1972

Variations in some serum constituents of the blue crab, Callinectes sapidus

Maurice P. Lynch
College of William and Mary - Virginia Institute of Marine Science

VARIATIONS IN SOME SERUM CONSTITUENTS OF THE BLUE CRAB *Callinectes sapidus*

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

---

by

Maurice Patrick Lynch

1972

APPROVAL SHEET

This dissertation is submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Maurice Patrick Lynch

Approved, January 1972

Kenneth L. Webb

Willard A. Van Engel

Robert E. L. Black

Dexter S. Haven

Charlotte P. Mangum, Biology Department

William J. Hargis, Jr.

TABLE OF CONTENTS

ACKNOWLEDGMENTS

I wish to express my deep appreciation to all those persons at the Virginia Institute of Marine Science who assisted me in my work. I am particularly grateful to Dr. K. L. Webb who was my advisor during this study. His support and encouragement were unflagging. Particular thanks must also go to Mr. W. A. Van Engel and Dr. P. A. Haefner, Jr. who assisted in the processing of the monthly samples. The knowledge of blue crab biology passed on by them during those sessions aided greatly in the interpretation of the data. I am grateful to the many technicians in the Physiology Department who assisted in the serum analysis. Particular thanks must go to Mrs. Rebecca Goldstein, Miss Carolyn Watson and Miss Rosemary Green for their efforts.

This study was supported by funds provided by the Commonwealth of Virginia and by the Virginia Institute of Marine Science Sea Grant program (P. L. 89-688) in 1969 by grant number GH67 administered by the National Science Foundation, in 1970 by grant number GH37 administered by NSF and the National Oceanic and Atmospheric Administration (NOAA) of the U. S. Department of Commerce, and in 1971 by grant number 1-36032 administered by NOAA.

In addition, animals supplied by commercial crabbers were purchased from funds provided by the Bureau of Commercial Fisheries, U. S. Department of Interior, now National Marine Fisheries Service, NOAA, U. S. Department of Commerce under the cooperative crab disease study program directed by Dr. John A. Couch, National Marine Fisheries Service Biological Laboratory, Oxford, Maryland for the period February 1969 - December 1970.

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Variation of serum chloride, osmotic concentration, protein, glucose and total ninhydrin positive substances (TNPS) has been determined in mature blue crabs, Callinectes sapidus from a range of environmental conditions in Virginia. Serum chloride and serum osmotic concentration in crabs from essentially the same salinity conditions varied cyclically with season, with highest values in winter and lowest values in summer. In summer and fall, serum chloride was regulated hyperionic to the medium below 21 o/oo, isoionic between 21-25 o/oo and hypoionic above 25 o/oo. Serum osmotic concentration during the same period was hyperosmotic below 25 o/oo and isosmotic between 25-31 o/oo.

Variation in serum protein was primarily associated with ovary development. Newly matured female crabs had lower serum protein levels than either male crabs or older females. The increase in serum protein associated with developing ovaries occurring simultaneously with the migration of mature females towards higher salinity waters for spawning created a probably spurious positive correlation between salinity and serum protein in female crabs.

Serum glucose levels were essentially the same in male and female crabs. A seasonal high in early summer was followed by a seasonal low in late summer and early fall. Salinity did not appear to affect serum glucose. Hyperglycemia was induced by holding crabs out of water for periods of 2-12 hours.

Serum TNPS was very variable, essentially no differences were found between male and female crabs. A positive correlation was found between salinity and serum TNPS in female crabs. Higher serum TNPS at higher salinities is felt to be an acclimation phenomena associated with the migration of females to higher salinities. Glycine, taurine, alanine, arginine and proline accounted for 70-90% of the serum free amino acids and 30-70% of the serum TNPS.

Levels of serum constituents in crabs thought stressed by red tide, DDT and heat were compared to the values determined under normal conditions. Serum glucose was elevated under all stress conditions, the other serum constituents varied in their response, depending upon the specific stress. The technique of comparing levels of serum constituents in populations of crabs thought to be under stress with baseline values determined from "normal" populations is thought to be a promising method of developing physiological indices with which to monitor population condition.

VARIATIONS IN SOME SERUM CONSTITUENTS OF THE BLUE CRAB *Callinectes sapidus*

INTRODUCTION

Populations of the blue crab, Callinectes sapidus, Rathbun are noted for wide fluctuation in density from year to year (Pearson, 1948; Van Engel, 1958). Attempts to determine the reason for these fluctuations have not been successful. Pearson (1948) suggested that excessively cold weather and low salinity in spawning areas due to high river discharge might be responsible for low abundance, but the evidence he presented was not conclusive.

Blue crabs in Virginia waters are found from about 30-32 o/oo to essentially fresh water (Churchill, 1919; Van Engel, 1958). Female crabs migrate to higher salinity waters to spawn upon reaching maturity. Immature crabs of both sexes migrate towards brackish water. As a result, higher salinites have a higher proportion of mature females compared with mature males, while the reverse is true at lower salinities. The molt of the female crab at maturity is a terminal molt, but that of the male is not (Van Engel, 1958).

Jeffries (1964) suggested that the use of chemical or physiological indices might prove to be a valuable method of predicting abundance. This approach was attempted with restricted populations of blue crabs from

Rhode Island, without much success (Jeffries, 1966). The use of physiological indices should prove to be of value for management of resources provided that proper indices are chosen and "baseline" or normal values can be established. This paper is a report of an attempt to establish the normal variation of several serum constituents and serum osmotic concentration in mature hard blue crabs (molt stages C_4 and C_t, Passano, 1960) under the various environmental conditions of salinity and temperature found in Virginia. The serum constituents chosen for study were chloride, total protein, glucose, total ninhydrin positive substances (TNPS) and free amino acids.

SERUM CHLORIDE AND OSMOTIC CONCENTRATION

Osmotic and ionic regulation have been extensively studied in many species of crustaceans (Krogh, 1939; Robertson, 1960; Lockwood, 1962; Schoffeniels & Gilles, 1970), but essentially only under laboratory conditions in the blue crab (Odum, 1953; Gifford, 1962; Tan & Van Engel, 1966; Mantel, 1967; Ballard & Abbott, 1969; Tagatz, 1971). These studies were in agreement that at low and intermediate salinities blue crabs maintain the blood or serum hyperosmotic to the environment. There is some disagreement, however, as to the type of regulation found in salinities approaching full strength seawater (30-35 o/oo).

Ballard & Abbott (1969) suggested that temperature could be a major factor in discrepancies reported by

various authors, although Tagatz (1971) found no temperature effect on osmoregulatory ability. Sexual differences in regulatory ability were reported by Tan & Van Engel (1966), Ballard & Abbott (1969) and Tagatz (1971).

SERUM PROTEIN

Serum protein was selected for analysis because of the success of Canadian scientists' studies relating serum protein to physiological condition and ecology of the lobster, Homarum americanus. Serum protein was found to be a reliable indicator of muscle weight (Stewart et al. 1967b), an indicator of diet (Stewart, et al., 1967a; Stewart et al. 1967b) and related to specific areas from which lobsters were taken (Stewart & Li, 1969).

The subject of serum proteins in crustaceans has been reviewed by Florkin (1960a; 1960b) and Passano (1960). Passano (1960) discussed the fluctuation of serum protein during the various stages of the molt cycle. In general, serum protein concentration increases immediately prior to molting, decreases at molt and gradually rises to its intermolt level. Florkin (1960a) reported blood proteins amounted to only a few percent of the constituents of blood (mean 4%) in the decapod crustaceans studied. The most concentrated protein was hemocyanin, with fibrinogen (in unclotted blood) the next most concentrated protein. Florkin (1960b) discussed a few of the conditions that may alter crustacean blood proteins, e.g., respiratory stress (low oxygen) which caused an increase in hemoglobin in

some cladocera and Sacculina infection which caused an increase in the serum protein in Carcinus maenas. Uglow (1969c), however, found Sacculina infection did not affect total serum protein levels in C. maenas and Macropipus holsatus.

A wide range of values of total serum protein (9-132 mg/ml) has been reported in the blue crab (Leone, 1953; Horn & Kerr, 1963). Differences in levels of serum proteins are found between various stages of the molt cycle (Leone, 1953), males and females, and egg-bearing (sponge) and non-sponge females (Horn & Kerr, 1963). Electrophoretic studies on blue crab serum indicate quantitative and qualitative differences in protein fractions related to sex (Maxwell & Baker, 1963; Horn & Kerr, 1969) and to the presence of a sacculinid (Manwell & Baker, 1963). Wood et al (1958), however, failed to find any differences in blue crab serum protein fractions related to sex or stage of the molt cycle.

In other crustaceans, total serum protein or serum protein fractions are decreased by starvation (Adiyode, 1969a; Stewart et al., 1967a; Stewart et al., 1967b; Uglow, 1969b; Djangmah, 1970). The starvation effects are less at lower temperatures (Stewart et al., 1967b). Temperature affected serum protein differently in different species. Serum protein levels were highest during winter months in Orconectes limosus (Andrews, 1967), but higher at higher temperatures in Uca pugilator

(Dean & Vernberg, 1966). Sex related differences in serum protein fractions are found in Paratelphusa hydrodromous (Adiyode, 1968), but sex does not affect total serum protein in Carcinus maenas (Uglow, 1969a) and Crangon vulgaris (Djangmah, 1970).

SERUM GLUCOSE

Blood glucose levels in crustaceans have been reviewed by Florkin (1960a), Hohnke & Scheer (1970) and Jeuniaux (1971). Florkin (1960a) reported values of total reducing substances ranging from 3- 182 mg/100 ml in freshly caught animals and 0-24 mg/100 ml in fasting animals. Fermentable reducing substances varied from 0-21 mg/100 ml in starved and 7-22 mg/100 ml in fed or freshly captured animals. Glucose in reported as the major reducing sugar in crustaceans. The other reducing sugars found to date are discussed by Hohnke & Scheer (1970) and Jeuniaux (1971).

Variations in blood sugar levels are attributed to several factors. Hyperglycemia is induced by handling stress (Abramowitz et al., 1944; Riegel, 1960; Telford, 1968a & 1968b), asphyxia (holding out of water or chloroform in the water) (Kleinholz & Little, 1949; Kleinholz et al., 1950), high temperature (Dean & Vernberg, 1965b), early ovogenesis (Dean & Vernberg, 1965a; Telford, 1968c) and diurnal changes (Dean & Vernberg, 1965a). Telford (1965) reported female blood glucose levels higher than male

blood glucose levels in Homarus americanus, but later indicated no differences between male and female glucose levels (Telford, 1968b), attributing the earlier reported difference to stress. A combined seasonal - sex effect results in elevated glucose in female Orconectes limosus compared to males in winter (Andrews, 1967). No seasonal effect on serum glucose was found in H. americanus (Telford, 1968a), Cancer borealis (Telford, 1968c) or Orconectes virilis (McWhinnie & Saller, 1960). Fasting generally decreased serum glucose levels (Florkin, 1960a) but is reported not to affect serum glucose levels in Libinia emarginata (Kleinholz & Little, 1949) or cold acclimated Uca pugilator (Dean & Vernberg, 1965b).

Dean & Vernberg (1965a) reported that glucose makes up 20-25% of the total reducing substances in blue crab blood. Mean serum glucose ranged from 10.5 to 37.6 mg/100 ml in animals held 3 days without feeding in the laboratory. Other reducing substances found were maltotetraose, maltotriose, maltose, galactose, mannose and a galactan derivative (Dean & Vernberg, 1965b). Total reducing sugars in the blue crab are reported to range from 4.2 to 182 mg/100 ml in freshly caught crabs and 8.6 to 24.0 mg/100 ml in fasted animals (Morgulis, 1922; Abramowitz et al., 1944; Kleinholz et al., 1950; Jeffries, 1966).

SERUM TOTAL NINHYDRIN POSITIVE SUBSTANCES AND FREE AMINO ACIDS

Serum non-protein nitrogen in decapod crustaceans has been reported to vary from 8-37.5 mg/100 ml, with amino-nitrogen, making up approximately 30% of the non-protein nitrogen ranging from 2.2 - 12.0 mg/100 ml (Florkin, 1960a). Serum free amino acids have been determined in several species, total serum amino acids range from 26-80 mg/100 ml with glycine, alanine, glutamic acid, proline, serine, ornithine and taurine making up the greater portion of the free amino acid pool with different amino acids being the most concentrated in different crustaceans (Camien *et al.*, 1951; Duchateau - Bosson & Florkin, 1961; Stevens *et al.*, 1961; Stewart *et al.*, 1966; Vincent-Marique & Gilles, 1970).

Little information is available on the variability of blood or serum non-protein nitrogen, free amino acids or TNPS in a single species. Delaunay (1931) reported blood amino N ranged from 1.6 - 8.4 mg/100 ml in *Maja squinado*. Stewart *et al.* (1966) reported a twofold variation of serum amino N in *Homarus americanus* sampled at different times during the year, (35.8 mg/100 ml in May; 74.7 mg/100ml in January). Glycine showed the greatest change (0.45 µ moles/ml in May; 2.40 µ moles/ml in January). Vincent-Marique & Gilles (1970) found up to a 600% increase in serum proline in *Eriocheir sinensis* 4 days after transfer from seawater to freshwater. After 8 days in fresh water

blood free amino acids (including proline) were below the levels found in seawater acclimated animals.

In the blue crab, blood non-protein nitrogen was reported to vary from 24.7 mg/100 ml in freshly caught animals to 9.0 mg/100 ml in animals held two days in the laboratory, indicating the serum non-protein nitrogen is dependent upon the nutritional state of the animal (Morgulis, 1922). Jeffries (1966) reported plasma non-protein nitrogen ranged from 8.6 - 102.9 mg/100 ml in male and 12.8 - 42.9 mg/100 ml in female blue crabs. He reported an inverse correlation between serum non-protein nitrogen and serum chloride.

MATERIALS AND METHODS

GENERAL APPROACH

The study was subdivided into two major and one minor program. The first major program consisted of a seasonal study of crabs from an area of intermediate salinity that was fished throughout the entire year by commercial crabbers. The second major program consisted of a study of serum constituents in crabs taken along the salinity gradient found in Virginia. Early in the study, a minor program of intensive sampling from a very restricted area (a small creek) was undertaken to provide information on short term variation in certain of the constituents. Miscellaneous sampling or experiments were undertaken as the opportunity or need arose.

COLLECTION OF CRABS

Crabs used to determine seasonal variation in serum constituents were obtained from commercial crabbers. All of these crabs were taken from the York Spit area of lower Chesapeake Bay (Fig. 1). Crabs obtained during the winter months (December-March) were taken by crab dredge, those obtained during the remainder of the year were taken in crab pots (Van Engel, 1962). Samples were obtained at monthly intervals from February 1969 through July 1971 with the exception of May 1971.

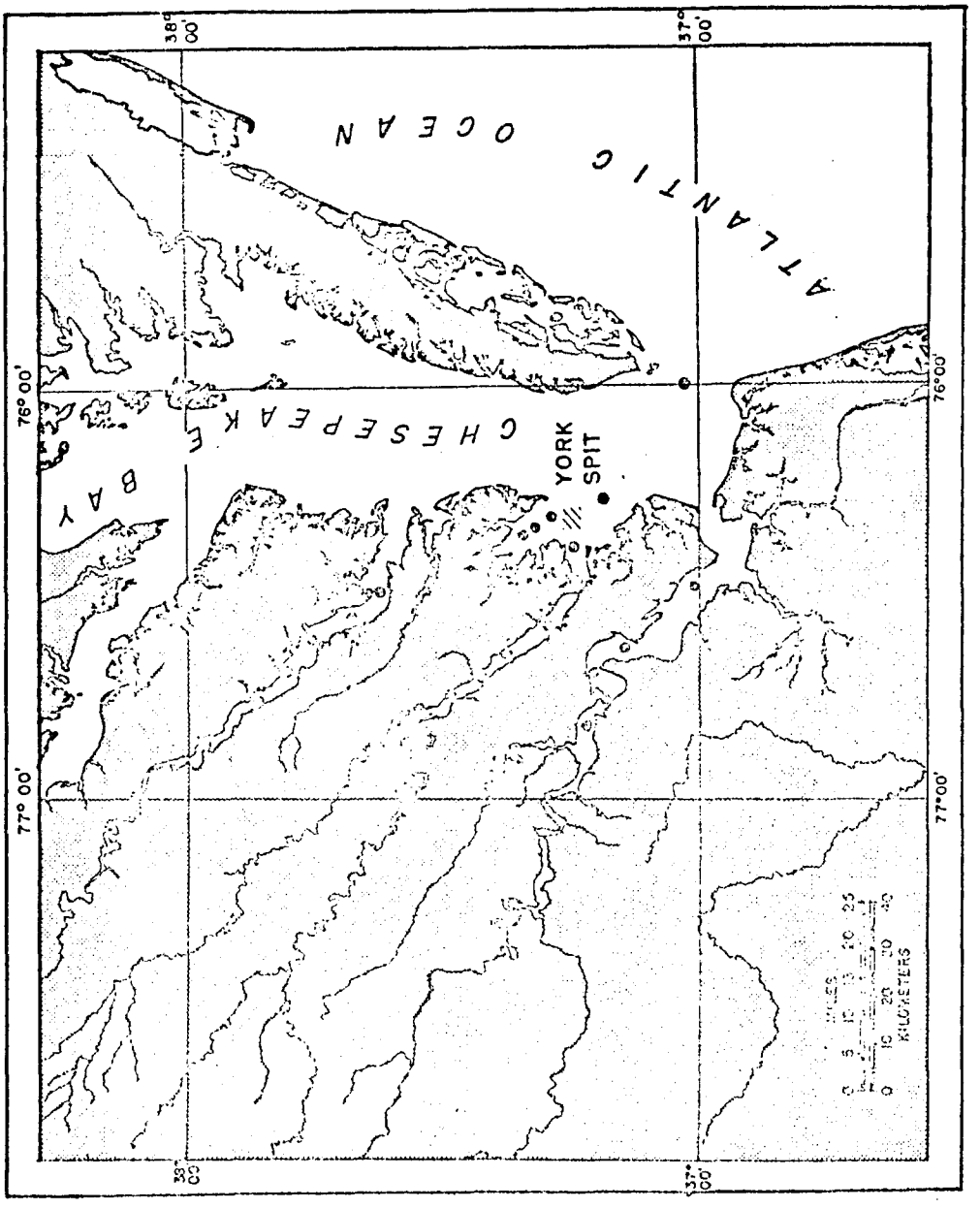

Fig. 1. Location of sampling areas from which blue crabs, Callinectes sapidus were taken during the serum chemistry study conducted February 1969-July 1971.

Crabs used to determine variation in serum constituents along the salinity gradients found in Virginia waters were taken primarily by trawling with a 30-foot semi-balloon trawl from R/V Pathfinder. The salinity gradient sampling program was begun in 1969 with sampling from the Rappahannock River and the Eastern Shore of Virginia. In 1970 sampling was extended to include the York River, James River, Pamunkey River, Mobjack Bay and Lower Chesapeake Bay (Fig. 1). A few crabs for the salinity gradient studies and miscellaneous studies were taken in pots and by dip nets. These samples were obtained during 1969 and 1970, primarily in summer and early fall.

During the period 2 July - 25 August 1969, serum of 149 male and 3 female mature, hard crabs from the North River, Mathews County, Virginia, was analyzed to determine short term variation of serum constituents. All of the crabs were caught in pots.

ENVIRONMENTAL DATA

Water samples were collected by a variety of methods (bucket, Kemmerer bottle, Frautsche bottle). Temperatures were measured to the nearest 0.1° C. Salinities were determined to the nearest 0.01o/oo with an Industrial Instruments Laboratory salinometer (RS-7A). Environmental data were obtained simultaneously with trawling samples and most of the miscellaneous samples. Environmental data

to accompany the samples from commercial sources were obtained during the monthly trawl surveys conducted by the Virginia Institute of Marine Science.

BLOOD SERUM

Crab blood was obtained by cutting through the merus of one of the rear walking legs and collecting the dripping blood in a heavy-walled test tube or a centrifuge tube. The blood samples were cooled (by refrigeration or placing in crushed ice) prior to further processing. In the laboratory, the clot which was normally present was broken up with a glass stirring rod and the blood centrifuged in an International (PRC-2) centrifuge at 2,000 g for 25 minutes at 5° C. The expressed serum was pipetted from the clot and stored in a polyethylene-capped vial. All analyses were performed on this serum. Serum was refrigerated, or if analyses were delayed, frozen until used. No differences in results were found using fresh serum or serum frozen and thawed once.

MORPHOMETRIC MEASUREMENTS AND LIFE STAGE DATA

At the time of bleeding, or after the last bleeding if several samples were taken from the same crab, the long width, i.e., the distance between lateral spine tips, was measured to the nearest millimeter. At the same time sex, maturity, year class (if females) and state of the molt cycle were determined. Sex and maturity were determined using the criteria of Van Engel (1958), i.e., mature females had a broad rounded abdomen free of

the ventral shell while immature females had a triangular abdomen sealed to the body. Mature males had a T-shaped abdomen either hanging free from the ventral shell or held in place against this shell by a pair of "snap-fastener-like tubercles". Immature males had a T-shaped abdomen sealed tightly to the ventral shell.

The year class of mature females was determined using the criteria described by Hopkins (1947). Earlier year class crabs (older) which had already spawned, possessed adult-sized nemerteans (Carcinomertes sp.) on the gills and usually eroded or fouled carapaces. Later year class (younger) crabs generally had bright, unfouled carapaces and no, or only very small nemerteans on the gills.

SERUM CHLORIDE

Serum chloride was determined either directly in the serum or, to conserve serum for other analyses, on deproteinized serum with an American Instrument Company chloride titrator according to Cotlove (1961). Deproteinization involved addition of 0.2 ml serum to 2.0 ml distilled water, swirling to mix, addition of 1.0 ml 0.3 N NaOH, swirling to mix, addition of 1.0 ml 5% $ZnSO_4.7H_2O$, vigorous mixing and centrifugation. Comparison of results using deproteinized serum as opposed to serum indicated no differences greater than the reproducibility of the method.

SERUM OSMOTIC CONCENTRATION

Serum osmotic concentrations were determined with 0.2 ml serum samples on a Precision Instrument Company freezing point osmometer (Osmette model).

TOTAL SERUM PROTEIN

Fresh serum or serum which had been frozen, stored and thawed once was diluted with distilled water (0.1 ml serum: 4.0 ml distilled water). Serum protein was determined by the biuret method (Layne, 1957) on a 1.0 ml aliquot of the diluted serum. Bovine serum albumin was used as a standard. Horn & Kerr (1963) have reported indistinguishable standard curves using bovine serum albumin and lyophilized hemocyanin using the method of Lowry et al. (1951). Steward & Li (1969) recommended the biuret method for lobster serum proteins over direct spectrophotometric techniques at 278 nm because of low levels of tyrosine in lobster serum proteins and wide fluctuations in serum free amino acids previously reported by Stewart, et al. (1966).

SERUM GLUCOSE

Serum glucose was determined by the modification of the Raabo & Terkildsen (1960) colorimetric glucose oxidase-peroxidase method described by Sigma (1969). Reagents were purchased in "Kit" form from Sigma Chemical Company (St. Louis, Mo. USA). The analyses were performed on protein free supernatants prepared by gently mixing

0.2 ml serum in 2.0 ml distilled water, adding 1.0 ml 0.3 N BaOH, swirling to mix, adding 1.0 ml 5% $ZnSO_4.7H_2O$, mixing thoroughly and centrifuging at 2,000 g for 15 minutes.

Fales (1963) found that $Zn(OH)_2$ filtrates are essentially free of materials interferring with the colorimetric glucose oxidase method, and that this method very closely approximates the true glucose level of blood.

SERUM TOTAL NINHYDRIN POSITIVE SUBSTANCES (TNPS)

Fresh serum or serum which had been stored, frozen and thawed once was deproteinized by adding four parts of absolute ethanol (100%) to one part of serum (the usual volumes used were 0.2 ml serum and 0.8 ml ethanol) and centrifuging at 2000 g for 10 minutes.

An aliquot of the deproteinized serum (usually 0.5 ml) was mixed with 1.0 ml of ninhydrin reagent (Moore and Stein, 1954) brought to 2.0 ml of solution with distilled water, heated in a boiling water bath for 20 minutes in a capped test tube, cooled to room temperature, diluted with 5.0 ml 50% ethanol and thoroughly mixed. A standard (0.5 ml, 1.0 millimolar leucine) and blank (2.0 ml distilled water) were treated the same way. Absorbance was determined at 570 nm. In practice the ninhydrin reagent used was drawn from the reservoir used with a Technicon (Ardsley, N.Y.) amino acid analyzer. Total NPS was calculated as micromoles leucine equivalents/ml original serum.

SERUM FREE AMINO ACID

A qualitative and quantitative analysis of the free amino acids in serum was made with a semi-automatic ion exchange analyzer (Technicon Auto-Analyzer). A volume of serum (0.2-1.0 ml) was mixed with four volumes of 100% ethanol and centrifuged as above. The entire supernatant was decanted and treated as described by DuPaul & Webb (1970).

OVARY WEIGHT

From March 1970 through April 1971, the ovaries from all the female crabs taken during the monthly samplings were removed and weighed to the nearest 0.1 g. Ovary weights of crabs sampled during the salinity gradient studies were not determined.

HOLDING EXPERIMENTS

A series of experiments were performed to determine the effect of holding crabs out of water on serum constituents. In 1969 a number of crabs taken in pots were divided into two groups. One group was bled approximately 3 hours after removal from the water, while the remaining animals were bled after holding for 12 hours at 5°C in air. A second experiment conducted in 1970 consisted of bleeding some crabs caught with a dip net immediately upon capture (within 2 minutes), those crabs not bled were held for approximately 12 hours at 5° C in air and bled the next morning. In addition, the crabs bled immediately upon

capture were also held at 5°C in air and rebled the next morning. Other crabs taken during salinity gradient studies were also bled and held overnight (12-15 hours) and bled again. In another experiment, crabs from a sea table were bled, returned to the sea table, and then rebled after a 10-11 hour period.

STATISTICAL ANALYSIS

Mean values of the various serum constituents in the samples were compared using student "t" tests for small samples described by Snedecor (1956). Differences between mean values were assumed if "t" fell outside the 95% confidence interval of a two-tailed distribution ($P < 0.05$).

Significant correlations were determined by comparing the correlation coefficient (r) to the appropriate table in Snedecor (1956). Significance was assumed if r were greater or equal to the r listed for the 95% confidence interval for the proper degree of freedom ($P \leq 0.05$).

RESULTS

ENVIRONMENTAL DATA

Salinity and temperature in the York Spit area (the seasonal study) varied from 17.3 to 27.0 o/oo and 0.5 to 26.8°C. The salinity and temperature ranges during the summer and fall salinity gradient studies were 1.0 to 30.6 o/oo and 21.1 to 29.0° C.

SERUM CHLORIDE

In the seasonal study, mean serum chloride ranged from a low of 323-343 meq/liter in summer months to 446-471 meq/liter in winter months (Table 1).

No significant differences were found between mean serum chlorides of males and females sampled during the same month, with the exception of two months (April 1969 and January 1971). No differences were found between serum chloride values of different year class females or between female crabs with or without egg masses (sponges) sampled during the same month (Table 2). A distinct seasonal cyclical variation in serum chloride was found (Fig. 2). This variation was not correlated with the salinity variation during the same period ($r = 0.10$). A strong negative correlation ($r = -0.72$) was found between serum chloride and temperature.

TABLE 1-SEASONAL VARIATION OF MEAN SERUM CHLORIDE CONCENTRATION IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS, FROM THE YORK SPIT AREA, CHESAPEAKE BAY, VIRGINIA

SAMPLE DATE DA MO YR	SALINITY (0/00)	TEMPERATURE (C)	SERUM CHLORIDE + OR - STANDARD ERROR (MEQ/LITER) MALES	(N)	OLD FEMALES	(N)	NEW FEMALES	(N)	MALES X OLD F	MALES X NEW F	OLD F X NEW F
					1967	CLASS	1968	CLASS			
26 3 69	20.91	3.2			446	5(11)					
22 4 69	21.37	10.5	431	9 (5)	394	2 (7)			*		
20 5 69	23.00	16.8	365	1 (2)	364	3 (8)					
23 6 69	23.50	22.9	302	52 (2)	318	12(12)	369	5 (2)			
23 7 69	22.49	26.8	347	11 (6)	371	(1)	356	5 (6)			
26 8 69	23.40	26.2	357	5 (5)			359	4(11)			
23 9 69	24.99	25.7	374	(1)			367	3(13)			
15 10 69	23.72	20.1	394	31 (4)			400	11 (8)			
19 11 69	25.53	13.8	445	7 (4)			455	8(19)			
18 12 69	24.92	8.9	456	11 (4)			445	8(26)			
					1968	CLASS	1969	CLASS			
23 1 70	22.70	4.2	464	10(19)	448	7(11)					
19 2 70	22.20	2.8	471	(1)	445	6(29)					
17 3 70	20.10	6.3			399	10(30)					
22 4 70	18.08	13.4			370	3(30)					
15 5 70	20.19	15.2			414	3(30)					
23 6 70	19.80	23.8	361	9 (3)	366	3(17)	364	29 (3)			
20 7 70	23.83	23.3	334	6 (5)	346	5(21)	340	3 (4)			
18 8 70	27.01	24.1			326	11 (6)	362	6(24)			
23 9 70	24.06	24.1	363	8 (5)	362	6 (7)	368	4(18)			
20 10 70	23.50	21.6	390	9 (5)	402	14 (6)	391	4(19)			
12 11 70	22.48	14.9	402	13 (5)			414	9(25)			
8 12 70	22.02	7.6	440	2 (3)			431	5(26)			
21 1 71	19.94	4.8	473	5 (6)	413	14 (2)	449	6(22)	*	*	
19 2 71	22.34	0.5			454	13 (4)	474	6(26)			
17 3 71	17.28	6.2	479	(1)	434	8 (2)	458	4(29)			
13 4 71	21.22	8.8	432	16 (5)			406	10(24)			
					1969	CLASS	1970	CLASS			
2 6 71	24.26	19.7	353	4 (3)	368	4(21)	358	10 (3)			
21 7 71	21.31	24.1	374	5 (8)	370	11 (7)	362	(1)			

* SIGNIFICANT DIFFERENCE

TABLE 2 - COMPARISON OF SERUM CONSTITUENT LEVELS IN EGG BEARING (SPONGE) AND NON-EGG BEARING (CLEAN) 1968 YEAR CLASS CALLINECTES SAPIDUS.

Sample Date	State	(N)	Serum Chloride (meq/liter)	Serum Osmotic Concentration (milliosmols) mean ± S.E.	Total Serum Protein (mg/ml)	Serum glucose (mg/100 ml)	Total Ninhydrin Positive Substances (μ moles/ml)
Jun 70	Sponge	(12)	368 ± 3	804 ± 5	55.9 ± 3.5	91.1 ± 6.3	8.7 ± 1.1
			n.s.	n.s.	n.s.	n.s.	n.s.
	Clean	(5)	361 ± 8	794 ± 14	53.6 ± 18.8	72.7 ± 26.3	8.2 ± 2.0
Jul 70	Sponge	(8)	340 ± 8	791 ± 7	70.0 ± 6.2	166.8 ± 25.5	14.9 ± 2.8
			n.s.	n.s.	*	*	n.s.
	Clean	(13)	350 ± 5	802 ± 11	53.1 ± 4.0	103.6 ± 14.1	14.6 ± 2.0
Aug 70	Sponge	(2)	300 ± 10	770 ± 4	46.6 ± 5.3	33.4 ± 10.6	13.9 ± 0.8
			n.s.	n.s.	n.s.	*	*
	Clean	(4)	338 ± 10	792 ± 20	60.6 ± 4.5	70.9 ± 6.0	5.9 ± 1.0

n.s. no significant difference * significantly different ($P < 0.05$)

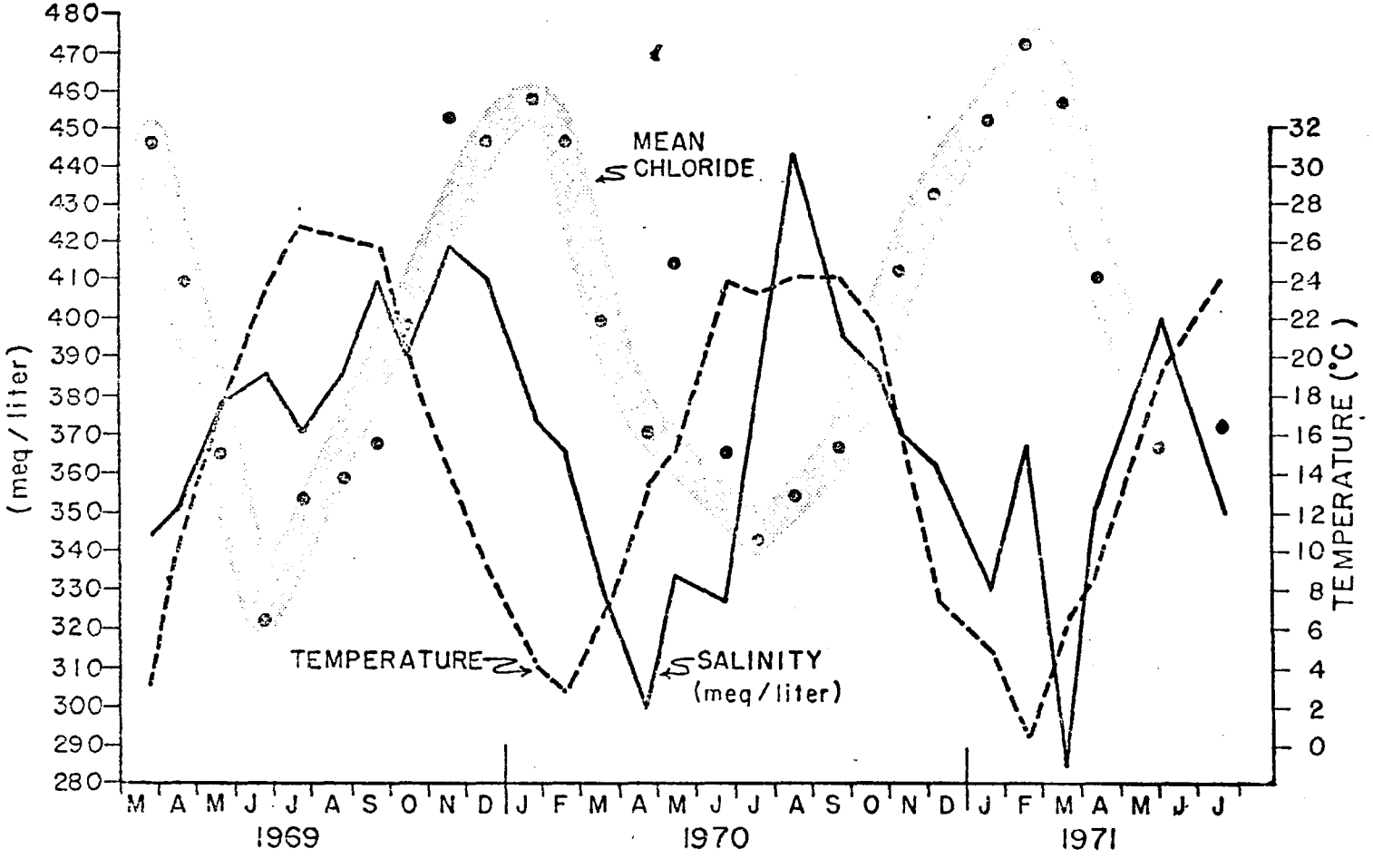

Fig. 2. Seasonal variation in mean serum chloride in mature blue crabs, Callinectes sapidus from the York Spit area of Chesapeake Bay, Virginia.

In the salinity gradient study, mean serum chlorides ranged from 290 meq/liter to 446 meq/liter (Table 3). Serum chloride was hyperionic to the medium below a salinity approximately 21 o/oo, essentially isoionic from 21-25 o/oo and hypoionic above that point (Fig. 3). Samples from the Rappahannock River taken in 1969 indicate significantly lower serum chlorides in male crabs compared to female crabs from the same stations below 15%. At higher salinities, no differences were found between serum chlorides in males and females. In 1970 no differences were found between male and female crabs collected during the trawl transects, with the exception of one station in 23.5 o/oo at which males had lower serum chloride than the females (Table 3).

SERUM OSMOTIC CONCENTRATION

In the seasonal study of serum osmotic concentration, mean serum osmotic concentration ranged from a low of 730-790 milliosmoles in summer to a high of 970-1030 milliosmoles in winter (Table 4). Significant differences were found between serum osmotic concentration of male and female crabs in January and April 1971 and between the different year class females in July 1970, January 1971 and March 1971. No differences were found in serum osmotic concentrations of egg-bearing and non-egg bearing females taken during the same month (Table 2). The seasonal trend of serum osmotic concentration is similar to that of serum chloride (Fig. 4). Negative

TABLE 3- MEAN SERUM CHLORIDE CONCENTRATION IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS, COLLECTED FROM VARIOUS SALINITIES IN VIRGINIA.

SAMPLE DATE DA	MO	YR	AREA	SALINITY (O/OO)	TEMPERATURE (C)	SERUM CHLORIDE + OR - STANDARD ERROR (MEQ/LITER) MALES	(N)	FEMALES	(N)
19	8	69	RR	19.00	27.5	309	15 (5)	327	6(13)
19	8	69	RR	17.50	27.6	314	18 (2)		
19	8	69	RR	14.00	28.0	324	3(12)*	336	3(11)*
19	8	69	RR	11.50	28.2	317	2(11)*	336	7 (9)*
19	8	69	RR	6.40	28.0	302	5(12)*	321	4(12)*
19	8	69	RR	1.30	28.0	301	(1)		
21	8	69	ES	27.50	22.0	386	8(16)	372	8 (4)
21	8	69	ES	18.90	22.0	353	17(72)	359	7 (4)
14	7	70	RR	19.11	24.0	352	(1)		
14	7	70	RR	16.50	24.6	348	(1)		
14	7	70	RR	13.82	24.9	319	(1)		
14	7	70	RR	10.08	25.6	320	9 (5)		
14	7	70	RR	4.75	26.4	326	(1)		
16	7	70	YR	24.00	26.0	283	(1)	340	(1)
16	7	70	MB	21.69	25.6	315	(1)		
16	7	70	MB	19.07	26.8	328	9 (9)	337	(1)
16	7	70	MB	18.71	25.8	343	1 (2)		
3	8	70	JR	21.25	27.2	339	7 (2)		
3	8	70	JR	10.71	28.4	302	(1)		
3	8	70	JR	4.62	28.8	307	(1)		
3	8	70	JR	2.84	29.0	288	23 (2)		
7	8	70	CB	30.38	22.2			388	6(13)
7	8	70	YR	27.01	24.1	385	15 (3)	346	2 (2)
7	8	70	CB	26.82	24.0			397	9 (3)
11	8	70	RR	15.34	25.7	340	3 (4)	338	(1)
11	8	70	RR	15.02	25.8	339	7 (8)	357	11 (2)
11	8	70	RR	14.66	26.4	336	5 (5)		
11	8	70	RR	13.90	25.6	345	12 (4)		
11	8	70	RR	11.49	25.8	325	7 (7)	322	4 (2)
11	8	70	RR	9.68	25.7	344	15 (2)	329	8 (4)
13	8	70	YR	19.28	26.6	344	(1)	416	(1)
13	8	70	YR	18.49	26.5	373	19 (3)	377	14 (5)
13	8	70	YR	16.00	25.8			370	7(13)
13	8	70	YR	13.90	25.8	404	67 (2)	365	7 (9)
13	8	70	PR	7.13	26.3	356	(1)		
14	9	70	CB	30.65	22.5			446	5(16)
14	9	70	CB	27.18	23.9			415	(1)
14	9	70	YR	24.06	24.1			399	3 (8)
17	9	70	YR	21.93	25.4			376	3 (5)
17	9	70	YR	20.42	25.7	362	16 (2)	372	2 (4)
17	9	70	YR	18.21	26.1	370	7 (2)	372	(1)
17	9	70	YR	17.00	26.0			367	3 (8)
17	9	70	PR	12.94	26.0			354	(1)
17	9	70	PR	8.97	26.3	300	(1)		
17	9	70	PR	.89	26.8	288	11 (4)		
8	10	70	PR	12.41	21.6	379	(1)		
8	10	70	PR	4.26	21.4	317	7 (4)	304	1 (2)
12	10	70	CB	29.74	21.1			387	7 (8)
12	10	70	YR	23.50	21.6	369	4 (7)*	406	(1)*

AREA CODE-- RR-RAPPAHANNOCK RIVER, YR-YORK RIVER, MB-MOBJACK BAY, JR-JAMES RIVER, CB-CHESAPEAKE BAY, PR-PAMUNKEY RIVER, ES-EASTERN SHORE(SEA SIDE)

* SIGNIFICANT DIFFERENCE

TABLE 4-SEASONAL VARIATION OF MEAN SERUM OSMOTIC CONCENTRATION IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS, FROM THE YORK SPIT AREA, CHESAPEAKE BAY, VIRGINIA

SAMPLE DATE DA	MO	YR	SALINITY (0/00)	TEMPERATURE (C)	SERUM OSMOTIC CONCENTRATION + OR - STANDARD ERROR (MILLIOSMOLES) MALES	(N)	OLD FEMALES	(N)	NEW FEMALES	(N)	MALES X OLD F	MALES X NEW F	OLD F X NEW F
							1968	CLASS	1969	CLASS			
23	6	70	19.80	23.8	799	9 (3)	801	5(17)	785	11 (3)			
20	7	70	23.83	23.3	789	14 (5)	798	7(21)	743	7 (4)			*
18	8	70	27.01	24.1			785	14 (6)	778	5(24)			
23	9	70	24.06	24.1	816	16 (5)	817	11 (7)	807	6(18)			
20	10	70	23.50	21.6	839	12 (5)	871	29 (6)	852	7(18)			
12	11	70	22.48	14.9	856	8 (5)			913	16(25)			
8	12	70	22.02	7.6	973	16 (3)			973	10(26)			
21	1	71	19.94	4.8	960	8 (6)	897	3 (2)	949	6(22)	*		*
19	2	71	22.34	0.5			994	36 (4)	1038	17(25)			
17	3	71	17.28	6.2	959	(1)	848	40 (2)	921	5(29)			*
13	4	71	21.22	8.8	818	7 (5)			845	5(25)		*	
							1969	CLASS	1970	CLASS			
2	6	71	24.26	19.7	737	10 (3)	782	5(21)	739	20 (3)			
21	7	71	21.31	24.1	768	5 (8)	773	4 (7)	744	(1)			

* SIGNIFICANT DIFFERENCE

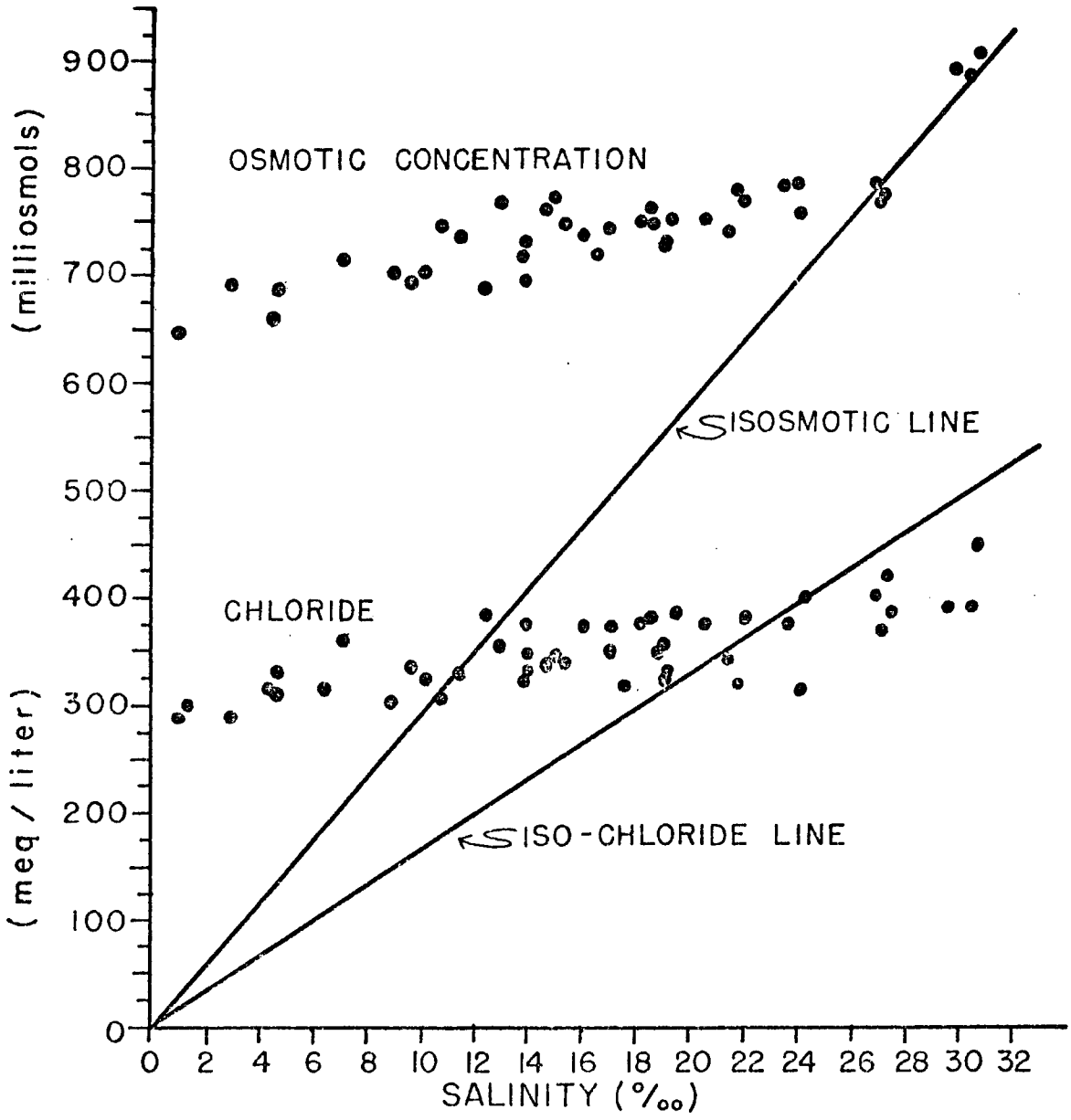

Fig. 3. Mean serum chloride and serum osmotic concentration of mature blue crabs taken in August 1969 and June-October 1970 in various waters in Virginia.

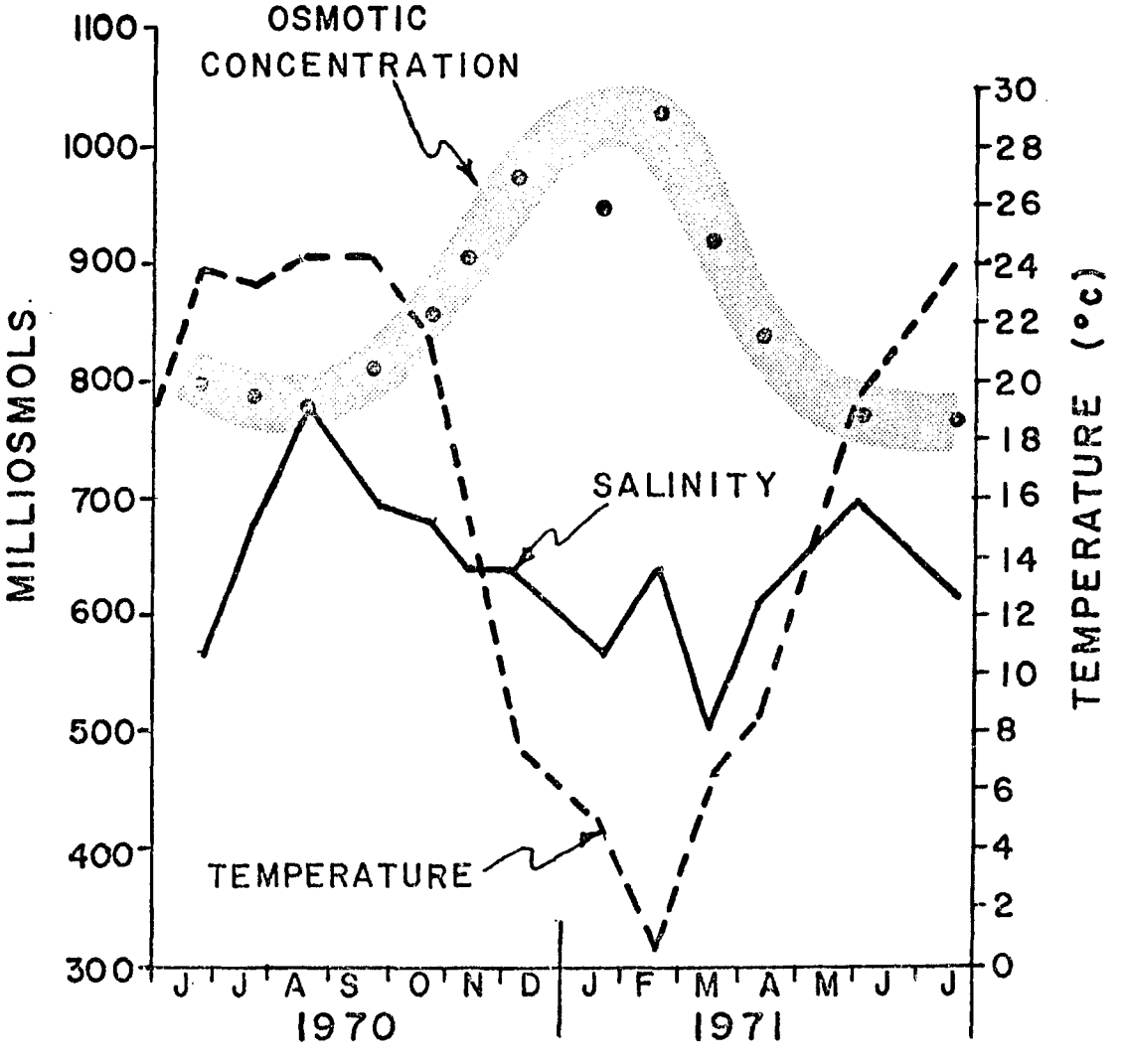

Fig. 4. Seasonal variation of mean serum osmotic concentration in mature blue crabs, Callinectes sapidus from the York Spit area of Chesapeake Bay, Virginia.

correlation between serum osmotic concentration and temperature (r = -0.83) was higher than the positive correlation with salinity (r = 0.41). The correlation between serum chloride and serum osmotic concentration was also high (r = 0.84).

In the salinity gradient study, mean serum osmotic concentrations ranged from 645 to 903 milliosmoles. With the exception of one sample of crabs from 18.9 o/oo, no differences were found between male and female crabs taken at the same station (Table 5). Below approximately 25 o/oo, serum osmotic concentration is maintained hyperosmotic to the environment. At salinities greater than 25 o/oo serum osmotic concentration is essentially isosmotic with the environment (Fig. 3).

TOTAL SERUM PROTEIN

In the seasonal study, mean serum protein ranged from about 20-80 mg/ml in male crabs and 35-100 mg/ml in female crabs (Table 6). Significant differences were found between serum protein levels of male and female crabs in 10 of the 23 months in which comparisons were possible. In 8 of these months serum protein levels were lower in males than in females. Differences between serum protein levels in older year class females and younger year class females were found during the periods June-September each year in months where comparisons were possible except for July 1971. With this one exception, mean serum protein

TABLE 5-MEAN SERUM OSMOTIC CONCENTRATION IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS, COLLECTED FROM VARIOUS SALINITIES IN VIRGINIA.

SAMPLE DATE DA MO YR	AREA	SALINITY (O/OO)	TEMPERATURE (C)	SERUM OSMOTIC CONCENTRATION ± CR - STANDARD ERROR (MILLIOSMOLES) MALES	(N)	FEMALES	(N)
14 7 70	RR	19.11	24.0	722	(1)		
14 7 70	RR	16.50	24.6	717	(1)		
14 7 70	RR	13.82	24.9	714	(1)		
14 7 70	RR	10.08	25.6	705	13 (5)		
14 7 70	RR	4.75	26.4	692	(1)		
16 7 70	YR	24.00	26.0	777	(1)	782	(1)
16 7 70	MB	21.69	25.6	774	(1)		
16 7 70	MB	19.07	26.8	731	7 (9)	713	(1)
16 7 70	MB	18.71	25.8	744	8 (2)		
3 8 70	JR	21.25	27.2	735	8 (2)		
3 8 70	JR	10.71	28.4	744	(1)		
3 8 70	JR	4.62	28.8	685	(1)		
3 8 70	JR	2.84	29.0	692	6 (2)		
7 8 70	CB	30.38	22.0			878	8
7 8 70	YR	27.01	24.0			778	10
7 8 70	CB	26.82	24.1	761	10 (3)	760	12
11 8 70	RR	15.34	25.7	752	12 (4)	729	(1)
11 8 70	RR	15.02	25.8	760	11 (8)	801	35 (2)
11 8 70	RR	14.66	26.4	757	19 (5)		
11 8 70	RR	13.90	25.6	695	12 (4)		
11 8 70	RR	11.49	25.8	740	9 (7)	722	20 (2)
11 8 70	RR	9.68	25.7	703	31 (2)	688	10 (4)
13 8 70	YR	19.28	26.6	755	(1)	740	(1)
13 8 70	YR	18.94	26.5	801	23 (3)*	734	4 (5)*
13 8 70	YR	16.00	25.8			737	5(13)
13 8 70	YR	13.90	25.8	737	4 (2)	726	8 (9)
13 8 70	PR	7.13	26.3	714	(1)		
14 9 70	CB	30.65	22.5			903	41(16)
14 9 70	CB	27.18	23.9			769	(1)
14 9 70	YR	24.06	24.1			753	3 (8)
17 9 70	YR	21.93	25.4			765	10 (5)
17 9 70	YR	20.42	25.7	749	13 (2)	745	5 (4)
17 9 70	YR	18.21	26.1	746	4 (2)	746	(1)
17 9 70	YR	17.00	26.0			741	5 (8)
17 9 70	PR	12.94	26.0			765	(1)
17 9 70	PR	8.97	26.3	700	(1)		
17 9 70	PR	0.89	26.8	645	11 (4)		
8 10 70	PR	12.41	21.6	684	(1)		
8 10 70	PR	4.26	21.4	657	6 (4)	656	6 (2)
12 10 70	CB	29.74	21.1			891	8 (8)
12 10 70	YR	23.50	21.6	774	6 (7)	795	(1)

AREA CODE-- RR-RAPPAHANNOCK RIVER, YR-YORK RIVER, MB-MOBJACK BAY, JR-JAMES RIVER, CB-CHESAPEAKE BAY, PR-PAMUNKEY RIVER

* SIGNIFICANT DIFFERENCE

TABLE 6-SEASONAL VARIATION OF MEAN SERUM PROTEIN CONCENTRATION IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS , FROM THE YORK SPIT AREA, CHESAPEAKE BAY, VIRGINIA

SAMPLE DATE DA	MO	YR	SALINITY (0/00)	TEMPERATURE (C)	SERUM PROTEIN + OR - STANDARD ERROR (MG/ML) MALES	(N)	OLD FEMALES	(N)	NEW FEMALES	(N)	MALES X OLD F	MALES X NEW F	OLD F X NEW F
							1967	CLASS	1968	CLASS			
25	2	69	20.80	4.4	72.40	11.30 (2)	86.79	3.18(10)					
26	3	69	20.91	3.2			86.89	4.16(11)					
22	4	69	21.37	10.5	49.52	5.90 (5)	86.67	3.87 (7)			*		
20	5	69	23.00	16.8	80.48	19.32 (2)	100.69	5.97 (8)					
23	6	69	23.50	22.9	20.20	11.89 (2)	82.11	4.77(12)	26.72	12.06 (2)	*		*
23	7	69	22.49	26.8	58.42	9.94 (6)	81.52	(1)	42.25	3.92 (6)			*
26	8	69	23.40	26.2	56.58	7.10 (5)			35.36	3.90(11)		*	
23	9	69	24.99	25.7	51.42	7.05 (2)			98.05	14.30(13)			
15	10	69	23.72	20.1	33.55	5.45 (4)			49.92	11.56 (8)			
19	11	69	25.53	13.8	26.63	2.87 (4)			38.12	4.42(19)			
18	12	69	24.92	8.9	54.08	12.53 (4)			71.56	4.82(26)			
							1968	CLASS	1969	CLASS			
23	1	70	22.70	4.2	35.39	2.70(19)	68.58	8.94(11)			*		
19	2	70	22.20	2.8	23.42	(1)	55.21	2.57(29)			*		
17	3	70	20.10	6.3			42.71	2.79(30)					
22	4	70	18.08	13.4			61.98	3.18(30)					
15	5	70	20.19	15.2			50.79	2.76(30)					
23	6	70	19.81	23.8	48.79	11.05 (3)	55.33	4.74(17)	26.34	2.24 (3)			*
20	7	70	23.83	23.3	55.96	6.01 (5)	59.57	3.78(21)	31.20	6.28 (4)		*	*
18	8	70	27.01	24.1			55.98	4.31 (6)	34.88	2.75(24)			*
23	9	70	24.06	24.1	37.21	4.02 (5)	61.41	6.60 (7)	45.82	3.44(18)	*		*
20	10	70	23.50	21.6	41.39	9.28 (5)	64.49	8.41 (6)	52.88	3.30(19)			
12	11	70	22.48	14.9	30.46	5.39 (5)			59.12	4.85(25)		*	
8	12	70	22.02	7.6	78.67	2.40 (3)			91.05	3.21(26)			
21	1	71	19.94	4.8	58.64	3.41 (6)	67.12	3.40 (2)	80.80	3.79(22)		*	
19	2	71	22.34	0.5			38.08	1.35 (4)	39.46	1.39(26)			
17	3	71	17.28	6.2	72.60	(1)	77.95	7.45 (2)	77.82	3.04(28)			
							1969	CLASS	1970	CLASS			
13	4	71	21.22	8.8	50.12	12.05 (5)	54.88	3.31(25)					
2	6	71	24.26	19.7	43.21	26.02 (3)	80.51	3.90(21)	46.27	10.01 (3)	*		*
21	7	71	21.31	24.1	38.87	5.73 (8)	40.92	(1)	42.36	7.41 (7)			

* SIGNIFICANT DIFFERENCE

was always higher in older year class female crabs during these months. Comparison of mean serum protein of different year classes during the months October to May showed no differences.

The seasonal pattern of mean total serum protein for female crabs is shown in Figure 5. With the exception of the September 1969 and February 1970 samples, the same general pattern appears in each year. Serum protein in female crabs starts at a low level (20-30 mg/ml) when the newly matured crabs first appear in June. The concentration of serum protein increases to a high of 65-90 mg/ml in December-January, after which a slight drop occurs with a subsequent leveling off around a value of approximately 50-70 mg/ml, although the protein levels in the 1967 year class of crabs appeared to level off at approximately 90 mg/ml. No seasonal effects on serum protein levels were discernable in male crabs.

Serum protein increased concurrently with increasing ovary weight until the ovary weight reached 5 or 6 g. Above 5 or 6 g ovary weight, serum protein varied between about 65-90 mg/ml (Fig. 6). Mean ovary weight and mean serum protein in the 1969 year class crabs showed an overall increase during the months June 1970 through January 1971. These increases appear directly related. In contrast, changes in mean ovary weight and serum protein in older (1968) year class crabs did not appear to be directly related (Fig. 7).

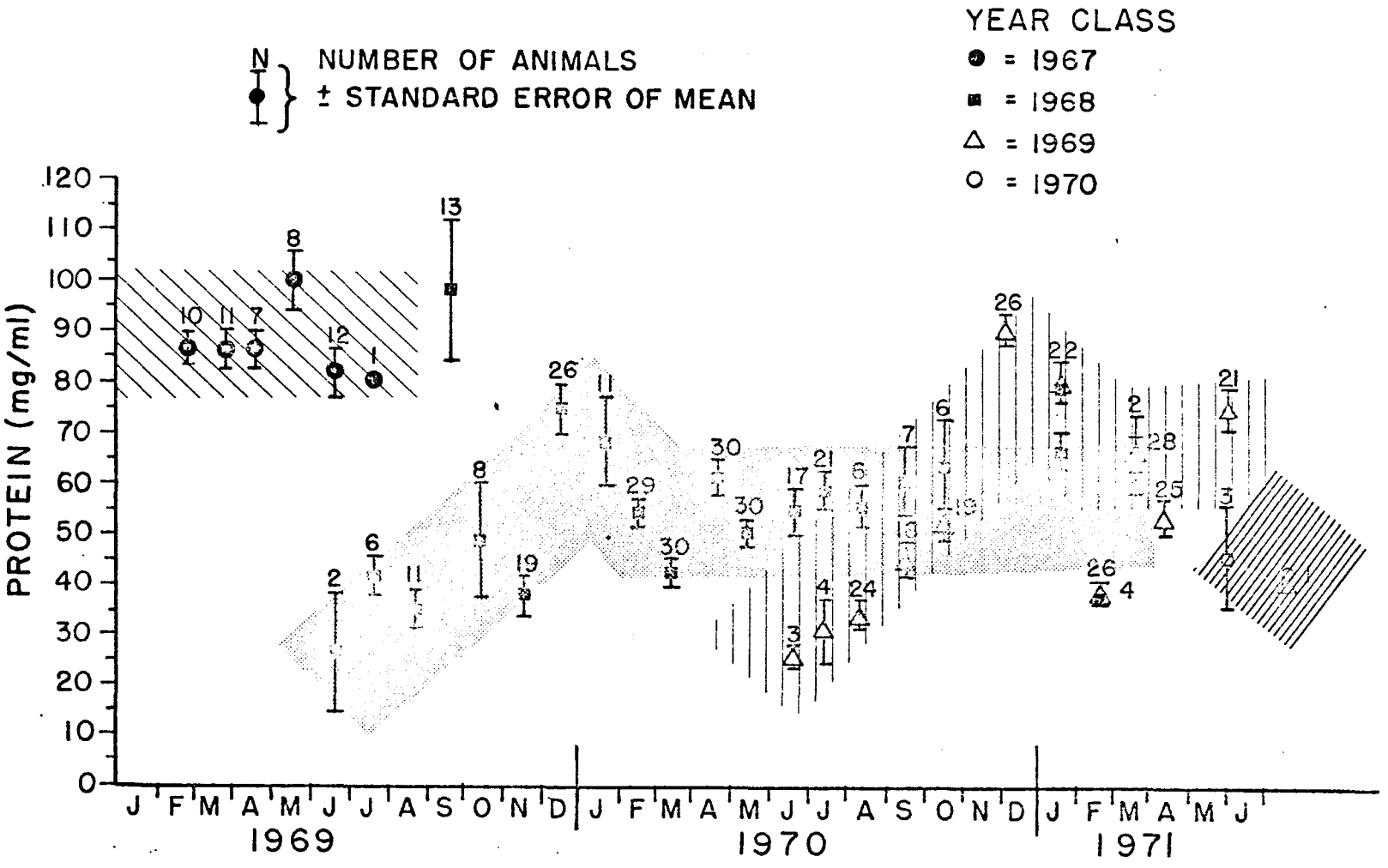

Fig. 5. Seasonal variation of mean total serum protein in mature female blue crabs, Callinectes sapidus from the York Spit area, Chesapeake Bay, Virginia.

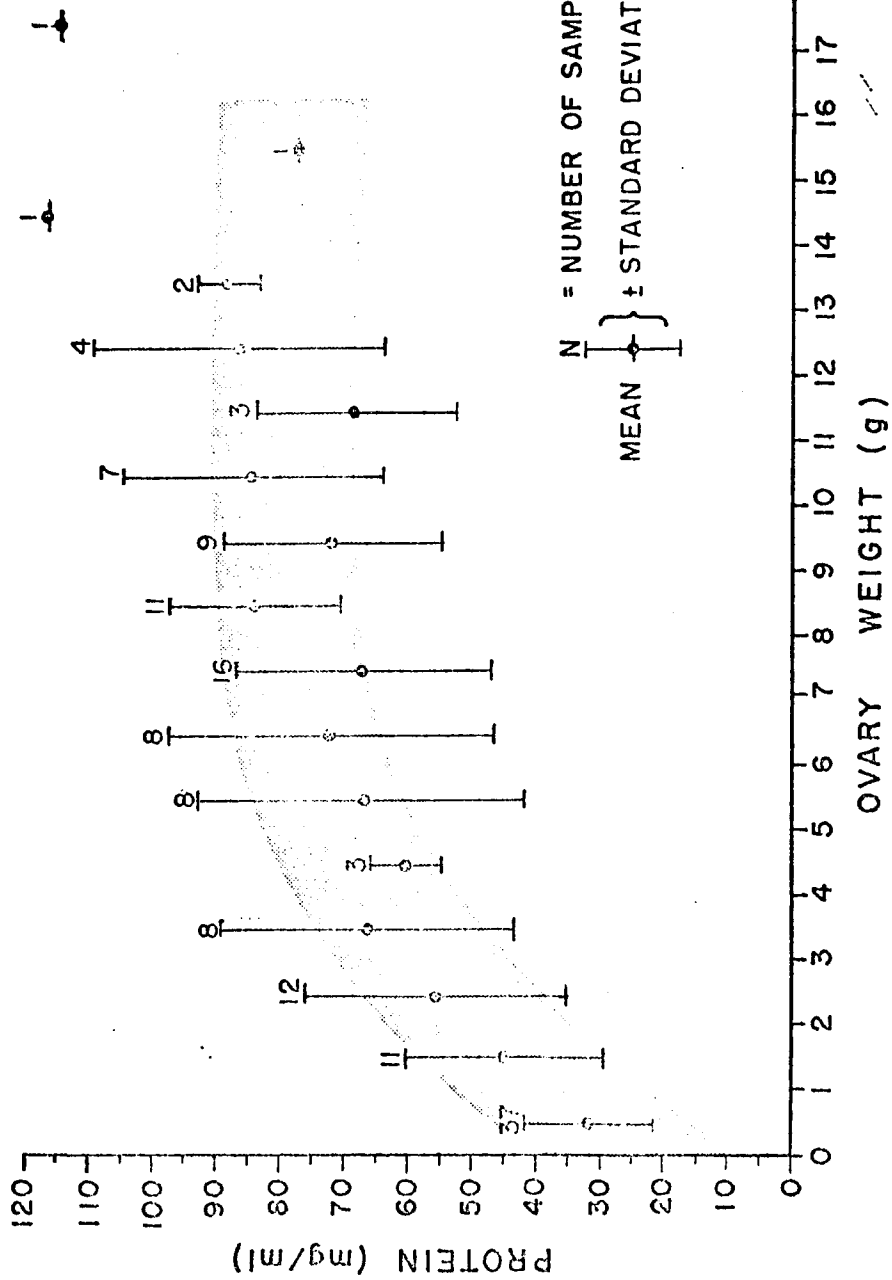

Fig. 6. The relationship between total serum protein and ovary weight in mature 1969 year class blue crabs, Callinectes sapidus taken during the period June 1970 to January 1971.

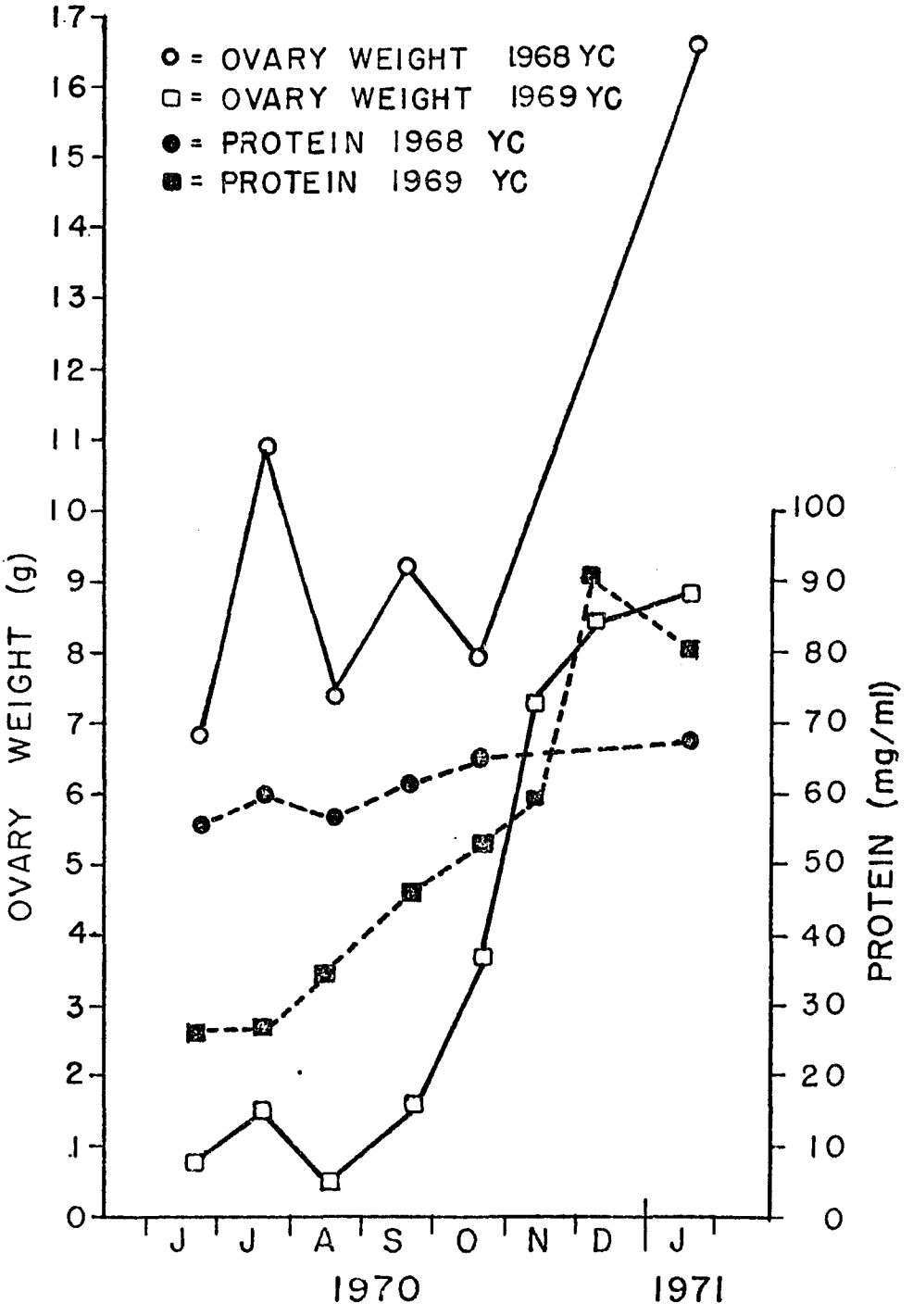

Fig. 7. Mean total serum protein and mean ovary weight of mature 1968 and 1969 year class blue crabs, Callinectes sapidus from the York Spit area, Chesapeake Bay, Virginia.

During the months of June, July and August 1970, 1968 year class crabs were taken with and without egg masses; comparison of means of serum protein yielded mixed results. No differences were found during June and August, but in July sponge bearing females had higher mean serum protein (Table 2).

In the salinity gradient study, mean serum protein ranged from 15-231 mg/ml in males and from 11-110 mg/ml in females (Table 7). Variability was extremely high. The coefficient of variation (C) for the various samples ranged from 2.5 to 109% with a mean of 45.6%.

For 1970, there was a significant positive correlation ($r = 0.548$) between serum protein in females and salinity. The correlation between serum protein in males and salinity ($r = -0.199$) was not significant.

SERUM GLUCOSE

In the seasonal study, serum glucose ranged from 2.3 - 125.5 mg/100 ml in male and 1.3 - 305.3 mg/100 ml in female blue crabs. Mean serum glucose ranged from 12.0 - 127.7 mg/100 ml (Table 8). Serum glucose could not be detected in 2 of 566 crabs. No differences were found between serum glucose levels of male blue crabs and female blue crabs in 14 of 15 months, or between old and new year class crabs in 7 of 9 months in which ample numbers of each sex were obtained to enable comparison. In June 1971 male crabs had lower serum glucose than the older (1969) year class females, and in June 1970 and

TABLE 7- MEAN SERUM PROTEIN CONCENTRATION IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS, COLLECTED FROM VARIOUS SALINITIES IN VIRGINIA.

SAMPLE DATE DA	MO	YR	AREA	SALINITY (O/OO)	TEMPERATURE (C)	SERUM PROTEIN + OR - STANDARD ERROR (MG/ML) MALES	(N)	FEMALES	(N)
19	8	69	RR	19.00	27.5	103.38	23.89(5)	51.75	5.74(13)
19	8	69	RR	17.50	27.6	230.85	25.05(2)		
19	8	69	RR	14.00	28.0	91.02	19.19(9)	58.67	7.19(11)
19	8	69	RR	11.50	28.2	110.27	7.09(12)	110.70	32.05(9)
19	8	69	RR	6.40	28.0	120.88	7.15(12)*	49.22	3.47(12)*
19	8	69	RR	1.30	28.0	91.75	32.45(2)		
21	8	69	ES	27.50	22.0	58.38	6.09(17)	71.38	14.98(4)
21	8	69	ES	18.90	22.0	104.32	8.07(73)	55.22	16.09(4)
14	7	70	RR	19.11	24.0	15.68	(1)		
14	7	70	RR	16.50	24.6	17.72	(1)		
14	7	70	RR	13.82	24.9	58.02	(1)		
14	7	70	RR	10.08	25.6	58.18	10.80(5)		
14	7	70	RR	4.75	26.4	58.02	(1)		
16	7	70	YR	24.00	26.0	92.06	(1)	30.10	(1)
16	7	70	MB	21.69	25.6	41.99	(1)		
16	7	70	MB	19.07	26.8	56.41	9.59(9)	18.54	(1)
16	7	70	MB	18.71	25.8	71.43	54.80(2)		
3	8	70	JR	21.25	27.2	36.58	14.80(2)		
3	8	70	JR	10.71	28.4	110.65	(1)		
3	8	70	JR	4.62	28.8	42.70	(1)		
3	8	70	JR	2.84	29.0	65.91	1.15(2)		
7	8	70	CB	30.38	22.2			67.15	3.53(13)
7	8	70	YR	27.01	24.1	31.65	8.74(3)	47.32	28.77(2)
7	8	70	CB	26.82	24.0			51.06	6.21(3)
11	8	70	RR	15.34	25.7	43.12	12.36(4)	11.11	(1)
11	8	70	RR	15.02	25.8	46.41	7.90(8)	15.61	2.27(2)
11	8	70	RR	14.66	26.4	38.70	14.51(5)		
11	8	70	RR	13.90	25.6	35.97	9.47(4)		
11	8	70	RR	11.49	25.8	50.21	6.31(7)	28.68	5.96(2)
11	8	70	RR	9.68	25.7	38.06	17.28(2)	16.90	1.37(4)
13	8	70	YR	19.28	26.6	16.15	(1)	32.10	(1)
13	8	70	YR	18.49	26.5	84.93	22.08(3)	22.37	1.21(5)
13	8	70	YR	16.00	25.8			36.57	3.16(12)
13	8	70	YR	13.90	25.8	50.97	13.77(2)	29.02	2.96(9)
13	8	70	YR	7.13	26.3	59.60	(1)		
14	9	70	CB	30.65	22.5			49.14	3.64(16)
14	9	70	CB	27.18	23.9			22.73	(1)
17	9	70	YR	24.06	24.1			27.50	3.66(8)
17	9	70	YR	21.93	25.4			37.08	16.48(5)
17	9	70	YR	20.42	25.7	55.05	26.52(2)	29.19	4.62(4)
17	9	70	YR	18.21	26.1	57.10	19.39(2)	35.36	(1)
17	9	70	YR	17.00	26.0			33.23	3.91(8)
17	9	70	YR	12.94	26.0			377.10	(1)
17	9	70	YR	8.97	26.3	124.85	(1)		
17	9	70	YR	0.89	26.8	64.38	21.04(4)		
8	10	70	YR	12.41	21.6	14.95	(1)		
8	10	70	YR	4.26	21.4	31.17	4.77(4)	45.66	24.88(2)
12	10	70	CB	29.74	21.1			55.16	4.31(8)
12	10	70	YR	23.50	21.6	22.77	4.20(7)	18.54	(1)

AREA CODE-- RR-RAPPAHANNOCK RIVER, YR-YORK RIVER, MB-MOBJACK BAY, JR-JAMES RIVER, CB-CHESAPEAKE BAY, PR-PAMUNKEY RIVER, ES-EASTERN SHORE,SEA SIDE

* SIGNIFICANT DIFFERENCE

TABLE 8-SEASONAL VARIATION OF MEAN SERUM GLUCOSE CONCENTRATION IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS , FROM THE YORK SPIT AREA, CHESAPEAKE BAY, VIRGINIA

SAMPLE DATE DA MO YR	SALINITY (0/00)	TEMPERATURE (C)	SERUM GLUCOSE + OR - STANDARD ERROR (MG/100ML) MALES	(N)	OLD FEMALES	(N)	NEW FEMALES	(N)	MALES X OLD F	MALES X NEW F	OLD F X NEW F
					1968 CLASS		1969 CLASS				
26 08 69	23.40	26.2	23.37	7.82 (5)	12.05	2.53(11)					
23 09 69	24.99	25.7	65.71	51.02 (2)	70.32	8.61(13)					
15 10 69	23.72	20.1	33.99	4.72 (4)	39.72	5.17 (8)					
19 11 69	25.53	13.8	30.63	11.54 (4)	33.63	3.03(19)					
18 12 69	24.92	8.9	30.14	2.86 (4)	33.44	2.48(25)					
23 01 70	22.70	4.2	40.31	3.17(19)	43.45	3.12(11)					
17 03 70	20.10	6.3			55.87	3.97(30)					
22 04 70	18.08	13.4			49.61	3.00(29)					
15 05 70	20.19	15.2			58.67	6.85(30)					
23 06 70	19.80	23.8	79.06	27.16 (3)	85.67	8.63(17)	17.77	4.95 (3)			*
20 07 70	23.38	23.3	73.79	19.51 (5)	127.66	14.42(21)	56.41	12.03 (4)			
18 08 70	27.01	24.1			58.38	9.18 (6)	36.57	5.19(24)			
23 09 70	24.06	24.1	18.16	3.74 (5)	32.71	9.02 (7)	22.97	2.92(18)			
20 10 70	23.50	21.6	13.47	6.11 (5)	47.77	13.85 (6)	44.93	9.27(19)			
12 11 70	22.48	14.9	41.62	11.85 (5)			68.88	5.54(25)			
8 12 70	22.02	7.6	44.96	16.92 (3)			41.85	4.42(26)			
21 01 71	19.94	4.8	39.48	3.19 (6)	53.64	4.82 (2)	46.76	2.77(22)			
19 02 71	22.34	0.5			71.07	10.57 (4)	91.77	15.20(25)			
17 03 71	17.28	6.2	0.00	(1)	13.41	5.87 (2)	88.45	16.24(29)			
					1969 CLASS		1970 CLASS				
13 04 71	21.22	8.8	69.12	8.49 (5)	89.91	6.16(25)					
2 6 71	24.26	19.7	22.52	3.64 (3)	99.03	10.65(21)	33.14	10.20 (3)	*		*

* SIGNIFICANT DIFFERENCE

1971 new year class females had lower serum glucose levels than the older year class crabs. Comparison of serum glucose in egg-bearing (sponge) females versus non-sponge (clean) females gave mixed results. Significant differences were found in two of the three months, however, the hierarchy was reversed with sponge females having higher values in August (Table 2). Despite high variability, (mean C = 52%) certain trends are apparent in seasonal variation of serum glucose (Fig. 8). The lowest levels of serum glucose were found in late summer and early fall (August-September) from this low point serum glucose increased to yearly high values in early and middle summer (June-July). With the exception of the September to November samples the ovary weight of the new year class crabs and serum glucose levels did not seem related (Fig. 9). No relation between ovary weight and serum glucose levels was apparent for the older year class.

In the salinity gradient study, serum glucose ranged from 0.9 - 69.5 mg/100 ml in females and 1.8 - 140.8 mg/100 ml in males. Mean serum glucose from different stations ranged from 2.8 - 79.2 mg/100 ml (Table 9). No significant correlation was found between salinity and serum glucose levels in males ($r = 0.05$, $N = 82$) or females ($r = -0.12$, $N = 101$). Serum glucose levels were somewhat more variable in male (mean C = 64%) than in female crabs (mean C = 46%). Serum glucose levels of

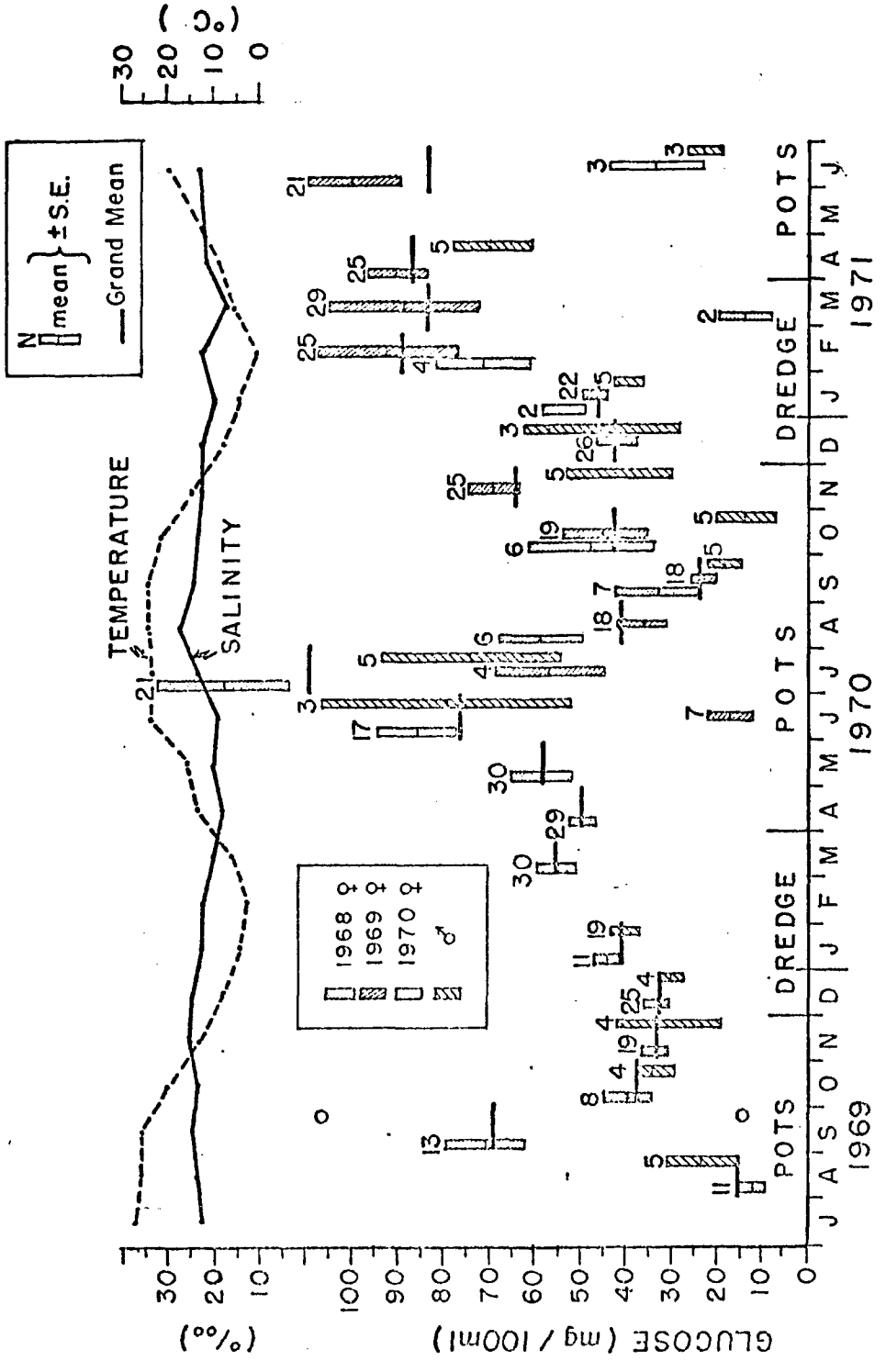

Fig. 8. Seasonal variation in mean serum glucose in mature blue crabs, Callinectes sapidus from the York Spit area, Chesapeake Bay, Virginia.

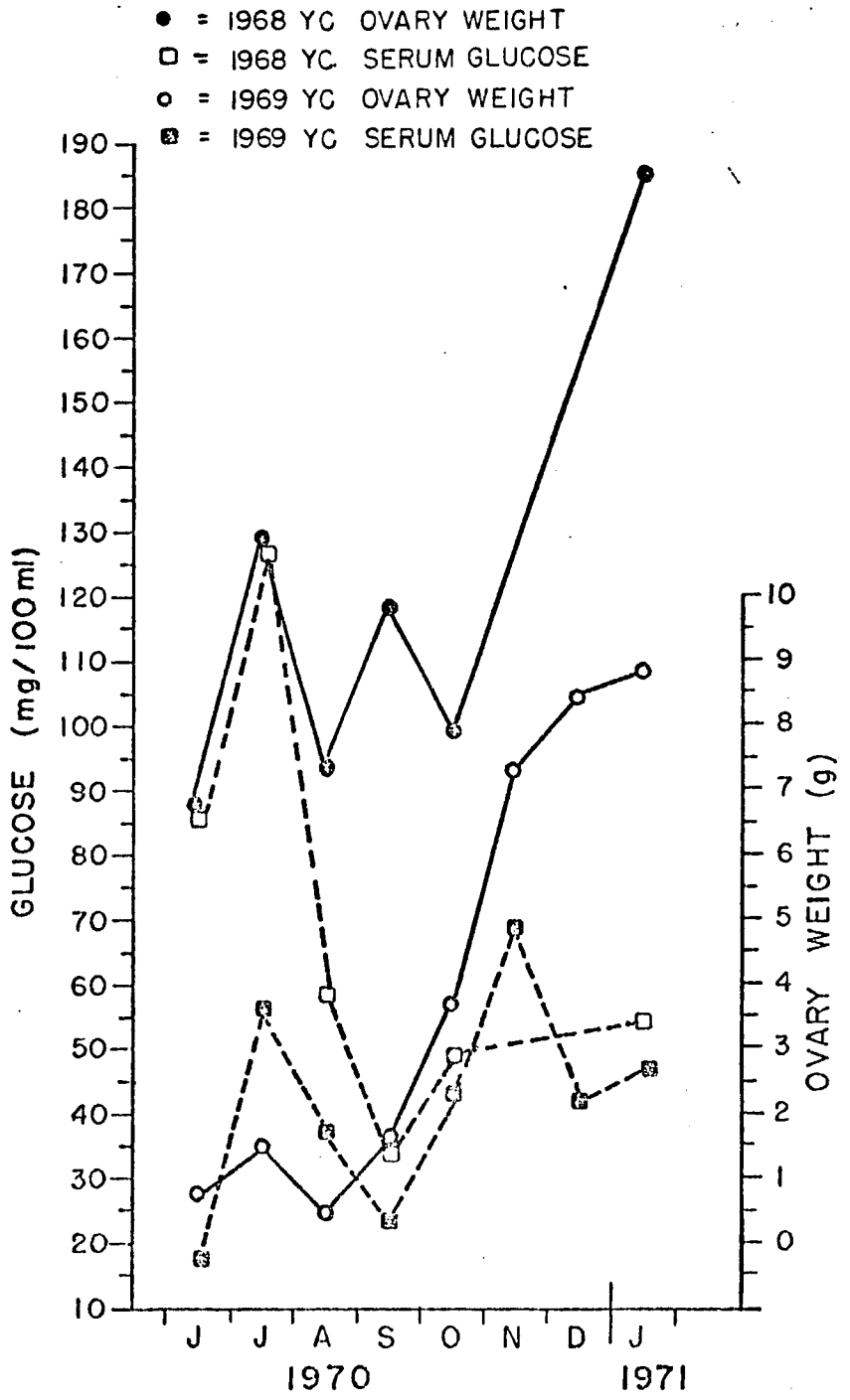

Fig. 9. Mean serum glucose and mean ovary weight of mature 1968 and 1969 year class blue crabs, Callinectes sapidus from the York Spit area of Chesapeake Bay, Virginia.

TABLE 9- MEAN SERUM GLUCOSE CONCENTRATION IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS, COLLECTED FROM VARIOUS SALINITIES IN VIRGINIA.

SAMPLE DATE DA MO YR	AREA	SALINITY (O/OO)	TEMPERATURE (C)	SERUM GLUCOSE + OR - STANDARD ERROR (MG/100ML) MALES	(N)	FEMALES	(N)
14 7 70	RR	19.11	24.0	5.46	(1)		
14 7 70	RR	16.50	24.6	21.96	(1)		
14 7 70	RR	13.82	24.9	137.59	(1)		
14 7 70	RR	10.08	25.6	79.16	18.42(5)		
14 7 70	RR	4.75	26.4	19.23	(1)		
16 7 70	YR	24.00	26.0	3.36	(1)		
16 7 70	MB	21.69	25.6	100.00	(1)		
16 7 70	MB	19.07	26.8	24.43	7.82(5)	14.76	(1)
16 7 70	MB	18.71	25.8	25.99	(1)		
3 8 70	JR	21.25	27.2	29.58	(1)		
3 8 70	JR	10.71	28.4	35.44	(1)		
3 8 70	JR	4.62	28.8	6.44	(1)		
3 8 70	JR	2.84	29.0	17.84	4.77(2)		
7 8 70	CB	30.38	22.2			36.92	5.47(13)
7 8 70	CB	26.82	24.0			8.03	2.16(3)
7 8 70	YR	27.01	24.1	6.04	3.07(3)	5.38	0.60(2)
11 8 70	RR	15.34	25.7	16.29	5.24(4)	8.91	(1)
11 8 70	RR	15.02	25.8	32.04	5.76(8)	40.83	11.79(2)
11 8 70	RR	14.66	26.4	41.99	6.44(5)		
11 8 70	RR	13.90	25.6	12.44	3.04(4)		
11 8 70	RR	11.49	25.8	46.96	9.62(7)*	29.67	2.84(2)*
11 8 70	RR	9.68	25.7	37.50	32.34(2)	11.79	3.32(4)
13 8 70	YR	19.28	26.6	9.13	(1)	5.67	(1)
13 8 70	YR	18.49	26.5	41.71	9.66(3)*	11.08	2.55(5)*
13 8 70	YR	16.00	25.8			18.20	2.05(13)
13 8 70	YR	13.90	25.8	27.54	12.10(2)	9.33	1.15(9)
13 8 70	YR	7.13	26.3	6.47	(1)		
14 9 70	CB	30.65	22.5			5.04	0.42(7)
14 9 70	CB	27.18	23.9			2.35	(1)
14 9 70	YR	24.06	24.1			6.58	1.10(7)
17 9 70	YR	21.93	25.4			6.35	1.94(5)
17 9 70	YR	20.42	25.7	6.50	1.89(2)	2.77	0.65(4)
17 9 70	YR	18.21	26.1	7.51	4.74(2)	2.77	(1)
17 9 70	YR	17.00	26.0			4.76	1.14(8)
17 9 70	PR	12.94	26.0			39.51	(1)
17 9 70	PR	8.97	26.3	1.85	(1)		
17 9 70	PR	0.89	26.8	6.76	1.92(4)		
8 10 70	PR	12.41	21.6	31.43	(1)		
8 10 70	PR	4.26	21.4	8.36	2.32(4)	11.46	5.42(2)
12 10 70	CB	29.74	21.1			9.83	2.16(8)
12 10 70	YR	23.50	21.6	5.75	1.34(7)	7.37	(1)

AREA CODE-- RR-RAPPAHANNOCK RIVER, YR-YORK RIVER, MB-MOBJACK BAY, JR-JAMES RIVER, CB-CHESAPEAKE BAY, PR-PAMUNKEY RIVER

* SIGNIFICANT DIFFERENCE

male and female blue crabs were not significantly different in 10 of 12 samples in which comparisons were possible. Serum glucose levels were higher in male blue crabs than female blue crabs in two samples (11 Aug 1970, Rappahannock River, 11.5 o/oo; 13 Aug. 1970, York River, 18.5 o/oo). Serum glucose levels were significantly lower in September and October than in July and August.

In the short term variability study, mean serum glucose in males varied from 7.6 - 67.2 mg/100 ml. Variation was high (mean C = 58%). Mean serum glucose levels were generally significantly lower in samples from the latter part of August than those in mid-July (Table 10). The serum glucose levels in females varied from 25.4 - 51.9 mg/100 ml. So few females were taken that no trends were apparent.

Glucose was the only serum constituent which varied significantly in the various holding experiments. The results of the various holding experiments are given in Table 11. Serum glucose levels increased in crabs held out of water. The mean increase was 2.5 times the original value. Blood glucose did not continue to increase indefinitely. The results of the experiment where one group of crabs were initially bled after 2-1/2 hours and another after an additional 12 hours indicated glucose decreased slightly (but not significantly) during this period.

TABLE 10. MEAN SERUM GLUCOSE IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS, COLLECTED FROM THE NORTH RIVER, MATHEWS COUNTY, VIRGINIA, JULY-AUGUST 1969.

Date	Salinity o/oo	Temperature °C	Sex	Serum Glucose ± S.E. (mg/100 ml)	N
2 Jul.			M	30.90± 3.06	(22)
3 Jul.			M	54.41± 6.57	(19)
7 Jul.			M	39.12± 6.41	(14)
9 Jul.	21.00	27.0	M	55.84± 9.61	(6)
15 Jul.			M	58.72± 5.40	(13)
16 Jul.			M	67.20± 7.5	(19)
17 Jul.			F	25.43	(1)
17 Jul.			M	48.94± 7.00	(5)
8 Aug.			F	51.95	(1)
8 Aug.			M	40.59± 6.22	(29)
14 Aug.	18.90	28.0	F	25.42	(1)
14 Aug.			M	13.09± 1.74	(9)
18 Aug.			M	19.59± 7.06	(7)
25 Aug.			M	7.64± 3.33	(6)

TABLE 11 - EFFECTS OF HOLDING BLUE CRABS, CALLINECTES SAPIDUS, OUT OF WATER ON SERUM GLUCOSE LEVELS.

Date Bled	Time out of water	Sex	Previously Bled	Serum glucose mean ± S.E. (mg/100 ml)	(N)
9 Jul 69	2-1/2 hrs	M	No	55.84 ± 9.61	(6)
9 Jul 69	14-1/2 hrs	M	No	36.13 ± 3.51	(7)
19 Jul 69	2 min	M	No	9.49 ± 2.77	(5)
20 Jul 70	12 hrs	M	Yes	48.87 ± 12.01	(5)
20 Jul 70	12 hrs	M	No	41.81 ± 16.56	(4)
19 Jul 70	2 min	F	No	10.24 ± 4.94	(2)
20 Jul 70	12 hrs	F	Yes	25.60 ± 5.01	(2)
20 Jul 70	12 hrs	F	No	31.55	(1)
13 Aug 70	2-5 min	M+F	No	14.25	(20)
13 Aug 70	12-15 hrs	M+F	Yes	36.61	(20)
10 Oct 70	2-5 min	M+F	No	8.21	(5)
10 Oct 70	12-15 min	M+F	Yes	22.67	(5)
15 Nov 70	1 min	F	No	4.08	(7)
15 Nov 70	10-11 hrs	F	Yes	8.28	(7)[1]

[1]These crabs were returned to the sea table directly after initial bleeding. The difference between before and after glucose values was not significant at the 0.05 level.

SERUM TOTAL NINHYDRIN POSITIVE SUBSTANCES (TNPS)

During the seasonal study, mean serum TNPS ranged from 0.8 - 16.1 u moles/ml in male crabs and 1.0-30.1 u moles/ml in female crabs (Table 12). TNPS were found in the serum of all the crabs sampled. Variability was high within each sample (the mean C was 52%). Month to month variation was also high (Fig. 10). The highest serum TNPS values appeared in January 1970), September 1970, February 1971 and April 1971. Mean serum TNPS was higher in males in 2 months and in females in 5 months. In the other 13 months in which comparisons were possible, no differences were found. Overall, there was probably no difference between serum TNPS levels in male and female blue crabs. Differences in serum TNPS between year classes of female crabs were found in 2 months (August 1970 and July 1971). In these months old year class females had significantly higher TNPS than new year class females. In the other 9 months in which 2 year classes of female crabs were sampled, no differences were found. Overall there was probably no difference between serum TNPS levels in older and younger year class crabs taken at the same time.

The higher level of TNPS in the older year class in August 1970 was due primarily to higher serum TNPS in 2 egg bearing (sponge) crabs. In June and July 1970, the sponge crabs and non-egg bearing (clean) crabs had no significant

TABLE 12-SEASONAL VARIATION OF MEAN SERUM TOTAL NINHYDRIN POSITIVE SUBSTANCES IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS FROM THE YORK SPIT AREA, CHESAPEAKE BAY, VIRGINIA

SAMPLE DATE DA	MO	YR	SALINITY (O/OO)	TEMPERATURE (C)	TNPS + CR - STANDARD ERROR (MICROMOLES/ML) MALES	(N)	OLD FEMALES	(N)	NEW FEMALES	(N)	MALES X OLD F	MALES X NEW F	OLD F X NEW F
							1967 CLASS		1968 CLASS				
23	7	69	22.49	26.8	4.33	0.98 (6)	4.18	(1)	4.06	0.57 (6)			
26	8	69	23.40	26.2	4.18	1.25 (4)			3.12	0.24(11)		*	
23	9	69	24.99	25.7	4.53	(1)			1.48	0.34(13)		*	
15	10	69	23.72	20.1	0.79	0.29 (4)			0.97	0.22 (8)			
19	11	69	25.53	13.8	3.68	0.41 (4)			5.50	0.45(12)		*	
18	12	69	24.92	8.9	5.64	1.94 (4)			8.47	1.91(21)			
							1968 CLASS		1969 CLASS				
23	1	70	22.70	4.2	22.90	1.63(19)	21.92	2.19(11)					
19	2	70	22.20	2.8	14.00	(1)	7.20	0.43(27)			*		
22	4	70	18.08	13.4			5.47	0.48(30)					
15	5	70	20.19	15.2			1.68	0.43(29)					
23	6	70	19.80	23.8	12.99	1.14 (3)	8.54	0.93(17)	8.22	0.88 (3)	*	*	
20	7	70	23.83	23.3	5.42	0.73 (4)	7.38	0.80(18)	5.57	0.82 (3)			
18	8	70	27.01	24.1			8.57	1.81 (6)	4.61	0.40(24)			*
23	9	70	24.06	24.1	16.08	7.44 (5)	17.76	4.53 (7)	16.24	2.76(17)			
20	10	70	23.50	21.6	9.05	2.08 (5)	6.65	1.41 (6)	10.99	1.64(19)			
8	12	70	22.02	7.6	6.24	0.91 (3)			6.72	0.73(26)			
21	1	71	19.94	4.8	6.21	1.39 (6)	5.92	0.10 (2)	7.39	0.64(22)			
19	2	71	22.34	0.5			27.08	4.31 (4)	30.77	3.06(26)			
17	3	71	17.28	6.2	3.31	(1)	2.89	2.47 (2)	6.97	0.82(28)			
13	4	71	21.22	8.8	15.36	1.61 (5)			23.60	1.52(25)		*	
							1969 CLASS		1970 CLASS				
2	6	71	24.26	19.7	6.39	0.94 (3)	6.94	0.83(21)	5.72	1.42 (3)			
21	7	71	21.31	24.1	2.41	0.26 (8)	7.07	(1)	2.13	(7)	*		*

* SIGNIFICANT DIFFERENCE

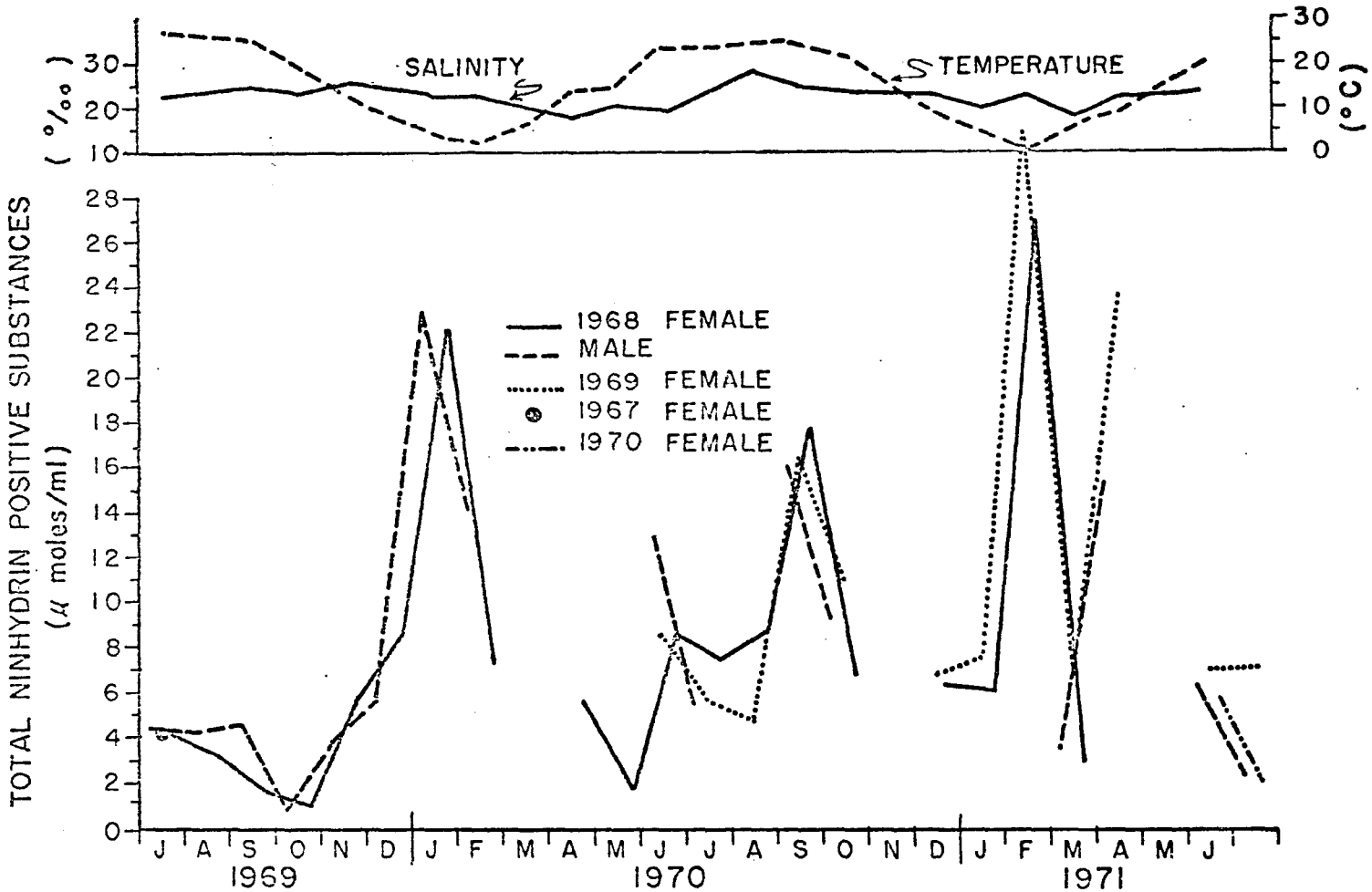

Fig. 10. Seasonal variation in mean serum total ninhydrin positive substances in mature blue crabs, Callinectes sapidus from the York Spit area of Chesapeake Bay, Virginia.

differences in serum TNPS. In August, however, the serum TNPS levels were significantly higher in the sponge females (Table 2).

In the salinity gradient study, serum TNPS was found to range from about 1.0 - 19.8 μ moles/ml in males and 1.9 - 32.1 μ moles/ml in females. Serum TNPS was detected in all crabs sampled. Mean serum TNPS ranged from 4.3 - 16.3 μ moles/ml in males and 4.1 - 21.4 μ moles/ml in females (Table 13). Variability was essentially the same for both sexes (mean C = 23.4% for females, 27.3% for males). Serum TNPS levels were significantly higher in male compared to female crabs in 3 of 18 possible comparisons. No differences were found in the other 15 comparisons. Serum TNPS in female crabs was positively correlated with salinity ($r = 0.56$, $n = 152$). The correlation between serum TNPS and salinity in male crabs was not significant ($r = 0.18$, $n = 131$).

In the short term variability study, serum TNPS in male crabs ranged from 0.9 - 14.8 μ moles/ml in males and 4.6 - 6.2 μ moles/ml in females. Mean TNPS varied from 4.2-8.5 μ moles/ml during this period. Day to day variation was high. In one 3-day period (15-17 July 1969) serum TNPS varied from 4.9 μ moles/ml to 8.5 μ moles/ml (Fig. 11). Mean C = 37.4% for these samples.

Serum TNPS and serum chloride were positively correlated in female crabs of the salinity gradient studies ($r = 0.44$, $n = 152$). Serum TNPS and serum glucose are

TABLE 13- MEAN SERUM TNPS CONCENTRATION IN MATURE, HARD BLUE CRABS, CALLINECTES SAPIDUS, COLLECTED FROM VARIOUS SALINITIES IN VIRGINIA.

SAMPLE DATE DA	MO	YR	AREA	SALINITY (O/OO)	TEMPERATURE (C)	SERUM TNPS + OR - STANDARD ERROR (MICROMOLES/ML) MALES	(N)	FEMALES	(N)
19	8	69	RR	19.00	27.5	5.97	0.76(5)	4.51	0.43(12)
19	8	69	RR	17.50	27.6	9.72	(1)		
19	8	69	RR	14.00	28.0	8.10	0.47(12)	7.03	0.46(11)
19	8	69	RR	11.50	28.2	6.85	0.42(10)*	5.06	0.40(7)*
19	8	69	RR	6.40	28.0	7.49	0.33(12)*	6.03	0.50(12)*
19	8	69	RR	1.30	28.0	6.36	0.92(2)		
21	8	69	ES	27.50	22.0	5.01	0.35(12)	5.11	0.55(4)
21	8	69	ES	18.90	22.0	5.24	0.21(69)	4.09	0.22(4)
14	7	70	RR	19.11	24.0	2.55	(1)		
14	7	70	RR	16.50	24.6	2.85	(1)		
14	7	70	RR	13.82	24.9	8.00	(1)		
14	7	70	RR	10.08	25.6	5.70	0.24(5)		
14	7	70	RR	4.75	26.4	5.30	(1)		
16	7	70	YR	24.00	26.0	13.57	(1)	9.18	(1)
16	7	70	MB	21.69	25.6	1.10	(1)		
16	7	70	MB	19.07	26.8	9.38	1.44(9)	8.58	(1)
16	7	70	MB	18.71	25.8	6.52	1.72(2)		
3	8	70	JR	21.25	27.2	7.00	0.67(2)		
3	8	70	JR	10.71	28.4	11.61	(1)		
3	8	70	JR	4.62	28.8	6.96	(1)		
3	8	70	JR	2.84	29.0	10.88	0.18(2)		
7	8	70	CB	30.38	22.2			21.43	1.40(13)
7	8	70	YR	27.01	24.1	16.33	2.33(3)*	5.64	0.74(2)*
7	8	70	CB	26.82	24.0			11.34	2.06(3)
11	8	70	RR	15.34	25.7	9.04	1.12(4)	6.31	(1)
11	8	70	RR	15.02	25.8	7.85	0.67(8)*	4.56	0.17(2)*
11	8	70	RR	14.66	26.4	7.74	1.45(5)		
11	8	70	RR	13.90	25.6	5.44	0.55(4)		
11	8	70	RR	11.49	25.8	9.02	1.28(7)	7.18	0.58(2)
11	8	70	RR	9.68	25.7	6.55	1.89(2)	4.78	0.81(4)
13	8	70	YR	19.28	26.6	9.20	(1)	9.74	(1)
13	8	70	YR	18.49	26.5	10.96	2.20(3)*	6.00	0.43(5)*
13	8	70	YR	16.00	25.8			9.09	0.67(13)
13	8	70	YR	13.90	25.8	9.36	1.38(2)	7.91	0.70(9)
13	8	70	PR	7.13	26.3	5.84	(1)		
14	9	70	CB	30.65	22.5			11.84	0.75(15)
14	9	70	CB	27.18	23.9			11.58	(1)
14	9	70	YR	24.06	24.1			7.58	0.65(8)
17	9	70	YR	21.93	25.4			6.39	1.25(5)
17	9	70	YR	20.42	25.7	5.92	0.66(2)	5.60	0.87(4)
17	9	70	YR	18.21	26.1	7.80	0.80(2)	8.60	(1)
17	9	70	YR	17.00	26.0			6.13	0.74(8)
17	9	70	PR	12.94	26.0			8.30	(1)
17	9	70	PR	8.97	26.3	7.13	(1)		
17	9	70	PR	0.89	26.8	7.49	0.65(4)		
8	10	70	PR	12.41	21.6	2.73	(1)		
8	10	70	PR	4.26	21.4	4.31	1.52(4)	2.96	0.45(2)
12	10	70	CB	29.74	21.1			6.95	0.86(8)
12	10	70	YR	23.50	21.6	5.52	0.96(7)	5.64	(1)

AREA CODE-- RR-RAPPAHANNOCK RIVER, YR-YORK RIVER, MB-MOBJACK BAY, JR-JAMES RIVER, CB-CHESAPEAKE BAY, PR-PAMUNKEY RIVER, ES-EASTERN SHORE(SEA SIDE)

* SIGNIFICANT DIFFERENCE

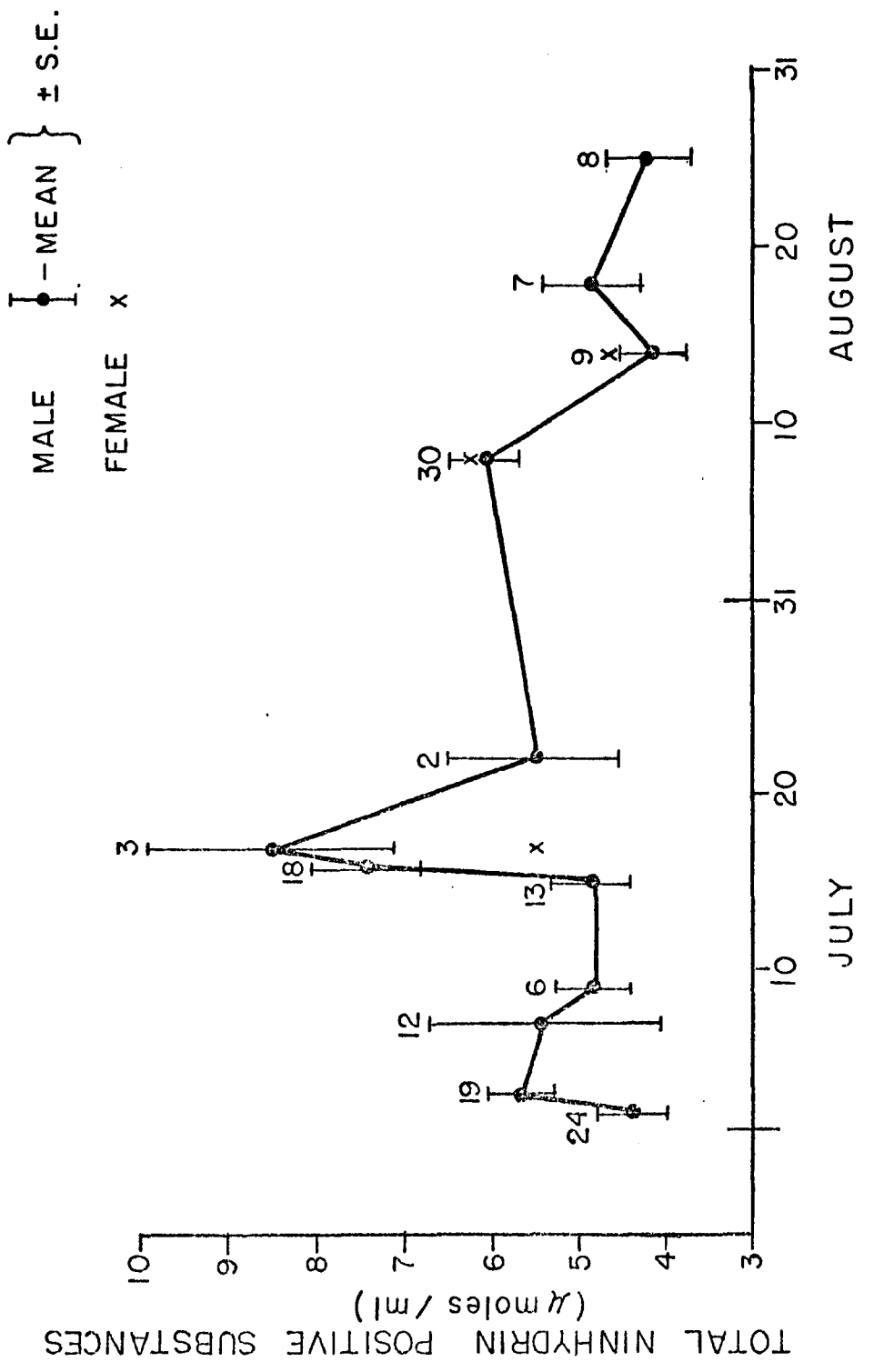

Fig. 11. Mean serum total ninhydrin positive substances in mature blue crabs, Callinectes sapidus from the North River, Mathews County, Virginia.

positively correlated in females of both the salinity gradient ($r = 0.60$, $n = 100$) and seasonal studies ($r = 0.16$, $n = 429$). All of these correlation coefficients were significant at the 99% confidence level ($P \leq 0.01$).

SERUM FREE AMINO ACIDS

Serum free amino acids were determined in 7 adult hard crabs. Glycine was the most abundant amino acid, followed by taurine, alanine, arginine and proline (Table 14). These 5 amino acids accounted for 72-90% of the total free amino acids measured and 39-70% of the total ninhydrin positive substances. A total of 14 serum free amino acid determinations (including serum from immature crabs and crabs from other stages of the molt cycle) were made (Fig. 12). The linear correlation between serum TNPS and the sum of the serum free amino acids was very high ($r = 0.96$).

TABLE 14 - CONCENTRATION OF INDIVIDUAL FREE AMINO ACIDS (FAA) IN THE SERUM OF THE BLUE CRAB, CALLINECTES SAPIDUS FROM VIRGINIA.

Sex	M	F	F	F	F	F	F
Salinity (o/oo)	16.5	19.5	19.5	21.0	21.0	21.0	21.0
Temperature (°C)	24.6	24.0	24.0	23.5	23.5	23.5	23.5
Amino Acids				(micromoles/ml)			
Cysteic acid	-	0.01	0.01	tr	0.01	0.01	0.03
Taurine	0.37	0.24	0.29	0.52	0.78	1.32	0.39
Aspartic acid	0.03	0.01	0.02	0.02	0.02	0.11	0.01
Threonine	0.09	0.05	0.04	0.23	0.07	0.13	0.05
Serine	0.08	0.03	0.04	0.15	0.09	0.18	0.04
Glutamic acid	0.05	0.03	0.05	0.05	0.06	0.07	0.02
Proline	0.16	0.13	0.06	0.75	0.29	0.62	0.18
Glycine	0.43	0.32	0.51	1.04	1.11	5.20	0.47
Alanine	0.28	0.15	0.24	0.69	0.58	0.90	0.27
Valine	0.05	0.01	0.02	0.06	0.07	0.10	0.03
Cystine	tr	-	-	0.01	-	tr	-
Methionine	0.02	tr	-	0.03	0.04	0.02	0.02
Isoleucine	tr	0.01	0.01	0.03	0.03	0.04	0.02
Leucine	0.04	0.01	0.01	0.05	0.05	0.07	0.03
Tyrosine	tr	0.01	0.01	0.01	0.04	0.04	0.01
Phenylalanine	0.03	0.01	0.02	0.05	0.04	0.07	0.04
Ornithine	0.05	0.02	0.02	0.01	0.02	0.02	0.01
Lysine	0.04	0.01	0.01	0.11	0.07	0.08	0.02
Tryptophan	-	-	-	0.02	-	-	0.02
Histidine	0.04	0.01	0.02	0.04	0.15	0.11	0.02
Arginine	0.12	0.19	0.16	0.35	0.94	1.96	0.18
Sum FAA	1.88	1.25	1.54	4.22	4.46	11.05	1.86
Total NPS	2.85	2.62	1.89	7.27	8.54	14.18	3.35

tr = trace

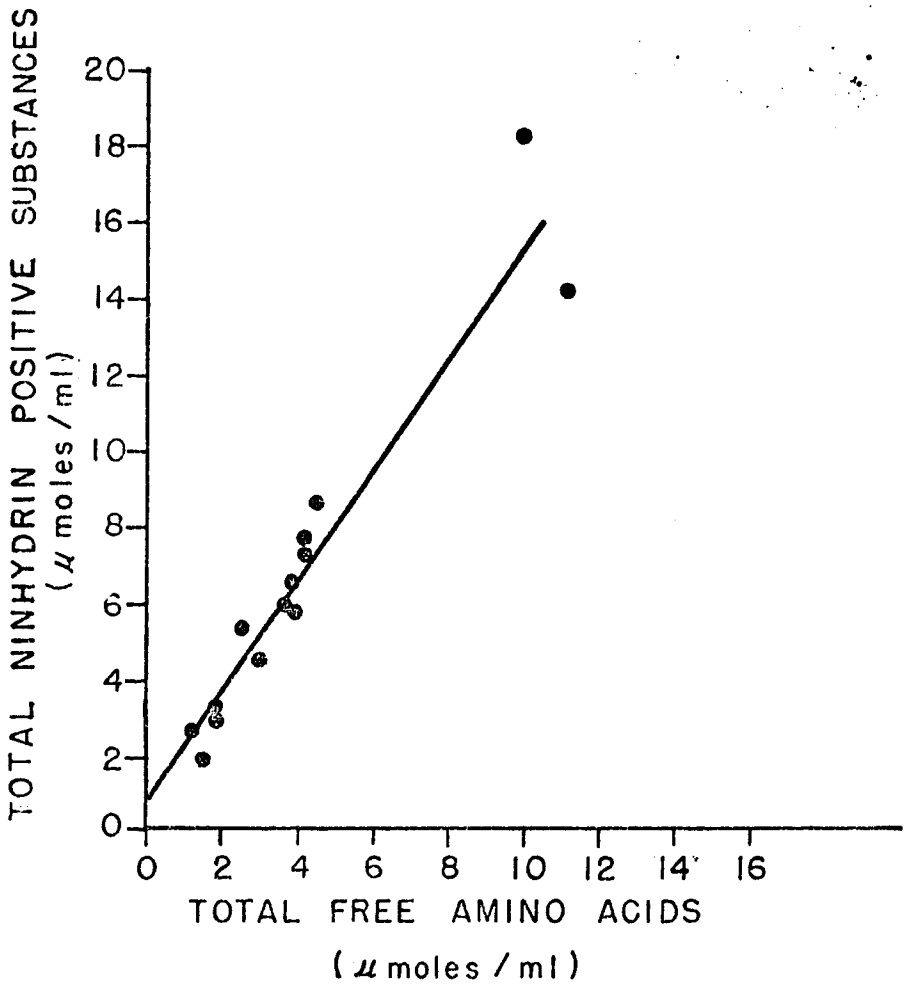

Fig. 12. Relationship between the free amino acids and total ninhydrin positive substances (NPS) in serum of the blue crab, Callinectes sapidus.

DISCUSSION

ENVIRONMENTAL VARIATION

The environmental ranges encountered during these studies were typical of those found in the lower Chesapeake Bay (Stroup & Lynn, 1963) and Seaside Eastern Shore, Virginia (Richards & Castagna, 1970).

SERUM CHLORIDE AND OSMOTIC CONCENTRATION

Vernberg & Vernberg (1970) categorize four basic types of osmotic adjustment to the medium. Over the salinity range encountered in this study, the blue crab appears to fall into the Type III category of hyperosmotic regulation in dilute medium and isomotic regulation in higher salinities (Fig. 3). Over the entire range for which serum or blood osmotic determinations are available, however, the blue crab is a Type IV, i.e. a hyper and hypoosmotic regulator (Fig. 13). One comment relative to the information compiled in Fig. 13 should be made. The "isotonic" line drawn in Fig. 13 is computed. Tagatz (1971 ; Fig. 2) reported serum osmotic concentration hypoosmotic to an external salinity of 34 o/oo. The "isotonic" line drawn in that figure was derived from measurements in the vapor pressure osmometer which was used to measure the osmotic concentration of the serum in his studies (Tagatz, personal communication). The remaining data compiled in Fig. 13 were determined by

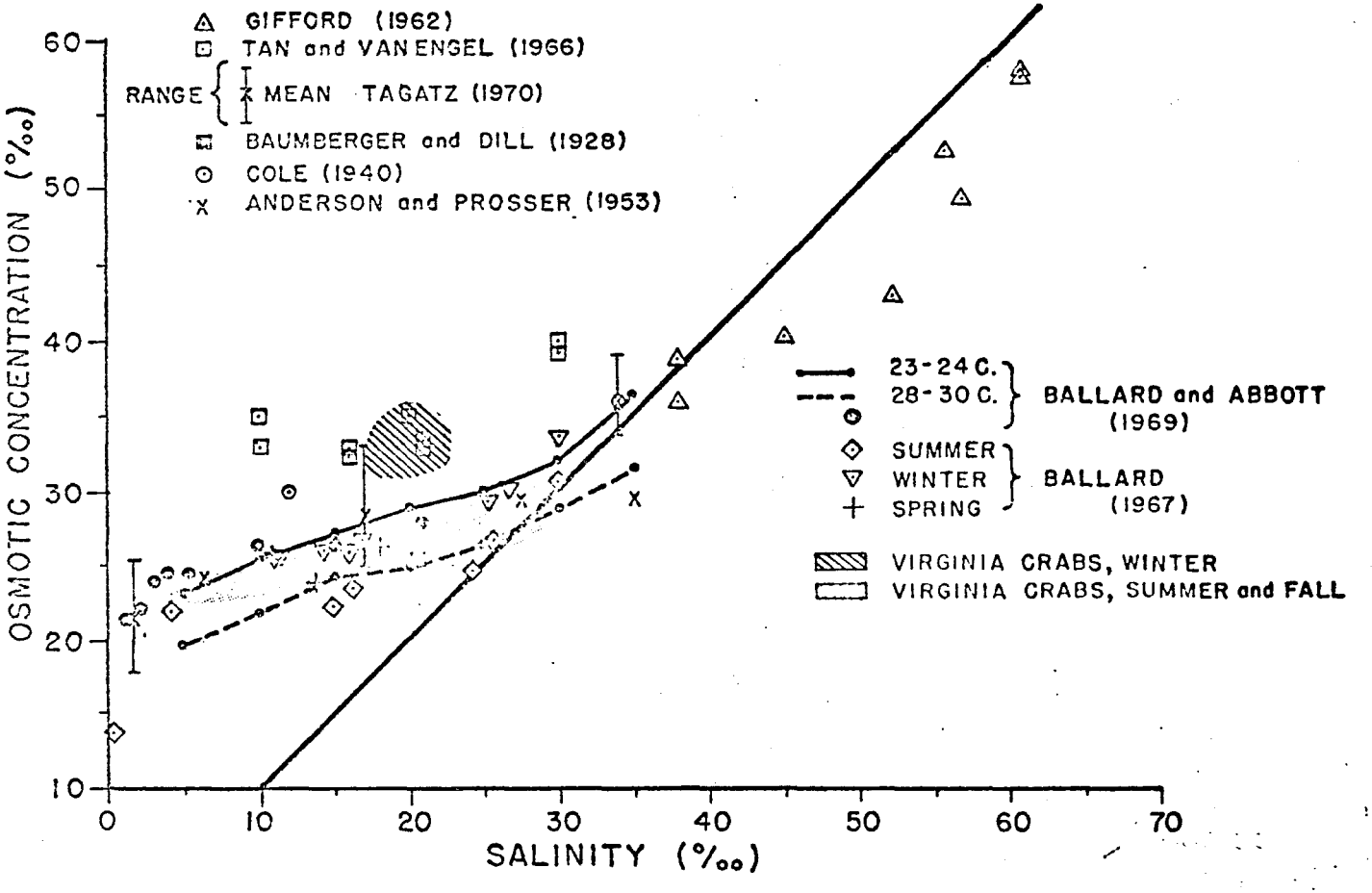

Fig. 13. Serum osmotic concentration of blue crabs, Callinectes sapidus as a function of environmental salinity. Data from sources indicated in legend.

various freezing point methods. His data, therefore, are not precisely comparable to the other data presented, and the indication of slight hyperosmocity at 35 o/oo is probably a misrepresentation.

Two generalities can be made regarding the data presented here in Fig. 13. First, winter values of blue crab serum osmotic concentration are generally higher than summer values from approximately equivalent salinities; and second, when this temperature effect is taken into consideration, the results of the various studies of osmotic regulation in blue crabs are all essentially in agreement.

Serum chloride regulation over the range of salinities encountered in Virginia is a Type IV regulation. Hyperionic regulation occurred below 21 o/oo, serum chloride and external chloride were essentially isoionic between 21-25 o/oo and above 25 o/oo hypoionic regulation occurred (Fig. 3). Odum (1953) found hyperionic regulation of chloride at 16 o/oo and below, isoionic regulation at approximately 30 o/oo and hypoionic regulation at salinities greater than full strength seawater. Gifford (1962) found hypoionic regulation at salinities above 38 o/oo.

Serum chloride did not appear to increase proportionally as much as the serum osmotic concentration increased in the range 25-31 o/oo (Fig. 3). The proportionally larger increase of serum osmotic concentration may have been due to a disproportionally higher increase in concentration of some organic constituent. Since

approximately 80 o/o of the animals from salinities greater than 20 o/o for which serum osmotic determinations were made were females (Table 5), the disproportionately larger increase in serum osmotic concentration compared to serum chloride concentration may be sex related. This hypothesis is also supported by significant positive correlations between both serum protein and serum TNPS and salinity in female crabs contrasted to a significantly lower correlation between serum TNPS and salinity and no significant correlation between serum protein and salinity in male crabs.

Tagatz (1971) reported no temperature effect on osmoregulation in the blue crab although he indicated his results would probably have been different with longer than 24 hours acclimation. The data on seasonal variation (Fig. 2) strongly support the latter contention. Lack of sufficient acclimation time might also explain the strongly hyperosmotic blood concentrations reported by Tan & Van Engel (1966). After only 4 days of acclimation at 20° C, they found blood osmotic equivalents of approximately 35, 36 and 40 o/oo at 10, 20, and 30 o/oo experimental salinity respectively, where we would expect to find blood osmotic equivalents of approximately 25, 26 and 30 o/oo in fully acclimated animals (Fig. 3). Tan & Van Engel's (1966) crabs were taken from approximately 25 o/oo at 5° C (Tan, 1962). Crabs in this study from the same area and season had blood osmotic concentrations equivalent to 31-36 o/oo (Fig. 12) suggesting incomplete acclimation in the

experimental animals used by Tan & Van Engel (1966).

Serum chloride is affected by temperature (Fig. 2) in a fashion similar to that of serum osmotic concentration. Serum chloride in Hemigrapsus nudus (Dehnel, 1967) and serum sodium in the blue crab (Mantel, 1967) also increase during winter months compared with summer months. Not all serum inorganic ions, however, increase with decreasing temperatures. Serum potassium decreased and magnesium remained constant with decreasing temperature in H. nudus (Dehnel, 1967).

Studies on several crustaceans (Wildmann, 1935; Otto, 1937; Panikkar, 1940, 1941; Dehnel, 1962; Todd, 1963; Andrews, 1967) have shown seasonal variation in serum osmotic concentration similar to that of the blue crab (Fig. 4), in which winter serum osmotic concentrations are higher than summer concentrations from approximately equivalent salinities. The ecological or adaptive significance of this relationship between serum osmotic concentration and temperature has not been demonstrated. Panikkar (1940) postulated that lowered serum osmotic concentration at warmer temperatures resulted in a wider tolerance to dilution in warmer regions, favoring colonization of fresh and brackish water. More recent investigators believe that the significance lies in the increased serum osmotic concentration at lower temperatures acting as an "anti-freeze" mechanism (Umminger, 1969). The relationship between temperature and osmotic concentration is not, however, as simple as presented above. In Ligia oceanica, for example, (Todd, 1963)

winter animals had higher serum osmotic concentrations than summer animals, but when summer animals were exposed to temperature of 5 and 15° C in the laboratory, the animals at 15° C had higher serum osmotic concentrations in 25 and 50% seawater and lower serum osmotic concentrations in 75 and 100% seawater than the animals at 5°C. Also, Crangon septemspinosa maintained at 15° C in the laboratory had higher serum osmotic concentrations than those held at 5° C (Haefner, 1969). Weber & Spaargaren (1970), Flugel (1963) and Broekma (1942) reported that the relationship between serum osmotic concentration and temperature differed depending upon the salinity in which the animals were maintained.

The disparate distribution of adult male and female blue crabs along the salinity gradient (Churchill, 1919; Pearson, 1948; Van Engel, 1958) has led to speculation that there is a difference in the osmoregulation ability of adult male and female crabs. Present data (Table 3) indicate that male crabs have lower serum chloride concentrations than female crabs in lower salinities (<15 o/oo). Ballard & Abbott (1969) also found this true except at extreme dilutions. Tagatz (1971) found differences only at 1.7‰, with female serum osmoconcentration significantly higher than male. Tan & Van Engel (1966) found significantly higher serum osmoconcentrations in males (rather than lower) compared to females at 10 and 20 o/oo. Their results appear to be aberrant since their results for blood sodium indicate a markedly lower value in males

as compared to females at 10 and 20 o/oo.

The evidence for a difference in regulatory ability between males and females is more conclusive in laboratory studies (Ballard & Abbott, 1969) than field results (compare 1969 and 1970 date in Table 3). This is not unexpected since environmental conditions can be controlled more closely in the laboratory, and mobile animals such as the blue crab may move several miles up or down the estuary in a day.

Gilbert (1959) reported a size effect on osmotic regulation in Carcinus maenas. He suggested that this size effect might be due to age, assuming larger crabs are older crabs. De Leersnyder (1967) was, however, unable to find size related differences in serum chloride concentrations in Eriocheir sinensis. No discernable size effects on serum osmotic were apparent in the mature crabs in this study. Neither were any significant differences found in serum chlorides of different age crabs (Table 1), however, significant differences were found between serum osmotic concentrations of different age crabs in 3 of 10 months in which comparisons were possible (Table 4). In two of these cases younger crabs had higher serum osmotic concentrations, in the third case, the older crabs had the higher concentration. The evidence is not conclusive enough to infer a size or age effect on serum chloride or serum osmotic concentration in mature blue crabs.

SERUM PROTEIN

Despite wide variability in serum protein levels in blue crabs, certain patterns or trends are apparent, particularly in female crabs. Serum protein in female crabs appears to coincide most closely with ovary development, particularly in newly matured crabs. In Chesapeake Bay, mating in blue crabs normally begins in May (Van Engel, 1958), immediately after the final molt for the female (Churchill, 1919; Van Engel, 1958). During the period of our sampling, these newly matured females began to appear in the June catches. The most complete series of samples is that for the 1969 year class which first was sampled in June 1970 (Fig. 5 and 7). The distinct increase in serum protein was very closely related to ovary weight increase (Fig. 7). After ovary weight reached about 8 g, however, serum protein levels did not appear related to ovary weight (Figs. 6 and 1968 year class data, Fig. 7). Kerr (1969) also found an increase in serum proteins of female crabs correlated with ovarian maturation. Highest serum protein concentrations were found in crabs with the most fully developed ovaries. After oocyte transfer from the ovary to the pleopods, a slight decrease in serum protein was found.

In female crustaceans that molt after reaching maturity, the serum protein concentrations fluctuate with stages of the molt cycle (Adiyode, 1968; Stewart & Li, 1969;

Djangmah, 1970). In Paratelphusa hydrodromous a sex protein which is found only in very low concentrations in males increased greatly in female crabs immediately prior to and in the later stages of vitellogenesis (Adiyodi, 1968). This protein may be similar to the lipoprotein found in female blue crabs by Kerr (1969). Adiyodi (1969b) reported that the sex protein of P. hydrodromous follows a cyclical pattern during the molt cycle, with highest concentrations occurring at the C_4 intermolt stage and then gradually disappearing during the premolt stages. If a similar sex protein was responsible for much of the increase in serum protein in C. sapidus during oocyte development, then the lack of molting phase between period of oocyte development in mature females probably maintained serum protein at a relatively constant level once the ovary developed.

The salinity gradient studies indicated that there was no significant correlation between serum protein and salinity in males, but that a significant positive correlation existed between salinity and serum protein in females. This correlation is probably spurious. The migration of mature female crabs to the higher salinities of lower Chesapeake Bay occurs after the final molt of females during the period when the ovary in the newly matured females is developing. The combination of active migration during the season of salinity gradient sampling and active ovary development in the migrating crabs

resulted in the higher serum protein in crabs in higher salinities. The 1969 data from the Rappahannock River and seaside Eastern Shore, Virginia (Table 7) which were taken during a two-day period (as contrasted to 5 months during 1970) indicated no significant correlation between serum protein and salinity.

Almost no information is available on the effects of temperature on serum proteins in decapod crustaceans. Andrews (1967) reported a seasonal variation in serum protein concentration in the crayfish, Orconectes limosus with highest values of serum protein in the cycle of a given year class of female crabs are found in winter just prior to the coldest months (Fig. 7). During the coldest months, the serum protein dropped and then increased to a more or less constant level. The peak found in a mature female's first winter may be the result of over production of proteins associated with vitellogenesis just at the time that decreasing temperatures caused a pause in vitellogenesis. During the non-feeding cold months, some of these proteins, taken from the blood, maintained basic metabolism. Adiyodi (1969a) has shown that under starvation conditions the serum proteins associated with vitellogenesis are among the first affected. Kerr (1969) reported a seasonal fluctuation of serum protein in female blue crabs. Serum protein was highest during the winter months, decreased until September and then increased to the winter high. Kerr (1969) felt that seasonal

fluctuation in serum protein was related to the reproductive physiology of the individual crabs. Kerr's (1969) data were not strictly comparable to the data in this study because the year classes of her crabs were not given.

Total serum protein levels in blue crabs from Virginia are very similar to those reported from other areas (Jeffries, 1966; Horn & Kerr, 1963). The report of consistently significantly lower serum protein concentrations for male crabs compared with female crabs (Horn & Kerr, 1963) could not be confirmed for Virginia blue crabs. When male serum protein levels were compared with serum protein levels in female crabs from low salinities or with levels in newly matured females, the males usually had higher levels (Tables 6 and 7). But, comparison of total serum proteins in male with those of female crabs from higher salinities usually indicates no differences between the two. Differences in serum protein levels between fully matured male and female blue crabs are probably not significant. In male crabs the changes of serum protein concentrations associated with the molting process complicate the picture. Van Engel (Personal communication) has found it extremely difficult to stage intermolt blue crabs until they reach the peeler stage. Male crabs called hard, intermolt may be in any stage between early intermolt through early proecdysis. The mature, hard, females, however are always

in the same stage of the molt cycle (C_t).

Despite the variability found in serum proteins in the blue crab, this constituent of the blood would probably prove valuable in determining the condition of a population, particularly if mature female crabs, properly aged, with known state of ovary development are used. Since temperature and salinity do not appear to affect serum protein concentrations, any variation of this constituent beyond those ranges discussed in this paper are probably the result of abnormal conditions.

The success of several investigators in separating crustacean serum proteins using electrophoretic techniques (Manwell & Baker, 1963; Adiyode, 1968, 1969a, 1969b; Horn & Kerr, 1969; Uglow, 1969a, 1969b, 1969c; Djangmah, 1970) suggests that this approach might provide more sensitive physiological indices than measurement of total serum protein. The apparent similarity in serum protein levels in widely separated areas increases the potential of this constituent as an ecological indicator. Discovery of critical environmental factors in one area would probably have relevancy in other areas.

SERUM GLUCOSE

Serum glucose levels are quite variable in blue crabs taken directly from the natural environment. Despite this large variability a seasonal trend in glucose levels was apparent. Glucose levels were highest in early and

mid summer and lowest in late summer and early fall. Figure 14 indicates this trend is apparent in both 1969 and 1970 in crabs handled in three different fashions. The trend is found in crabs of both sexes.

The only consistent difference in serum glucose levels between crabs of different life stages was a significantly lower glucose level in new year class female crabs compared to old year class female crabs during the first appearance of the new females in the samples (June of 1970 and 1971). No correlation such as between serum protein and ovary weight was found. The high correlation between increasing ovary weight and increasing serum glucose during September, October and November (Fig. 9) is probably just a coincidence, since a similar increase in serum glucose occurred in male blue crabs during this period (Fig. 8). Variation is slightly higher in male serum glucose levels than in female serum glucose levels. As mentioned earlier, this may be due to difficulties in determining the exact stage of intermolt males.

Essentially no significant differences were found between serum glucose levels of male and female blue crabs taken in field conditions, confirming the report of Dean & Vernberg (1965a) based upon animals held without food prior to sampling. Dean and Vernberg (1965a) reported significantly higher serum glucose levels in early sponge crabs, and indicated that female crabs with old sponges have lower glucose levels than mature males,

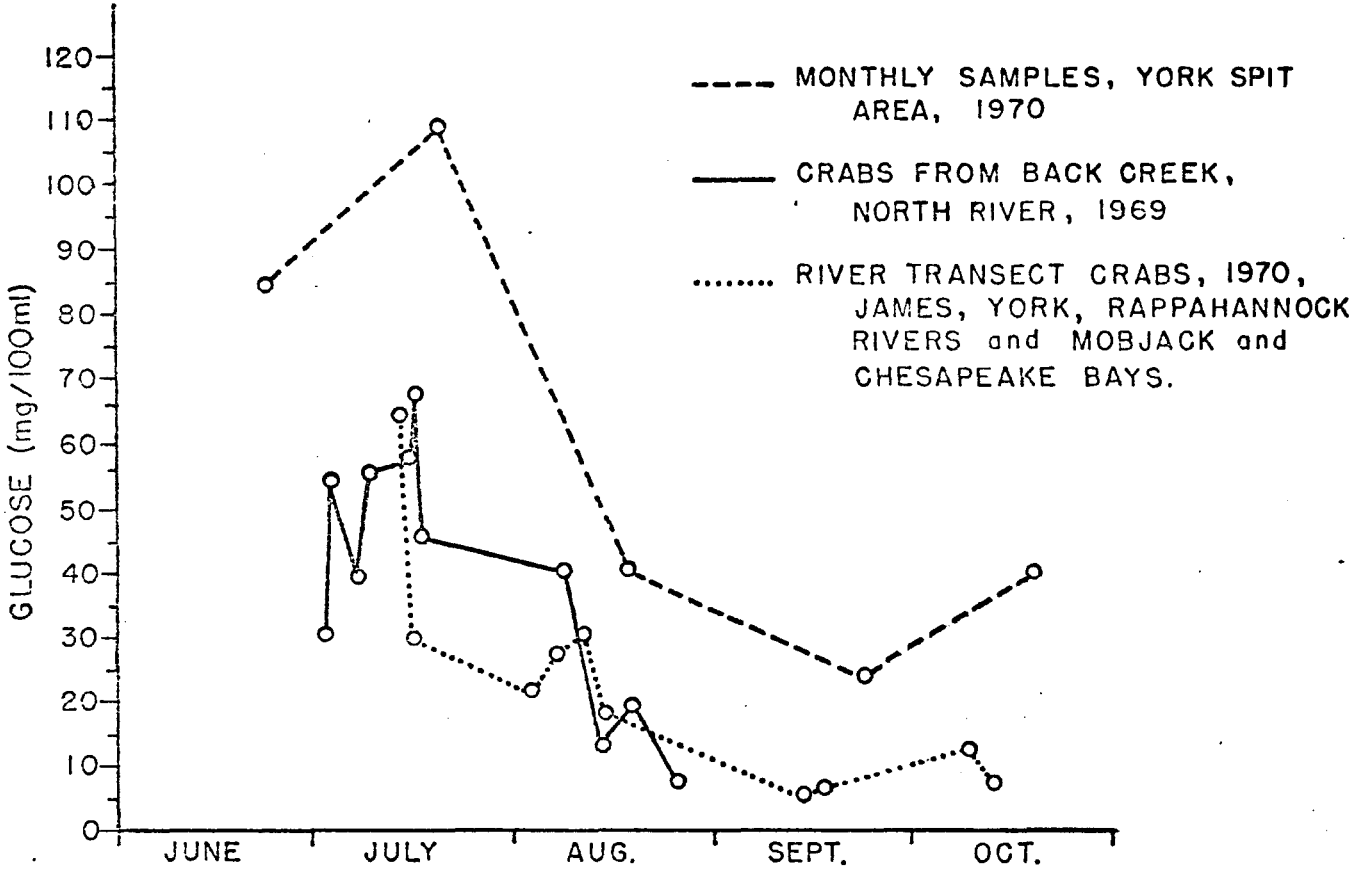

Fig. 14. Variation in mean serum glucose of blue crabs, Callinectes sapidus from various locations in Virginia during summer and fall months.

immature females and females with no eggs sampled early in the spring. This higher serum glucose level in crabs with early sponges compared to other type sponges or clean crabs could not be confirmed. The decrease of serum glucose in late August and September found during both 1969 and 1970 (Fig. 14) makes direct comparison with Dean and Vernberg's (1965a) data difficult. In addition, the animals sampled by Dean and Vernberg (1965a) had been starved for 3 days which should tend to reduce blood sugars (Florkin, 1960a; Morgulis, 1922).

It is possible that the serum values of Dean & Vernberg (1965b) were affected by seasonal variation unrelated to reproductive cycles, but since sampling dates were not provided in their paper, direct comparison cannot be made with data from this study. The mean serum glucose levels for their starved animals (10.52 $\pm$ 1.03 to 37.61 $\pm$ 2.77 mg/100 ml $\pm$ S.E.) are of the same magnitude as the levels obtained in this study with relatively unstressed animals (Table 9) except for those crabs sampled in September 1970 which had significantly lower serum glucose (6.13 $\pm$ 0.92 mg/100 ml $\pm$ S.E., N = 43) than any of Dean & Vernberg's (1965a) groups of crabs.

Applying Dean & Vernbergs (1965a) estimate of the fraction (20-25%) of glucose in the total reducing sugar in blue crab serum to the data of Morgulis (1922) and Jeffries (1966) results in a range of 12.9 - 45.5 and 0.9 - 11.6 mg/100 ml of serum glucose respectively.

Morgulis's (1922) values are similar to the values found in this study during early and mid-summer. Jeffries' (1966) values represent the mean of all stages of crabs collected over a period extending from May to December. It is impossible to compare his values directly with the present data except to note that they appear somewhat low. The inability to compare the results of this study directly to the results of Dean & Vernberg (1965a) and Jeffries (1966) indicates the importance of determining the effect of environmental variability, including seasonal variability, upon specific constituents. Although this study indicates that salinity appears not to effect serum glucose levels, there is a significant seasonal effect upon serum glucose levels. This seasonal effect in turn indicates the importance of fully specifying environmental information, when reporting biological data collected in the field.

The results of the holding experiments (Table 11) confirms the reports in the literature (Kleinholz & Little, 1949; Kleinholz et al., 1950; Roche & Dumazert, 1935) that holding crabs out of water induces hyperglycemia. The increase in serum glucose due to holding crabs out of water appears to be relatively constant factor of the original glucose concentration, (Approx. 2.5). If the mean serum glucose levels of the seasonal samples (Table 8) taken during July through October 1970 are reduced by this factor of 2.5, the reduced values very closely approximate

the mean serum glucose levels obtained during the salinity gradient studies (Table 9) on approximately the same days (Table 15), indicating that corrections might possibly be made for some types of stress.

SERUM TOTAL NINHYDRIN POSITIVE SUBSTANCES AND FREE AMINO ACIDS

Both Morgulis (1922) and Jeffries (1966) have reported values for serum non-protein nitrogen in the blue crab. An approximate conversion of NPN to TNPS was made by using a 30% amino-N fraction in NPN (Florkin, 1960) and assuming amino N accounts for 70% of the TNPS (Table 18). The converted values of Morgulis (1922) ranged from 7.4 μ moles/ml in a freshly caught crab to 2.7 μ moles/ml in a crab held for 2 days in an aquarium. Jeffries' (1966) values converted to a range of 2.6 - 30.8 μ moles/ml for males and 3.8 - 12.9 μ moles/ml for females. These values are within the ranges found in this study.

Jeffries (1966) reported that non-protein nitrogen in blue crab serum is inversely correlated with serum chloride. Unfortunately he combined data from both male and female crabs in various stages of the molt cycle to determine the correlation. In the present study distinct differences are found between the relationship of serum TNPS and serum chloride in male and female crabs. No significant correlation between serum TNPS and serum chloride is found in male crabs. In female crabs, however, a significant positive correlation is found in data taken

TABLE 15 - COMPARISON OF MEAN SERUM GLUCOSE LEVELS IN BLUE CRABS OBTAINED DURING SALINITY GRADIENT STUDIES AND MEAN SERUM GLUCOSE LEVELS IN BLUE CRABS OBTAINED DURING SEASONAL STUDIES CORRECTED FOR HYPERGLYCEMIA INDUCED BY HOLDING OUT OF WATER.

Date of Seasonal Sample	Mean Serum Glucose (mg/100 ml) Uncorrected Seasonal Sample	Corrected Seasonal Sample [1]	Salinity Gradient	Date of Salinity Gradient Sample
20 Jul 70	109.18	43.67	47.04	14, 16 Jul 70
18 Aug 70	40.93	16.37	18.98	13 Aug 70
23 Sep 70	24.44	9.78	6.13	14, 17 Sep 70
20 Oct 70	40.25	16.10	9.31	8, 12 Oct 70

1 Mean values corrected by multiplying by 0.4

during the salinity gradient studies. This correlation is not found in data from the seasonal studies.

Serum TNPS and salinity were not correlated for either male or female crabs in the seasonal studies, but in the salinity gradient study significant positive correlation is found between serum TNPS and salinity in crabs of both sex. Analysis of covariance indicated the correlation was significantly higher in females. Because of the lack of correlation between serum TNPS and salinity during the seasonal studies and the lack of correlation between serum TNPS and serum chloride in males in either phase of the present study, the relationship between salinity and serum TNPS in females found in the salinity gradient study is probably due to some factor other than different osmotic conditions.

Free amino acids are known to be intracellular osmotic effectors in euryhaline marine invertebrates (Lange, 1968; Schoffeniels & Gilles, 1970). Gilles & Schoffeniels (1964, 1968, 1969) have shown that the increase in tissue free amino acids occurs primarily by de novo synthesis within the cells. Several investigators have shown, however, that intracellular amino acids can be passed (or are leaked) to the extracellular spaces (Gilles & Schoffeniels, 1969; Vincent - Marique & Gilles, 1970), particularly during volume regulation (Fugelli, 1967). When isolated blue crab muscle fiber are exposed to a hypertonic medium, there is an immediate decrease in fiber

volume (Lang & Gainer, 1969). This volume reduction is due to an efflux of water from the cell to the surrounding fluid (Lange & Mostad, 1967). Volume readjustment is accomplished by increasing the intracellular concentration of organic solutes, primarily free amino acids. Gilles & Schoffeniels (1969) have demonstrated two mechanisms to accomplish this increase in intracellular free amino acids, modifications of the permeability of membranes and modifications of pathways responsible for biosynthesis and degradation of these compounds. Female blue crabs begin migrating towards higher salinity waters upon reaching maturity, whereas male crabs do not have such a migratory pattern (Van Engel, 1958). Female crabs captured along a salinity gradient during the periods of migration, therefore, are most likely in a state of active osmotic readjustment that involves increased amino acid biosynthesis. Upon reaching higher salinity waters, an equilibrium is reached, and the serum TNPS concentrations no longer reflect the higher rate of metabolic activity required during passage along the salinity gradient. The secondary peak of TNPS found in the fall of 1970 (Fig. 10) occurs at about the same time of year as the peak of the migration of mature females to higher salinity waters. This peak might be a reflection of active osmotic adjustment.

The correlation between TNPS and salinity in females is not thought to be related to ovogenesis or

ovary development as is the correlation between total serum protein and salinity since no significant correlation was found between serum protein and serum TNPS, and essentially no differences were found between serum TNPS levels in different year classes.

The cause for the differences in the relationship between serum TNPS and serum glucose in males and females is not readily apparent. Since there is no significant correlation between serum glucose and salinity, the significant correlation between serum TNPS and serum glucose in females taken along the salinity gradient cannot be explained by the migratory behavior of the mature females, particularly as a significant correlation between these constitutents is also found in the seasonal samples.

The seasonal variation in serum TNPS (Fig. 10) is marked by a high value in each winter (1970 and 1971) of the study. These high values coincide relatively closely with the highest serum chloride values found during the year. A distinct difference is seen, however, in that serum chloride varies continuously during the year, closely related to the environmental temperature. Serum TNPS, however, increase sharply during one month and decrease just as sharply the following month.

Duchâteau & Florkin (1955) have demonstrated lower concentrations of tissue free amino acid in Eriocheir sinensis at 1-3°C than at 10-11°C. One possible explaination for the sharp increase in TNPS in blue crab serum is that

at some low temperature, approximately 5°C, the permeability of cell membranes to free amino acids changes drastically allowing intracellular free amino acids to leak out to the surrounding medium. As is the case with the sudden extrusion of intracellular free amino acids to the serum of Eriocheir sinensis transferred from seawater to fresh water (Vincent-Marique & Gilles, 1970), the higher amino acid levels are detectable in the serum for several days after the imposed stress.

Free amino acid distribution in blue crab serum is generally similar to the free amino acid distribution reported in other crustaceans (Duchâteau-Bosson & Florkin, 1961; Camien et al., 1951). The major difference appears to be that serine, which is among the five most concentrated amino acids in Cancer irroratus, Eriocheir sinensis and Homarus americanus is not a major portion of the free amino acids pool in Callinectes sapidus (Stevens et al., 1961; Stewart et al., 1966; and Vincent-Marique et al., 1970).

CONCLUSIONS

Under normal environmental conditions the concentrations of the several serum constituents studied vary widely. Some of these variations are attributed to temperature and salinity variation of the environment, while others are attributed to age, sex or life stage of the organism.

Temperature and salinity appear to affect serum chloride and serum osmotic concentration to a greater extent than the other constituents. Sex appears to have an effect on certain constituents, particularly in combination with other factors. For example, a sex-maturity combination of factors is the dominant factor in determining serum protein concentration, and sex-salinity combinations have an effect on variation serum chloride and serum osmotic concentration. The information gathered during this study provides a baseline of "normal" values against which levels of serum constituents of organisms suspected of being under stress may be compared. What is needed now is a study of the blood constituents of known diseased or stressed crabs, so that deviations from "normal" values can be properly evaluated when found.

The response of serum glucose to stresses as shown both in this and other studies, indicates that this constituent might, as part of a routine monitoring program, indicate a stress condition. When combined with deviations from the baselines of other constituents it might pinpoint the reason for the elevated glucose. A biological monitoring program based upon changes in physiological factors could provide a system for determining effects of alterations to the natural system prior to the appearance of mass mortalities of the species concerned.

REFERENCES

Abramowitz A. A., Hisaw F. L. & Papandrea D. N. (1944) The occurrence of a diabetogenic factor in the eyestalks of crustaceans. Biol. Bull. mar. biol. Lab., Woods Hole 86, 1-5.

Adiyodi R. G. (1968) Protein metabolism in relation to reproduction & moulting in the crab, Paratelphusa hydrodromous (Herbst): Part I - Electrophoretic studies on the mode of utilization of soluble proteins during vitellogenesis. Indian J. exp. Biol. 6, 144-147.

Adiyodi R. G. (1969a) Protein metabolism in relation to reproduction & moulting in the crab, Paratelphusa hydrodromous: Part III - RNA activity & protein-yolk biosynthesis during normal vitellogenesis & under conditions of acute inanition. Indian J. exp. Biol. 7, 13-16.

Adiyodi R. G. (1969b) Protein metabolism in relation to reproduction & moulting in the crab, Paratelphusa hydrodromous (Herbst): Part IV - moulting. Indian J. exp. Biol. 7, 135-137.

Anderson J. D. & Prosser C. L. (1953) Osmoregulating capacity in populations occurring in different salinities. Biol. Bull. mar. biol. Lab., Woods Hole 105, 369. (Abstract)

Andrews P. (1967) Uber den blutchemismus des flubkrebses Orconectes limosus und seine veranderung im laufe des jahres. A. vergl. Physiol. 57, 7-43.

Ballard B. S. (1967) Osmotic accomodation in Callinectes sapidus Rathbun. Ph.D. Thesis, Mississippi State University, State College, Mississippi, U.S.A.

Ballard B. S. & Abbott W. (1969) Osmotic accomodation in Callinectes sapidus Rathbun. Comp. Biochem. Physiol. 29, 671-687.

Baumberger J. P. & Dill D. B. (1928) A study of the glycogen and sugar content and the osmotic pressure of crabs during the molt cycle. Physiol. Rev. 1, 545-549.

Broekema M. M. M. (1942) Seasonal movements and the osmotic behavior of the shrimp Crangon crangon L. Arch. neerl. Zool. 6, 1-100.

Camien M. N., Sarlet H., Duchâteau G. L. & Florkin M. (1951) Non-protein amino acids in muscle and blood of marine and fresh water crustacea. J. Biol. Chem. 193, 881-885.

Churchill E. P. Jr. (1919) Life history of the blue crab. Bull. Bur. Fish., Wash. 36, 91-128.

Cole W. H. (1940) The composition of fluids and sera of some marine animals and of the seawater in which they live. J. gen. Physiol. 23, 575-584.

Cotlove E. (1961) Chloride. In Standard Methods of Clinical Chemistry (Edited by Seligson D.), Vol. 3, pp. 81-92. Academic Press, New York.

Dean J. M. & Vernberg F. J. (1965a) Variations in the blood glucose level of crustacea. Comp. Biochem. Physiol. 14, 29-34.

Dean J. M. & Vernberg F. J. (1965b) Effects of temperature acclimation on some aspects of carbohydrate metabolism in decapod crustacea. Biol. Bull. mar. biol. Lab., Woods Hole 129, 87-94.

Dean J. M. & Vernberg F. J. (1966) Hypothermia and blood of crabs. Comp. Biochem. Physiol. 17, 19-22.

Dehnel P. A. (1962) Aspects of osmoregulation in two species of intertidal crabs. Biol. Bull. mar. biol. Lab., Woods Hole 122, 208-227.

Dehnel P. A. (1967) Osmotic and ionic regulation in estuarine crabs. In Estuaries (Edited by Lauff G. H.), pp. 541-547. Am. Assoc. Adv. Sci., Washington, D. C.

Delaunay H. (1931) L'excretion azotee des invertebres. Biol. Rev. 6, 265-301.

DeLeersynder M. (1967) Le milieu interieur d'Eriocheir sinensis Milne-Edwards et ses variations. I. - Etude dans les milieu naturel. Cah. Biol. Mar. 8, 195-218.

Djangmah J. S. (1970) The effects of feeding and starvation on copper in the blood and hepato-pancreas, and on blood proteins of Crangon vulgaris (Fabricius). Comp. Biochem. Physiol. 32, 709-731.

Duchâteau G. L. & Florkin M. (1955) Influence de la temperature sur l'etat stationnaire du pool des amines non proteiques des muscles d'Eriocheir sinensis Milne Edwards Arch. int. Physiol. Biochim. 63, 213-221.

Duchâteau-Bosson G. L. & Florkin M. (1961) Change in intracellular concentration of free amino acids as a factor of eury halinity in the crayfish Astacus astacus. Comp. Biochem. Physiol. 3, 245-249.

DuPaul W. D. & Webb K. L. (1970) The effect of temperature on salinity-induced changes in the free amino acid pool of Mya arenaria. Comp. Biochem. Physiol. 32, 785-801.

Fales F. W. (1963) Glucose (enzymatic). In Standard Methods of Clinical Chemistry (Edited by Seligson D.), Vol. 4, pp. 101-112. Academic Press, New York.

Florkin M. (1960a) Blood chemistry. In The Physiology of Crustacea (Edited by Waterman T. H.) Vol. 1, pp. 141-159. Academic Press, New York.

Florkin M. (1960b) Ecology and metabolism. In The Physiology of Crustacea (Edited by Waterman T. H.) Vol. 1, pp. 395-410. Academic Press, New York.

Flugel H. (1963) Electrolytregulation und temperatur bei Crangon crangon L. und Carcinus maenas L. Kieler Meeresforsch. 19, 189-195.

Fugelli K. (1967) Regulation of cell volume in flounder (Pleuronectes flesus) erythrocytes accompanying a decrease in plasma osmolarity. Comp. Biochem. Physiol. 22, 253-260.

Gifford C. A. (1962) Some aspects of osmotic and ionic regulation in the blue crab, Callinectes sapidus, and the ghost crab, Ocypode albicans. Publs. Inst. mar. Sci. Univ. Tex. 8, 97-125.

Gilbert A. B. (1959) The composition of the blood of the shore crab, Carcinus moenas Pennant, in relation to sex and body size I. Blood conductivity and freezing point depressions. J. exp. Biol. 36, 113-119.

Gilles R. & Schoffeniels E. (1964) Action de la veratrine de la cocaine et de la stimulation electrique sur la synthese et sur le pool des

acides amines de la chaine nerveuse ventrale du homard. Biochim. biophys. Acta 82, 525-537.

Gilles R. & Schoffeniels E. (1968) Fixation de $^{14}CO_2$ par les acides amines de la chaine nerveuse ventrale du crustace Homarus vulgaris M. Edw. Arch. int. Physiol. Biochim. 76, 441-451.

Gilles R. & Schoffeniels E. (1969) Isosomotic regulation in isolated surviving nerves of Eriocheir sinensis Milne Edwards. Comp. Biochem. Physiol. 31, 927-939.

Haefner P. A. Jr. (1969) Osmoregulation of Crangon septemspinosa Say (Crustacea: Caridea). Biol. Bull. mar. biol. Lab., Woods Hole 137, 438-446.

Hohnke L. & Scheer B. T. (1970) Carbohydrate metabolism in crustaceans. In Chemical Zoology (Edited by Florkin M. & Scheer B. T.), Vol. 5, Arthropoda, Part A. pp. 147-166. Academic Press, New York.

Horn E. C. & Kerr M. S. (1963) Hemolymph protein and copper concentrations of adult blue crabs (Callinectes sapidus Rathbun). Biol. Bull. mar. biol. lab., Woods Hole 125, 499-507.

Horn E. C. & Kerr M. S. (1969) The hemolymph proteins of the blue crab, Callinectes sapidus - I. Hemocyanins and certain other major protein constituents. Comp. Biochem. Physiol. 29, 493-508.

Hopkins S. H. (1947) The nemertean Carcinomertes as an indicator of the spawning history of the host,

Callinectes sapidus. J. Parasit. 33, 146-150.

Jeffries H. P. (1964) Indices of ecological condition in marine organisms--A trial study. In Symposium on Experimental Marine Ecology (Edited by Marshall N., Jeffries H. P., Napora T. A. and Sieburth J. NcN.) pp. 59-68. Occasional Publ. No. 2. Grad. School. Oceanogr., Univ. of Rhode Island, Kingston.

Jeffries H. P. (1966) Internal condition of a diminished blue crab population (Callinectes sapidus). Chesapeake Sci. 7, 164-170.

Jeuniaux C. (1971) Hemolymph - Arthropoda. In Chemical Zoology (Edited by Florkin M. & Scheer B. T.), Vol. 6, Arthropoda, Part B. pp. 64-118. Academic Press, New York.

Kerr M. S. (1969) The hemolymph proteins of the blue crab, Callinectes sapidus II. A lipoprotein serologically identical to oocyte lipovitellin. Develop. Biol. 20, 1-17.

Kleinholz L. H., Havel V. J. & Reichart R. (1950) Studies in the regulation of blood-sugar concentration in crustaceans. II. Experimental hyperglycemia and the regulatory mechanisms. Biol. Bull. mar. biol. Lab. Woods Hole 99, 454-468.

Kleinholz L. H. & Little B. C. (1949) Studies in the regulation of blood-sugar concentration in crustaceans. I. Normal values and hyperglycemia in Libinia emarginata. Biol. Bull. mar. biol. Lab., Woods Hole 96, 218-227.

Krogh A. (1939) Osmotic regulation in aquatic animals, 242 pp. Cambridge University Press, London.

Lang M. & Gainer H. (1969) Volume control by muscle fibers of the blue crab. Volume readjustment in hypotonic salines. J. gen. Physiol. 53, 323-341.

Lange R. (1968) Isosmotic intracellular regulation. Nytt Mag. Zool. 16, 1-13.

Lange R. & Mostad A. (1967) Cell volume regulation in osmotically adjusting marine animals. J. exp. mar. Biol. Ecol. 1, 209-219.

Layne E. (1957) Spectrophotometric and turbidometric methods for measuring proteins. In Methods in Enzymology (Edited by Colwick S. P. & Kaplan N. O.) Vol. III, pp. 447-454. Academic Press, New York.

Leone C. A. (1953) Preliminary observations on intra-specific variation of the levels of total protein in the sera of some decapod crustacea. Science, N. Y. 118, 295-296.

Lockwood A. P. M. (1962) The osmoregulation of crustacea. Biol. Rev. 37, 257-305.

Lowry O. H., Rosebrough N. J., Farr A. L. & Randall R. J. (1951) Protein measurement with the Folin phenol reagent. J. biol. Chem. 193, 265-275.

Mantel L. H. (1967) Asymmetry potentials, metabolism and sodium fluxes in gills of the blue crab, Callinectes sapidus. Comp. Biochem. Physiol. 20, 743-753.

Manwell C. & Baker C. M. A. (1963) Starch gel electrophoresis of sera from some marine arthropods: Studies on the heterogeneity of hemocyanin and on a "ceruloplasmin-like protein". Comp. Biochem. Physiol. 8, 193-208.

McWhinnie M. A. & Saller P. N. (1960) Analysis of blood sugars in the crayfish, Orconectes virilis. Comp. Biochem. Physiol. 1, 110-122.

McWhinnie M. A. & Scheer B. T. (1958) Blood glucose of the crab, Hemigrapsus nudus. Science, N. Y. 128, 90.

Morgulis S. (1922) A study of the non-protein constituents in blood of some marine invertebrates. J. biol. Chem. 50, lii-liv.

Moore S. & Stein W. H. (1954) A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. biol. Chem. 211, 907-913.

Odum H. T. (1953) Factors controlling marine invasion into Florida fresh waters. Bull. mar. Sci. Gulf Caribb. 3, 134-156.

Otto J. P. (1937) Uber den einflus der temperatur auf den osmotischen wert der blutflussigkeit bei der wollhandkrabbe (Eriocheir sinensis H. Milne-Edwards). Zool. Anz. 119,98-105.

Panikkar N. K. (1940) Influence of temperature on osmotic behavior of some crustacea and its bearing on problems of animal distribution. Nature, Lond. 146, 366-367.

Panikkar N. K. (1941) Osmoregulation in some paleomonid prawns. J. mar. biol. Ass. U. K. 25, 317-359.

Passano L. M. (1960) Molting and its control. In The Physiology of Crustacea (Edited by Waterman T. H.) Vol. 1, pp. 473-536. Academic Press, New York.

Pearson J. C. (1948) Fluctuations in the abundance of the blue crab in Chesapeake Bay. Res. Rep. U. S. Fish. Wildl. Serv. No. 14, 26 pp.

Raabo E. & Terkildsen T. C. (1960) On the enzymatic determination of blood glucose. Scand. J. Clin. Lab. Invest. 12, 402-407.

Richards C. E. & Castagna M. (1970) Marine fishes of Virginia's Eastern Shore (inlet and marsh, seaside waters). Chesapeake Sci. 11, 235-248.

Riegel J. A. (1960) Blood glucose in crayfishes in relation to moult and handling. Nature, Lond. 186, 727.

Robertson J. D. (1960) Osmotic and ionic regulation. In The Physiology of Crustacea (Edited by Waterman T. H.), Vol. I, pp. 317-339. Academic Press, New York.

Roche J. & Dumazert C. (1935) Sur la glycemie de Cancer pagurus. Nature des substances reductrices et facteurs de variation de la glycemie. Compt. Rend. de Biol. Paris. 120, 1225-1227.

Schoffeniels E. & Gilles R. (1970) Osmoregulation in aquatic arthropods. In Chemical Zoology (Edited by Florkin M. and Scheer B. T.), Vol. 5, Athropoda, Part A. pp. 255-286. Academic Press, New York.

Sigma (1969) The enzymatic/colorimetric determination of glucose in whole blood, plasma or serum at 425-475 nm. Tech. Bull. 510. Revised May 1969. Sigma Chemical Co., St. Louis, Mo.

Snedecor G. W. (1956) Statistical methods applied to experiments in agriculture and biology. p. 534. Iowa State University Press, Ames.

Stewart J. E., Cornick J. W. & Dingle J. R. (1967a) An electronic method for counting lobster (Homarus americanus Milne-Edwards) hemocytes and the influence of diet on hemocyte numbers and hemolymph proteins. Can. J. Zool. 45, 291-304.

Stewart J. E., Cornick J. W., Foley D. M., Li M. F. & Bishop C. M. (1967b) Muscle weight relationship to serum proteins, hemocytes and hepatopancreas in the lobster, Homarus americanus. J. Fish. Res. Bd. Can. 24, 2339-2354.

Stewart J. E., Dingle J. R. & Odense P. H. (1966) Constituents of the hemolymph of the lobster, Homarus americanus Milne-Edwards. Can. J. Biochem. 44, 1447-1459.

Stewart J. E. & Li M. F. (1969) A study of lobster (Homarus americanus) ecology, using serum protein concentration as an index. Can. J. Zool. 47, 21-28.

Stevens T. M., Howard C. E., & Schlesinger R. W. (1961) Free amino acids in sera of the marine invertebrates, Cancer irroratus, Limulus polyphemus, and Homarus americanus. Comp. Biochem. Physiol. 3, 310-314.

Stroup E. D. & Lynn R. J. (1963) Atlas of salinity and temperature distributions in Chesapeake Bay 1952-1961 and seasonal averages 1949-1961. Graphical Summary Report No. 2. Reference 63-1, Chesapeake Bay Institute, The Johns Hopkins University, Baltimore, Maryland.

Tagatz M. E. (1971) Osmoregulatory ability of blue crabs in different temperature-salinity combinations. Chesapeake Sci. 12, 14-17.

Tan Eng-Chow (1962) Studies on osmotic and ionic regulation in the blue crab, Callinectes sapidus Rathbun. M.A. Thesis, College of William and Mary, Williamsburg, Virginia, 66 pp.

Tan Eng-Chow & Van Engel W. A. (1966) Osmoregulation in the adult blue crab, Callinectes sapidus Rathbun. Chesapeake Sci. 7, 30-35.

Telford M. (1965) Carbohydrates in the blood of the lobster, Homarus americanus. Can. J. Zool. 43, 503-507.

Telford M. (1968a) Changes in blood sugar composition during the molt cycle of the lobster, Homarus americanus. Comp. Biochem. Physiol. 26, 917-926.

Telford M. (1968b) The effects of stress on blood sugar composition of the lobster, Homarus americanus. Can. J. Zool. 46, 819-826.

Telford M. (1968c) The identification and measurement of sugars in the blood of three species of Atlantic crabs. Biol. Bull. mar. biol. Lab., Woods Hole 135, 574-584.

Todd M. E. (1963) Osmoregulation in Ligia oceanica and Idotea granulosa. J. exp. Biol. 40, 381-392.

Uglow R. F. (1969a) Haemolymph protein concentrations in portunid crabs - I. Studies on adult Carcinus maenas. Comp. Biochem. Physiol. 30, 1083-1090.

Uglow R. F. (1969b) Haemolymph protein concentrations in crabs - II. The effects of imposed fasting

on Carcinus maenas. Comp. Biochem. Physiol. 31, 959-967.

Uglow R. F. (1969c) Haemolymph protein concentrations in portunid crabs - III. The effect of Sacculina. Comp. Biochem. Physiol. 31, 969-973.

Umminger B. L. (1969) Physiological studies on supercooled killifish (Fundulus heteroclitus) II. Serum organic constituents and the problem of supercooling. J. exp. Zool. 172, 409-424.

Van Engel W. A. (1958) The blue crab and its fishery in Chesapeake Bay. Part I - Reproduction, early development, growth and migration. Comml. Fish. Rev. 20, 6-17.

Van Engel W. A. (1962) The blue crab and its fishery in Chesapeake Bay. Part 2 - Types of gear for hard crab fishing. Comml. Fish. Rev. 24, 1-10.

Vernberg F. J. & Vernberg W. B. (1970) The animal and the environment, 398 pp. Holt, Rinehart & Winston, Inc. New York.

Vincent-Marique C. & Gilles R. (1970) Modification of the amino acid pool in blood and muscle of Eriocheir sinensis during osmotis stress. Comp. Biochem. Physiol. 35, 479-485.

Weber R. E. & Spaargaren D. H. (1970) On the influence of temperature on the osmoregulation of Crangon crangon and its significance under estuarine conditions. Neth. J. Sea Res. 5, 108-120.

Widmann E. (1935) Osmoregulation bei einheimischen wasser-und feuchtluft-crestaceen. Z. wiss. Zool. 147, 132-169.

Woods K. R., Paulsen E. C., Engle R. L. and Pert J. H. (1958) Starch gel electrophoresis of some invertebrate sera. Science, N. Y. 127, 519-520.

APPENDIX

The Use of Serum Constituents of the Blue Crab *Callinectes Sapidus* as Indicators of Physiological Condition

by

Maurice Patrick Lynch

INTRODUCTION

One of the more pressing problems facing managers and scientists are the effects of materials that enter aquatic ecosystems as run off, waste discharges or as inadvertent introduction. Presently recommended water quality criteria are primarily based upon bioassays in which lethal limits (normally 96 hr TL_m) are used with an application factor to indicate safe concentrations in the receiving waters. Among the recommendations for setting water quality criteria based upon TL_m was that chronic exposure tests be conducted to demonstrate the validity of these criteria (U.S. Dept. Interior, 1968).

Jeffries (1964) suggested that physiological criteria might prove to be sensitive indicators of population condition, and that development of physiological indices might provide management agencies with more pertinent information than presently available. The use of physiological indices to evaluate population condition is not an entirely academic subject. Oyster biologists, for example use a condition index (CI) based upon a ratio of meat weight to shell cavity volume as a measure of the quality of oyster meats in a population. (Haven, 1962). CI in oysters, in addition to providing

an index of overall quality in a population, may serve as a guide to management by indicating when oysters from a certain region should be harvested for the most profitable yield. No other indices of condition in marine or estuarine invertebrates are in routine use.

Several reports of physiological variation in estuarine invertebrates due to artificial or natural stress have been made. Mengebier & Wood (1967; 1969) report depressed activity in specific respiratory enzymes of the oyster when infected with the haplosporidian, Minchinia nelsoni or in the presence of "red tide" blooms of the dinoflagellate Cochlodinium helicoides. Tissue free amino acid patterns in two mollusks and a crustacean are found to differ depending upon whether the animals were taken from polluted or nonpolluted areas (Schafer, 1961, 1963). Jeffries (1969, 1970) found that free amino acid and lipid composition of zooplankton vary with the physiological conditions of the dominant organisms. Hemolymph free amino acids and total protein are significantly decreased in snails infected with a schistosome (Gilbertson et al., 1967). Hemolymph free amino acids are also depleted in oysters infected with Minchinia nelsoni (Feng et al., 1970; K. L. Webb, personal communication).

Serum constituents of the lobster, Homarus americanus are indicators of physiological condition. Telford (1968) found elevated serum glucose in lobsters

held in commercial pounds. Total serum protein is a reliable indicator of muscle weight (Stewart et al., 1967b), an indicator of diet (Stewart et al., 1967a) and related to specific areas from which lobsters were taken (Stewart & Li, 1969).

Jeffries (1964, 1966) attempted to determine indices of ecological condition in the crabs Cancer irroratus, Cancer borealis and Callinectes sapidus. His attempts were not very successful primarily because no normal or "baseline" data were available for comparison with experimental values. Jeffries (1964) was able, however, to demonstrate a decrease in serum nitrogen and an increase in serum chloride associated with the stress of prolonged exercise.

In 1969 the decision was made to begin a pilot study to determine if the establishment of physiological indices of condition were feasible. The subject chosen for this study was the blue crab, Callinectes sapidus, Rathbun. This species is noted for wide fluctuations in populations, the causes of which have not been adequately explained (Pearson, 1948; Van Engel, 1958). The blue crab is widely distributed in the waters of Virginia (Churchill; 1919) and is readily available throughout the year (Van Engel, 1958; 1962). To eliminate or minimize the variation in blood constituents associated with different stages of the molt cycle (Florkin, 1960; Passano, 1960; Jeuniaux, 1971), only mature, hard crabs

were used in so far as possible. In female blue crabs, the molt to maturity is terminal, but the male blue crab continues to molt after reaching maturity (Van Engel, 1958).

It was hoped that physiological indices, if established would assist in the determination of causes of population fluctuations in the blue crab, and provide the criteria which could be used in chronic exposure tests of various material which might be added to the ecosystem.

MATERIALS AND METHODS

GENERAL

This study was divided essentially into two parts, the development of the baselines and the determination of serum constituents under stressed conditions. The first part of the study is reported in the main paper. The second part is reported in this appendix. The pilot study eventually involved five serum fractions, total protein, chloride, glucose, total ninhydrin positive substances (TNPS) and serum osmotic concentration. Collection of crabs, environmental data, morphometric measurements, life stage data and blood serum are described in the main section. Details of the analytical methods and of the sampling program are also given in the main paper. Only the details of the stress studies are presented here.

FIELD STUDIES:

A relatively minor fish and blue crab "kill", probably caused by a bloom of the dinoflagellate Cochlodinium heterolobotom, occurred in the Elizabeth River, Norfolk, Virginia, 4 June 1970 (R. J. Huggett, Va. Inst. Mar. Sci., unpublished report). A number of live blue crabs

from the area of the "kill" were returned to the laboratory for analysis. A sample of blue crabs from Swash Bay, Eastern Shore, Virginia was collected on the same day. The Swash Bay sample served as an unstressed "control" for comparison.

On 18 July 1970, four live mature, hard male blue crabs were obtained from Nassawaddox Creek on the bayside of the Eastern Shore. An extensive blue crab kill had occurred at the site one week previously where DDT (and derivatives) had inadvertently been sprayed during agricultural operations.

LABORATORY STUDIES

On 20 November 1970, a group of eight mature female crabs were subjected to a relatively rapid (approximately 1°C/hr) temperature rise. The crabs were maintained in an aerated aquarium, equipped with a 1000 watt quartz immersion heater and a small electrically driven water stirrer. At periodic intervals temperature was recorded with a stem thermometer and individual crabs examined. The experiment was terminated when 50% of the crabs appeared moribund, i.e., did not move when prodded with a glass stirring rod and did not exhibit movement of gill bailers when removed from the water. Crabs were bled just before heating and again when the experiment was terminated or when a crab became moribund. A control group of eight crabs was bled at the start of the experiment, returned alive to an aquarium at ambient

temperature and bled again at the termination of the experiment. Ambient temperature and salinity at the start of this experiment were 13.9°C and 21.7 o/oo, respectively. Experimental animals were subjected to an approximately linear increase in temperature to 35.7-36.0°C over an 11 hour period (Fig. 1). Control animals remained at the same temperature. No appreciable salinity change occurred in either control or experimental aquaria during the course of the experiment.

On 8 December 1970, sixteen mature female crabs which had been held seven days in running York River water in the laboratory were bled and then transferred to a closed aquarium at the same ambient salinity and temperature (20.5 o/oo, 8.0°C). Water in the aquarium was continuously filtered and circulated with an airlift pump. Additional aeration was provided with air stones. The water was allowed to come to room temperature (19°C) in 24 hours. The aquarium was checked periodically each day and late each evening and dead crabs removed as soon as noticed. At the end of a 15 day period, the surviving crabs were bled. Serum was not taken from dead crabs.

ANALYSIS OF DATA

Mean values of the various serum constituents in the stressed samples were compared to either mean values from control samples or to the baseline values developed during the main study. Comparisons were made

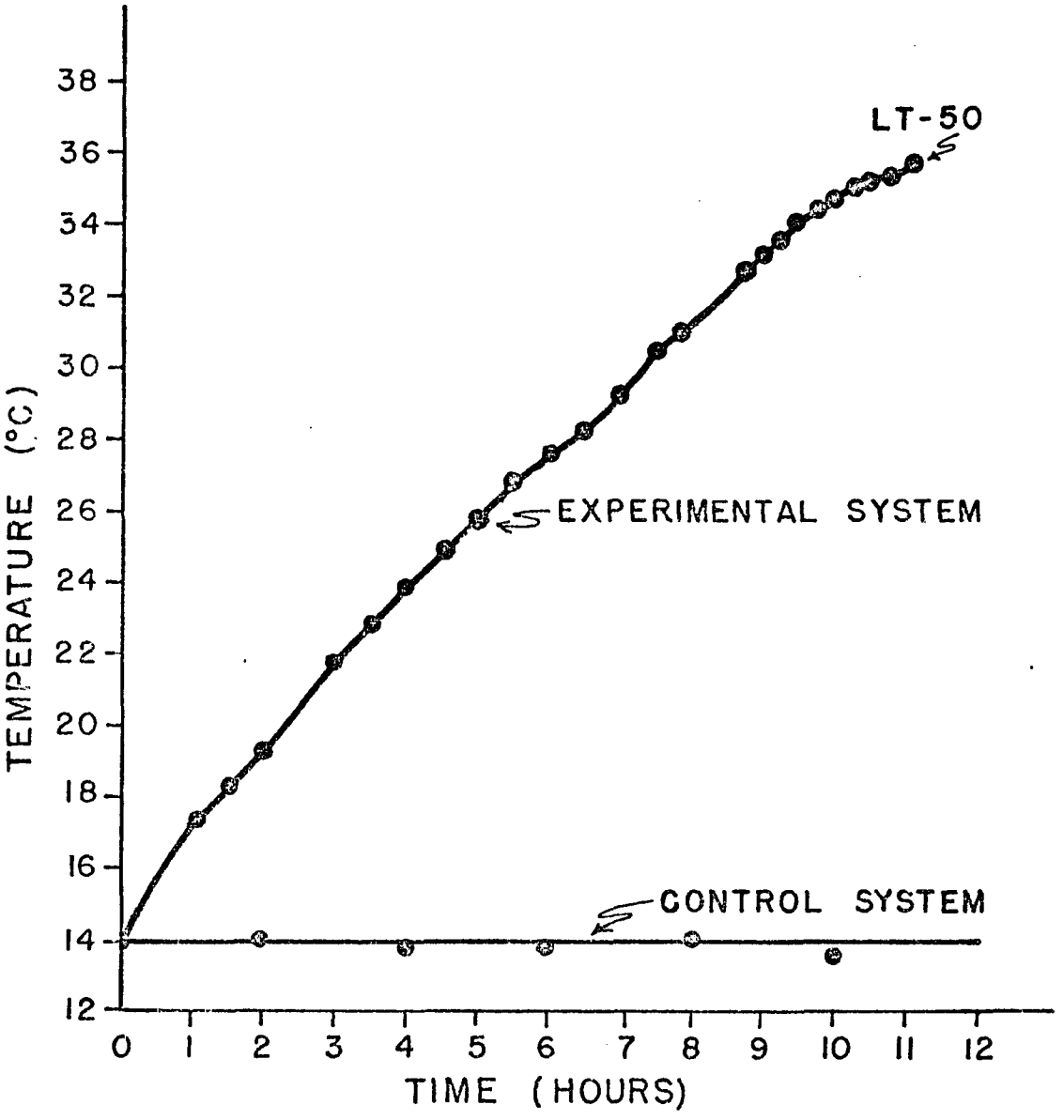

Fig. 1. Heating curve for thermal stress experiment, 20 November 1970.

using Student "t" tests for small samples described by Snedecor (1956). Differences between mean values were assumed if "t" fell outside the 95% confidence interval of a two-tailed distribution ($p < 0.05$).

Serum glucose values obtained from crabs which had been out of water were corrected for hyperglycemia (multiplying by the factor 0.4 derived during the baseline study) prior to comparison with serum glucose from crabs bled immediately upon removal from the water.

RESULTS

Results of the baseline sampling program are provided in the main paper. Specific portions of the "baselines" are provided for comparison with results from stressed crabs (Figs. 2, 3, 4 and 5).

ELIZABETH RIVER SAMPLES

Mean values of serum constituents of samples from the Elizabeth River and Swash Bay are presented in Table 1. No significant differences were found between mean concentrations of any of the serum constituents of hard male and papershell female blue crabs. The means of these two groups were combined for some of the other analyses. Mean serum glucose, total serum portein and TNPS in the Elizabeth River sample are higher than in sera from the Swash Bay samples. Because of the salinity difference, the serum chloride levels in the two samples were not compared.

When the serum constituents of Elizabeth River samples are compared with baseline values (Fig. 1), differences are apparent only in mean serum protein and serum glucose. Serum glucose in male crabs from the Elizabeth River is not different from that in the males or older year class females in the baseline samples.

TABLE 1. COMPARISON OF SERUM CONSTITUENTS OF MATURE BLUE CRABS, *CALLINECTES SAPIDUS* FROM AN AREA OF THE ELIZABETH RIVER, VA. EXPERIENCING A BLOOM OF THE DINOFLAGELLATE *COCHLODINUM HETEROLOBOTOM* AND FROM SWASH BAY, EASTERN SHORE, VA. 4 JUNE 1970.

Area	Crabs	Oxygen (PPM)	Salinity (o/oo)	Temperature (°C)	Chloride (meq/liter)	Protein (mg/ml) Mean ± S.E. (N)	TNPS (umoles/ml)	Glucose (mg/100 ml)
Swash Bay	1968 YC Females		29.0		468±5 (20)	47.6± 6.9 (20)	3.0±0.2 (18)	34.8± 7.4 (20)
	Males (hard)				347±4 (5)	124.8±19.7 (5)	6.3±0.7 (5)	78.8±11.9 (5)
Elizabeth River	1969 YC Females (Papershell)	2.4	14.0	26.0	347±8 (3)	72.1±25.8 (3)	5.2±0.8 (3)	65.9±14.8 (3)
	Combined				347±4 (8)	105.0±17.4 (8)	5.9±0.6 (8)	74.0± 8.9 (8)

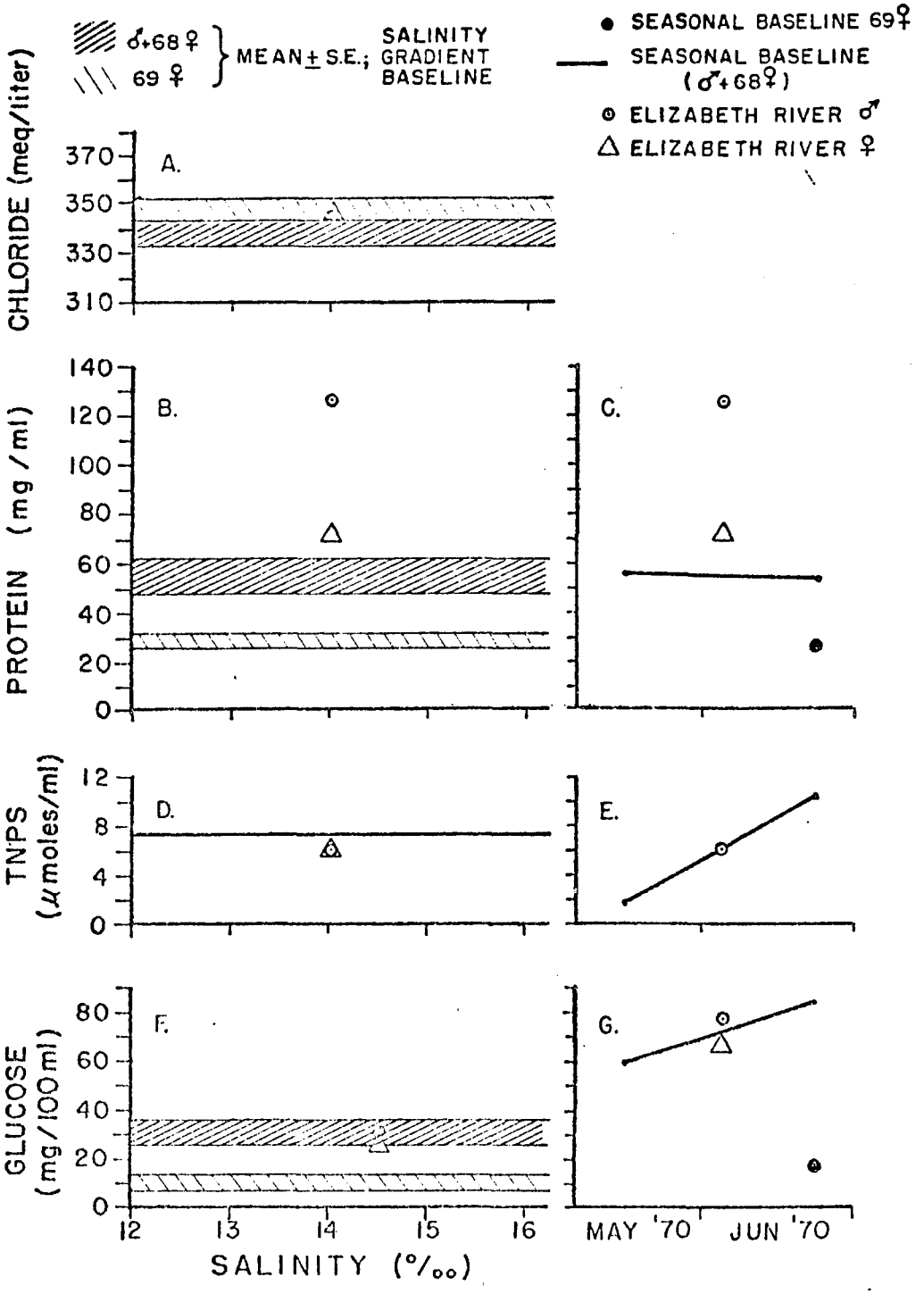

Fig. 2. Comparison of serum constituents of the blue crab, Callinectes sapidus from red tide waters (Elizabeth River) with baseline values.

Serum glucose in the new year class females from the Elizabeth River is significantly higher than that in new year class baseline female crabs (Fig. 2g and Fig. 2f). The Elizabeth River serum glucose was corrected for hyperglycemia prior to comparison with the salinity gradient baseline serum glucose values. Mean serum protein of new year class female and male crabs from the Elizabeth River are significantly higher, respectively than mean serum proteins in new year class females and males and old year class females in both baselines (Fig. 2b and Fig. 2c).

NASSAWADDOX CREEK SAMPLES

Mean values of serum constituents of the Nassawaddox Creek crabs are presented in Table 2. When the mean serum constituent values of the Nassawaddox Creek crabs are compared with the baseline data (Fig. 3), the mean serum protein and mean serum osmotic concentration of the baseline were significantly higher. No differences between sample serum mean values and baseline values were found for serum chloride, TNPS or glucose. Coefficient of variability of the serum glucose of Nassawaddox crabs (117%) was approximately twice that of the salinity gradient baseline crabs (57%) or the seasonal baseline crabs (59% in each month).

TABLE 2 - SERUM CONSTITUENTS OF MATURE, HARD, MALE BLUE CRABS CALLINECTES SAPIDUS FROM NASSAWADDOX CREEK, EASTERN SHORE, VA., 18 JULY 1970 (SALINITY 16.6 o/oo; TEMPERATURE, 32.0°C).

Constituent	Units	Mean ± S. E.	(N)
Chloride	meq/1.	339 ± 5	(4)
Osmotic Concentration	milliosmols	669 ± 13	(4)
Total Protein	mg/ml	21.5 ± 7.8	(4)
Glucose	mg/100	39.2 ± 23.1	(4)
TNPS	umoles/ml	4.8 ± 0.6	(4)

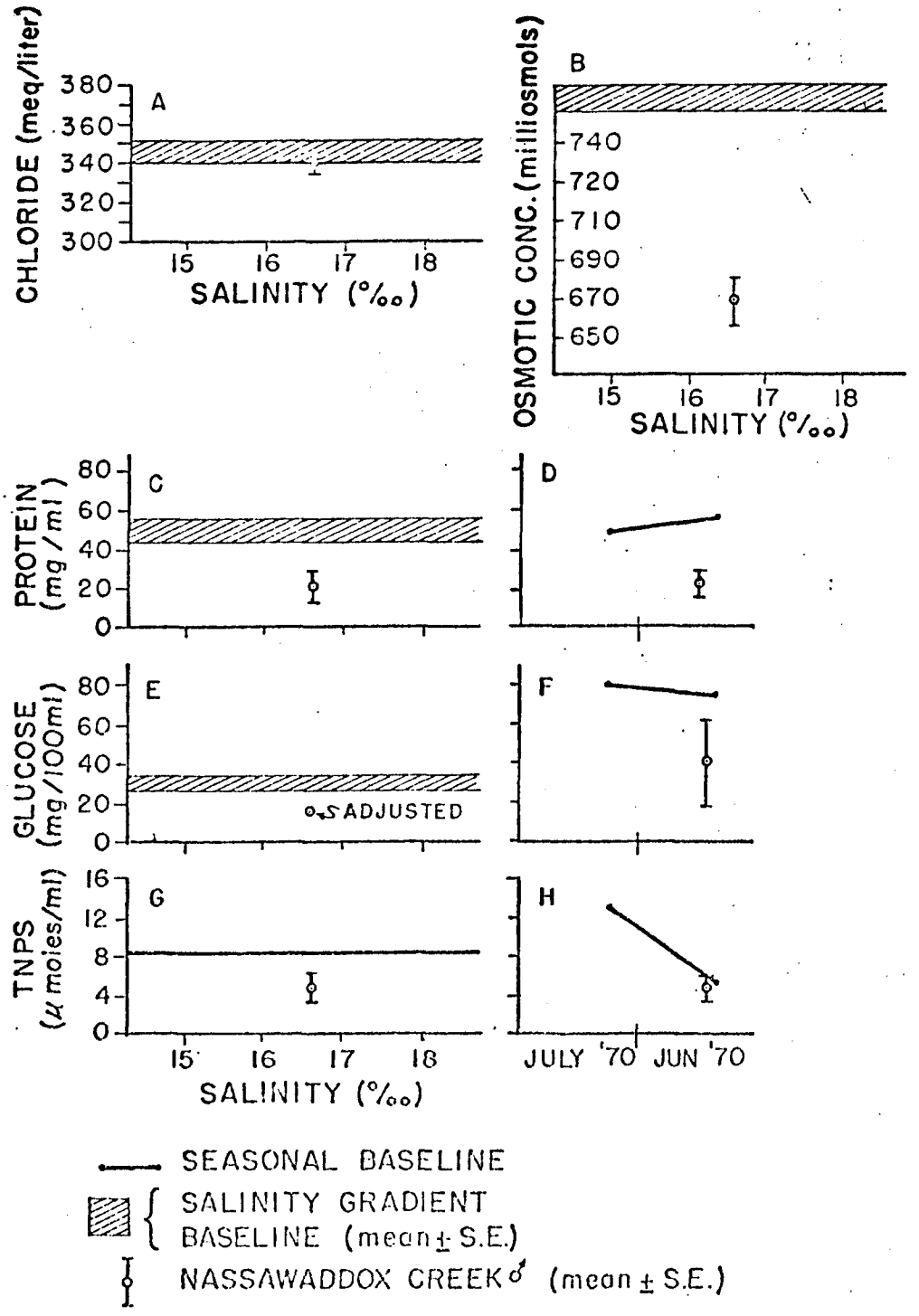

Fig. 3. Comparison of serum constituents of mature male blue crabs from the area of a recent DDT caused crab kill (Nassawaddox Creek) with baseline values.

LABORATORY STUDIES

The analyses of serum constituents in the 20 November 1970 thermal stress experiment are summarized in Table 3. No differences were found between means of serum constituents in the control and experimental groups at the start of the study, nor between means in the control animals at the start and end. A significant increase in mean serum glucose and a significant decrease in mean serum chloride occurred in the experimental crabs during the study. Significantly higher mean serum TNPS and osmotic concentration are found in experimental animals compared to control animals at the end of the study.

No differences are found between serum chloride, glucose, protein or TNPS in experimental animals and baseline animals at the start of the study. Serum osmotic concentration in experimental animals was significantly lower than the baseline value (Fig. 4). At the end of the experiment, mean serum TNPS and glucose were higher in stressed animals than baseline values. Approximately the same level of hyperglycemia is found in experimental crabs and the unadjusted baseline values (Fig. 4d).

Results of the thermal experiment conducted in December 1970 are presented in Table 4. Comparison of mean serum values with baseline values are shown in Fig. 5. Significantly lower mean serum protein, TNPS and osmotic concentration are found in the experimental group compared with the baseline at the start of the study.

TABLE 3 - SERUM CONSTITUENTS OF MATURE FEMALE BLUE CRABS, CALLINECTES SAPIDUS USED IN A THERMAL STRESS EXPERIMENT ON 20 NOVEMBER 1970.

	CONTROL			EXPERIMENTAL		
	Mean ± S. E. (N) Start	End	Difference[1]	Mean ± S. E. (N) Start	End	Difference[1]
Salinity (o/oo)	21.2	21.2	0.0	21.2	21.5	+ 0.3
Temp (°C)	13.9	13.9	0.0	13.9	35.8	+21.9
Protein (mg/ml)	47.2 ± 8.5 (8)	40.2 ± 7.5 (8)	-7.0 ± 5.6 (8)	50.2 ± 7.9 (8)	42.7 ± 6.6 (8)	-8.1 ± 1.7 (8)
Chloride (meq/liter)	418 ± 9 (8)	440 ± 8 (8)	26 ± 8 (8)	428 ± 4 (8)	399 ± 10 (8)	-29 ± 7 (8)
Glucose (mg/100 ml)	5.3 ± 1.7 (8)	8.3 ± 2.8 (7)	4.2 ± 1.9 (7)	11.7 ± 4.5 (8)	47.1 ± 7.7 (8)	35.4 ± 7.6 (8)
Ninhydrin Positive substances (umoles/ml)	3.9 ± 2.3 (5)	2.0 ± 0.4 (8)	-1.9 ± 1.7 (5)	27 ± 0.7 (2)	21.8 ± 4.5 (7)	11.9 (1)
Osmotic concentration (milliosmols)	834 ± 7 (8)	828 ± 4 (8)	-14 ± 8 (8)	818 ± 14 (8)	854 ± 7 (8)	36 ± 17 (8)

[1] End value - Start value: Figure represents mean of individual differences not difference of means.

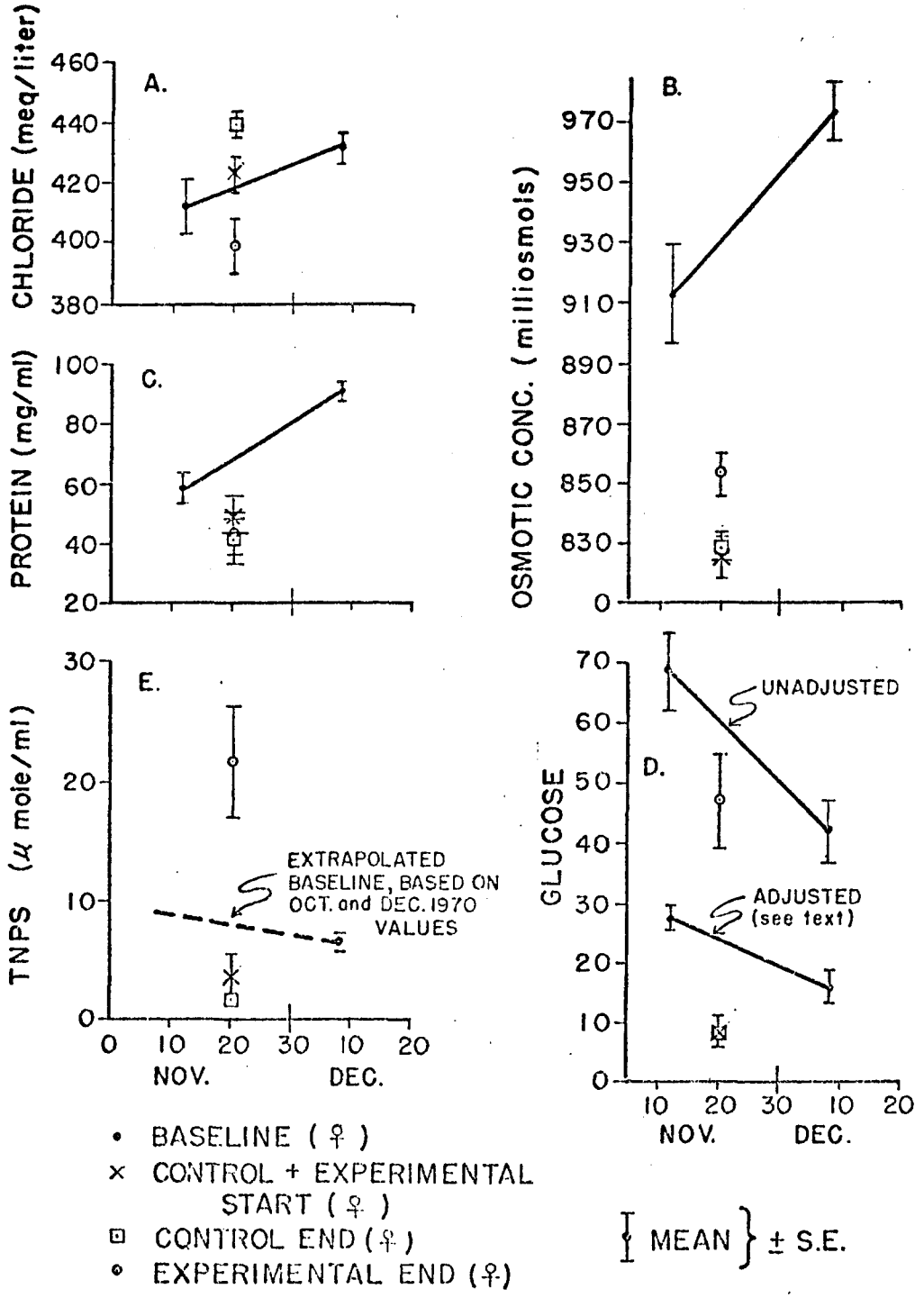

Fig. 4. Comparison of serum constituents of mature female blue crabs, Callinectes sapidus used in a thermal stress experiment 20 November 1970 with baseline values.

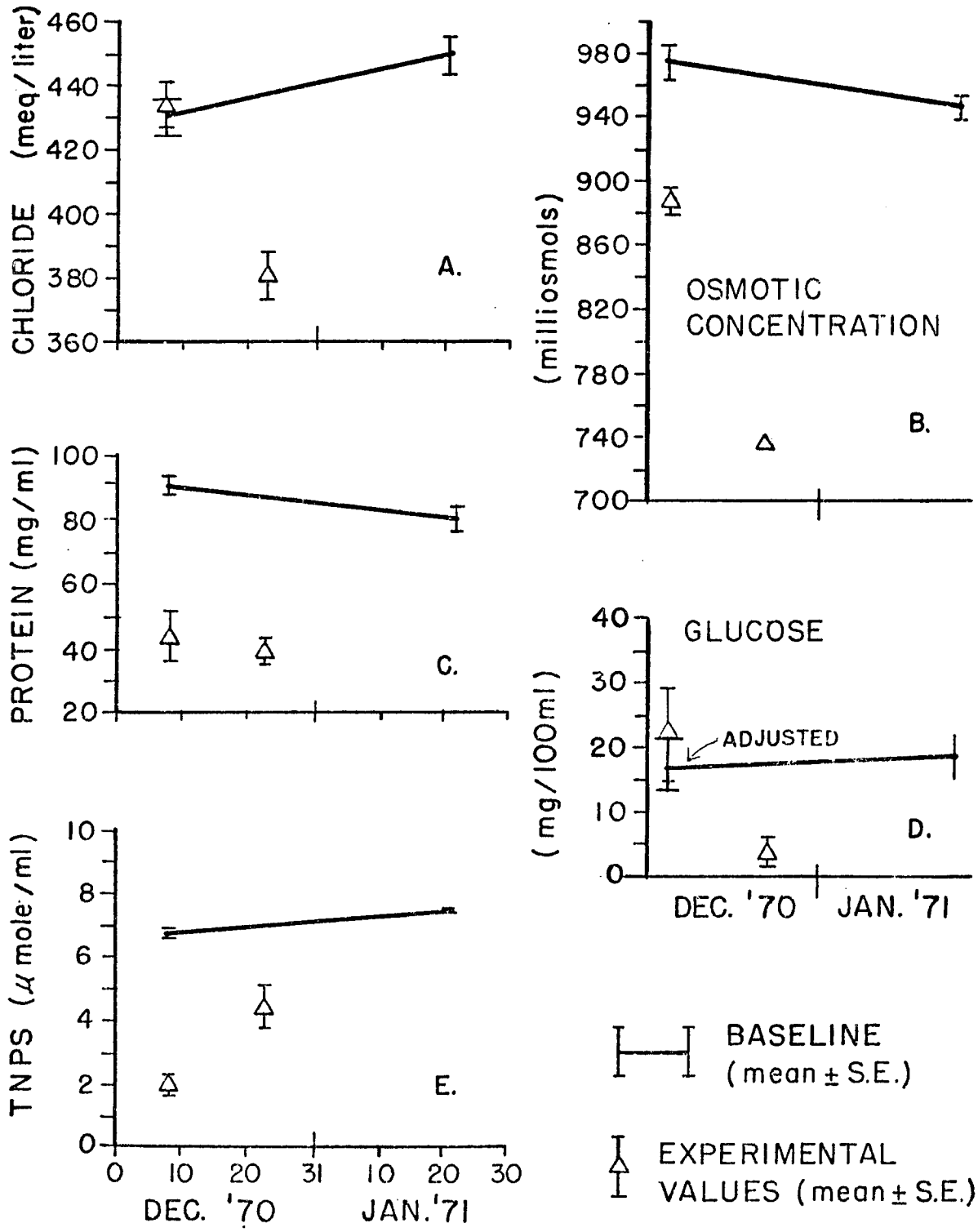

Fig. 5. Comparison of serum constituents of mature female blue crabs, Callinectes sapidus used in thermal experiment 8-23 December 1971.

TABLE 4 - SERUM ANALYSIS OF MATURE FEMALE BLUE CRAB, CALLINECTES SAPIDUS BROUGHT FROM AN AMBIENT TEMPERATURE OF 8.0°C TO 19.0°C IN 24 HOURS AND MAINTAINED FOR 15 DAYS AT 19.0°C.

	Start of Experiment Mean ± S. E. (N)	End of Experiment Mean ± S. E. (N)
Salinity (o/oo)	20.5	20.5
Temperature (°C)	8.0	19.0
Protein (mg/ml)	41.6 ± 3.9 (16)	39.3 ± 4.1 (5)
Chloride (meq/liter)	434 ± 7 (16)	380 ± 6 (5)
Glucose (mg/100 ml)	23.5 ± 7.4 (16)	3.6 ± 2.2 (5)
Ninhydrin Positive Substances (umoles/ml)	2.0 ± 0.4 (14)	4.4 ± 0.8 (5)
Osmotic Concentration (milliosmols)	883 ± 9 (16)	737 ± 4 (5)

At the end of the 15 day period, mean serum chloride, osmotic concentration and protein are significantly lower than the baseline. When means at the beginning and end of the study are compared, serum chloride and osmotic concentration are significantly lower and TNPS is significantly higher at the end. The coefficient of variation (106%) for the initial glucose values of the crabs that died during the first 24 hours of the study was approximately four times as high as the coefficient of variation (27%) of the initial glucose values of the crabs that survived the full 15 days (Table 5).

TABLE 5 - MEAN SERUM GLUCOSE LEVELS OF MATURE FEMALE BLUE CRABS, CALLINECTES SAPIDUS USED IN THE THERMAL EXPERIMENT CONDUCTED 8 - 23 DECEMBER 1970.

	(N)	mg/100 ml ± S. E.	Coefficient of variation %
Initial values all crabs	16	23.5 ± 7.4	125
Initial values crabs that died first 24 hours	7	38.6 ± 15.5	106
Initial values crabs that survived 15 days	5	11.4 ± 1.4	27
Final values crabs that survived 15 days	5	4.4 ± 0.8	40
Adjusted values for crabs taken from the York Spit area 8 Dec 1970	26	16.7 ± 1.8	54

DISCUSSION

Although no causal relationships have been demonstrated, there is evidence that different stress conditions can alter the levels of some serum constituents of mature blue crabs. Of the constituents examined in this study, serum glucose appears to be the most reliable indicator of stress. Serum glucose levels were elevated by holding the crabs out of water (Kleinholz et al., 1950), by thermal stress, by association with "red tide" organisms, and by holding under laboratory conditions.

Other serum constituents did not respond in the same manner to different stresses. Total serum protein levels were elevated in crabs associated with a red tide bloom, but depressed in crabs from the area where DDT had caused a crab kill. Thermal stress appeared to have no effect on serum protein levels. TNPS appeared unaffected by red tide and DDT, but was elevated by thermal stress. Serum chloride also appeared unaffected by red tide and DDT, but decreased under thermal stress, a response which follows the normal temperature induced seasonal pattern of chloride variation found in the baseline studies. Serum osmotic concentration, however, did not follow this normal response pattern when subjected to thermal stress. The increase in serum osmotic concentration

under conditions of thermal stress indicates that some additional component or components are being added to the blood in relatively large amounts or that water (and chloride, since chloride responds "normally") is being removed from the blood. The depressed levels of osmotic concentration (and to a lesser extent serum protein) found in crabs being held in the laboratory indicates that these crabs are physiologically different from those in the field.

The success in demonstrating abnormal values of certain serum constituents in blue crabs associated with stress conditions, in contrast with Jeffries (1966) study is due mainly to the existence of "baseline" values for comparison. Future studies aimed at developing physiological indices of condition should include the determination of baseline values.

It is possible at this time to develop an index of condition for blue crabs based on serum glucose concentration. Although, higher and more variable serum glucose levels in crabs from one area compared to another area (or a baseline) would indicate a possible stressed population, the cause of the stress would not be known. The depressed serum osmotic concentration in crabs held for a week in the laboratory compared to serum osmotic concentration of field animals, if further substantiated, might provide an index, along with serum glucose when blue crabs are truly, if ever, "acclimated" to laboratory conditions.

To associate specific causes of stress with a physiological index, it will probably be necessary to develop an index which incorporates a combination or profile of different constituents. Future efforts to develop more sensitive physiological indices should concentrate on specific entities making up some of the serum fractions examined in this study, such as the individual free amino acids in the TNPS pool or individual proteins in the total serum protein fraction. Evidence has already been reported that some stress conditions, including disease and parasitism, are associated with specific changes in free amino acid pools (Feng et al., 1970; Schafer, 1961, 1963; Senft, 1967) and specific enzyme activity (Mengebier & Wood, 1967, 1969), in other invertebrates.

Development of physiological indices of condition should provide both resource managers and resource scientists with an important technique for the study or understanding of ecological relationships. Some specific phenomena which might be recognized at an early stage, when mitigating actions might be possible, could include development of susceptibility ot specific diseases, deterioration of water quality due to addition of various pollutants, presence of naturally produced material in the water (e.g. toxins from "red tide" blooms) and stress caused by abnormal environmental conditions (e.g. unusual temperature, salinity or oxygen levels or combinations).

REFERENCES

Churchill E. P. Jr. (1919) Life history of the blue crab. Bull. Bur. Fish., Wash. 36, 91-128.

Feng S. Y., Kairallah E. A. & Canzonier W. J. (1970) Hemolymph-free amino acids and related nitrogenous compounds of Crassostrea virginica infected with Bucephalus sp. and Minchinia nelsoni. Comp. Biochem. Physiol. 34, 547-566.

Florkin M. (1960) Blood chemistry. In The Physiology of Crustacea (Edited by Waterman T. H.), Vol. 1, pp. 141-159. Academic Press, New York.

Gilbertson D. E., Etges F. J. & Ogle J. D. (1967) Free amino acids of Australorbis glabratus hemolymph: Comparison of four geographic strains and effect of infection by Schistosoma mansoni. J. Parasitol. 53, 565-568.

Haven D. (1962) Seasonal cycle of condition index of oysters in the York and Rappahannock Rivers. Proc. Natl. Shellfish. Assoc. 51, 42-66.

Jeffries H. P. (1964) Indices of ecological condition in marine organisms -- a trial study. In Symposium on Experimental Marine Ecology (Edited by Marshall N., Jeffries H. P., Napora T. A. and

Sieburth J. NcN.) pp. 59-68. Occasional Publ. No. 2 Grad. School. Oceanogr., Univ. of Rhode Island, Kingston.

Jeffries H. P. (1966) Internal condition of a diminishing blue crab population (Callinectes sapidus). Chesapeake Sci. 7, 164-170.

Jeffries H. P. (1969) Seasonal composition of temperate plankton communities: free amino acids. Limnol. Oceanogr. 14, 41-52.

Jeffries H. P. (1970) Seasonal composition of temperate plankton communities: fatty acid. Limnol. Oceanogr. 15, 419-426.

Jeuniaux C. (1971) Hemolymph - Arthropoda. In Chemical Zoology (Edited by Florkin M. & Scheer B. T.), Vol. 6, Arthropoda, Part B. pp. 64-118. Academic Press, New York.

Kleinholz L. H., Havel V. J. & Reichart R. (1950) Studies in the regulation of blood-sugar concentration in crustaceans. II. Experimental hyperglycemia and the regulatory mechanisms. Biol. Bull. mar. biol. Lab. Woods Hole 99, 454-468.

Mengebier W. L. & Wood L. (1967) Respiratory enzymes in mantle homogonates of Crassostrea virginica and the effects of Minchinia nelsoni (MSX) and Cochlodinium helicoides on activity levels. Comp. Biochem. Physiol. 21, 611-617.

Mengebier W. L. & Wood L. (1969) The effects of *Minchinia nelsoni* infection on enzyme levels in *Crassostrea virginica* - II. Serum phosphohexose isomerase. Comp. Biochem. Physiol. 29, 265-270.

Passano L. M. (1960) Molting and its control. In *The Physiology of Crustacea* (Edited by Waterman T. H.) Vol. 1, pp. 473-536. Academic Press, New York.

Pearson J. C. (1948) Fluctuations in the abundance of the blue crab in Chesapeake Bay. Res. Rep. U.S. Fish. Wildl. Serv. No. 14, 26 pp.

Schafer R. D. (1961) Effects of pollution on the free amino acid content of two marine invertebrates. Pac. Sci. 15, 49-55.

Schafer R. D. (1963) Effects of pollution on the free amino acid content of *Mytilus edulis*. Pac. Sci. 17, 246-250.

Senft A. W. (1967) Studies in arginine metabolism by schistosomes - II. Arginine depletion in mammals and snails infected with *S. mansoni* or *S. hematobium*. Comp. Biochem. Physiol. 21, 299-306.

Snedecor G. W. (1956) Statistical methods applied to experiments in agriculture and biology. p. 534. Iowa State University Press, Ames.

Stewart J. E., Cornick J. W. & Dingle J. R. (1967a) An electronic method for counting lobster (*Homarus*

americanus Milne-Edwards) hemocytes and the influence of diet on hemocyte numbers and hemolymph proteins. Can. J. Zool. 45, 291-304.

Stewart J. E., Cornick J. W., Foley D. M., Li M. F. & Bishop C. M. (1967b) Muscle weight relationship to serum proteins, hemocytes and hepatopancreas in the lobster, Homarus americanus. J. Fish. Res. Bd. Can. 24, 2339-2354.

Stewart J. E. & Li M. F. (1969) A study of lobster (Homarus americanus) ecology, using serum protein concentration as an index. Can. J. Zool. 47, 21-28.

Telford M. (1968) The effects of stress on blood sugar composition of the lobster, Homarus americanus. Can. J. Zool. 46, 819-826.

U. S. Department of Interior (1968) Water Quality Criteria: Report of the National Technical Advisory Committee to the Secretary of the Interior. U.S. Government printing Office Washington, D. C. 234 p.

Van Engel W. A. (1958) The blue crab and its fishery in Chesapeake Bay. Part I - Reproduction, early development, growth and migration. Comml. Fish. Rev. 20, 6-17.

Van Engel W. A. (1962) The blue crab and its fishery in Chesapeake Bay, Part 2 - Types of gear for hard crab fishing. Comml. Fish. Rev. 24, 1-10.

VITA

Maurice Patrick Lynch

Born in Boston, Massachusetts, 24 February 1936. Graduated East Bridgewater High School, East Bridgewater, Massachusetts, June 1953. Received A.B. (Biology) Harvard College, Cambridge, Massachusetts, June 1957. Served with U.S. Navy July 1957-June 1962. Received M.A. (Marine Science) College of William and Mary in Virginia, August 1965. Instructor Biology Department, College of William and Mary in Virginia February-June 1966. National Institute of Health Pre-Doctoral Fellow July 1966-July 1968. Graduate Research Assistant, Virginia Institute of Marine Science August 1968-October 1962. Accepted position as Associate Marine Scientist, Virginia Institute of Marine Science 1 November 1971.