1985

Metabolic Responses of the Estuarine Gastropod Thais Haemastoma to Hypoxia (Energy Charge, Opine Dehydrogenase, survival, Adaptation, Respiration).

Martin A. Kapper
Louisiana State University and Agricultural & Mechanical College

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METABOLIC RESPONSES OF THE ESTUARINE GASTROPOD THAIS HAEMASTOMA TO HYPOXIA

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Physiology

by

Martin A. Kapper
B.S., St. Lawrence University, 1976
M.A., State University of New York at Buffalo, 1980
August, 1985
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ABSTRACT

The hypoxia tolerance (LC-50) of *Thais haemastoma* was inversely related to acclimation temperature but was not related to acclimation salinity between 10 and 30°C and 10 and 30°/ooS. *T. haemastoma* is moderately tolerant to anoxia, the LT-50 (time to 50% mortality) being 12-22 days. Oxyregulatory ability varies from poor to good, and varies inversely with temperature. The oxygen consumption rate was lower after 28 days exposure to hypoxic conditions than at full oxygen saturation at all salinities at 20 and 30°C. Oxygen consumption was low at 10°C regardless of ambient PO₂. Oxyregulatory ability in *T. haemastoma* did not show acclimation after 28 days exposure to 53 mm O₂ at 30° and 30°/ooS. Likewise, no induction of the activities of the pyruvate oxidoreductase enzymes in foot tissue occurred after 28 days exposure to 10, 33, and 67% of oxygen saturation. The ratio of mean activities of alanopine dehydrogenase:lactate dehydrogenase:strombine dehydrogenase:octopine dehydrogenase was 3.8:1.2:1.0:0. Alanopine dehydrogenase activity nearly doubled on the first day but had returned to the control level by day 2, and no change occurred in strombine dehydrogenase and lactate dehydrogenase activity over 6 days exposure to anoxia. The total concentration of cellular adenylates remained constant, but the adenylate energy charge, ([ATP]+.5[ADP]/([ATP]+[ADP]+[AMP])), was reduced at low PO₂'s. Arginine phosphate concentrations were lower in snails exposed to 10 and 33% oxygen saturation than in those at 67 and 100% of oxygen saturation. Energy charge declined from 0.79 to 0.58 after one day of anoxia at 30°C and 30°/ooS while the arginine phosphate concentration declined by 95%. There was no further decrease in either variable with longer exposure to anoxia. Hypoxia tolerance of *T. haemastoma* is due to a reduction in...
metabolic demand, especially at low temperature, with ATP production maintained at the expense of arginine phosphate while metabolism switches to anaerobic pathways. The opine pathway is probably only used as a transitional mode before use of long-term anaerobic pathways is initiated.
INTRODUCTION

The primary mode of metabolism of most metazoans is aerobic, yet many marine invertebrates have been recognized as having a substantial capacity for anaerobic metabolism during exposure to hypoxia (de Zwaan and Wijsman, 1976; Livingstone, 1982). There have been several recorded instances of hypoxic or anoxic conditions developing in the Atlantic Ocean off New Jersey (Garlo, et al., 1979), in the Chesapeake Bay (Officer, et al., 1984; Seliger, et al., 1984) and in the Gulf of Mexico (Harper et al., 1981; Turner and Allen, 1982). Although most of these recorded hypoxic events have been offshore, recurrent hypoxic episodes have been documented in the inshore bays and estuaries of the northern Gulf of Mexico between Texas and Alabama (Rabalais, et al., 1985).

Although many species of benthic marine molluscs are extremely resistant to anoxia, with LT-50's (time to 50% mortality) of up to 35 days (Theede, et al., 1969), few species can survive indefinitely without oxygen. During a three month hypoxic episode (<2 ppm, or 23 mm of oxygen) off the Texas coast, macrobenthic species diversity and population density declined precipitously, although populations of the bivalve *Abra aequalis* appeared to be little effected (Harper, et al., 1981).

The respiratory response of marine molluscs to declining oxygen tension and hypoxia has received considerable attention over the past 15 years (Bayne, 1971, 1973; Bayne and Livingstone, 1976; Mangum and van Winkle, 1973;). Most investigators have concentrated on either the changes in the rate of oxygen consumption with acutely declining PO$_2$ or on the anaerobic biochemical pathways used during anoxia exposure. Animals have traditionally been classified as either oxyregulators if their oxygen consumption rate remains constant with declining PO$_2$ or
oxyconformers, if the oxygen consumption rate is proportional to ambient PO\textsubscript{2} (Mangum and van Winkle, 1973). Realistically, oxyregulation and oxyconformity (or oxygen independence and oxygen dependence) describe opposite ends of a spectrum.

Unlike most vertebrates, lactate is not the sole end product of anaerobiosis in marine molluscs. Depending on the species and the duration of anoxia, such varied molecules as alanine, succinate, and propionate will accumulate (Fields, 1983; Gade, 1983; Livingstone, 1982). Recently, another class of molecules, the opines, have begun to attract attention. These molecules are formed by the condensation of an amino acid with pyruvate, oxidizing NADH. The opine dehydrogenase enzymes along with lactate dehydrogenase are classified as pyruvate oxidoreductases. Octopine dehydrogenase, which uses arginine as the amino acid substrate, alanopine dehydrogenase which uses alanine, and strombine dehydrogenase, which uses glycine, are functionally analogous to lactate dehydrogenase, and result in the same total ATP formation (Livingstone 1983). High activities of these enzymes are have been found in many species (Livingstone et al., 1983).

The adenylate energy charge represents the state of energetic balance in the cell. It's value can be thought of as being related to the balance between energy-yielding and energy-requiring metabolic processes (Atkinson, 1968). When the concentration of ATP is high relative to the concentrations of ADP and AMP, key enzymes in glycolysis and the Krebs cycle are allosterically inhibited. Conversely, when ATP concentrations are low with the resultant increase in ADP and AMP concentrations, these same enzymes are allosterically activated (Atkinson, 1968). In normally metabolizing cells, adenylate energy charge is regulated to an average value of 0.85 (Ivanovici, 1980).
The southern oyster drill, *Thais haemastoma* is the dominant subtidal gastropod on oyster reefs and other hard substrates in the northern Gulf of Mexico. It is responsible for losses of up to half the yearly oyster crop in Louisiana (Cake, 1983). *T. haemastoma* is a remarkably euryhaline species, the adults surviving for at least four weeks in the lab at 5°C (Garton and Stickle, 1980; Hildreth and Stickle, 1980), and maintains a positive energy budget from 15 to 30°C and 7.5 to 35°C (Stickle, 1985) although its low-salinity distributional limit in the field is 15°C (St. Amant, 1938).

*T. haemastoma* is exposed to diurnal fluxes of dissolved oxygen; for example, a the daily range from 25 to 120% of saturation was observed over a 24-hour period (Stickle, unpublished data). The snail will burrow into the azoic zone of sediments in the winter, and intermittently in the summer. The only previous reference regarding the ability of *T. haemastoma* to survive hypoxia is from a fisheries report that states that the snail will "survive, experimentally at least, oxygen levels too low to support most other marine life" (Butler, 1954).

The specific objectives of this study are 1) to determine the hypoxia tolerance of the southern oyster drill, *Thais haemastoma* over its normal temperature and salinity range, 2) to determine the degree of regulation of the aerobic respiratory rate of *T. haemastoma* subjected to acutely declining oxygen tension, and to determine if this response will acclimate to prolonged hypoxia, 3) to determine the activities of the pyruvate oxidoreductase enzymes, and determine if these enzymes are induced over the course of chronic hypoxia, and 4) to determine the changes in the phosphoadenylate and arginine phosphate pool and adenylate energy charge as an indicator of physiological condition in *T. haemastoma* exposed to chronic hypoxia. These data will serve as a
starting point for understanding the mechanisms by which *T. haemastoma*

is able to withstand variation in one of the most important abiotic

factors in its environment, oxygen.

MATERIALS AND METHODS

**Oyster Drill Collection and Maintenance**

*Thais haemastoma* were collected throughout the year from bulkheads

and jetties in the vicinity of Caminada Pass, Grand Isle, La. The snails

were brought back to the lab and placed in 38-liter glass aquaria using

*Instant Ocean*(TM) artificial seawater at the same temperature and

salinity as occurred at the collection site. Aquaria were housed in a

temperature-controlled environmental room under constant illumination.

Live oysters (*Crassostrea virginica*) obtained from local seafood

suppliers were continually available to the snails as prey items. When

necessary, acclimation temperatures of 10, 20 and 30°C were reached by

changing the temperature of the environmental room by 1°C per day. Final

acclimation salinities of 10, 20, and 30°/ooS were reached by adding

either 40°/ooS artificial seawater or deionized water to the aquaria to

change salinity by 2°/oo per day. Snails were held for two weeks at the

final temperature-salinity combination before being used in experiments.

**Hypoxia Exposure System**

The hypoxia exposure system (Fig. 1) consisted of three sets of

five flow-through aquaria, one set each at 10, 20 and 30°/ooS. Water was

pumped from a large filtration unit into each of five 38-liter glass

tanks at each salinity by peristaltic pumps at 20-55 ml·min⁻¹, a rate

sufficient to ensure one to two complete turnovers of the water in each

tank in a day. The water level was maintained in each tank by a

constant-level siphon, which drained back into the filtration unit. The

filter units were large (55-gallon) partitioned plywood boxes lined with
fiberglass resin. The boxes contained two beds of crushed oyster shell as the filtration material. Effluent water from each tank passed over both beds in succession before being returned to the tanks.

Target oxygen tensions of 107, 53, 10, and 0 mm O$_2$ corresponding to 67, 33, 10 and 0% of oxygen saturation were created and maintained by mixing oxygen and nitrogen in Matheson model 7402T gas mixers along with 0.03% carbon dioxide to maintain pH near 7.8. Each gas mixer was connected to an outlet manifold and fed three tanks, one at each salinity. The outlet manifolds were connected to the standpipes of the standard under-gravel filters of each tank. Aeration of control tanks (air-saturation = 156-157 mm O$_2$) was by diaphragm aquarium air pumps. Each tank was covered by a form fitting plexiglass lid with holes drilled in it to accommodate gas lines, water lines, and the siphon. All tanks were located in a temperature-controlled water bath. P0$_2$, pH, and ammonia concentration were monitored daily as indices of water quality. P0$_2$ was always within 10-15% of the target value at the three higher levels, while the 10% oxygen saturation tank was within 5 mm O$_2$ of the target, and the P0$_2$ of the 0% oxygen saturation tanks was usually in the range of 3-8 mm O$_2$. The pH was in the range 7.6-8.1, and ammonia levels were consistently below 25 μmoles·l$^{-1}$ (Table I).

**Hypoxia Bioassays**

Survival at each P0$_2$ was determined daily for 28 days at each temperature-salinity combination. A snail was considered dead if both the siphon and the foot failed to respond to tactile stimulation. LC-10, LC-50, and LC-90 values, the P0$_2$ at which 10, 50, and 90% of the snails had died after a defined period of time, were calculated daily by the SAS Probit procedure (SAS Institute, 1982), or by the Spearman-Karber technique (Hamilton et al., 1977) if mortality in at least two of the
five $P_{O_2}$'s was not between 0 and 100%.

Oxygen Consumption and Indices of Oxyregulation

Oxygen consumption rates at constant $P_{O_2}$'s were measured in a flow-through system as described by Stickle et al. (1985), with the following modifications: Incurrent water was bubbled with a gas mixture of the appropriate $P_{O_2}$ from a gas mixer identical to those used in the hypoxia exposure system. Water was pumped directly from the reservoir into the distribution manifold at a low flow rate. Flow through the individual glass animal chambers was regulated to 5–10 ml • min$^{-1}$ by regulating the speed of the pump and by using different size hypodermic needles on the outlet ends of the animal chambers. The entire apparatus was placed inside a temperature controlled environmental room. After the snails were placed in the chambers and the flow rates adjusted, one hour was allowed for the animals to acclimatize to the apparatus. Water samples were taken anaerobically with a 5-ml glass syringe, and the $P_{O_2}$ was measured using the Radiometer thermostatted cell housing a Radiometer model E-5046 oxygen electrode connected to a Radiometer PHM71 Mk II acid-base analyzer. Oxygen consumption rates were calculated by subtracting the $P_{O_2}$ from the outlet of an animal chamber from the average of the $P_{O_2}$'s of the two blank chambers in the system divided by the air-saturated $P_{O_2}$ at that temperature and salinity. That value was multiplied by the oxygen content of air saturated water as determined from the nomograph of Strickland and Parsons (1968) and the individual chamber's flow rate in liters • hr$^{-1}$ to yield microliters of oxygen consumed per hour.

Dry snail weight was determined by cracking the snail's shell with a hammer and removing the soft tissues which were then frozen in liquid nitrogen, lyophilized, and weighed. The relationship between oxygen
consumption rates and dry weight at constant PO\textsubscript{2} was described by the allometric form:

$$\mu l O_2 \cdot hr^{-1} = a \cdot (\text{dry weight})^b$$

where a and b are the slope and intercept of the regression of log\textsubscript{10} oxygen consumption on log\textsubscript{10} dry weight. For Q\textsubscript{10} determinations the individual regression lines at 100% oxygen saturation were compared by analysis of covariance with temperature and salinity as main effects and weight as the covariate. The salinity term was non-significant, and the weight effect was identical for all three temperatures, therefore, the data were pooled and a common slope was calculated. Oxygen consumption rates at 100% saturation are expressed as $\mu l O_2 \cdot gram\ dry\ tissue^{-0.954}$. To compare rates of oxygen consumption at different PO\textsubscript{2}'s within temperatures, a second set of log\textsubscript{10} oxygen consumption to log\textsubscript{10} dry weight regressions was calculated and the lines subjected to analysis of covariance with temperature, salinity, and PO\textsubscript{2} as main effects and dry weight as the covariate.

Respiration rates under conditions of acutely declining oxygen tension were measured in a closed system respirometer. The respirometer vessel used at 20 and 30\textdegree C consisted of a 1000 ml Kontes reaction flask which had a plexiglas platform covering a magnetic stirring bar on the bottom. The vessel was completed by attaching a four-holed flask top to the bottom. The ground glass joint between top and bottom was sealed with a small amount of silicon vacuum grease and secured by a clamp. The center hole of the respirometer top was occupied by a Radiometer model E-5046 macro oxygen electrode mounted in a rubber stopper. The other three holes contained an inlet port that delivered water to the bottom of the chamber and two outlet ports, providing exhaust from the top. The inlets and outlets were equipped with stopcocks. The oxygen electrode
was attached to a Radiometer PHM71 Mk II acid-base analyzer. Because the respiratory rate of T. haemastoma is extremely low at 10°C, it was necessary to construct a 500 ml respirometer.

A declining oxygen tension experiment consisted of placing a snail in the bottom of the vessel, sealing the vessel, and placing it into a temperature controlled water bath over a magnetic stirrer. Air-saturated water was pumped into the vessel from a continually aerated reservoir. Once the respirometer vessel was filled with water and all air bubbles removed, the magnetic stirrer was turned on and water was allowed to flow through the system for an hour. After this period of acclimation to the apparatus, the pump was turned off, all stopcocks were closed, and the decline in PO$_2$ in the chamber was followed on an Omniscribe Model 5212-15 strip chart recorder connected to the Radiometer PHM71 Mk II Acid-Base analyzer. Oxygen consumption rates were calculated as follows: The total amount of oxygen (ml) in the respirometer vessel at full oxygen saturation was calculated by taking the volume of the chamber minus the volume of the snail and multiplying by the oxygen solubility in ml O$_2$ ml water$^{-1}$ (Strickland and Parsons, 1968). By dividing this value by the PO$_2$ at full saturation, μl O$_2$ mm O$_2$$^{-1}$ is obtained. The decline in PO$_2$ for a 30-minute interval was converted to oxygen consumption in μl O$_2$ hr$^{-1}$. The respiration rate thus calculated is considered to be the oxygen consumption rate at the midpoint PO$_2$ of the interval. The response to acutely declining oxygen tension was measured in snails acclimated to 10, 20 and 30°C and 10, 20 and 30°/ooS (n=10).

A second set of experiments was conducted to determine if the response to hypoxia is altered after chronic exposure. The response to acutely declining PO$_2$ was first measured on ten snails acclimated to 30°C and 30°/ooS. These animals were then marked with a non-toxic
indelible marker and placed into an aquarium being maintained at 51.6 ±
3.3 (± 95% C.I., n=28) mm O₂. The response to declining PO₂ of each
snail was measured again after exposure to chronic hypoxia for 28 days.

Three indices of respiratory response to declining PO₂ were
calculated for each temperature-salinity combination run. The critical
oxygen tension, or Pc, is the PO₂ where the respiratory rate changes
from being relatively oxygen-independent to being relatively oxygen-
dependent. The Pc was calculated for each experiment by running an
iterative partial linear regression program on the PO₂-oxygen
consumption rate data. Briefly, the program assumes that the PO₂-oxygen
consumption rate curve can be approximated by two straight lines,
representing the PO₂'s where the individual reacts more as an
oxyconformer (below the Pc) and more as an oxyregulator (above the Pc).
Suggested values for the Pc were supplied, and linear regressions
calculated based on the data points lying between the origin and the
putative Pc. This procedure was iterated until a maximum coefficient of
determination (R²) was obtained.

The second calculated index of respiratory oxygen dependence is
K₁/K₂ (Bayne, 1971; Tang, 1933). K₁ and K₂ are respectively the Y-
intercept and slope of a linear-transformed hyperbola fit of weight-
specific oxygen consumption to PO₂:

\[ \frac{PO₂}{VO₂} = K₁ + (K₂ \times PO₂) \]

When K₁ is large compared to K₂, the oxygen consumption rate is
proportional to PO₂, oxygen dependence. When K₁ is small compared to K₂,
the oxygen consumption rate approaches a constant, oxygen independence.
The magnitude of the index K₁/K₂ is inversely proportional to the degree
of oxygen independence of the aerobic respiration rate.

When the oxygen consumption rate at different oxygen tensions is
expressed as a percentage of the respiratory rate at full saturation, the data often fit the quadratic curve defined by:

\[
\text{Standardized } \dot{V}O_2 = B_0 + (B_1 \times P_{O_2}) + (B_2 \times P_{O_2}^2).
\]

When plotted in this manner, \(B_2\), the coefficient of the \(P_{O_2}^2\) term describes the departure from linearity. The stronger the degree of oxygen independence, the more negative \(B_2\) will be (Mangum and van Winkle, 1973).

Each of these indices was calculated for each declining oxygen tension experiment. Two way analyses of variance for temperature and salinity for each index were performed using the General Linear Models procedure of SAS (SAS Institute, 1982), with individual differences detected using the Duncan's Multiple Range Test and least squares means options. Correlation and regression analyses were performed to compare the three indices of respiratory response to declining \(P_{O_2}\); \(P_c\), \(K_1/K_2\), and \(B_2\). Paired-sample t-tests on the differences between the values of each index before and after chronic exposure to hypoxia were made by the SAS procedure Means (SAS Institute, 1982).

Biochemical Variables

Activities of the pyruvate oxidoreductase enzymes lactate dehydrogenase (LDH-E.C. 1.1.1.27), octopine dehydrogenase (ODH-E.C. 1.5.1.11), alanopine dehydrogenase (ADH-E.C. 1.5.1.x), and strombine dehydrogenase (SDH-E.C. 1.5.1.x) were determined in foot tissue of snails exposed for 28 days at 156-157, 107, 53, and 16 mm \(O_2\) at 30° and 30°/ooS using an NADH-linked spectrophotometric assay technique (Livingstone et al., 1983). Enzyme activities were also measured in foot tissue of snails on days 0, 2, 4, and 6 of exposure to anoxia.

Concentrations of ATP, ADP, AMP, and arginine phosphate were measured in foot tissue according to the methods in Lowry and Passonneau
(1972), substituting lobster arginine phosphokinase for creatine phosphokinase (Nicchitta and Ellington, 1983). Arginine phosphate and adenylates were measured after the same experimental conditions as used for the opine dehydrogenase enzymes. The adenylate energy charge (AEC) was calculated as \( \frac{[\text{ATP}]+0.5[\text{ADP}]}{[\text{ATP}]+[\text{ADP}]+[\text{AMP}]} \). Hexokinase was purchased from Boehringer, and all other biochemicals were purchased from Sigma. Foot tissue was used for the biochemical analyses because muscle tissues have the highest activities of opine dehydrogenase enzymes (Eberlee, et al., 1983; Plaxton and Storey, 1982) and because the foot of _Thais haemastoma_ is the easiest tissue to dissect rapidly before proteolysis and ATP degradation set in.

**RESULTS**

**Tolerance**

Hypoxia tolerance increased with decreasing temperature, but there was no clear trend across salinities (Fig. 2). As shown by an increase in the LC values over time, hypoxia tolerance decreased with increasing length of exposure to low oxygen tension.

_Thais haemastoma_ is very tolerant of moderate hypoxia for more than 28 days within the seasonal temperature and salinity range to which it is normally exposed. There were only six deaths recorded at 67% oxygen saturation over the entire course of the study, and those were all at 30°. At 10°, the snails tended to be moribund regardless of the PO\(_2\), and it was difficult to determine when an individual had died. Only the snails at 67 and 100% oxygen saturation had their foot extended at 10°. No feeding was observed at 10°, although fresh oysters were continually made available. At 20 and 30°C snails had their foot extended, were attached to the substrate and were actively crawling about the tanks at PO\(_2\) exposures down to 33% oxygen saturation for all
28 days of the hypoxia exposure. The snails exposed to 0 and 10% of oxygen saturation became inactive after two to three days of exposure and did not feed. These snails either had the foot extended, but limp and unattached to the substrate, or had the opercula tightly closed with the siphon showing in the siphonal canal of the shell. Occasionally the foot of a snail in a 0 or 10% oxygen saturation tank would appear to be swollen for several days before the snail died. A snail was considered to be alive if the foot or siphon would retract after tactile stimulation.

**Oxygen Consumption and Indices of Oxyregulation**

The rate of oxygen uptake at full oxygen saturation was directly related to the acclimation temperature (Fig. 3). At 10°C, the oxygen consumption rate was very low, indicating cold torpor, and the animals tended to be moribund. Q10's were 3.88 between 10 and 20°C, and 1.95 between 20 and 30°C. The overall Q10 between 10 and 30°C was 2.75.

After 28 days exposure to low PO2, the relationship between log10 oxygen uptake and log10 dry weight was different at each temperature, but was consistent across salinities and PO2's within temperatures. Oxygen consumption was directly related to acclimation temperature, and except for the group at 30°C and 10°C/oo, the tendency was either for oxygen consumption to be directly related to PO2 or for there to be no statistically significant difference in oxygen consumption rates across PO2's (Fig. 4).

In the declining oxygen tension experiments, snails extracted all detectable oxygen from the respirometer vessel in all nine temperature-salinity conditions used. Aerobic shutdown, as described by Mangum and van Winkle (1973) never occurred. At 20 and 30°C, the responses of individual snails to declining PO2 ranged from excellent oxyregulation,
with Pc's as low as 5 mm O₂ to poor oxyregulators with Pc's as high as 97 mm O₂. At 20 and 30°C snails were able to extract all oxygen from the respirometer in 8-12 hr. The 2-way analysis of variance of Pc on temperature and salinity indicated no significant difference in Pc with salinity. Pc was significantly higher at 10°C than at either 20 or 30°C, where Duncan's Multiple Range Test indicated no significant difference (Table II). Qualitatively, the overall response patterns of the oxygen consumption rate to acutely declining P0₂ were identical at 20 and 30°C (Table II). For both the relatively good regulator (Fig. 5a) and the relatively poor regulator (Fig. 5b) as P0₂ decreases, the oxygen consumption rate remains relatively constant until the Pc is approached, and as P0₂ continues to decrease, oxygen consumption becomes proportional to the oxygen partial pressure.

When the pump was turned off after the acclimation period in the declining P0₂ experiments at 10°C, the indicated P0₂ dropped by 10-20 mm O₂ within the first 10-15 minutes. This decline in P0₂ translated to an oxygen consumption rate in excess of 1000 μl g dry weight⁻¹ hr⁻¹, nearly ten times the average oxygen consumption rate at 10°C determined in the flow through system at full oxygen saturation. The rapid decline in P0₂ is very likely an artifact due to the change in hydrostatic pressure on the oxygen electrode membrane. For the calculation of the oxyregulation indices Pc, B2, and K₁/K₂ at 10°C, full oxygen saturation was considered to be in the range of 130-140 mm O₂.

At 10°C oxygen consumption remained fairly constant between 50 and 150 μl g dry weight⁻¹ hr⁻¹ over most of the P0₂ range (Fig. 5C). Pc's varied widely among different individuals, some as low as 10 mm O₂ and some as high as 130 mm O₂ (Table II). The K₁/K₂ values were uniformly low at 10°C (Table II), indicating good oxyregulation, as could also be
inferred from the consistency of oxygen consumption rates over the large range of $P_{O_2}$. This consistency, however, made the relationship between $P_{O_2}$ and standardized oxygen consumption linear, the average slope being 0.12, with the result that the quadratic term, or $B^2$ value at $10^\circ$ was not significantly different from 0 (Table II), and would indicate that the snails are not regulating at this temperature. There was not a significant difference in any of the calculated oxyregulation indices with salinity at $10^\circ$.

According to a two-way analysis of variance, oxyregulatory ability as measured by the quadratic coefficient of the second degree polynomial, $B^2$, relating standardized oxygen consumption to $P_{O_2}$ was highly significant for temperature ($P<0.0001$), but not significant for salinity ($P=0.1402$) when considered across all temperature-salinity combinations. Duncan's Multiple Range test for temperature indicated that $B^2$ was not different between 20 and $30^\circ$, but that a significant difference existed between the two higher temperatures and $10^\circ$.

The magnitude of $K_{1}/K_2$ is inversely related to oxyregulatory ability. There was a highly significant ($P<0.0001$) difference in the mean value of $K_{1}/K_2$ with temperature, the best regulation occurring at $10^\circ$, the poorest at $30^\circ$. There was also a salinity factor, regulation at 20 and $30^\circ/\text{o}o$ being poorer ($P=0.0061$) than at $10^\circ/\text{o}o$.

The only correlation between oxyregulation indices was between Pc and $B^2$, ($P=0.0002$) with a correlation coefficient ($r$) of 0.39 ($n=90$). There was not a significant correlation between Pc and $K_{1}/K_2$ or between $B^2$ and $K_{1}/K_2$.

Eight out of ten snails showed no evidence of acclimation to hypoxic conditions at $30^\circ$ and $30^\circ/\text{o}o$ as a shift of the $P_{O_2}$-oxygen consumption curve to the left. The oxygen tension-oxygen consumption
curves generated before and after 28 day's exposure to 53 mm O$_2$ were either superimposed or slightly shifted to the right (Fig. 6). Paired-sample t-tests indicate no significant differences in either the B2 or $K_1/K_2$ index before and after chronic hypoxia regardless of whether the index for the pre-exposure run had been calculated over the PO$_2$ range 0-156 mm O$_2$ or over the range of 0-53 mm O$_2$.

**Biochemical Variables**

The total adenylate pool ([ATP]+[ADP]+[AMP]) remained constant in foot tissue of *Thais haemastoma* at 30°C and 30°C/ooS after 28 days exposure to hypoxia between 10 and 100% of oxygen saturation (Fig. 7). ATP concentrations were significantly lower, and ADP and AMP concentrations were significantly higher in the hypoxia-exposed animals than in the normoxic controls (Fig. 7). There was a slight, but statistically significant decrease in the AEC in the hypoxia-exposed snails relative to the controls (Fig. 8). There was not, however, a significant difference in AEC among the three groups exposed to 10, 33, and 67% of oxygen saturation (Fig. 8). The concentration of arginine phosphate was the same in animals exposed to either 100 or 67% oxygen saturation, but had declined by half at 53 and 10% of oxygen saturation (Fig. 8).

In snails exposed to anoxia (<5 mm O$_2$) both the AEC and arginine phosphate concentration showed a significant decline within one day (Fig. 9). After the first day of anoxia, concentrations of arginine phosphate were not significantly different from zero. The AEC also did not show significant decreases after day one. The total adenylate pool remained constant over the six days of anoxic exposure (Fig. 10). The concentration of ATP declined over the first two days of exposure, while ADP and AMP concentrations continued to rise, with the greatest increase
occurring over the first two days of exposure (Fig. 10).

Alanopine dehydrogenase had the highest activity among the opine dehydrogenase class of pyruvate oxidoreductases regardless of either the duration or degree of hypoxia. Octopine dehydrogenase activity was not detected in any treatment. After 28 days exposure to 33, 67, or 100% of oxygen saturation, analysis of variance showed no significant difference in any of the pyruvate oxidoreductase activities with PO₂ (Table III). The analysis of variance for alanopine dehydrogenase activity was not significant, P=0.0547. None of the snails exposed to 0 or 10% oxygen saturation survived for the 28 day exposure period.

During six days exposure to anoxia, the lactate dehydrogenase activity showed a slight, but significant increase in activity on the fourth and sixth day. There was no change in strombine dehydrogenase activity, and alanopine dehydrogenase activity nearly doubled on day one, but had returned to the control activity by day two and did not change over the next four days (Fig. 11).

DISCUSSION

_Thais haemastoma_ survives for more than 28 days at oxygen tensions approaching zero, placing it in about the mid range of molluscan anoxia tolerance (Table IV). It is extremely tolerant of mild hypoxia, surviving indefinitely at PO₂-s as low as one-third of oxygen saturation. There is no apparent difference in hypoxia tolerance with salinity, and tolerance is inversely related to temperature (Fig. 4). The Caminada Pass-Barataria Bay estuary, where this population of _T. haemastoma_ is found, is characterized by seasonal and diurnal fluctuations of temperature, salinity, and dissolved oxygen (Barrett, 1971). Over a 24-hour period, the dissolved oxygen levels at one of the collection sites varied from a low of 25% saturation at 6:30 A.M. to
nearly 120% saturation in the early afternoon of September 27, 1980 (Stickle, unpublished data). Seasonally, the snails are subject to long-term hypoxia, burrowing into the sediment in the winter, when the ambient temperature falls below 20°C. Snails emerge from the sediment during the winter during intermittent warm spells which last long enough to increase the temperature in this shallow estuarine system. They will also occasionally burrow into the sediments in the summer when the temperature exceeds 30°C (personal observations). Snails have been collected in mid-August covered with black, hydrogen sulfide-laden mud from around the bases of pilings.

The hypoxia tolerance of *T. haemastoma* at 10° does not vary much with duration of exposure (Fig. 2), the 28-day LC-50 is 10-16 mm O₂, and the anoxia LT-50 (time to 50% mortality) is 12-22 days. These data are indicative of the high degree to which *T. haemastoma* is adapted to its environment. The ability of the snail to overwinter in anoxic sediments is determined by its high hypoxia tolerance and low metabolic demand (100 μl O₂ · g dry weight⁻¹ · hr⁻¹) at low temperature. It is during the winter months that *T. haemastoma* is the most likely to encounter chronic hypoxia. The response of *T. haemastoma* to anoxia while buried in hydrogen sulfide-laden sediments may be different than the response to anoxia in the water column due to a possible negatively synergistic interaction of anoxia and hydrogen sulfide on survival. Theede et al. (1969) found that the anoxia tolerance of several benthic species declined when hydrogen sulfide was added to the system.

In an encyclopedic study, Theede et al. (1969) determined the anoxia LT-50 in several species of benthic invertebrates from the Baltic and North Seas under anoxic conditions. They found that species from soft muddy substrates such as the bivalves *Cyprina islandica*,
Scrobicularia plana, and Mya arenaria, or those intertidal species that were regularly exposed to air, such as Mytilus edulis were able to survive longer in the absence of oxygen than were species inhabiting hard, submerged substrates such as the asteroid, Asterias rubens, or the shrimp, Crangon crangon. In general, the hypoxia tolerance of species from microhabitats where the dissolved oxygen concentration varies is greater than the hypoxia tolerance of species living in environments where dissolved oxygen is stable and at high concentrations over the long term (Vernberg, 1972). Habitat differences appear to be more closely related to differences in hypoxia tolerance than are phylogenetic relationships (Theede, et al., 1969; Vernberg, 1972). Sessile or slow-moving species show a greater tolerance to hypoxia than do highly active pelagic species (Stickle and Kapper, unpublished data; Vernberg, 1972).

One response of mobile animals to declining oxygen tension is to migrate from the area. A common behavior of T. haemastoma during the declining oxygen tension experiments was to crawl to the top of the respirometer and remain there for the duration of the experiment. Southern oyster drills are often found in the field close to the air-water interface, where dissolved oxygen levels are at a maximum. T. haemastoma is only rarely found out of the water.

Acclimation can be defined as the return of a rate function towards its original value after an alteration in some environmental parameter (Prosser, 1973). For oxygen consumption under chronic hypoxia, acclimation occurs if the oxygen consumption rate at low PO$_2$ is higher after the acclimation period than before. If the oxygen consumption rates before and after chronic exposure to hypoxia are the same, then complete (100%) acclimation has occurred.
The oxygen consumption rates of *T. haemastoma* exposed to long-term hypoxia stress exhibit the same general pattern as the oxygen consumption rates with acutely declining oxygen tension (Figs. 4, 5); as the PO$_2$ declines, so does the rate of oxygen consumption. This pattern is most pronounced at 30°C, and is suggested at 20°C. At 10°C, the snails are in a state of cold torpor and do not respond to most stimuli. The fact that the oxygen consumption rate of oyster drills exposed to low PO$_2$ for 28 days is lower than that at full oxygen saturation and the fact the oxygen consumption-PO$_2$ curve does not shift to the left after 28 days exposure to 53 mm O$_2$ at 30°C and 30% ooS indicates that *T. haemastoma* does not exhibit acclimation to moderate hypoxia. *T. haemastoma* is slightly heat-stressed at 30°C, the scope for growth (excess of energy absorbed over energy expended through metabolic processes) under normoxic conditions at 30° and 30%/oo is approximately 350 joules·day$^{-1}$ for a 1 g dry weight snail compared to 1500 joules·day$^{-1}$ at 20° and 30%/oo (Stickie, 1985). Even though the oxygen consumption rate of *T. haemastoma* did not acclimatize to moderate hypoxia, the snails did not show any behavioral evidence of physiological stress at 53 mm O$_2$. They continued to move about the aquaria and feed throughout the four week experiment, even with the reduction in the adenylate energy charge from 0.86 in the controls to 0.68 after 28 days exposure. Total metabolic demand of the snail at 33 and 10% oxygen saturation is likely decreased, as reflected by a reduced rate of oxygen consumption (Gade, 1983). Reduction of the total metabolic output of marine molluscs during anoxia can be considerable, anaerobic heat production of *Littorina irrorata* (Pamatmat, 1978) and *Littorina littorea* (Hammen, 1908) is only 5% of aerobic heat production, *Modiolus demissus* produces 7.5% of its aerobic heat output while under anoxia (Pamatmat, 1979), and *Mytilus*
produces only 7-10% of its aerobic heat production when kept under a nitrogen atmosphere (Shick et al., 1983).

In contrast to the lack of acclimation of oxyregulation by *T. haemastoma*, Bayne and Livingstone (1977) noted a distinct shift of the oxygen consumption–PO\textsubscript{2} curve of *Mytilus edulis* to the left after five days at 53 mm O\textsubscript{2}. The shift was accompanied by a decrease in the oxyregulation index B2 from $-0.049 \times 10^{-3}$ to $-0.345 \times 10^{-3}$ over the course of 17 days. Acclimation of the rate of oxygen consumption in *M. edulis* was evident at both 10 and 17°C. In contrast, Bayne et al., (1976) found no evidence of acclimation of oxygen consumption in *Mytilus californianus* maintained at 58 mm O\textsubscript{2} for up to 13 days, a response similar to that of *T. haemastoma*.

One strategy used to maintain an adequate oxygen supply to the gills during hypoxia is increased ventilation (Herreid, 1980). Increased energy use for ventilation results in an increased rate of oxygen consumption, which, as PO\textsubscript{2} continues to decline, leads to further increases in ventilation. Eventually (at the Pc) the benefit of the greater effort put into ventilation is exceeded by its the energetic cost and both ventilation and oxygen consumption decline. An increase in ventilatory effort might explain the transient increase in the oxygen consumption rate at the Pc of the snail in Fig. 5A.

The measurement of ventilation and excurrent PO\textsubscript{2} in gastropods is difficult because of the continual movement of the siphon and the diffuse nature of the exhalant stream. One cannot put a respiratory mask on a snail as is commonly done with crabs (Sabourin, 1984) or place flow sensors and oxygen electrodes by a well-defined exhalant siphon, as has been done with bivalves (Taylor and Brand, 1975a). In larger snails, these technical problems are surmountable, and Mangum and Polites (1980)
were able to measure ventilation and the P0₂ of the exhalant current in
the whelk *Busycon canaliculatum* under declining oxygen tension. *B. canaliculatum* is a very large snail, the dry soft tissue weight of
adults reaching 20 grams or more as compared to *Thais haemastoma*, where
the dry weight of the soft tissues of a large individual may be 3–4 g.
Oxygen consumption in *B. canaliculatum* is regulated between 40 and 120
mm O₂ and is proportional to the P0₂ on either side of that range. The
changes in oxygen consumption are reflected in the ventilation rate and
percent oxygen extraction. As the oxygen consumption rate declines with
declining P0₂ from 150 to 120 mm O₂ ventilation and percent extraction
both decline. As the P0₂ continues to decline, the percentage of oxygen
extracted from the ventilatory current rises concurrent with an increase
in the ventilation rate, having the effect of maintaining the overall
rate of oxygen consumption constant (Mangum and Polites, 1980).

Among the factors that have been shown to affect oxyregulatory
ability in molluscs are temperature, salinity, and size (Herreid, 1980).
Many authors have found that within a species large individuals are
better regulators than are small individuals (Bayne, 1971; Famme, 1980;
Murdoch and Shumway, 1980; Taylor and Brand, 1975a,b). This relationship
cannot be made for this set of experiments with *T. haemastoma* since the
snails used for the declining oxygen tension experiments were selected
for a uniform, large size.

The lower mean value for K₁/K₂ at 20° than at 30° (Table II)
indicates that *T. haemastoma* is better able to regulate its oxygen
consumption at 20°C than at 30°C, where it is under a slight degree of
heat stress (Stickle, 1985). The average B2 values of *T. haemastoma* at
10°C are not significantly different from zero, indicating minimal
oxyregulation. The oxygen tension–oxygen consumption curve at 10° has a
slope of $0.12\, \mu l \cdot g \, dry \, weight^{-1} \cdot hr^{-1} \cdot mm \, O_2^{-1}$ for nearly the entire range of $P_0$ (Fig. 6C), which, along with the low value of $K_1/K_2$ (Table II) indicates excellent regulation.

At 20°C, both $K_1/K_2$ and $B_2$ indicate better oxyregulation in $T_. haemastoma$ at 10 and 30°/ooS than at 20°/ooS (Table II). In the marine pulmonate $Amphibola crenata$, which occurs in salinities between fresh water and 40°/ooS, $K_1/K_2$ is lowest and $B_2$ is the most negative between 0 and 25°/ooS indicating the best oxyregulation at the lowest salinities (Shumway, 1981). In contrast, the oxyregulatory ability of the bivalves $Anadra granosa$ and $Gelonia ceylonica$ were reduced at the lower end of their salinity ranges (Bayne, 1973).

It should be kept in mind that the terms "oxyregulation" and "oxyconformity" do not describe a dichotomy, but rather the two extremes of a continuum (Mangum and van Winkle, 1975), and that the two oxyregulation indices, $K_1/K_2$ and $B_2$ are only mathematical constructs that indicate approximately where an animal's response pattern lies on that continuum. Each index appears to have validity, and has been calculated for several molluscan species (Table V). Only rarely, however, have both indices been calculated from the same declining oxygen tension experiments as in these experiments. The failure of these oxyregulation indices to show strong correlations in this study dictates that care must be used in their interpretation. In some species, the oxygen consumption rate under declining $P_0$ does not follow a simple convex curve as is assumed for the calculation of $P_c$, $K_1/K_2$, and $B_2$. Sometimes, as with $Busycon canaliculatum$, a region of strong oxyregulation will be "sandwiched" in between two regions of oxyconformity (Mangum and Polites, 1980). Whenever the actual response of the respiration rate to declining oxygen tension departs sufficiently
from the initial assumptions of the model that the index is based on, the calculated result will be meaningless (van Winkle and Mangum, 1975).

The opine dehydrogenase enzymes are functionally equivalent to lactate dehydrogenase, anaerobically condensing an amino acid with pyruvate to produce an opine compound while oxidizing NADH (Livingstone, 1983). Their occurrence is extremely widespread throughout the animal kingdom (Livingstone, et al., 1983). Strombine, the condensation product of glycine and pyruvate, has been demonstrated to accumulate in the posterior adductor muscle of *Mytilus edulis* during air exposure (de Zwaan and Zurburg, 1981), and in the adductor muscle of *Crassostrea virginica* exposed to anoxia (Eberlee, et al., 1983), although in both cases the increase in the concentration of strombine was less than that of alanine, indicating that the opine pathway is quantitatively less important during anaerobiosis than the alanine pathway. The concentration of strombine continued to increase in *M. edulis* (de Zwaan and Zurburg, 1981) and *C. virginica* (Eberlee et al., 1983) adductor muscle during recovery before returning to control levels. Similarly, alanopine accumulated in the adductor muscle of *Modiolus squamosus* during air exposure, and after a brief drop at the onset of recovery, continued to accumulate for six hours of a twelve hour recovery period (Nicchitta and Ellington, 1983). Continued use of anaerobic pathways during aerobic recovery has been hypothesized by de Zwaan et al. (1983) to supplement aerobic pathways during the replenishment of the arginine phosphate pool when ATP requirements are high.

Although octopine, alanopine, and strombine were not assayed for, some preliminary hypotheses may be drawn regarding the possible use of these molecules during exposure to hypoxia or anoxia in *T. haemastoma*. Firstly, unlike *Thais lapillus*, which has a very high octopine
dehydrogenase activity (Livingstone, 1982), the absence of any
detectable octopine dehydrogenase activity in T. haemastoma (Table III)
rules out octopine as a metabolite during either anoxia or a subsequent
recovery period. Secondly, since the activities of the opine
dehydrogenases are the same in snails that have been exposed to low
oxygen levels for 28 days as in snails maintained for 28 days at
normoxia, (Table III) it is unlikely that the synthesis of these enzymes
is induced as a response to chronic hypoxia. Thirdly, the transient
increase in alanopine dehydrogenase activity on the first day of anoxia
(Fig. 11) suggests that alanopine is being produced during the
transition from the use of aerobic to anaerobic metabolic pathways.
Alanine, the amino acid substrate of alanopine dehydrogenase, is a major
end product of anaerobic metabolism in T. haemastoma (Ellington,
personal communication). If, in fact, the alanopine dehydrogenase
pathway is activated during the onset of hypoxia, it may be one of the
mechanisms by which T. haemastoma is able to successfully cope with the
large diurnal fluxes in PO₂ that characterize its environment during the
spring and summer. Since the detection of an enzyme activity in vitro
does not verify its role in vivo, these hypotheses remain to be verified
experimentally for T. haemastoma.

Although the adenylate energy charge has not fulfilled its early
promise as an absolute quantitative indicator of stress (Ivanovici,
1979), it is still useful quantitatively for intraspecific and
qualitatively for interspecific comparisons when coupled with knowledge
of muscle phosphagen concentrations. That T. haemastoma is slightly
stressed by mild hypoxia is evident from the decline in energy charge
from 0.86 in snails remaining at 100% oxygen saturation to 0.72 in
snails exposed to 67% oxygen saturation for 28 days (Fig 8). Use of
energy charge alone can be deceiving. When oxygen saturation is less than 100%, there is not a significant difference in either the concentrations of adenylate phosphates or in the adenylate energy charge (Fig. 7) which would suggest that there is no difference in the magnitude of stress incurred by snails exposed to several partial pressures of dissolved oxygen. Use of the adenylate energy charge as a stress index is misleading without concurrent knowledge of the concentration of the muscle phosphagen. There is a significant 59% decrease in the concentration of arginine phosphate between 67 and 33% oxygen saturation which indicates that the snails are at a lower energy level in the more hypoxic condition.

In snails exposed to anoxia (<5-8 mm O₂) the decline in energy charge and arginine phosphate concentrations occurred within one day, an identical response as observed in the whelk, Nassa mutabilis (Gade, et al., 1984) the mussel Mytilus edulis (Ebberink and de Zwaan, 1980; Wijsman, 1976), the cockle, Cardium tuberculatum (Gade, 1980), the anemone Bunodosoma cavernata (Ellington, 1981) and others (Table VI). During this initial exposure to anoxia, metabolism is changing from dependence on high output aerobic pathway to low output anaerobic pathways (de Zwaan, 1977; Gade, 1983). Evidently this transition takes some time, because ATP levels are maintained at the expense of arginine phosphate. Arginine phosphate is the primary source of high-energy phosphate during muscular activity in molluscs (Gade, 1980; Grieshaber, 1978). The extremely low level of motor activity in T. haemastoma at very low oxygen tensions may be related to the depletion of the phosphagen pool.

The energy charge in foot tissue of T. haemastoma after one day of anoxia was 0.58, a 28% decline from the control while arginine phosphate
concentration had fallen by 95%. In contrast, in snails exposed to 10% oxygen saturation for 28 days, energy charge had declined by 20% and arginine phosphate concentration by only 54%. The threshold between the zone of capacity adaptation (the environmental conditions where the animal can survive indefinitely) and the zone of resistance adaptation (where, unless environmental conditions change for the better, death is inevitable) (Vernberg and Vernberg, 1972) can be better predicted by a significant decline in the concentration of the phosphagen, arginine phosphate, than by a decline in the adenylate energy charge in foot tissue of T. haemastoma.

A temporary period of anoxia or hypoxia often results in a transient increase in both the aerobic respiration rate and degree of oxyregulation during the recovery period (Herreid, 1980). Both length and intensity of hypoxia exposure are correlated with the magnitude of increase in oxygen consumption and oxyregulation relative to the pre-exposure state (Shumway, 1981; Bayne and Livingstone, 1977). The presence of an elevated rate of oxygen consumption after anoxia and increased evidence of oxyregulation is most pronounced in animals living in microhabitats regularly exposed to hypoxic conditions (McMahon and Russell-Hunter, 1978). Higher than normal oxygen consumption rates after a hypoxic episode have been compared to the classical oxygen debt (Herreid, 1980), where the increased oxygen consumption is used to metabolize the accumulated end products of anaerobiosis. If such an oxygen debt is present in T. haemastoma after short term exposure to anoxia it would indicate that the responses to acute and chronic hypoxia are very different. It would be useful to determine if T. haemastoma incurs an oxygen debt after acute hypoxia, and if so, if it is associated with an accumulation of alanopine or strombine during the
recovery phase. Better understanding of the oyster drills response to short term anoxia would be gained by a fine tuning of the time course of change in tissue arginine phosphate concentration and adenylate energy charge and the recovery of these parameters during re-exposure of oyster drills to normoxic conditions.

It has been established that Thais haemastoma is very tolerant to low oxygen conditions within its normal range of temperatures and salinities. At 20 and 30°C the pattern of oxygen consumption with declining PO\textsubscript{2} is variable, but most individuals tend to be oxyconformers. At 10°C, the aerobic respiration rate is extremely low (100 μl O\textsubscript{2} · g dry weight\textsuperscript{-1} · hr\textsuperscript{-1}), but is constant over the PO\textsubscript{2} range of 20-140 mm O\textsubscript{2} reflecting the torpid state of the snail at low temperatures.

Although T. haemastoma survives for 28 days at 33% of oxygen saturation, the animals do not show acclimation of the aerobic metabolic rate during hypoxia. Finally, the opine dehydrogenase pathway is probably not being used to maintain ATP production during chronic hypoxia, but may be active during the initial transition to anaerobic metabolism at the onset of hypoxic stress.
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**TABLE I**

Mean weekly water quality variables for the 30°C-30‰ bioassay experiment. Values are running means from day 0 up through the indicated day. Underlined values for ammonia and pH are the extremes recorded. These are representative of the complete set of hypoxia tolerance experiments.

<table>
<thead>
<tr>
<th>Target PO2 (mm Hg)</th>
<th>0-7</th>
<th>0-14</th>
<th>0-21</th>
<th>0-28</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>9</td>
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<td>7</td>
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<td>107</td>
<td>18</td>
<td>13</td>
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<td>16</td>
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<td>7.6</td>
<td>7.5</td>
<td>7.5</td>
<td>7.4</td>
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</tbody>
</table>
Indices of oxyregulation in *Thais haemastoma* exposed to acutely declining oxygen tension. \( P_c \), the critical oxygen tension, was calculated from the breakpoint of the curve relating \( P_O_2 \) to oxygen consumption as calculated from the iterative partial regression technique. \( B2 \times 10^3 \) is the quadratic coefficient relating standardized oxygen consumption to \( P_O_2 \) (Mangum and van Winkle, 1973). \( K_1/K_2 \) is calculated as the slope divided by the y-intercept of the linear-transformed hyperbola relating weight-specific oxygen consumption to \( P_O_2 \) (Bayne, 1971). Data are given as \( x \pm S.E., n=10 \).

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<th>TEMP. INDEX</th>
<th>SALINITY (%ooS)</th>
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<th>30</th>
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<tr>
<td>10</td>
<td></td>
<td>( P_c )</td>
<td>-0.022 ± 0.022</td>
<td>-0.032 ± 0.022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( B2 )</td>
<td>17 ± 5</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>( P_c )</td>
<td>57 ± 5</td>
<td>50 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( B2 )</td>
<td>-0.100 ± 0.016</td>
<td>-0.047 ± 0.007</td>
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<tr>
<td></td>
<td></td>
<td>( K_1/K_2 )</td>
<td>43 ± 6</td>
<td>82 ± 13</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>( P_c )</td>
<td>50 ± 7</td>
<td>55 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( B2 )</td>
<td>-0.069 ± 0.010</td>
<td>-0.043 ± 0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( K_1/K_2 )</td>
<td>62 ± 10</td>
<td>95 ± 12</td>
</tr>
</tbody>
</table>
### TABLE III

Activities (μmoles·g wet tissue⁻¹·min⁻¹) of the pyruvate oxidoreductase enzymes in foot tissue of *Thais haemastoma* exposed to hypoxia at 30°C and 30°C/oo for 28 days (X ± S.E., n=5)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>% Oxygen Saturation</th>
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<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>2.08 ± 0.13</td>
</tr>
<tr>
<td>Octopine Dehydrogenase</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Alanopine Dehydrogenase</td>
<td>6.73 ± 1.34</td>
</tr>
<tr>
<td>Strombine Dehydrogenase</td>
<td>2.00 ± 0.24</td>
</tr>
<tr>
<td>Species</td>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td><em>Littorina littorea</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Littorina saxatilis</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Nassarius obsoletus</em></td>
<td>22</td>
</tr>
<tr>
<td><em>Nassarius trivittatus</em></td>
<td>22</td>
</tr>
<tr>
<td><em>Thais haemastoma</em></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td><em>Cardium edule</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Crassostrea virginica</em></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10-15</td>
</tr>
<tr>
<td></td>
<td>15-20</td>
</tr>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td><em>Crassostrea virginica</em></td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td><em>Cypria islandica</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Mulinia lateralis</em></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td><em>Mya arenaria</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Mytilus edulis</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Scrobicularia plana</em></td>
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## TABLE V

Values of the oxyregulation indices B2 and $K_1/K_2$ in marine molluscs.

<table>
<thead>
<tr>
<th>Species</th>
<th>B2</th>
<th>$K_1/K_2$</th>
<th>Reference</th>
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<tr>
<td><strong>Gastropods</strong></td>
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<tr>
<td>Acmaea testudinalis</td>
<td>-0.8180</td>
<td></td>
<td>McMahon &amp; Russell-Hunter, 1978</td>
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<tr>
<td>Amphibola crenata</td>
<td>-0.0497</td>
<td>96</td>
<td>Shumway, 1981</td>
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<tr>
<td>Crepidula fornicata</td>
<td>-0.0868</td>
<td>9-26</td>
<td>Newell et al., 1978</td>
</tr>
<tr>
<td>Lacuna vincta</td>
<td>-0.0603</td>
<td></td>
<td>McMahon &amp; Russell-Hunter, 1978</td>
</tr>
<tr>
<td>Littorina littorea</td>
<td>-0.0403</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Littorina obtusata</td>
<td>-0.0513</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Littorina saxatilis</td>
<td>-0.0503</td>
<td></td>
<td></td>
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<td>Mitrella Lunata</td>
<td>-0.0320</td>
<td>to +0.0100</td>
<td>17-34</td>
</tr>
<tr>
<td>Thais baenata (10°)</td>
<td>-0.0466</td>
<td>to -0.1001</td>
<td>41-82</td>
</tr>
<tr>
<td>&quot; (20°)</td>
<td>-0.0428</td>
<td>to -0.0734</td>
<td>62-99</td>
</tr>
<tr>
<td>&quot; (30°)</td>
<td>-0.1180</td>
<td></td>
<td>Mangum &amp; van Winkle, 1973</td>
</tr>
<tr>
<td><strong>Bivalves</strong></td>
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<td></td>
</tr>
<tr>
<td>Anadara granosa (33°/oo)</td>
<td>-0.0097</td>
<td>to +0.0575</td>
<td>Mackay &amp; Shumway, 1980</td>
</tr>
<tr>
<td>&quot; (20°/oo)</td>
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<td>to -0.0756</td>
<td>McMahon, 1979</td>
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<tr>
<td>&quot;</td>
<td>-0.1200</td>
<td>to -0.2320</td>
<td>Shumway &amp; Koehn, 1982</td>
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<tr>
<td>&quot;</td>
<td>-0.0106</td>
<td>to +0.0489</td>
<td>Bayne, 1973</td>
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<tr>
<td>&quot; (20°/oo)</td>
<td>-0.2280</td>
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<tr>
<td>&quot;</td>
<td>-0.1200</td>
<td>to -0.2320</td>
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<td>&quot;</td>
<td>-0.1384</td>
<td>to -0.0735</td>
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<td>&quot; (20°)</td>
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<tr>
<td>&quot;</td>
<td>-0.0234</td>
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<td>-0.0695</td>
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<td>to +0.0062</td>
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<td>Tissue</td>
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<td>entire</td>
<td>0.88</td>
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<td>0.80</td>
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<td>entire</td>
<td>0.85</td>
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<td>P.A.M.</td>
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P.A.M. = posterior adductor muscle
FIGURE LEGENDS

1. Diagram of the hypoxia bioassay system showing path of water through filter unit, pump, exposure tank, and aquarium; and gas mixer connected to aquarium under-gravel filter.

2. 95% confidence bands around LC-90 (XXX), LC-50 (XX), and LC-10 (X) of Thais haemastoma over 28 days exposure to hypoxia at 10, 20 and 30°C and 10, 20, and 30°/ooS.

3. Rates of oxygen consumption in Thais haemastoma acclimated to different temperatures. (μl O₂ • g dry weight⁻¹ • hr⁻¹, ±S.E. n=30).

4. Rates of oxygen consumption (μl O₂ • g dry weight⁻¹ • hr⁻¹, ±S.E., n=10) at 10, 20, and 30°C and 10, 20, and 30°/ooS of Thais haemastoma after 28 days exposure to hypoxia. Points within temperature-salinity groups with the same letters from Duncan's Multiple Range test are not significantly different from each other. For 10°C, b=1.0024, for 20°C, b=0.6400, for 30°C, b=0.4800.

5. Oxygen consumption rate (μl O₂ • g dry weight⁻¹ • hr⁻¹) of Thais haemastoma as a function of ambient P0₂ (mm O₂) for (A) a relatively good oxyregulator; K₁/K₂=15, B2x10⁻³=0.121, Pc=31; and (B) and a relatively poor oxyregulator, K₁/K₂=140, B2x10⁻³=0.026, Pc=55. These are typical of snails run at 20 and 30°C. (C) Typical curve relating oxygen consumption rate (oxygen consumption in μl•S dry weight⁻¹•hr) as a function of ambient P0₂ (mm O₂) at 10°C; K₁/K₂=6, B2x10⁻³=0.005, Pc=134.
6. Representative response of oxygen consumption to declining oxygen tension before (○) and after (■) 28 days exposure to 53 mm O₂ at 30°C and 30°/ooS. The points are superimposed on each other, indicating no acclimation of the respiratory rate chronic hypoxia. This pattern was seen in 8 out of 10 individuals.

7. Concentrations of total adenylates, ATP, ADP, and AMP in foot tissue (μmoles • g wet weight⁻¹) of *Thaïs haemastoma* exposed to 10, 33, 67, and 100% oxygen saturation at 30°C and 30°/ooS for 28 days. (x±S.E., n=5).

8. Adenylate energy charge (AEC) and arginine phosphate concentration in foot tissue (μmoles • g wet weight⁻¹) of *Thaïs haemastoma* exposed to 10, 33, 67, and 100% oxygen saturation at 30°C and 30°/ooS for 28 days. (x±S.E., n=5).

9. Adenylate energy charge (AEC) and arginine phosphate concentration in foot tissue (μmoles • g wet weight⁻¹) of *Thaïs haemastoma* exposed to anoxia for 0, 1, 2, 4, and 6 days at 30°C and 30°/ooS for 28 days. (x±S.E., n=5).

10. Concentrations of total adenylates, ATP, ADP, and AMP concentration (μmoles • g wet weight⁻¹) in foot tissue of *Thaïs haemastoma* exposed to anoxia for 0, 1, 2, 4, and 6 days at 30°C and 30°/ooS for 28 days. (x±S.E., n=5).

11. Activities of pyruvate oxidoreductase enzymes (μmoles • g wet weight⁻¹ • min⁻¹) in foot tissue of *Thaïs haemastoma* at 30° and 30°/oo after 0, 1, 2, 4, and 6 days exposure to anoxia (<5 mm O₂). (x±S.E., n=5). ADH=alanopine dehydrogenase; LDH=lactate dehydrogenase; SDH=strombine dehydrogenase.
Fig. 1.

PO_{2} CONTROL

WATER TABLE

BIOFILTRATION UNIT

OYSTER CHIPS

OYSTER CHIPS
Fig. 2

Legend for the graph:
- 10% O₂
- 20% O₂
- 30% O₂

Graph showing oxygen levels in mm Hg across different temperature ranges (10°, 20°, 30°).
OXYGEN CONSUMPTION

(μl·g dry weight⁻⁰·⁹⁵₄·hr⁻¹)

Fig. 3
Fig. 4

% OXYGEN SATURATION

OXYGEN CONSUMPTION (μl g dry weight⁻¹ hr⁻¹)

PO₂ (mm O₂)

10°C
20°C
30°C

A
B
AB

400
300
200
100

1000
500
0

50 100
50 100
50 100

0 30 60 90 120 150
0 30 60 90 120 150
0 30 60 90 120 150
Fig. 7

% Oxygen Saturation

Concentration (μmoles g wet weight⁻¹)

PO₂ (mm Hg)

- Total
- ATP
- ADP
- AMP
Fig. 8

% Oxygen Saturation

Arginine Phosphate (μmoles·g wet weight⁻¹)

PO₂ (mm Hg)

AEC

ARG-P

Adenylate Energy Charge
ENZYME ACTIVITY
($\mu$ moles·g wet weight$^{-1}$·min$^{-1}$)

Fig. 11
VITA
MARTIN A. KAPPER

Personal:

Date of Birth: August 10, 1954
Birthplace: Bay Shore, N.Y.
Not Married

Home Address:
824 W. Chimes St. #3
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Education:

B.S. Biology, 1976. St. Lawrence University, Canton, N.Y. 13617

M.A. Biological Sciences, 1980. State University of New York at Buffalo, Buffalo, N.Y. 14260. Major Professor, C.A. Privitera. Project title: "The ability of the chiton Mopalia muscosa to extract oxygen from water and from air"

Ph.D. Physiology, August, 1985 (anticipated). Louisiana State University, Baton Rouge, La. 70803. Major Professor, W.B. Stickle, Jr. Dissertation title: "Metabolic responses of the estuarine gastropod Thais haemastoma to hypoxia"

Professional Experience:

1976-78: Teaching Assistant SUNY/Buffalo: Animal Physiology, Comparative Anatomy, Embryology, General Biology

1978-79: Technical Specialist, SUNY/Buffalo: Neurophysiology

1979-Present: Teaching Assistant, LSU: Comparative Physiology, Environmental Physiology of Estuarine Organisms, Elementary Physiology (lab), Human Physiology (lecture), General Zoology (majors), General Biology (non-majors)


Awards & Honors:

Robert A. LeFleur/Petroleum Refiners Environmental Council of Louisiana Graduate Fellow, 1980-84
Professional Societies:

American Society of Zoologists
American Society for the Advancement of Science

Papers Presented:


Publications:


Publications in Preparation:

Shirley, T.C., M.A. Kapper, W.B. Stickle, C. Brodersen, and S.D. Rice. Tolerance and Bioenergetics of Alaskan king crab, Paralithodes camtschatica, exposed to the WSF and oil contaminated sediment of Cook Inlet crude oil.

Stickle, W.B., M.A. Kapper, T.C. Shirley, M.G. Carls, and S.D. Rice. Tolerance and Bioenergetics of the pink shrimp (Pandalus borealis) during long term exposure to the water soluble fraction and oil contaminated sediments of Cook Inlet crude oil.

Stickle, W.B. and M.A. Kapper. Tolerance limits of several species of Northern Gulf of Mexico invertebrates to chronic hypoxia.

Kapper, M.A. and W.B. Stickle. Metabolic responses of the muricid gastropod Thais haemastoma to hypoxia.


DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Martin A. Kapper

Major Field: Physiology

Title of Dissertation: Metabolic Responses of the Estuarine Gastropod Thais haemastoma to Hypoxia

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

July 22, 1985