MIDGUT GLAND RESPIRATION IN THE ESTUARINE CRAB, HEMIGRAPSUS NUDUS (DANA)

by

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ABSTRACT

Weight-specific oxygen consumption of midgut gland tissue of Hemigrapsus nudus has been investigated at three levels of salinity (35%, 75% and 125% sea water), two levels of experimental temperature (5°C and 20°C) and four acute (Warburg) temperatures (5°, 10°, 15° and 20°C) in all combinations for each season (summer and winter).

Metabolic-temperature curves reveal that at standard baseline conditions where the animals are held 24 hr at their respective seasonal temperature and salinity, midgut gland respiration is highest at all acute temperatures in the summer animals. Acutely measured metabolic-temperature curves for midgut gland tissue show that winter animals acclimated to their opposite seasonal conditions of temperature and salinity for 10 days demonstrate the greatest degree of acclimation.

The effect of experimental temperature is statistically and biologically significant. The highest respiration rate is at 5°C. Low temperature (5°C) may provide a greater thermal stress than a high temperature (20°C) resulting in a higher rate of oxygen consumption. Experimental temperature also influences the seasonal respiratory response of midgut gland tissue to salinity. In summer animals there is no correlation of midgut gland respiration to salinity at 5°C. There is a increase in respiration rate as the osmotic gradient between the blood and medium

increases at the seasonal baseline temperature of 20° C. Winter animals held at the seasonal baseline temperature of 5° C demonstrate a "V-shaped" relationship to salinity with the lowest respiratory response in 75% sea water where the gradient between the blood and medium is minimal. Animals held at 20° C increase respiration with an increase in salinity.

It is suggested that the metabolic activity of midgut gland from summer animals may be related to the maintenance of a osmotic gradient between the blood and medium or alternatively to the energy demands associated with new exoskeleton formation. The proposal is put forth that midgut gland respiration in winter animals may indicate osmotic work being done to maintain the osmotic gradient between the blood and medium. The production of a urine hypotonic to the blood may also assist winter animals in regulation of blood electrolytes.

The regression coefficients of weight-specific oxygen consumption as a function of body weight were not significantly different from zero at the 0.01 probability level.

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INTRODUCTION

The midgut gland is physiologically one of the most important organs in crustacea. It secretes digestive enzymes, absorbs and transforms food and is the major depot for the storage of mineral and food reserves. Travis (1955, 1957) and Weel (1955) have demonstrated these functions in the spiny lobster, Panulirus argus, and in the brachyuran crab, Atya spinipes, by observations on histological and histochemical changes of the gland and connective tissue.

The midgut gland itself is composed of a number of blind-ending tubules seperated from one another by connective tissue. The tubules open into secondary secretion ducts which, in turn, open into a primary or collecting duct through which the digestive fluid is poured into the midgut (Travis, 1955; Weel, 1955).

There are very few studies which have concentrated upon an examination of metabolic activity in excised invertebrate tissue. Hopkins (1930) found in both red and white muscle of the posterior adductor of the clam, Venus mercenaria, a decrease in respiration from young to old clams when respiration measurements were made at 27.5°C. The body size and number of annual growth rings were used as criteria of age. In a later paper Hopkins (1946) showed, that in excised gill tissue of Venus mercenaria, respiration was higher in the cold-adapted animal (below 20°C) than the warm-adapted animal (27°C) when measured at a inter-

mediate temperature of 25° C. Vernberg (1956) demonstrated a relationship between oxygen consumption of excised gill tissue with habitat and activity of several species of marine decapod crustacea at 27° C. Roberts (1957b) found acclimation in muscle tissue only at a high temperature (23.5° C) and not at all in isolated brain tissue in the striped rock crab, Pachygrapsus crassipes. Weight-specific oxygen consumption of excised gill in Hemigrapsus nudus and H. oregonensis showed that tissue from summer animals respires at a higher rate than tissue from winter animals over the physiological temperature range of 5° C to 20° C (Dehnel and McCaughran, 1964). King (1965) demonstrated an increase in oxygen consumption of excised gill in the crab, Callinectes sapidus (brackish) and C. sapidus (marine), by 30% and 10%, respectively, when transfered from 80% to 50% sea water. Recently, Vernberg and Vernberg (1966) found in heart, muscle and brain tissue of tropical and temperate zone fiddler crabs, Uca sp., a common tendency for respiration measurements to be higher in warm-adapted animals when rate determinations were made between 10° C or 15° C and 25° C.

Respiration studies on midgut gland tissue are even more sparsely documented. Belding et al. (1942) established that there are no metabolic gradients in midgut gland for the kelp crab, Pugettia producta, when comparing the anterior, median and hind parts of the gland. The Q_{O_2} was found to vary as an inverse function of body size (carapace length) with a negative regression value of -0.285 for both sexes

at 15°C. Weymouth *et al.* (1944) demonstrated at 15°C in the midgut gland of the same crab a weight-specific oxygen consumption regression to body weight of -0.203. The oxygen consumption of excised midgut gland from nine species of marine decapod crustacea has shown a direct correlation with activity when comparing animals within any one habitat (Vernberg, 1956). Minamori (1964) found that the activity of hepatopancreas catalase at 0°C showed a positive regression b value to body weight of 0.80 to 0.81 in three races of the loach fish, *Cobitis taenia striata*.

To adequately evaluate environmental effects on tissue or whole animal respiration one should use a multi-factorial approach where several environmental factors are examined simultaneously. Most studies have concentrated on a uni-factorial analysis on which to base conclusions. As Kinne (1963) points out, such an analysis may give conclusions that have no validity ecologically since the organism responds to the whole environment, not to isolated single factors. The purpose of the present study is to consider those factors which are assumed to be the most important in defining the relationship of midgut gland respiration to the intact animal and in turn to the environment. A multi-factorial design is utilized to accomplish this end. The effect of temperature, salinity and seasonal changes in these factors is examined in terms of weight-specific oxygen consumption of excised midgut gland of the shore crab, *Hemigrapsus nudus*, over the physiological temperature

range of 5°C to 20°C.

The term "main effect" will be used to describe effects of a single factor (eg. salinity, temperature) averaged for all acute temperatures, experimental temperatures and salinities and both seasons. The term "interaction" (eg. salinity-temperature combination) refers to the combined effect of factors where differences in response to one factor varies with the level of another factor which is applied simultaneously (Steel and Torrie, 1960). A further discussion of the concept of interaction will follow.

The acute temperatures (5°, 10°, 15° and 20°C) refer to the Warburg water-bath temperatures to which the tissue samples are equilibrated and at which oxygen consumption values are recorded. Experimental temperatures (5°C and 20°C) and experimental salinities (35%, 75% and 125% sea water) are the physical parameters to which the animals are acclimated for 10 days or held at for 24 hr for standard baseline measurements.

Acclimation, as used in the context of this study, will refer to a phenotypic alteration in metabolic activity due to change in salinity, temperature or seasonal changes in these factors when measured over the physiological temperature range of 5°C to 20°C. This definition will include also the liability for genotypic change as reflected in a phenotypic alteration of metabolism. Compensation and adaptation will be used with the same connotation as acclimation and will include the concept of homeostasis. Homeostasis is a mechanism by which the animal physiologically maintains internal constancy

despite changes in the environment.

The term hepatopancreas is a misnomer when applied to crustacea and will not be used in the text of this study. Instead, the term midgut gland will be used in place of hepatopancreas. Belding et al. (1942) point out, "Since it (midgut gland) is without homology in the mammal, the term ' "liver" ' and ' "hepatopancreas" ' are unjustified. Because it develops from the midgut, the term ' "midgut gland" ' identifies it without chance of error and with no misleading implications as to function."

MATERIAL AND METHODS

The crab, Hemigrapsus nudus, was obtained from Spanish Bank (Lat. 49° 17'N.; Long. 123° 07'W.) Vancouver, British Columbia. Summer animals were collected from June through August and winter animals were collected from November through March. The summer period is characterized by a average temperature of 20°C and a salinity of 35‰ (11‰) sea water. The winter season is characterized by a relatively stable temperature of 5°C and a salinity of 75‰ (24‰) sea water. The average summer and winter temperatures and salinities serve as standard baseline conditions for the respective seasons.

The standard sea water of 100‰ (32‰) used in this study is based on a chlorinity of 17.65‰ and a salinity of 31.88‰. The proportions of the major ions in 100‰ sea water, as determined by laboratory analysis, are as follows:

Na:	433.0	mEq./l.
K :	10.1	" "
Ca:	25.6	" "
Mg:	97.9	" "
Cl:	497.0	" "

The ions are complexed as chlorides, plus the sulfate of sodium. Two experimental temperatures, 5°C and 20°C (±1°C) and three experimental salinities, 35% (11‰), 75% (24‰) and 125% (40‰) sea water (±1% sea water) were used in all combinations for each season (summer and winter). The animals were held 5, 10 and 15 days at these experimental combinations to determine the time period that resulted in the maximum degree of acclimation. A 10 day time period gave the maximal acclimation response.

Only adult intermolt male crabs were selected for study. They ranged in weight from 6.0 to 10.0 g. The wet weight of the whole animal after damp drying was weighed to the nearest 0.01 g. In the laboratory the animals were immediately placed in plastic containers holding 3.5 liters of the appropriate (35%, 75% or 125%) sea water. Sea water was aerated and the containers were placed in a refrigerator at constant temperature (5°C or 20°C) in total darkness. The water was changed daily. At standard baseline conditions the animals were held 24 hr. Those animals acclimated in the laboratory were held 10 days and not fed. Only 10-12 animals were held in each container at any one time to minimize mortality due to crowding. Crabs that molted during the 10 day acclimation period were discarded.

The direct method of Warburg was used to measure respiration rates of midgut gland (Umbriet, Burris and Stauffer,

1957). The Gilson Medical Electronics (Warburg) respirometer was made available for this work.

Tissue samples excised from the animals ranged in weight from 0.3 to 0.6 g. The excised tissue samples were placed directly in pre-weighed aluminum pans and weighed to the nearest 0.1 mg. The tissue samples were not damp dried on filter paper prior to weighing. The delicate nature of the tubles made it difficult to pick the gland off filter paper without fragmenting the tissue with subsequent loss of substrate and enzyme fluids. After weighing, the tissue samples were placed in the Warburg reaction flasks containing 3.0 ml of physiological saline prepared as follows:

850.0	ml	of	0.52 M NaCl
51.0	"	"	0.35 M $MgCl_2$
35.0	"	"	0.35 M $CaCl_2$
35.0	"	"	0.42 M Na_2SO_4
17.6	"	"	0.38 M H_3BO_3
1.3	"	"	0.48 M NaOH

pH = 7.8 $\quad\quad$ Δ_{FP} °C = -1.61

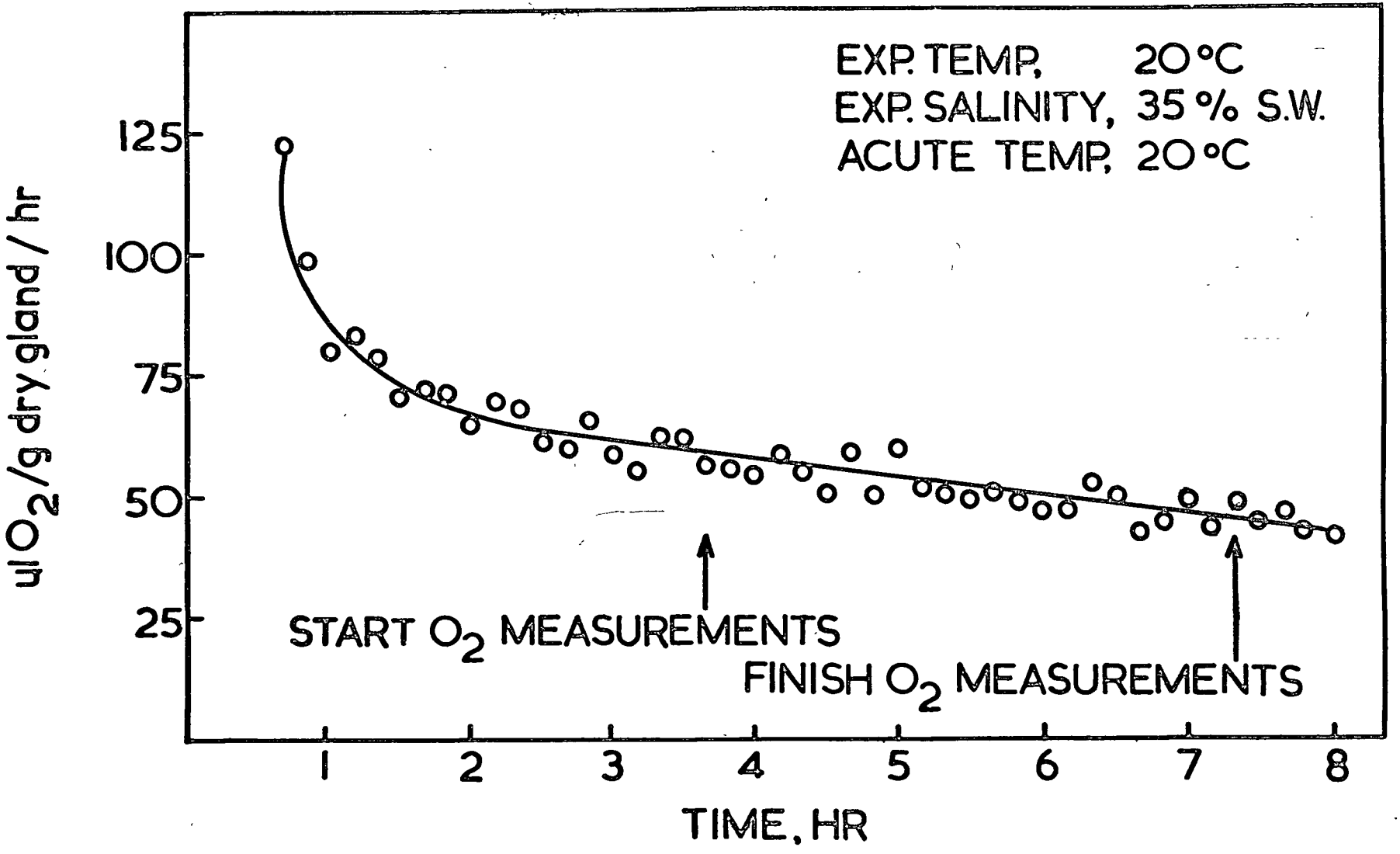
The center wells of the reaction flasks contained 0.2 ml of 15% KOH. During the dissection the flasks were kept in an ice-bath. Three hours and ten minutes after excision of the final tissue sample, the flasks were taken out of the ice-bath and attached to the manometers. The attached flasks were then placed in the constant temperature water-bath (±0.1° C) of the Warburg. The seventeen tissue samples were allowed to equilibrate for 15 min, during which time they were gassed with oxygen for 10 min. The respiratory rate was measured at four acute temperatures (5°, 10°, 15° and 20°C) for each set of experimental conditions. An exami-

nation of Figure 1 reveals that one set of tissue samples could respire at two acute temperatures since the slope of the weight-specific oxygen consumption rate curve with time shows a small, but non-significant decrease over the time interval of measurement. This is the portion of the curve between the arrows. The first set of tissue samples respired at 5°C and 10°C and the second set of tissue samples respired at 15°C and 20°C. Each set of tissue samples respired 90 min at each of the two acute temperatures with a 40 min time lapse between metabolic measurements at the first and second acute temperatures. It took 25 min to change the temperature of the water-bath 5°C and 15 min to equilibrate the tissues. The tissue samples were shaken at a constant rate of 120 oscillations/min. Nine hours from the time of dissection were required to complete respiration measurements at two acute temperatures. One is justified in measuring the respiration of the tissue at two acute temperatures, since measurement of tissue respiration in the reverse direction (10° and 5°C or 20° and 15°C) does not significantly alter the slope of the metabolic-temperature (M-T) curves.

With the completion of an experimental run, the tissue samples and saline were placed in pre-weighed aluminum pans and dried in an oven at 100-106°C for 24 hr. Dry body weights were obtained by the same procedure. The data were expressed in $ul O_2/g$ dry gland/hr at N.T.P.

A statistical analysis of the data was performed with the aid of the 7040 IBM Computer. The data were processed

Figure 1. Weight-specific oxygen consumption as a function of time for midgut gland of Hemigrapsus nudus. Each point represents amount of oxygen consumed during a 10 min time interval. Weight of crab used for oxygen consumption measurement was 7 g. Curve is eye-fitted.


EXP. TEMP, 20°C
EXP. SALINITY, 35 % S.W.
ACUTE TEMP. 20°C
125
100
75
50
25
μl O2/g dry gland / hr
START O2 MEASUREMENTS
FINISH O2 MEASUREMENTS
1
2
3
4
5
6
7
8
TIME, HR

using the Fortran Program.

Weight-specific oxygen consumption was plotted as a function of whole body weight on double logarithmic graph paper. The data gave a straight line that took the form:

$$\frac{O_2}{W} = aW^{b-1}$$

Reduce to: $\log O_2 = \log a + b(\log W)$

where O_2 = $ul O_2/g$ dry gland/hr, a = intercept, b = slope of line and W = whole body weight. The exponent (b-1) by which body weight is raised to a given value proportional to metabolism is referred to as the regression coefficient. Slope values were considered significant at the 0.01 probability level.

Using a equal sample size of twelve, an analysis of variance was performed on the data (see Table IV). The level of significance at which the Null Hypothesis was accepted that there is no difference between treatment means was the 0.01 probability level. The values of the sum of squares and mean squares in Table IV are expressed in natural logarithms. The data upon which the statistic was performed are presented in tabulated form in Tables I and II. Only the mean values of the main effects and interactions for weight-specific oxygen consumption are expressed in the tables. These mean values are presented graphically in the subsequent figures.

Table I: Mean values of main effects for weight-specific oxygen consumption of midgut gland tissue.

Source of Variance	Mean ($ul O_2$/g dry gland/hr)
A(Acute Temp.° C)	
5	185
10	259
15	427
20	631
B(Exp. Temp.° C)	
5	375
20	303
C(Exp. Salinity, %S.W.)	
35	351
75	319
125	341
D(Season)	
Winter	344
Summer	330

Table II: Mean values of first and second-order interactions for weight-specific oxygen consumption of midgut gland tissue.

Source of Variance		Mean ($ul O_2$/g dry gland/hr)
First-Order Interactions		
AB		
A(° C)	B(° C)	
5	5	206
10		295
15		465
20		694
5	20	165
10		228
15		392
20		573
AC		
A(° C)	C(%S.W.)	
5	35	190
10		277
15		450
20		643
5	75	175
10		239
15		404
20		617
5	125	189
10		264
15		429
20		632
BC		
B(° C)	C(%S.W.)	
5	35	403
	75	329
	125	396
20	35	306
	75	310
	125	294
AD		
A(° C)	D(Season)	
5	Winter	183
10		279
15		421
20		651

Table II: Continued

5		186
10	Summer	241
15		433
20		611

BD

B(° C)	D(Season)	
5	Winter	383
20		309
5	Summer	366
20		297

CD

C(%S.W.)	D(Season)	
35		350
75	Winter	301
125		386
35		353
75	Summer	338
125		301

Second-Order Interactions

ABC

A(° C)	B(° C)	C(%S.W.)	
5			224
10	5	35	324
15			495
20			737
5			162
10	20	35	236
15			409
20			561
5			187
10	5	75	258
15			404
20			599
5			164
10	20	75	221
15			403
20			635
5			210
10	5	125	308
15			502
20			758
5			170
10	20	125	226
15			366
20			527

Table II: Continued

ABD			
A(° C)	B(° C)	D(Season)	
5			207
10	5	Winter	317
15			460
20			714
5			162
10	20	Winter	246
15			385
20			593
5			206
10	5	Summer	275
15			470
20			675
5			168
10	20	Summer	211
15			399
20			552

ACD			
A(° C)	C(%S.W.)	D(Season)	
5			181
10	35	Winter	286
15			443
20			652
5			170
10	75	Winter	223
15			355
20			586
5			199
10	125	Winter	326
15			474
20			723
5			200
10	35	Summer	267
15			457
20			634
5			180
10	75	Summer	245
15			458
20			649
5			180
10	125	Summer	214
15			388
20			553

BCD			
B(° C)	C(%S.W.)	D(Season)	
	35		457
5	75	Winter	292
	125		422

Table II: Continued

5	35	Summer	357
	75		369
	125		373
20	35	Winter	268
	75		311
	125		354
20	35	Summer	349
	75		310
	125		244

Table III: Standard error of mean weight-specific oxygen consumption of midgut gland tissue for each experimental combination of factors.

Experimental Combination of Factors				Mean ($ul O_2/g$ dry gland/hr)	Standard Error ($S_{\bar{x}}$)
Acute Temp.°C	Exp. Temp.°C	Salinity (%S.W.)	Season		
5				244	129
10	5	35	Winter	376	134
15				551	203
20				854	188
5				173	233
10	5	75	Winter	237	217
15				335	268
20				534	324
5				210	150
10	5	125	Winter	357	153
15				529	172
20				794	161
5				135	278
10	20	35	Winter	218	216
15				356	276
20				494	310
5				168	156
10	20	75	Winter	230	201
15				377	301
20				642	204
5				189	242
10	20	125	Winter	297	236
15				425	117
20				659	190

Table III: Continued

5				206	130
10	5	35	Summer	280	175
15				444	200
20				632	237
5				202	387
10	5	75	Summer	281	136
15				489	193
20				672	234
5				210	133
10	5	125	Summer	265	118
15				477	135
20				725	155
5				193	196
10	20	35	Summer	255	183
15				470	331
20				636	357
5				160	268
10	20	75	Summer	213	289
15				430	172
20				627	231
5				154	172
10	20	125	Summer	172	230
15				315	309
20				422	423

Table IV: Analysis of variance of weight-specific oxygen consumption for the factorial (4X 2X 3X 2X) experiment incorporating four acute temperatures (A), two experimental temperatures (B), three experimental salinities (C) and two seasons (D) (Steel and Torrie, 1960). The F-values are considered significant at the 0.01 probability level (**).

Source of Variance	df	Sum of Squares	Mean Squares	F
A (Acute Temp.)	3	126.56	42.185	753.3**
B (Exp. Temp.)	1	6.43	6.433	114.9**
C (Exp. Salinity)	2	0.91	0.457	8.2**
D (Season)	1	0.26	0.260	4.6**
AB	3	0.16	0.053	1.0
AC	6	0.17	0.028	0.5
BC	2	1.72	0.859	15.3**
AD	3	0.72	0.241	4.3**
BD	1	0.00	0.000	0.0
CD	2	3.35	1.674	29.9**
ABC	6	0.50	0.083	1.5
ABD	3	0.03	0.009	0.2
ACD	6	0.45	0.074	1.3
BCD	2	4.48	2.238	40.0**
Total Treatment	41	145.74	3.555	63.4**
Error	534	29.67	0.056	
Total	575	175.41		

Table V: Seasonal compensation of weight-specific oxygen consumption of midgut gland at all combinations of acute temperature (A), experimental temperature (B), experimental salinity (C) and season (D). The numbers in the body of the table represent the type of compensation: type 5, indicates that summer animals have the highest respiration rate; type 4, seasonal rates are equal; and type 3, winter animals have the highest respiration rate (Precht, 1951).

Experimental Temp. (°C)	Acute Temp. (°C)	Experimental Salinity (‰S.W.)		
		35	75	125
5	5	3	5	4
	10	3	5	3
	15	3	5	3
	20	3	5	3
20	5	5	3	3
	10	5	3	3
	15	5	5	3
	20	5	3	3

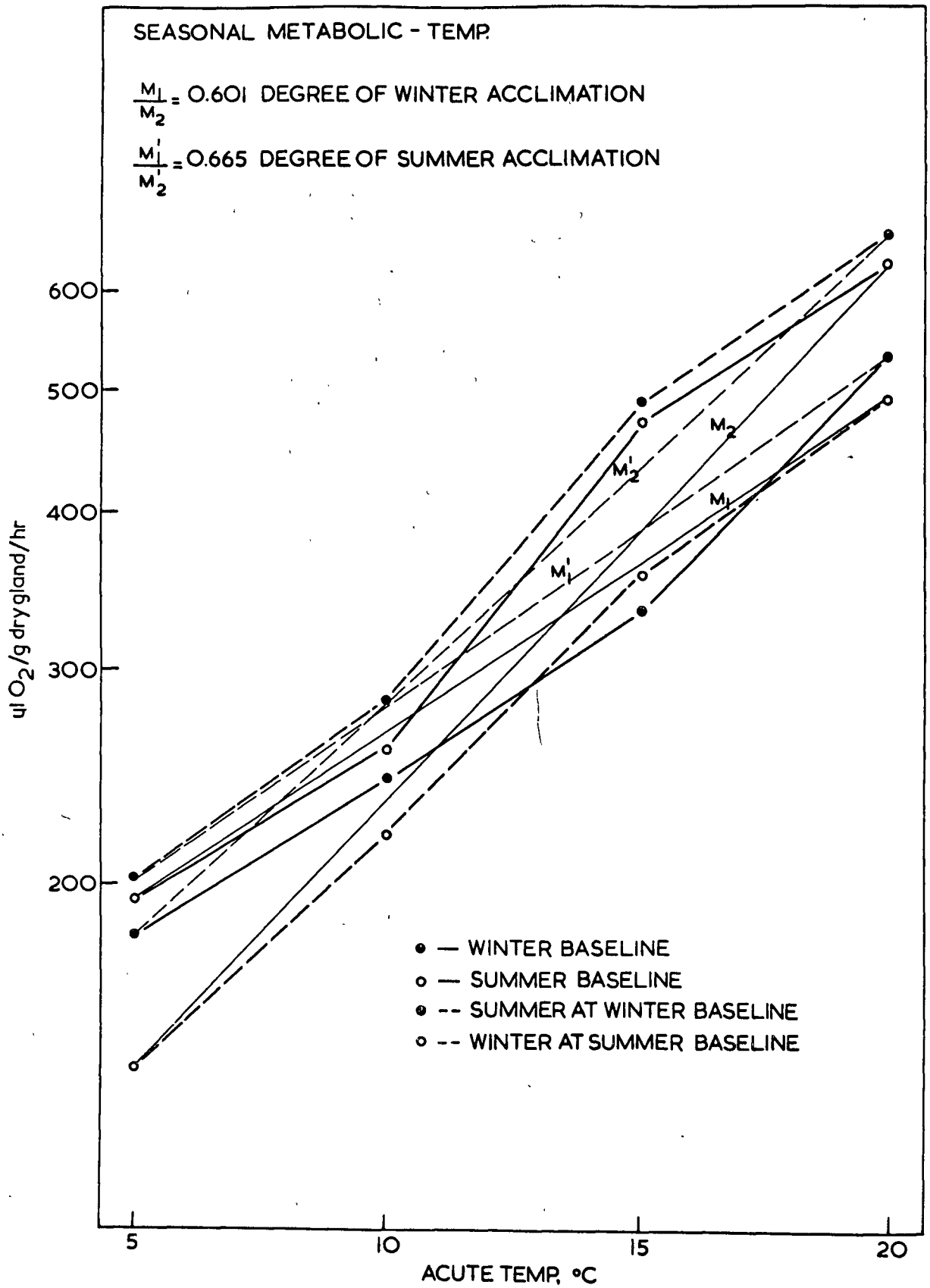
RESULTS

Seasonal Metabolic-Temperature Experiments

The data for a seasonal comparison of weight-specific oxygen consumption are presented in Table III. It may be seen by examining the standard errors that there is a great deal of variabliity in the respiratory measurements at any one acute temperature. In Figure 2 is presented a seasonal comparison of midgut gland respiration. The metabolic-temperature (M-T) curves reveal that Hemigrapsus nudus demonstrates inverse seasonal compensation, type 5 (Precht, 1951). This is illustrated by the fact that the summer baseline M-T curve (35% sea water, 20° C) is higher on the ordinate than the winter at summer baseline M-T curve (acclimated to summer baseline conditions) by 43% at 5°C, 17% at 10°C, 32% at 15°C and 29% at 20°C acute temperature. Conversely, the winter baseline M-T curve (75% sea water, 5° C) is lower on the ordinate than the summer at winter baseline M-T curve (acclimated to winter baseline conditions) by 17%, 19%, 46% and 26% at the same respective acute temperatures.

Using Precht's (1951) classification scheme, the types of seasonal compensation are indicated in Table V. Type 5, indicates that summer animals have the highest respiration rate; type 4, seasonal rates are equal; type 3, winter animals have the highest respiration rate.

Figure 2. Seasonal-metabolic temperature curves, acutely measured, for midgut gland of Hemigrapsus nudus. Winter (75% sea water, 5°C) and summer (35% sea water, 20°C) baseline animals were held 24 hr prior to experimentation. The acclimated animals were held at the opposite seasonal conditions for 10 days prior to experimentation. Each point is the mean of weight-specific oxygen consumption at the respective acute temperatures. The ratio of the slopes M_1 to M_2 and M_1' to M_2' define the degree of acclimation shown by the winter and summer animals, respectively.


SEASONAL METABOLIC - TEMP.
$\frac{M_1}{M_2}$ = 0.601 DEGREE OF WINTER ACCLIMATION
$\frac{M_1'}{M_2'}$ = 0.665 DEGREE OF SUMMER ACCLIMATION
600
500
400
300
200
µl O_2/g dry gland/hr
M_2
M_2'
M_1
M_1'
● — WINTER BASELINE
○ — SUMMER BASELINE
◉ -- SUMMER AT WINTER BASELINE
◎ -- WINTER AT SUMMER BASELINE
5
10
15
20
ACUTE TEMP. °C

In comparing the degree to which acclimation has been achieved, the compensation temperature coefficient as first suggested by Roberts (1952) may be applied to the present data (see Rao, 1953). The coefficient value is a ratio between two slopes, M_1 and M_2. The slope M_1 is drawn between the highest respiration rate of the winter at summer baseline M-T curve and the lowest respiration rate of the summer baseline M-T curve. The slope M_2 is the reciprocal of slope M_1. The same graphing procedure applies to slopes M_1' and M_2'. In this case slope M_1' is drawn between the highest respiration rate of the winter baseline M-T curve and the lowest respiration rate of the summer at winter baseline M-T curve. The slope M_2' is the reciprocal of slope M_1' (Fig. 2). The ratio is less than one if there is any compensation and approaches zero as the degree of compensation increases.

The compensation coefficient between winter at summer baseline M-T curve and summer baseline M-T curve is 0.601. The coefficient value between the winter baseline M-T curve and summer at winter baseline M-T curve is 0.665. A comparison of these coefficient values indicates that winter animals show the greatest degree of compensation (lower coefficient value). This point is brought out in Table V.

Main Effects

Effect of Season (D)

The effect of season on midgut gland respiration is

significant (Table IV). This effect was determined by comparing the mean of all winter respiration data with the mean of all summer respiration data.

Midgut gland demonstrates inverse seasonal compensation, type 5, for all acute temperatures at standard baseline conditions of 35% sea water, 20°C (summer) and 75% sea water, 5°C (winter). Except for two cases, partial seasonal compensation, type 3, is demonstrated at all other experimental combinations of acclimation temperature or acclimation salinity (Table V).

Effect of Acute Temperature (A)

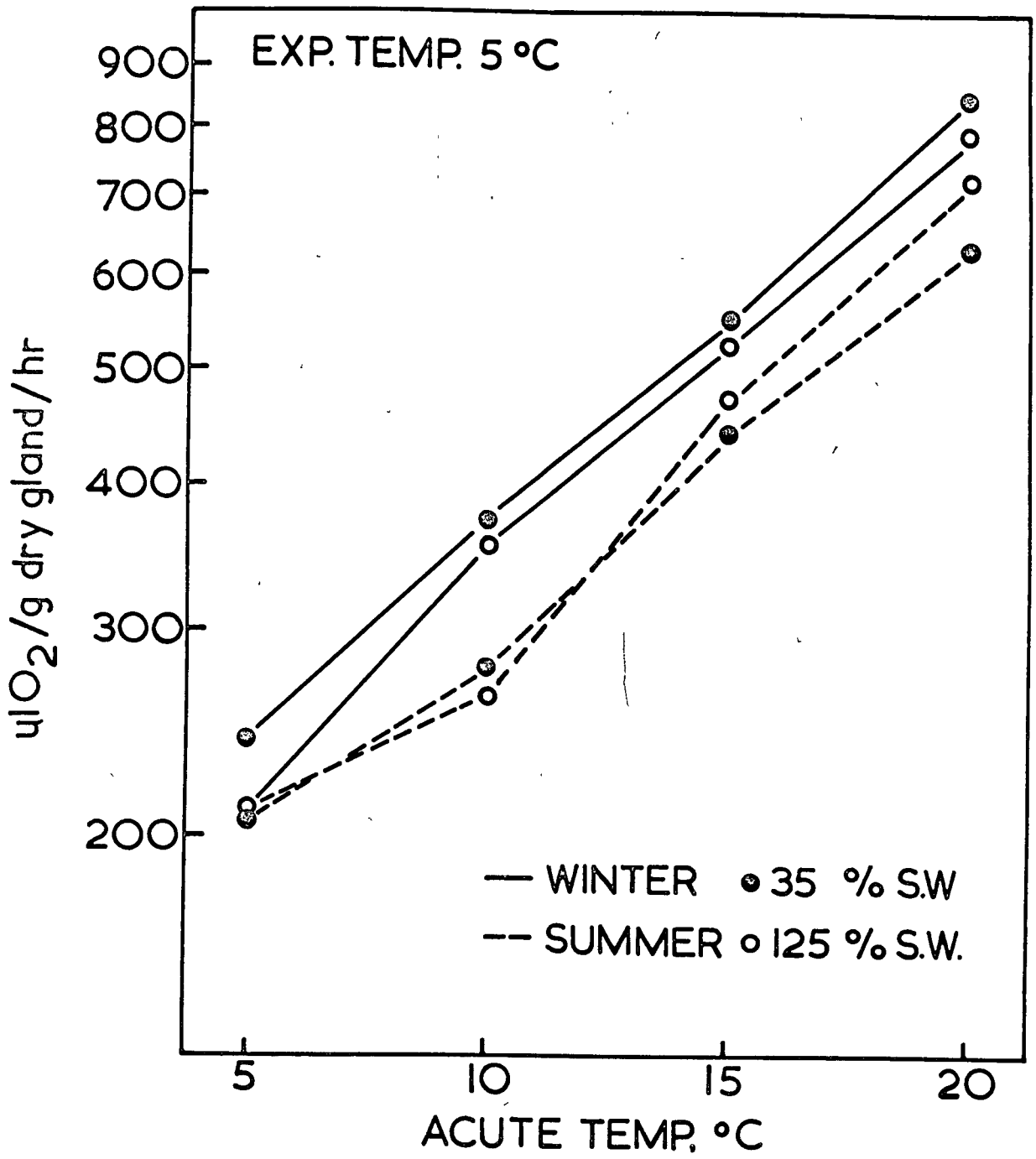
An examination of Table IV reveals that the main effect of acute temperature is significant. This effect was derived by comparing the means of the four acute temperatures after averaging all the respiration data at each acute temperature.

Figure 3 is a typical representation of acute temperature effects on midgut gland respiration. As the acute temperature rises the rate of tissue respiration is increased. It may be seen that the acutely measured metabolic-temperature curves are influenced by season, experimental temperature and experimental salinity interactions. These effects will be examined shortly.

Effect of Experimental Temperature (B)

The main effect of experimental temperature was determined

Figure 3. Metabolic-temperature curves, acutely measured, for midgut gland of Hemigrapsus nudus. The summer and winter animals were held 10 days in 35% and 125% sea water at 5°C prior to experimentation. The points on the curves represent the means of weight-specific oxygen consumption for each acute temperature.


EXP. TEMP. 5 °C
900
800
700
600
500
400
300
200
µlO2/g dry gland/hr
— WINTER • 35 % S.W
-- SUMMER ○ 125 % S.W.
5
10
15
20
ACUTE TEMP. °C

by averaging all respiration data at each experimental temperature and then comparing the means. This effect is significant (Table IV).

The main effect of experimental temperature is due to a higher respiratory rate at 5°C than at 20°C (Fig. 4). The fact that inverse, type 5, and partial, type 3, compensation is shown at 5°C and 20°C indicates that the respiratory response of summer and winter animals is being altered by the experimental temperature effect (Table V).

Effect of Experimental Salinity (C)

The main effect of experimental salinity was determined by comparing the means of the experimental salinities. The means were derived by averaging all respiration data at each experimental salinity. It is shown in Table IV that the main effect of experimental salinity is significant.

The respiratory response of midgut gland shows a "V-shaped" relationship to salinity. The lowest respiration rate for the main effect is in 75% sea water and the highest respiration rates in 35% and 125% sea water (Fig. 5). It is apparent that season and experimental temperature have an influence on the respiratory response of midgut gland tissue to experimental salinity (Fig. 6). The respiratory rate may increase, decrease, show no change or reveal a "V-shaped" relationship with a change in salinity. A further examination of these interactions will follow.

Figure 4. Experimental temperature-season interaction (BD) and main effect of experimental temperature (B) for midgut gland of Hemigrapsus nudus. Each point on the experimental temperature-season interaction curves is the mean of weight-specific oxygen consumption at that particular experimental temperature-season combination. The main effect is the mean of all weight-specific oxygen consumption data at the respective experimental temperatures.

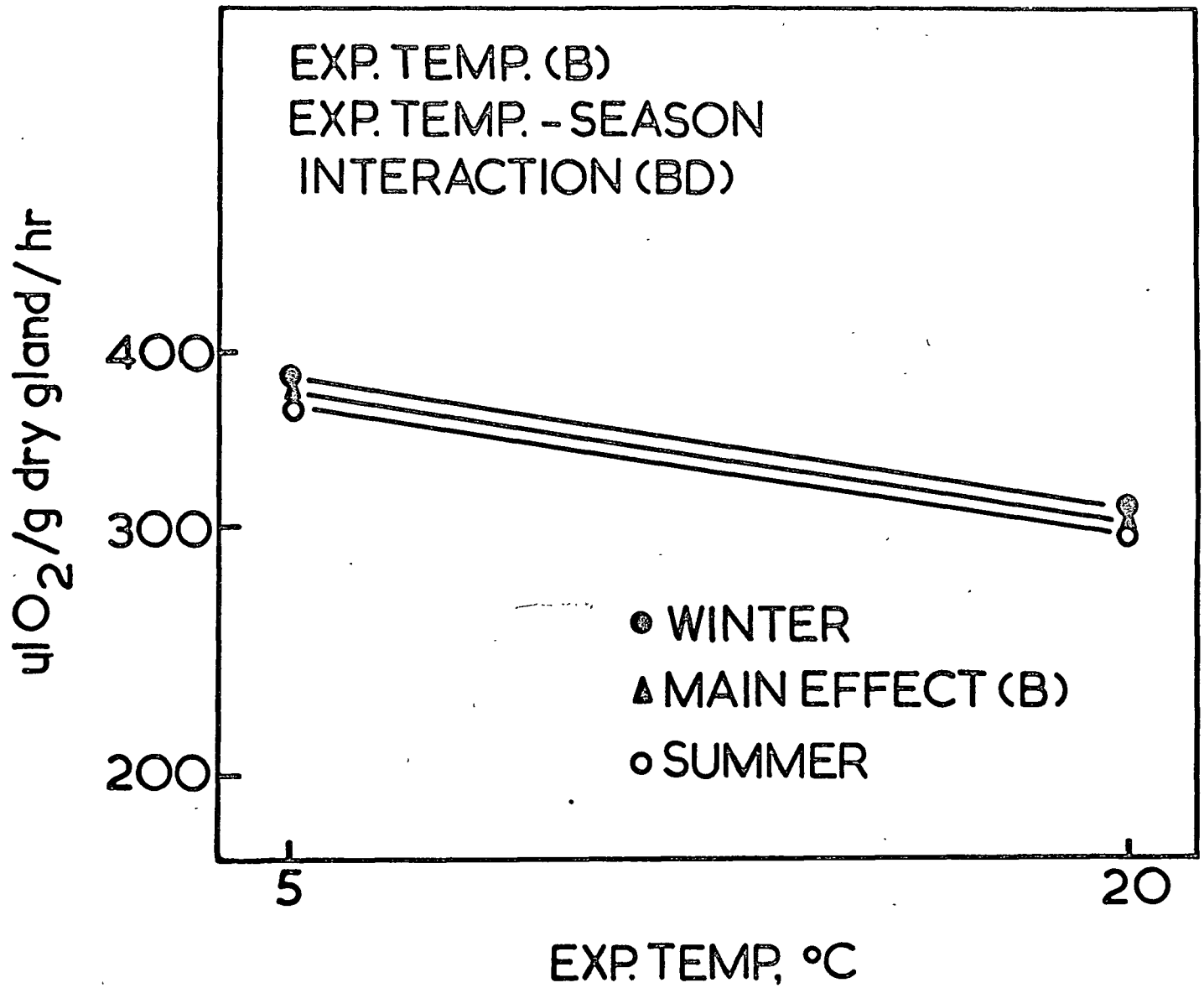

EXP. TEMP. (B)
EXP. TEMP. - SEASON
INTERACTION (BD)
400
300
200
$\mu l O_2/g$ dry gland/hr
WINTER
MAIN EFFECT (B)
SUMMER
5
20
EXP. TEMP, °C

Figure 5. Experimental salinity-season interaction (CD) and main effect of experimental salinity (C) for midgut gland of Hemigrapsus nudus. Each point on the experimental salinity-season interaction curves is the mean of weight-specific oxygen consumption at that particular experimental salinity-season combination. The main effect is the mean of all weight-specific oxygen consumption data at the respective experimental salinities.

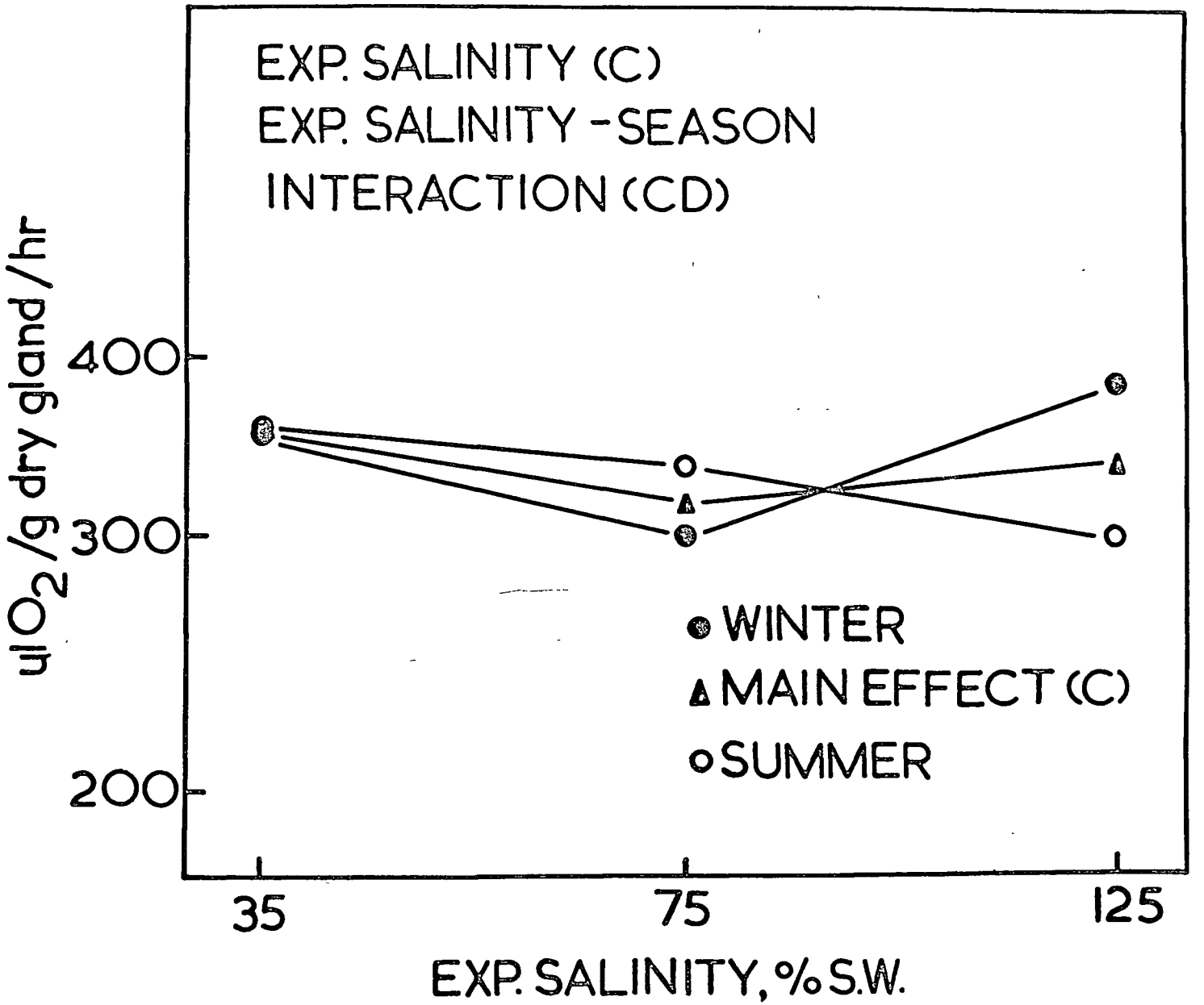
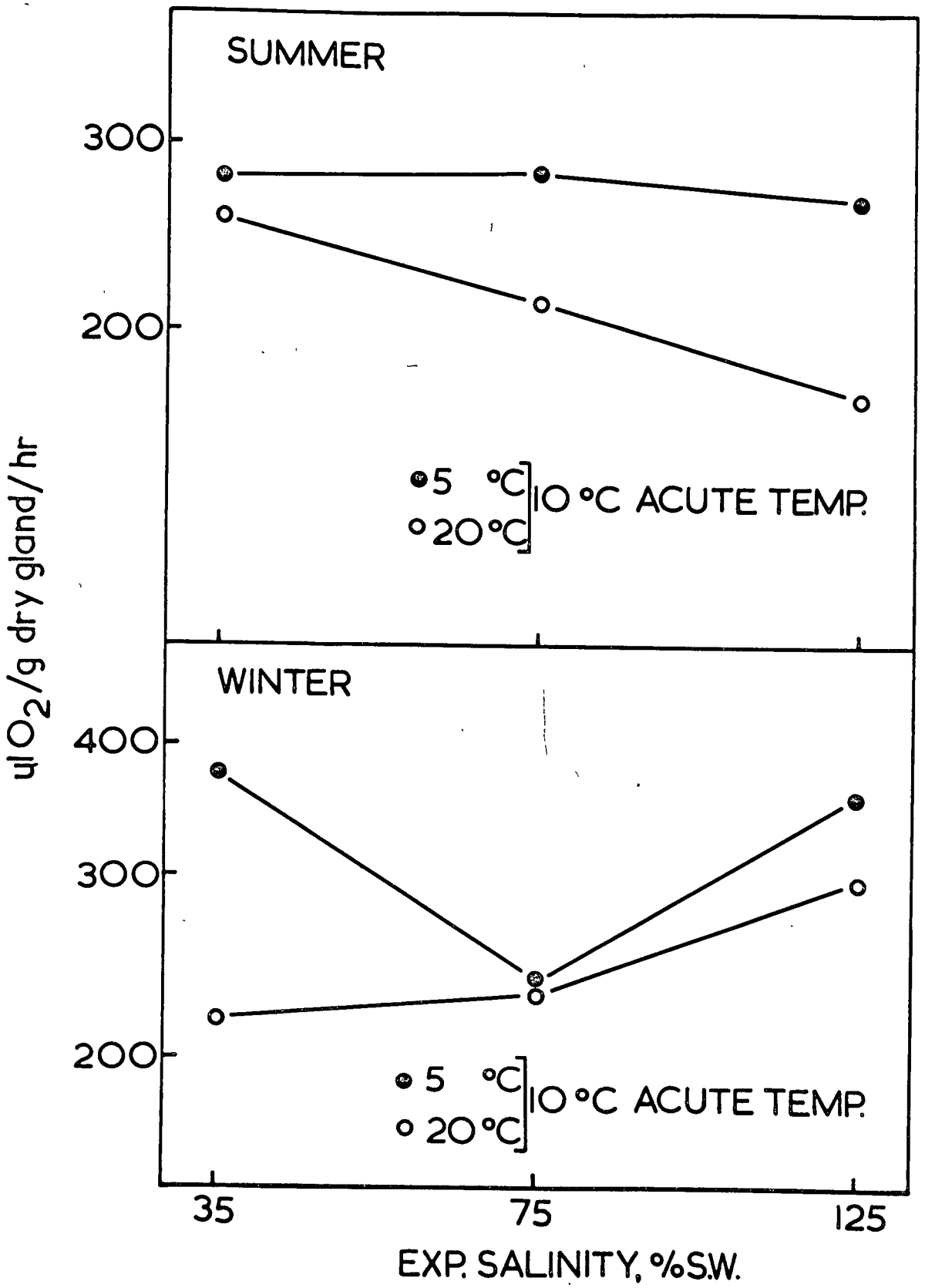

EXP. SALINITY (C)
EXP. SALINITY - SEASON
INTERACTION (CD)
μlO2/g dry gland/hr
400
300
200
WINTER
MAIN EFFECT (C)
SUMMER
35
75
125
EXP. SALINITY, % S.W.

Figure 6. Seasonal comparison of experimental salinity and experimental temperature effects on midgut gland respiration of Hemigrapsus nudus, measured at 10°C acute temperature. Each point on the curves is the mean of weight-specific oxygen consumption at that particular experimental salinity, experimental temperature and season.


SUMMER
300
200
5 °C
20 °C
10 °C ACUTE TEMP.
WINTER
400
300
200
5 °C
20 °C
10 °C ACUTE TEMP.
35
75
125
EXP. SALINITY, %S.W.
µl O2/g dry gland/hr

Interactions

Steel and Torrie (1960) define interaction "..... as a departure of the simple effects (factors) from an additive law or model based on main effects only." A significant interaction is one where the factors do not act independently of one another. Interactions involving two factors are called first-order interactions and interactions with three factors are referred to as second-order interactions.

First-Order Interactions

Acute Temperature-Experimental Temperature (AB)

The means of weight-specific oxygen consumption at four acute temperatures and two experimental temperatures are plotted in Figure 7. The metabolic-temperature (M-T) curve at 5°C is higher at all acute temperatures than the M-T curve at 20°C.

The interaction, however, is not significant (Table IV). This indicates that there is a great deal of variability in the respiration measurements at any one acute temperature-experimental temperature combination.

Acute Temperature-Season (AD)

The acute temperature-season interaction is significant (Table IV).

The means of the respiration data at all acute temperatures for each season are shown in Figure 9. There is no

consistent trend in a seasonal response to acute temperature. The differences are small and randomly distributed.

Acute Temperature-Experimental Salinity (AC)

This interaction is not significant (Table IV).

The means of the respiration data at four acute temperatures and three experimental salinities are plotted in Figure 8. The highest respiratory rate is in 35% sea water and the lowest respiration rate is in 75% sea water. The differences in the magnitude of response (position of slope on ordinate) are small.

Experimental Temperature-Experimental Salinity (BC)

This interaction is significant (Table IV).

In Figure 10 the means of the two experimental temperatures at three experimental salinities are graphically represented. The M-T curve at 5°C shows a "V-shaped" slope with the lowest respiratory rate in 75% sea water. The M-T curve at 20°C shows little change in slope with a change in salinity from 35% to 125% sea water.

Experimental Salinity-Season (CD)

The means of the respiration values for each experimental salinity and both seasons are plotted in Figure 5. The respiratory rate for the winter M-T curve shows a "V-shaped" relationship to salinity with the lowest respiration rate in 75% sea water. The summer respiratory rate is represented

Figure 7. Acute temperature-experimental temperature interaction (AB) for midgut gland of Hemigrapsus nudus. Each point on the acute temperature-experimental temperature interaction curves is the mean of weight-specific oxygen consumption at that particular acute temperature-experimental temperature combination.

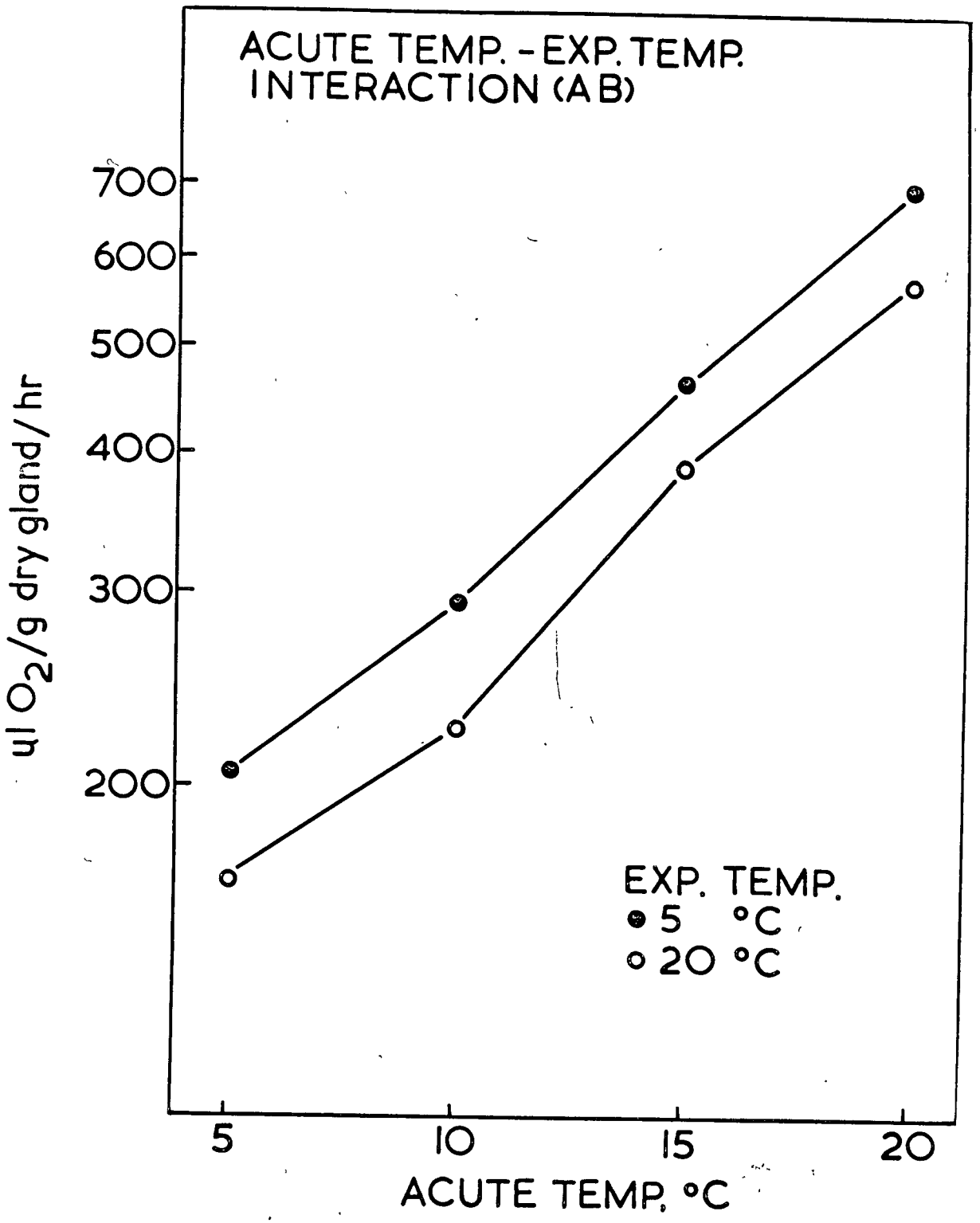

ACUTE TEMP. - EXP. TEMP.
INTERACTION (AB)
700
600
500
400
300
200
µl O2/g dry gland/hr
EXP. TEMP.
• 5 °C
o 20 °C
5
10
15
20
ACUTE TEMP. °C

Figure 8. Acute temperature-experimental salinity interaction (AC) for midgut gland of Hemigrapsus nudus. Each point on the acute temperature-experimental salinity interaction curves is the mean of weight-specific oxygen consumption at that particular acute temperature-experimental salinity combination.

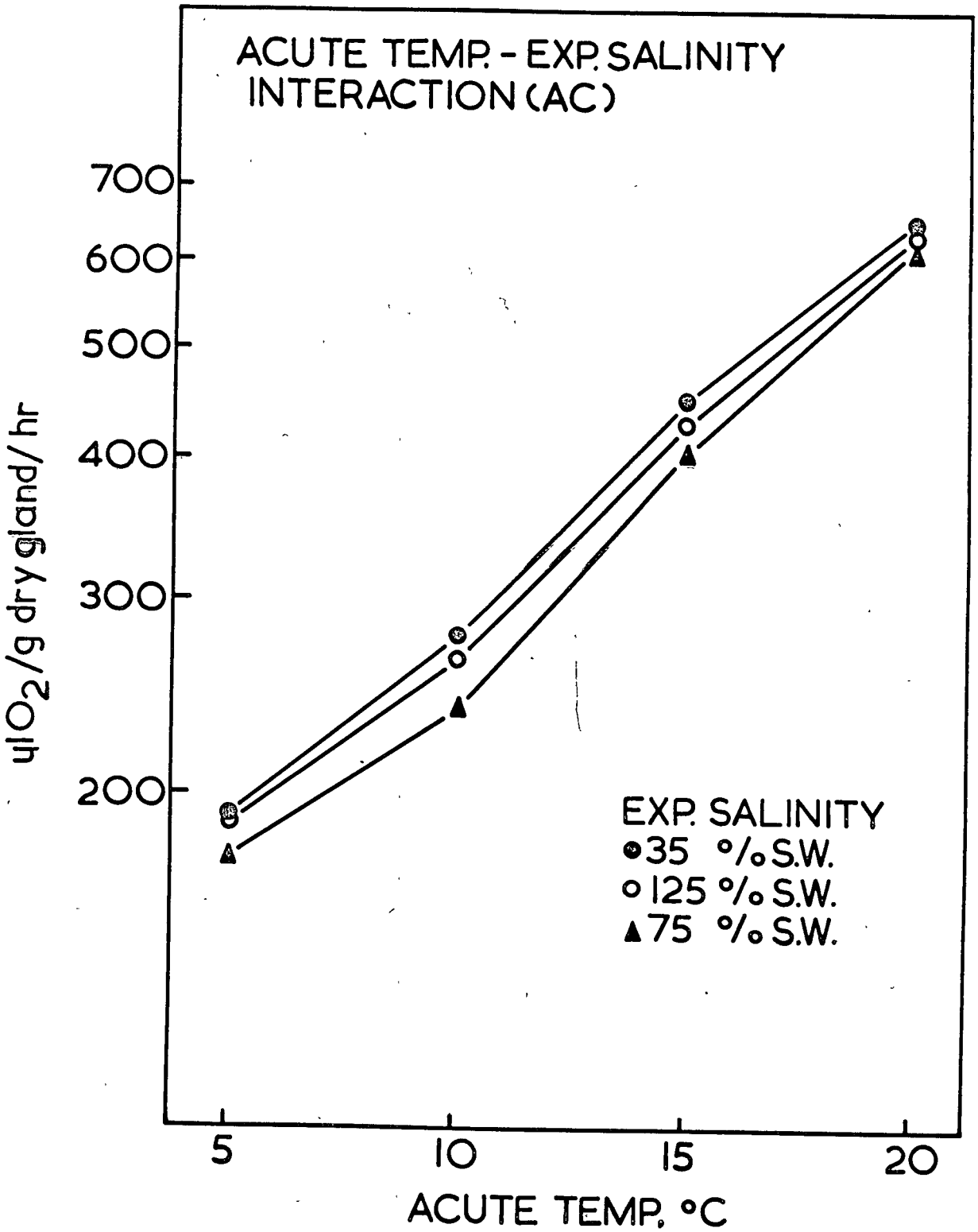

ACUTE TEMP. - EXP. SALINITY
INTERACTION (AC)
700
600
500
400
300
200
µlO2/g dry gland/ hr
EXP. SALINITY
●35 °/o S.W.
○125 °/o S.W.
▲75 °/o S.W.
5
10
15
20
ACUTE TEMP. °C

Figure 9. Acute temperature-season interaction (AD) and main effect of acute temperature (A) for midgut gland of Hemigrapsus nudus. Each point on the acute temperature-season interaction curves is the mean of weight-specific oxygen consumption at that particular acute temperature-season combination. The main effect is the mean of all weight-specific oxygen consumption data at the respective acute temperatures.

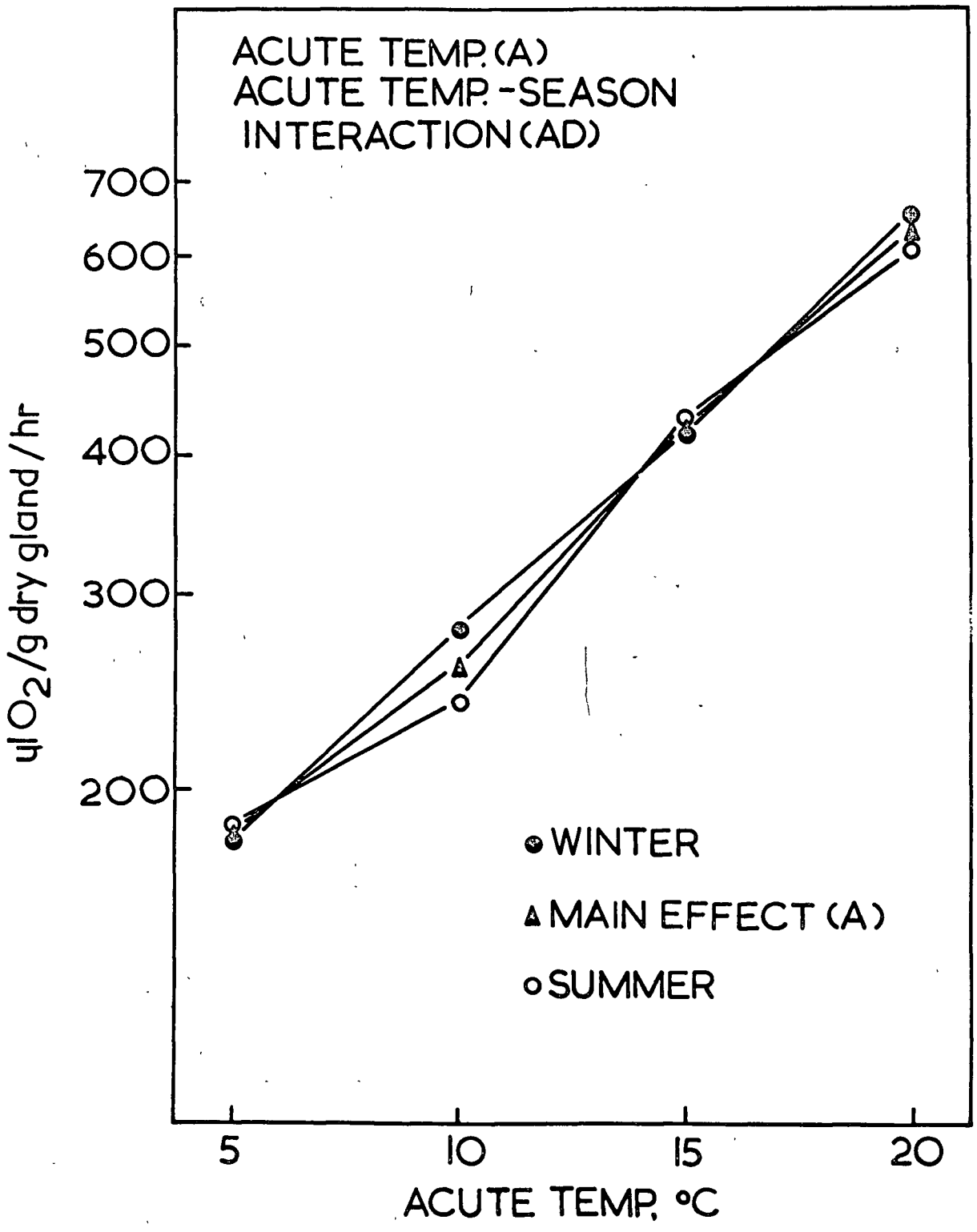
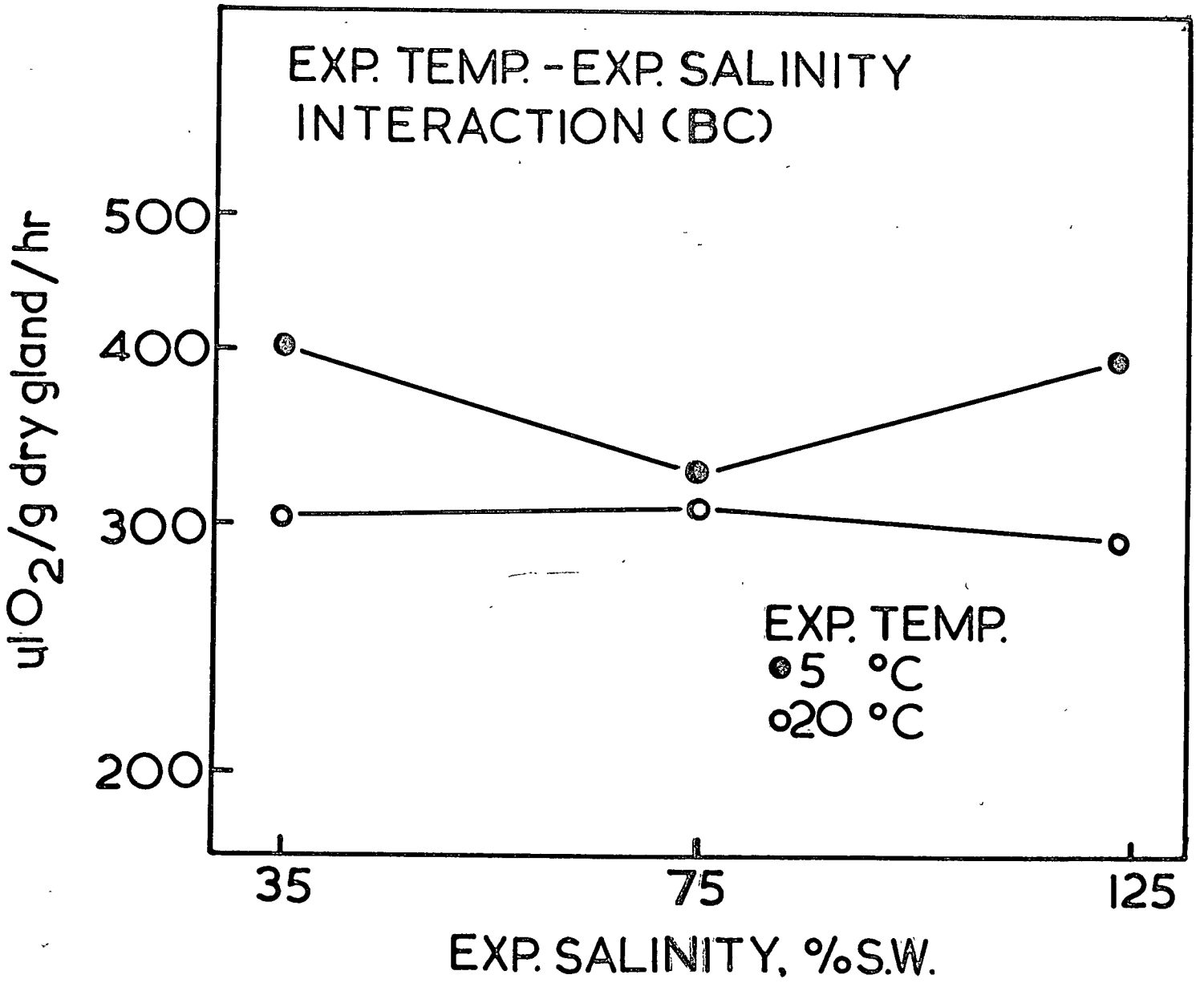

ACUTE TEMP.(A)
ACUTE TEMP.-SEASON
INTERACTION(AD)
700
600
500
400
300
200
µl O2/g dry gland/hr
WINTER
MAIN EFFECT (A)
SUMMER
5
10
15
20
ACUTE TEMP. °C

Figure 10. Experimental temperature-experimental salinity interaction (BC) for midgut gland of Hemigrapsus nudus. Each point on the experimental temperature-experimental salinity interaction curves is the mean of weight-specific oxygen consumption at that particular experimental temperature-experimental salinity combination.


EXP. TEMP. - EXP. SALINITY
INTERACTION (BC)
500
400
300
200
µlO2/g dry gland/hr
EXP. TEMP.
●5 °C
○20 °C
35
75
125
EXP. SALINITY, %S.W.

by a M-T curve in which the respiratory rate decreases with an increase in salinity.

As seen in Table IV, the interaction is significant.

Experimental Temperature-Season (BD)

A seasonal comparison of respiratory response to experimental temperature is not significant (Table IV).

In Figure 4 it may be seen that the means of the respiration values for each experimental temperature show only a small magnitude of difference seasonally. The winter rates of respiration are higher than the summer respiration rates by a factor of 5% at 5°C and 4% at 20°C experimental temperature.

Second-Order Interactions

Experimental Temperature-Experimental Salinity-Season (BCD)

This combination of factors results in the only significant second-order interaction (Table IV).

The means of all the respiratory data for each experimental temperature, experimental salinity and season are plotted in Figure 11. The three factor interaction is interpreted as an interaction of the interaction CD (experimental salinity-season) with factor B (experimental temperature). The winter M-T curves at the three experimental salinities have slopes which are different from the summer M-T curves. These M-T curves in turn have slopes which are influenced by experimental temperature. It is seen in

Figure 11. Experimental temperature-experimental salinity-season interaction (BCD) for midgut gland of Hemigrapsus nudus. Each point on the experimental temperature-experimental salinity-season interaction curves is the mean of weight-specific oxygen consumption at that particular experimental temperature-experimental salinity-season combination.

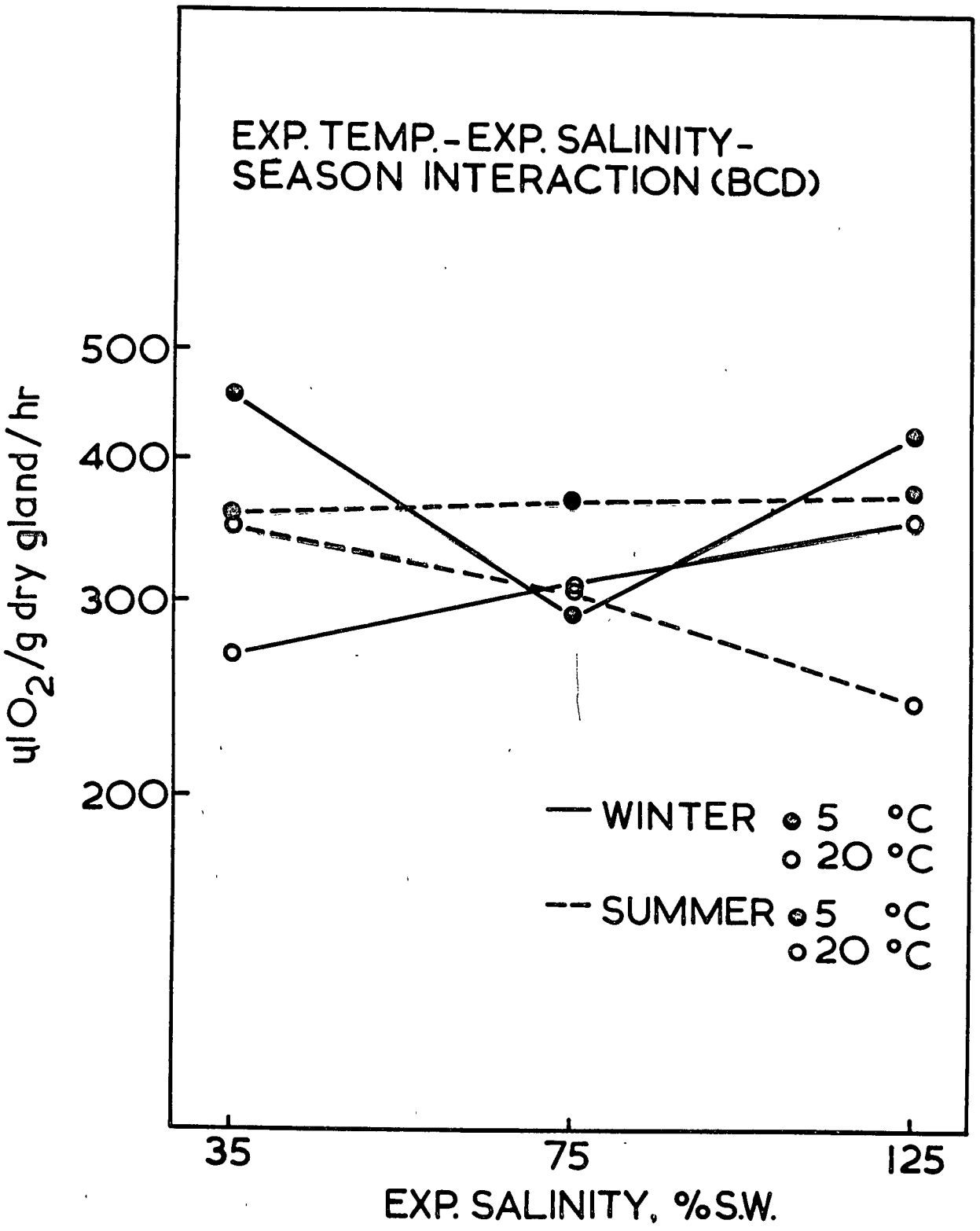

EXP. TEMP.-EXP. SALINITY-
SEASON INTERACTION (BCD)
500
400
300
200
μlO2/g dry gland/hr
WINTER 5 °C
20 °C
SUMMER 5 °C
20 °C
35
75
125
EXP. SALINITY, %S.W.

Figure 11 that the slope of the winter M-T curve at 5°C shows a "V-shaped" relationship to salinity. The lowest respiration rate is in 75% sea water. The winter M-T curve at 20°C shows an increase in slope as the salinity increases. Tissue samples from summer animals have M-T curves that also vary with the salinity due to a change in experimental temperature. The summer M-T curve at 5°C does not show a difference in the magnitude of response over the salinity range of 35% to 125% sea water. At 20°C, the slope of the M-T curve decreases with a increase in salinity.

Other Second-Order Interactions (ABC,ABD,ACD)

None of these interactions is significant (Table IV). The differences between the means are small and randomly distributed. The relatioships are not graphically represented in the text of this study.

Effect of Body Weight

The slopes of the regression lines of weight-specific oxygen consumption against body weight were not significantly different from zero for both seasons at the 0.01 probability level. At the 0.05 level of significance, three of twenty-four slopes were significantly different from zero for the winter animals and two of twenty-four slopes were significantly different from zero for the summer animals. These slope differences were considered to be due to chance alone and therefore were not statistically significant. This was confirmed when adjustment of the treatment means by the

analysis of covariance resulted in very small changes in the F-values.

DISCUSSION

Seasonal Metabolic-Temperature Experiments

The seasonal rate of oxygen consumption for midgut gland was found to be higher in summer animals at all acute temperatures from 5° C to 20° C (Fig. 2). This is interpreted as inverse seasonal compensation, type 5 (Precht, 1951). An identical pattern of response has been observed for excised gill tissue (Dehnel and McCaughran, 1964) and whole animal (Dehnel, 1960) over the same physiological temperature range. Vernberg and Vernberg (1966) have recently found that the most common type of seasonal adaptation in heart, muscle and brain tissue of temperate and tropical zone species of Uca at acute temperatures from 5° C to 35° C is inverse compensation.

Prosser (1958) suggests that inverse seasonal compensation may have no adaptive significance and appears to reflect a quantitative rather than a qualitative change in enzymes. That is, a particular enzyme might change in proportion to another enzyme in parallel or series. This is seen as a translation of the summer M-T curve to the left or above the winter M-T curve (Fig. 2). Prosser's suggestion has found support in the findings of Kanungo and Prosser (1959), Ekberg (1958) and Freed (1965) on intact animal, tissue and enzymes isolated from temperature ac-

climated goldfish.

Alternatively, the higher metabolic rate of summer animals may be due to the fact that many of the summer animals selected for study may have been physiologically in premolt, rather than intermolt. The rapid synthesis of organic material and mobilization of inorganic ions prior to and during exoskeleton formation could account for the higher respiration rate of summer animals. Skinner (1962) has noted that the synthesis of exoskeleton during late D_2 stage (premolt) increases the weight-specific oxygen consumption of the land crab, Gecarcinus lateralis, by 60% as compared to stage C_4 (intermolt).

An examination of the types of compensation found in Table V reveals that partial seasonal compensation (type 3) predominates at salinity and temperature combinations to which the animals are acclimated; not at standard baseline conditions. This indicates that osmotic and/or temperature stress may have a more depressant effect on the metabolic activity of the winter animals (see Fig. 3). To offset this depressant effect, winter animals increase metabolic activity above that shown in summer animals. This is correlated with the lower compensation coefficient of winter acclimated animals, indicating that winter animals demonstrate the greatest degree of acclimated response.

Metabolism-Body Weight Relationship

The relationship of whole animal and tissue respiration

to body weight has received considerable attention in reviews by Brody (1945), Krebs (1950) and Zeuthen (1953).

Bertalanffy (1951) proposed that the regression of metabolism on body weight could be expressed in terms of 2/3, 3/4 or 1 proportionality. Bertalanffy suggested that there is a species-specific power function that is fixed. Bertalanffy and Krywienczyk (1953) found that the surface law of 2/3 proportionality characteristic of crustacea could be applied to the metabolic-weight response of the brine shrimp, Artemia salina. Weymouth et al. (1944), Zeuthen (1953) and Scholander et al. (1953) have shown in other species of crustacea that the correlation between metabolism and body weight tends to assume the 3/4 power function. In the grapsoid crabs, Pachygrapsus crassipes, Hemigrapsus nudus and H. oregonensis, the regression coefficients rarely approached the 2/3 or 3/4 exponent (Roberts 1957a; Dehnel, 1960). Dehnel (1960) actually found in both species of Hemigrapsus a spread in weight-specific oxygen consumption regression values from -0.685 to -0.333.

In this study the weight-specific oxygen consumption to body weight regression of excised midgut gland was found to be independent of body weight. The same relationship was found by Vernberg and Gray (1953) for the Q_{O_2} of excised brain tissue of teleost fish. The independence of midgut gland respiration from body weight is in contrast to the mean -0.169 regression coefficient of excised gill tissue for both species of Hemigrapsus (Dehnel and McCaughran,

1964).

It appears that each tissue has its own unique pattern of response to changes in body weight. These changes have been reflected as differences in cellular enzyme activity-weight regressions for mammalian tissues (Rosenthal and Drabkin, 1943; Kunkel and Campbell, 1952; Fried and Tipton, 1953). An attempt to assign a fixed proportionality value to a whole animal and/or its tissues and enzyme systems is, therefore, invalid. The regression coefficient is dependent on the environmental history of the animal, effect of physical and biotic parameters, and techniques employed to measure responses. The fact that tissue samples when removed from the animal are no longer influenced by the central nervous system or by hormones could account in part for the discrepancies between tissue and whole animal metabolism to body weight regression.

Effect of Experimental Temperature

Temperature determines to a great extent the rates of chemical reactions and, thus, the rate of metabolism and activity. In this sense temperature is considered one of the most important of environmental parameters.

Bullock (1955) and Prosser (1955) have presented comprehensive reviews of temperature effects on rate functions dealing with such things as thermal limits of tissues and whole organisms, oxygen consumption, heart beat and ciliary pumping activity. Not only rate functions, but the metabolic

pathway utilized by an organism may be altered (Ekberg, 1958; Hochachka and Hayes, 1962; Dean and Vernberg, 1965).

There is a 24% decrease in metabolic activity of midgut gland with an increase in experimental temperature from 5°C to 20°C (Fig. 4). This percentage decrease holds for both summer and winter animals. The experimental temperature effect corresponds to Precht's type 3 (partial compensation). A similar effect is depicted in Figure 7 where the experimental temperature is averaged at each acute temperature (5°, 10°, 15° and 20°C) for both seasons and three experimental salinities (35%, 75% and 125% sea water). This type of compensation has been documented extensively by a number of investigators. Edwards and Irving (1943) demonstrated this effect in the sand crab, Emerita talpoida. Scholander et al. (1953) found the same response in aquatic fish and crustacea. Partial compensation has also been demonstrated by Clark (1955) for the terrestrial amphipod, Talitrus sylvaticus, by Kanungo and Prosser (1959) for goldfish and by Dehnel (1960) for both species of Hemigrapsus.

Dehnel and McCaughran (1964) found that excised gill tissue showed no experimental temperature effect for both species of Hemigrapsus. This effect was not considered biologically significant. The demonstration of partial compensation for excised midgut gland is significant statistically and biologically. The biological importance of this effect is interpreted on the grounds that low

temperature may provide a greater thermal stress than high temperature (Todd and Dehnel, 1960). To offset this temperature stress, the rate of oxygen consumption is increased above that experienced at 20° C. This interpretation is correlated in part with the observations of Freed (1965) who established that cytochrome oxidase activity increased in the cold acclimated (5° C) goldfish and decreased with acclimation to heat (30° C).

Effect of Experimental Salinity

Salinity is the other major environmental factor, besides temperature, which has a pronounced effect on the metabolic activity of aquatic invertebrate animals and their tissue.

The effect of salinity may alter the metabolic response by increasing respiration in sub- and supra-normal salinities. This has been demonstrated by Flemister and Flemister (1951) for the sand crab, Ocypode albicans, by Lofts (1956) in a salt marsh population of the prawn, Palaemonetes varians, and by Rao (1958) for the brackish-water species of the prawn, Metapenseus monoceros. There are other studies that have indicated that metabolic activity may be higher in sub-normal salinities. Dehnel (1960) found in both species of Hemigrapsus that the rate of respiration was highest in dilute sea water where the osmotic gradient between the blood and medium was greatest. Lance (1965) showed that the metabolic rate in 30% sea water was

double that in 100% sea water for the planktonic copepod, Acartia tonsa. King (1965) demonstrated in the crabs, Carcinus mediterraneus and Callinectes sapidus, a 33% and 53% increase in oxygen consumption, respectively, after the animals were transfered from 80% to 50% sea water. The conclusion reached by these authors is that when the blood concentration is no longer isotonic to the medium oxygen consumption is increased to maintain the osmotic gradient.

Several investigators have raised objections to the proposal that increased oxygen consumption reflects osmotic work to maintain the osmotic gradient between the blood and medium. Gross (1957) has suggested that the increase in respiration rate with an increase in osmotic gradient for the rock crab, Pachygrapsus crassipes, is related to an increase in activity. A comparison of respiratory and osmoregulatory data in both species of Hemigrapsus indicates that a increase in respiration rate does not necessarily reflect osmotic work (Dehnel, 1962). Dehnel and McCaughran (1964) found that the rate of oxygen consumption for excised gill tissue of winter species of Hemigrapsus did not show any correlation with salinity. King (1965) discovered that the excised gills of Carcinus, an osmoregulator, did not show a significant change in oxygen consumption when transfered from 80% to 50% sea water. In Maja, a crab that remains isoosmotic with the medium, there was a 6% increase in oxygen consumption of excised gill upon dilution of the suspending medium from 80% to 50% sea water.

To assess properly the effect of salinity on midgut gland respiration, one should first examine the extent to which the osmotic concentration of the blood and urine change with changes in salinity. The blood concentration of winter and summer Hemigrapsus nudus is hypertonic to the medium over the experimental salinity range from 25% to 125% sea water. The animals regulate their blood concentration in salinities from 25% to 75% sea water. Beyond this salinity range, regulation breaks down and the blood approaches isotonicity with the medium although still hypertonic to it. The osmotic concentration of the urine for summer animals is equal to the blood concentration over the experimental salinity range from 25% to 125% sea water and hypertonic to the medium. Winter animals have a urine which is hypotonic to the blood at all experimental salinities from 25% to 125% sea water and all experimental temperatures except 15°C. The urine is hypertonic to the medium below 90% sea water and hypotonic above this sea water concentration (Dehnel, 1962; Dehnel and Stone, 1964).

When these osmoregulatory data are compared with the respiratory response of excised midgut gland tissue, a seasonal effect is noted. In summer animals there is a decrease in midgut gland respiration with a increase in salinity from 35% to 125% sea water (Fig. 5). In 35% sea water there is a large osmotic gradient between the blood and medium. To maintain the blood concentration hypertonic

to the medium, active absorption of ions from the midgut gland may occur. Osmotic work would have to be performed to maintain this gradient and the rate of oxygen consumption would be high. Whole animal respiration of Hemigrapsus nudus, however, does not support this proposal (Dehnel, 1962). The blood approaches isotonicity with the medium in 75% sea water. Since the osmotic gradient is small and the animal is regulating its blood concentration to a minimal degree, these facts may account for the drop in midgut gland oxygen consumption in 75% sea water. In 125% sea water osmotic stress may increase mortality and cause a further drop in respiration. This is plausible since summer animals normally do not encounter such a high salinity in the field. Dehnel and McCaughran (1964) have demonstrated that the gills of summer Hemigrapsus sp. also appear to be important in osmotic regulation when the osmotic gradient between the blood and medium is maximal (35% sea water). As has been suggested earlier, metabolic activity of midgut gland from summer animals may be related more to the energy demands of new exoskeleton formation than to the maintenance of a osmotic gradient. This proposal is valid when it is recognized that many of the summer animals selected for study may have been physiologically in premolt, rather than intermolt. In winter animals there is a "V-shaped" relationship of midgut gland respiration to salinity with the lowest respiration rate in 75% sea water (Fig. 5). The low metabolic response in 75% sea water corresponds to the

point where the blood and urine concentrations approach isotonicity with the medium. The high respiratory response in 35% sea water may indicated work being done to maintain the osmotic gradient between the blood and medium. In Figure 8 a similar pattern of response is found when the experimental salinity effect is averaged at each acute temperature (5°, 10°, 15° and 20°C) for two experimental temperatures (5°C and 20°C) and both seasons. The midgut gland appears to be active in the winter in the maintenance of a osmotic gradient although evidence presented by Gross (1957) and Dehnel (1962) on whole animal would tend to confute this suggestion. The production of a urine hypotonic to the blood may also assist the midgut gland during the winter in salt regulation of the blood. Potts (1954) points out, however, that the production of a hypotonic urine yields a negligible saving of osmotic work in sea water below 50%.

Effect of Temperature-Salinity-Season Interactions

There are several studies which have assessed the effects of environmental factors acting simultaneously on whole animal and tissue. Panikkar (1940), Broekema (1941), Dehnel (1960) and Todd and Dehnel (1960) have examined the combined effects of seasonal changes in temperature and salinity on the mortality, metabolic activity and thermal limits of intact crustacea. Dehnel and McCaughran (1964) have determined temperature, salinity and seasonal effects on excised gill tissue in both species of Hemigrapsus.

Kinne (1963,1964) has presented a comprehensive review of studies demonstrating differential response effects to temperature and salinity.

In Figure 6 is presented a seasonal comparison of weight-specific oxygen consumption of midgut gland for the experimental temperature-salinity interaction at 10°C acute temperature. The interaction is statistically significant (Table IV). An experimental temperature effect is evident when comparisons are made within and between the seasons. In summer animals at 5°C there is no correlation of midgut gland respiration with salinity. At the summer baseline temperature of 20°C there is a decrease in oxygen consumption with an increase in salinity. This experimental temperature-salinity effect may be related to osmotic work being done in maintenance of a osmotic gradient between the blood and medium or to the energy demands of exoskeleton formation, since it is recognized that many of the summer animals used in this study may have been in premolt. In the winter animals at their winter baseline temperature of 5°C a "V-shaped" relationship to salinity is shown. The lowest respiration rate is in 75% sea water where the blood approaches isotonicity with the medium. The increased respiration rate in 35% sea water may indicate that the midgut gland is doing osmotic work in response to the osmotic gradient. The winter animals at 20°C gradually increase the rate of midgut gland respiration with an increase in salinity. An explanation of this response cannot be given

at this time. The same general trends are also evident in Figure 11 where seasonal changes in experimental temperature and salinity are averaged for all acute temperatures (5°, 10°, 15° and 20°C).

In a comparable study on excised gill tissue for both species of Hemigrapsus, Dehnel and McCaughran (1964) found that gill tissue from summer animals appeared to be active in the maintenance of an osmotic gradient between the blood and medium while the gills of winter animals showed no correlation in respiration rate with experimental salinity. On a seasonal basis, these findings differ from those observed for midgut gland.

Temperature and salinity both have biologically important effects on seasonal changes in midgut gland respiration. The metabolic activity of midgut gland in summer animals may be geared not only to maintenance of an osmotic gradient but also to energy requirements associated with new exoskeleton formation. In winter animals the midgut gland may play a role in regulating blood electrolytes. This fact together with the evidence of the production of a urine hypotonic to the blood may account in part for the mechanisms of osmotic regulation in winter animals.

SUMMARY

1. Weight-specific oxygen consumption of midgut gland tissue of Hemigrapsus nudus has been investigated at three levels of salinity (35%, 75% and 125% sea water), two levels of experimental temperature (5°C and 20°C) and four acute (Warburg) temperatures (5°, 10°, 15° and 20°C) in all combinations for each season (summer and winter). The data are evaluated and discussed in terms of midgut gland function in the intact animal.

2. Weight-specific oxygen consumption of midgut gland from summer animals held 24 hr at seasonal baseline conditions (35% sea water, 20°C) is higher at all acute temperatures of measurement (5°C to 20°C) than weight-specific oxygen consumption of midgut gland from winter animals held 24 hr at its seasonal baseline conditions (75% sea water, 5°C).

3. Acutely measured metabolic-temperature curves of midgut gland tissue from winter and summer animals held 10 days at their opposite seasonal conditions show that winter animals demonstrate the greatest degree of acclimation.

4. The effect of experimental temperature is statistically and biologically significant. There is a 24% decrease in metabolic activity of midgut gland with an increase in experimental temperature from 5°C to 20°C. Low temperature may provide a greater thermal stress than a high temperature resulting in a higher rate of oxygen

consumption. The experimental temperature effect corresponds to Precht's (1951) type 3 (partial compensation).

5. Experimental temperature effect is noted seasonally in the respiratory response of midgut gland to salinity. Summer animals at 5°C show no change in the ordinal position of the metabolic-temperature curve with a change in salinity. At the summer baseline temperature of 20°C there is a increase in respiration with a decrease in salinity. Winter animals at the seasonal baseline temperature of 5°C demonstrate a "V-shaped" relationship to salinity. The lowest rate of weight-specific oxygen consumption is in 75% sea water. The metabolic-temperature curve of winter animals at 20°C increases with an increase in salinity.

6. It is suggested that the metabolic activity of midgut gland in summer animals may be related to the maintenance of a osmotic gradient between the blood and medium. The highest rate of oxygen consumption is at summer baseline conditions (35% sea water, 20°C) where the osmotic gradient between the blood and medium is maximal. Alternatively, midgut gland respiratory activity may be geared to the energy demands associated with new exoskeleton formation. This proposal is valid since it is recognized that many of the summer animals selected for study may have been physiologically in premolt. Since premolt animals have a higher respiration rate than intermolt animals, the higher seasonal summer rates could be explained on this basis.

7. Winter animals at their seasonal baseline temperature (5°C) show a "V-shaped" relationship to salinity.

This relationship reflects the possibility that midgut gland tissue may be regulating blood salts. The high respiratory response in 35% sea water may indicate work being done to maintain the osmotic gradient between the blood and medium. In 75% sea water where the rate of oxygen consumption is minimal, the osmotic gradient between the blood and medium is at a minimum and little work would apparently have to be done to maintain the gradient. The production of a urine hypotonic to the blood also may assist winter animals in maintaining the blood concentration hypertonic to the medium.

8. The regression of weight-specific oxygen consumption as a function of body weight does not show a significant relationship. The slope values are not significantly different from zero at the 0.01 probability level.

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