	329.9	1.631	55%
148.0	180.7	—	7.640	10.4	0.884	705.1	321.6	—	—
145.5	165.4	—	7.560	15.5	0.486	662.4	319.6	—	—
145.5	163.9	—	7.638	12.0	0.536	707.6	319.9	1.521	48%
145.5	154.3	—	7.731	9.8	0.623	657.0	304.9	—	—
145.5	142.7	—	7.653	11.0	0.823	714.8	330.4	—	—
145.5	242.1	—	7.631	14.8	0.608	640.6	288.3	—	—
145.5	150.1	—	7.675	13.7	0.881	632.7	290.6	—	—

Mean Values ± S.E. n = 10

P_eO_2	Wt.	PH	P_vO_2	C_vO_2	OSMO	CL-
146.5±0.4	171.0±8.8	7.653±0.01	11.4±0.9	0.708±0.05	583.3±10.4	322.4±7.7

Summer 15°/oo Salinity

151.0	205.7	—	7.650	10.6	0.338	750.5	359.2	—	—
151.0	178.5	—	7.620	12.9	0.465	749.7	365.6	—	—
151.0	182.5	—	7.681	12.5	0.360	724.3	341.6	—	—
145.0	140.7	—	7.697	15.0	0.705	718.1	322.5	—	—
145.0	196.1	—	7.658	12.5	0.455	731.4	343.4	—	—
145.0	158.0	—	7.688	15.4	0.740	736.8	332.2	—	—
145.0	163.1	—	7.730	10.5	0.540	705.3	327.7	1.310	62%
145.0	191.5	—	7.704	13.6	0.740	702.0	319.0	—	—
139.8	216.2	—	7.621	8.5	0.620	756.8	375.1	—	—
139.8	149.1	—	7.681	12.5	0.430	712.4	352.8	—	—

Mean Values ± S.E. n = 10

P_eO_2	Wt.	PH	P_vO_2	C_vO_2	OSMO	CL-
145.8±1.5	178.1±7.9	7.673±0.01	12.4±0.7	0.539±0.05	728.8±6.2	343.9±6.0

Table II (con't)

Summer 25°/oo Salinity

mm Hg P_eO_2	Wt.	cm.	PH	P_vO_2	C_vO_2	OSMO	CL-	C_cO_2	S_v
153.5	215.9	—	7.677	10.1	0.610	792.0	392.1	1.665	49%
153.5	186.3	—	7.623	11.5	0.635	799.8	386.3	—	—
153.5	151.5	—	7.630	14.6	0.430	813.4	380.6	—	—
153.5	151.9	—	7.633	9.8	0.500	789.4	387.6	—	—
153.5	134.3	—	7.662	12.2	0.440	800.0	383.5	—	—
146.2	135.3	—	7.647	10.1	0.550	816.2	365.5	—	—
146.2	181.2	—	7.651	10.2	0.490	802.3	390.6	—	—
146.2	201.3	—	7.665	8.5	0.560	813.0	403.0	—	—
142.0	188.5	—	7.723	6.0	0.550	777.4	357.2	—	—
142.0	194.0	—	7.672	10.0	0.800	773.1	374.9	—	—

Mean Values ± S.E. n=10

P_eO_2	Wt.	PH	P_vO_2	C_vO_2	OSMO	CL-
149.0±1.0	174.0±9.1	7.658±0.009	10.3±0.71	0.557±0.03	797.7±4.7	382.1±4.2

Summer 35°/oo Salinity

141.6	236.8	—	7.613	5.6	0.433	968.0	500.8	1.371	48%
141.6	175.6	—	7.619	4.5	0.803	993.5	462.4	1.980	53%
141.6	183.3	—	7.654	11.5	0.526	959.1	507.7	—	—
141.6	179.1	—	7.640	11.6	0.401	1065.7	497.5	—	—
141.6	200.5	—	7.615	7.4	0.405	971.9	473.1	—	—
150.0	172.9	—	7.578	8.7	0.765	999.0	468.8	—	—
150.0	216.0	—	7.573	5.5	0.380	1082.9	506.5	—	—
150.0	156.0	—	7.526	11.1	0.420	992.1	502.8	—	—
150.0	175.7	—	7.533	11.5	0.430	999.4	491.6	—	—
150.0	184.2	—	7.578	13.0	0.430	1002.9	480.0	—	—
150.0	160.6	—	7.559	8.5	0.515	1018.0	495.3	—	—

Mean Values ± S.E. n=11

P_eO_2	Wt.	PH	P_vO_2	C_vO_2	OSMO	CL-
146.2±1.3	185.5±7.2	7.590±0.010	9.0±0.9	0.501±0.04	1004.8±11.6	489.8±4.8

Table II (con't)

Winter 5°/oo Salinity

mm Hg P_eO_2	Wt.	cm.	PH	P_vO_2	C_vO_2	OSMO	CL-	C_cO_2	S_v
157.2	209.9	7.4	7.584	8.3	0.700	685.8	327.1	—	—
147.1	224.0	7.5	7.688	9.5	0.976	669.6	328.8	—	—
154.1	215.5	7.0	7.567	18.1	0.945	661.4	326.5	—	—
154.1	202.1	7.5	7.634	19.2	0.766	648.4	328.3	—	—
159.7	195.7	6.9	7.690	13.2	0.669	704.8	377.6	—	—
154.0	230.0	6.8	7.642	16.1	0.450	657.0	312.5	—	—
154.0	175.2	6.6	7.580	15.9	0.600	658.6	308.1	—	—
152.0	215.5	6.8	7.603	13.4	0.403	692.8	359.4	—	—
152.0	180.0	6.5	7.663	9.9	0.630	679.8	331.6	—	—
152.0	215.5	6.8	7.647	13.8	0.731	716.9	366.8	—	—

Mean Values ± S.E. n = 10

P_eO_2	Wt.	PH	P_vO_2	C_vO_2	OSMO	CL-
153.6±1.0	206.3±5.7	7.630±0.01	13.7±62	0.687±0.06	677.5±7.1	336.7±7.3

Winter 15°/oo Salinity

147.9	144.8	7.0	7.523	18.3	0.800	720.4	326.6	—	—
152.0	133.6	6.7	7.521	11.5	0.500	783.3	360.9	—	—
159.4	207.2	7.0	7.440	15.7	0.374	751.3	372.6	—	—
159.4	226.6	7.5	7.584	10.6	0.387	707.2	356.8	—	—
154.0	190.1	6.8	7.597	19.5	0.825	687.1	333.7	—	—
154.0	178.3	6.6	7.577	—	—	681.0	351.2	—	—
138.5	209.0	6.6	7.584	11.2	0.436	764.9	374.0	—	—
138.5	208.5	6.9	7.617	8.1	0.598	777.3	403.2	—	—
138.5	170.2	6.5	7.598	12.3	0.688	763.7	370.2	—	—
138.5	161.2	6.3	7.621	11.7	0.650	755.6	392.1	—	—

Mean Values ± S.E. n = 10

P_eO_2	Wt.	PH	P_vO_2	C_vO_2	OSMO	CL-
148.0±2.7	182.9±9.7	7.566±0.02	13.2±1.3	0.584±0.06	739.2±11.8	364.1±7.5

Table II (con't)

Winter 25°/oo Salinity

P_eO_2	Wt.	cm.	PH	P_vO_2	C_vO_2	OSMO	CL-	C_cO_2	S_v
150.8	175.9	6.9	7.506	11.8	0.850	820.1	393.3	—	—
142.5	192.0	6.9	7.605	9.7	0.770	802.7	358.2	—	—
142.5	191.4	7.0	7.570	10.2	0.675	808.2	329.9	—	—
142.5	160.9	6.5	7.567	10.4	0.539	797.7	366.9	—	—
146.0	170.4	6.3	7.636	11.0	0.897	819.6	362.5	2.017	57%
146.0	237.1	7.1	7.601	12.7	0.909	820.7	350.6	1.893	62%
152.0	191.7	6.4	7.576	12.7	0.485	801.9	386.4	1.490	44%
147.0	179.8	6.5	7.600	11.9	0.672	830.9	399.0	—	—
152.0	214.3	6.8	7.678	10.9	0.485	827.9	418.2	1.219	62%

Mean Values ± S.E. n = 10

P_eO_2	Wt.	PH	P_vO_2	C_vO_2	OSMO	CL-
146.8±1.3	190.4±7.8	7.593±0.01	11.3±0.4	0.698±0.06	814.4±4.0	373.9±9.2

Winter 35°/oo Salinity

152.0	165.8	6.6	7.536	7.0	0.500	1045.0	555.7	—	—
146.5	196.0	6.8	7.509	11.4	0.723	1049.4	509.8	—	—
146.5	228.2	7.3	7.488	20.5	0.615	1037.3	521.6	—	—
144.9	202.3	7.0	7.399	16.8	0.531	1069.6	527.9	—	—
145.0	168.8	6.8	7.444	12.5	0.403	1085.9	587.0	—	—
148.5	211.6	7.5	7.526	14.9	0.511	1037.0	553.9	—	—
145.0	172.6	6.8	7.516	10.0	0.874	1034.6	529.4	—	—
155.0	157.3	6.3	7.566	10.5	0.468	1090.0	447.0	—	—
158.8	232.2	7.5	7.428	—	—	1027.2	—	—	—
158.8	310.2	7.8	7.475	—	—	1032.0	—	—	—
158.8	216.3	6.8	7.476	16.9	0.636	1021.4	537.8	—	—

Mean Values ± S.E. n = 10

P_eO_2	Wt.	PH	P_vO_2	C_vO_2	OSMO	CL-
149.1±1.6	202.8±12.2	7.488±.01	13.4±1.4	0.5845±0.05	1044.3±7.5	530.0±12.9

concentration would be expected between 35°/oo and 25°/oo with little increase in ammonia output between 25°/oo and the lower salinities. It is interesting that significant differences in PH are found only between 35°/oo and the three lower salinities. In fact the correlations of PH with both osmolality ($P<.01$) and chloride ($P<.001$) are highly significant.

Effects of Acclimation Salinity on Oxygen Transport

When the blue crab makes osmotic adjustments to dilute media, blood PH rises. If this change were unopposed, it would result in an increase in oxygen affinity and thus % oxyhemocyanin. At the same time, however, the salt content of the blood decreases. If this change occurred in isolation, the result would be a reduction in hemocyanin oxygenation.

Another variable that must be considered is oxygen consumption ($\dot{V}O_2$). Although $\dot{V}O_2$ changes as salinity changes in many crustaceans, Laird found that Callinectes sapidus exhibits no $\dot{V}O_2$ change at acclimation salinities between 30°/oo and 10°/oo (25° c). However, Mangum (1976) found that $\dot{V}O_2$ does increase at 5°/oo.

The blood oxygen parameters indicate little significant change in oxygen affinity over the salinity range (see Table I). The PH increase at the lower salinities apparently counters the effect of the lower ionic concentration on hemocyanin affinity. The compensation is not perfect, but the two factors seem to balance each other well enough so that the respiratory parameters remain within a relatively narrow range.

It appears that the opposite responses of the hemocyanin molecule to salt and PH allow for a remarkable homeostasis of respiratory function in the unstable estuarine environment.

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