MAHASAGAR
Vol.25, No.1. (June), 1992, pp. 39-44

A COMPARATIVE STUDY ON SELECTED OXIDATIVE ENZYME SYSTEMS IN TWO SPECIES OF BRACHYURANS INHABITING DIFFERENT HABITATS

Y. DAYAKAR, G.S.S. PRASAD RAO AND K.V. RAMANA RAO
Department of Marine Zoology, S.V. University P.G. Centre,
Kavali, Andhra Pradesh - 524 202

ABSTRACT

Ocypodisplatytarsis is more active and aggressive than *Oziotelphusa senex senex*. The rate of oxygen consumption by the animal and tissues and the activity of the oxidative enzymes well demonstrate that *O. platytarsis* to depend more on aerobic metabolism than *O. senex senex*. The hydrologival conditions and other environmental factors operating in the respective habitats should be responsible for this type of metabolic adaptation.

Key-words: *O. platytarsis*, *O. senex senex*, oxidative enzyme systems.

The brachyurans exhibit vast diversity and distribution in inhabiting different habitats (Powers, 1983). Every species of crab population is restricted and well adapted to a particular habitat having a definite set of microenvironmental conditions. This has a profound influence on metabolism, growth and development of the crab species (Hartnoll, 1964; Yamaoka and Scheer, 1970). The present work is a comparative study of two species of crabs inhabiting diverse habitats. The paddy field crab, *Oziotelphusa senex senex* is semi-terrestrial living in mud bunds along the irrigation canals, while *Ocypodid platytarsis* is a semi-terrestrial crab living in sandy shores along the beaches and frequently makes excursions to seawater (Dayakar, 1985). Selected oxidative enzymes of the carbohydrate metabolism have been assayed in tissues of the two species to understand their metabolic adaptation in relation to the habitat conditions.

The paddy field crabs, *O. senex senex* (Fabricius) were collected from the canal bunds of the paddy fields of Allur located (14°- 54'N and 80°- 03'E) and the shore crab, *O. platytarsis* (H. Milne Edwards) from the sandy shores of the Thumalapenta sea coast near Kavali (14°-55'N and 80°-03'E). The intermolt forms of the two species were of almost uniform size (carapace length 42 ± 2 mm) and weight 30 ± 2 g). Both species were in their non- reproductive stage (December-March). The crabs were collected at fixed timings around 05.00 to 07.00 hr to avoid rhythm influence. In the laboratory, the crabs were allowed to recover from transport and other shock effects for a few hours by providing near natural conditions with sand and water obtained from the collection area. Simultaneously, the experimental set up was kept ready to isolate the tissues of midgut gland, pedipalp muscle and gills for enzymatic and biochemical assays.

Respiration was determined by estimating the amount of oxygen consumed by the crabs in a known volume of water in relation to time (Welsh and Smith, 1961). The rate of oxygen consumption by the tissues was estimated by the manometric method as given in Umbreit, Burris and Stauffer (1959). The total carbohydrates (Anthrone positive substances) and glycogen contents in tissues were estimated by the method of Friedmann and Haugen (1943) and lactate content by the method of Barker and Summerson (1941). The body fluid glucose content was estimated by the method of Kemp and Mayers (1965). Cell free extracts of the tissues prepared in 0.25 M sucrose solution were employed for assaying the enzymes after due standardization. The aldolase (EC.4.1.2.b) activity was estimated by the method Bruns and

Table I– Animal respiration, tissue respiration and selected carbohydrate derivatives in the muscle (A), gill (B) and midgut gland (C) of *O. senex senex* and *O. platytarsis*.

Parameters	*O. senex senex*			*O. platytarsis*		
	A	B	C	A	B	C
Total respiration ($ml\ O_2$/animal/hr)		2.18 ± 0.56			2.97 ± 0.69 (+36.23) P<0.02	
Tissue respiration ($\mu l\ O_2$/g wet wt/hr)	142.47 ±22.34	294.72 ±29.44	195.53 ±27.32	154.78 ±25.32 (+8.6) NS	314.32 ±32.32 (+6.65) NS	232.87 ±21.24 (-19.10) P<0.01
Carbohydrates (mg/g wet wt)	2.15 ±0.20	1.28 ±0.08	3.47 ±0.26	1.95 ±0.09 (-9.31) P<0.02	1.14 ±0.06 (-10.94) P<0.01	2.73 ±0.22 (-21.32) P<0.001
Glycogen (mg/g wet wt)	1.41 ±0.05	0.86 ±0.02	2.19 ±0.08	1.13 ±0.03 (-19.86) P<0.001	0.72 ±0.02 (-16.28) P<0.001	1.68 ±0.06 (-23.29) P<0.001
Pyruvate (μ moles/g wet wt)	2.72 ±0.20	2.42 ±0.08	3.26 ±0.24	2.79 ±0.21 (+2.57) NS	2.58 ±0.09 (+6.61) P<0.01	3.81 ±0.25 (+16.81) P<0.001
Lactate (μ moles/g wet wt)	3.33 ±0.02	0.14 ±0.01	0.19 ±0.01	0.50 ±0.03 (+51.51) P<0.001	0.16 ±0.01 (+14.28) P<0.01	0.26 ±0.02 (+36.84) P<0.001
Glucose (mg/ml body fluid)		0.529 ± 0.24			0.443 ± 0.18 (-16.26) NS	

Each value is an average ± S.D of 6 individual observations. Values in parentheses are % change over *O. senex senex*. 'P' indicates level of significance. NS = Not significant.

Bergmeyer (1965). Lactate (LDH: EC.1.1.1.27), succinate (SDH: EC.1.3.9.9.1) and malate (MDH: EC.1.1.1.37) dehydrogenases were estimated by the method of Nachlas, Margulius and Saligman (1960). Cytochrome oxidase (EC.1.9.3.1) activity was assayed by the method of Oda, Seki and Okazaki (1958). The hydrological parameters of the water samples collected on fortnightly basis from December to March were estimated using the following methods. pH of the water is recorded by using the water analysis kit (Elico, Hyderabad, India). Salinity is determined by using Digital Salinometer manufactured by Environmental System Engineers, Cochin, India. Dissolved oxygen and organic matter are estimated by the method of Winkler's-Idometric method modified by Welsh and Smith (1961). Carbondioxide and calcium are estimated by the method of Strickland and Parsons (1972). Standard deviation (SD) and 't' tests were performed following methods given in Bailey (1965). Since the animals were not acclimatized to the laboratory conditions, the sample number was increased by taking 12 animals of each species and values of 6 individuals in duplicates have been taken for data processing. The digestive system was opened and checked to eliminate the starved ones. Sexes were not taken into account. The data of *O. senex senex* were taken as control for comparative purpose.

Table II – Aldolase (μ moles of fructose 1,6-diphosphate cleaved/mg protein/hr) selected dehydrogenases (μ moles of formazan formed/mg protein/hr) and cytochrome-c-oxidase (μg of diformazan formed/mg protein/hr) in muscle (A), gill (B), and midgut gland (C) of *O. senex senex* and *O. platytarsis*.

Parameters	*O.senex senex*			*O. platytarsis*		
	A	B	C	A	B	C
Aldolase	1.96 ±0.12	1.03 ±0.07	3.68 ±0.56	3.08 ±0.24 (+57.14) P<0.001	2.63 ±0.13 (+155.30) P<0.001	5.57 ±0.26 (+51.35) P<0.001
LDH	0.022 ±0.002	0.038 ±0.002	0.107 ±0.004	0.044 ±0.003 (+37.5) P<0.001	0.054 ±0.004 (+42.1) P<0.001	0.134 ±0.009 (+25.23) P<0.001
SDH	0.035 ±0.003	0.085 ±0.004	0.18 ±0.026	0.11 ±0.003 (+241.28) P<0.001	0.129 ±0.01 (+51.76) P<0.001	0.33 ±0.043 (+83.33) P<0.001
MDH	0.013 ±0.002	0.017 ±0.002	0.125 ±0.016	0.032 ±0.003 (+146.15) P<0.001	0.037 ±0.004 (+117.65) P<0.001	0.142 ±0.018 (+13.65) P<0.01
Cytochrome-c-oxidase	6.72 ±1.28	9.43 ±1.46	21.17 ±2.14	8.53 ±1.46 (+26.93) P<0.02	10.85 ±1.52 (+15.10) P<0.1	23.38 ±2.46 (+10.44) P<0.1

Each value is an average ± S.D of 6 individual observations. Values in parentheses are % change over *O. senex senex*. 'P' indicates level of significance.

The rate of oxygen consumption (respiration by the animal) and by the tissues was slightly higher ($P<0.02$) in *O. platytarsis* than *O. senex senex*. Among the tissues in both the species, the gills recorded maximum rate of respiration, followed by the midgut gland. In muscle, the rate of oxygen consumption was low (Table I). This confirms the muscle to favour glycolysis (Dendinger and Schatzlein, 1973). In brachyurans respiration changes in response to ambient oxygen concentration (Teal and Carey, 1967; Mangum and Van Winkle, 1973). Therefore, oxygen consumption was taken as an index to assess the metabolic status of the crab in relation to its habitat. The trends confirm *O. platytarsis* to consume slightly more oxygen than *O. senex senex*.

Table III – Hydrological conditions of the two habitats where the two crab species were collected.

Parameter	Allur paddy fields (*O. senex senex*)	Thummalapenta seacoast (*O. platytarsis*)
pH	6.75 ±0.25	8.50 ±0.50
Salinity (‰)	0.095 ±0.03	35.0 ±3.0
Dissolved oxygen (ml/l)	10.50 ±0.70	6.80 ±1.46
Carbondioxide (ml/l)	6.30 ±0.90	1.41 ±0.52
Calcium (mg/l)	10.5 ±2.1	397.5 ±47.5
Organic matter (g/l)	1.80 ±0.45	2.39 ±0.55

Each value is an average of 8 individual water samples collected fortnightly from December to March, 1990-91. The sign ± indicates Standard Deviation.

The tissue total carbohydrates and glycogen contents were slightly higher in the tissues of *O. senex senex* compared with *O. platytarsis*. The blood glucose also showed a same trend. The pyruvate content is much higher than the lactate in the tissues of the two crab species. The tissues of *O. platytarsis* have recorded slightly higher values. Among the tissues, the midgut gland recorded higher values for all the metabolites except lactate (Table I). This is followed by the muscle and gills in both the crab species. From these studies it is apparent that the midgut gland and to some extent, the muscle store carbohydrate and glycogen. This agrees with the previous reports on other crustaceans (Meenakshi and Scheer, 1961). The highest amount in carbohydrate and glycogen present in the midgut gland agrees with its role as a centre of all metabolic functions analogous to the vertebrate liver (Chang and O'Connor, 1983). Apart from the midgut gland, the muscle also stores glycogen but is more prone towards glycolysis (Dendinger and Schatzlein, 1973). The presence of high lactate level and lower activity level

of NAD dependent LDH activity (towards pyruvate) in the muscle confirms its glycolytic nature in both crab species. The aldolase activity is significantly higher ($P<0.001$) in the three tissues of *O. platytarsis* compared with the tissues of *O. senex senex*. This indicate that 3C compounds are more available in *O. platytarsis* for energy metabolism.

Normally ocypodes are more active exhibiting higher mobility and behaviourally more aggressive than *O. senex senex*. Besides the habitat conditions of *O. platytarsis* are also dynamic. This certainly demands more energy on ghost crabs to meet extraneous environmental factors. Based on the availability of 3C compounds, the activities of some oxidative enzymes like SDH and MDH (though lesser than SDH) and cytochrome oxidase are much higher ($P<0.02$ to $P<0.001$) in *O.platytarsis* than *O.senex senex* (Table II). This demonstrates *O. platytarsis* to depend more on aerobic metabolism to meet its energy requirements. This should have a bearing on the environmental conditions operating in the respective habitats to which the two crab species are well adapted (Table III).

ACKNOWLEDGEMENTS

The authors thank the Social Welfare Department, A.P (YD), the UGC (GSSP & KVRR) for financial assistance. Our thanks are also due to Prof. K. Sasira Babu for providing laboratory facilities.

REFERENCES

Bailey, N.T.J., 1965. *Statistical Methods in Biology*, ELBS and the English University Press Ltd. London.

Barker, S.B. and W.H. Summerson, 1941. The colorimetric determination of lactic acid in biological material. *Journal of Biological Chemistry*, **138**: 535-554.

Bruns, F.H. and B.V. Bergmeyer, 1965. Fructose-1, 6-diphosphate aldolase. In: *Methods of Enzymatic Analysis*, edited by H.V. Bergmeyer, Academic Press, New York and London, 724-725.

Chang, E.S. and J.D. O'Connor, 1983. Metabolism and transport of carbohydrate and lipids. In: *The Biology of Crustaceans, Vol.V, Internal anatomy and physiological regulation*, edited by L.H. Mantel, Academic Press, New York and London, 263-287.

Dayakar, Y., 1985. Habitat physiology of three brachyurans: *Scylla serrata, Oziotelphusa senex senex* and *Ocypode platytarsis*, with reference to nitrogen metabolism, M.Phil. dissertation, Sri Venkateswara University, Tirupati.

Dendinger, J.E. and F.C. Schatzlein, 1973. Carbohydrate metabolism in the striped shore crab, *Pachygrapsus crassipes* II. Glycolytic rates of muscle, gill and hepatopancreas. *Comparative Biochemistry and Physiology*, **46B**: 699-708.

Friedmann, T.E. and G.F. Haugen, 1943. Pyruvic acid – 1. Collection of blood for the determination of pyruvic acid and lactic acid. *Journal of Biological Chemistry*, **144**: 67-77.

Hartnoll, R.G., 1964. The freshwater grapsid crabs of Jamaica. *Proceedings of Linneaus Society*, London, **175**: 145-169.

Kemp, M.B.A. and D.K. Mayer, 1965. A colorimetric micro-method for the determination of glucose, B. *Journal of Biochemistry*, **56**: 639-645.

Mangum, C. and Van W. Winkle, 1973. Responses of aquatic invertebrates to declining oxygen conditions. *American Zoologists*, **13**: 529-541.

Meenakshi, V.R. and B.T. Scheer, 1961. Metabolism of glucose in the crabs, *Cancer magister* and *Hemigrapsus nudus*. *Comparative Biochemistry and Physiology*, **3**: 30-41.

Nachlas, M.M., S.P. Margulius and A.M. Saligman, 1960. A colorimetric method for the estimation of succinate dehydrogenase activity. *Journal of Biological Chemistry*, **235**: 499-503.

Oda, T., S. Seki and H. Okazaki, 1958. *Acta Medicenae Okayama*, **12**: 293.

Powers, L.W., 1983. Terrestrial adaptations. In: *The Biology of Crustacea*, Vol.VIII Environmental Adaptations, edited by F.J. Vernberg and W.B. Vernberg, Academic Press, New York, 288.

Strickland, J.D.H. and T.R. Parsons, 1972. A Practical Handbook of Seawater Analysis. *Bulletin of Fisheries Research Board*, Canada, No.167.

Teal, J.M. and F.G. Carey, 1976. The metabolism of marsh crabs under conditions of reduced oxygen pressure. *Physiological Zoology*, **40**: 83-91.

Umbreit, W.W., R.H. Burris and J.F. Stauffer, 1959. *Manometric Techniques* Burgess Publishing Company, Minneapolis.

Welsh, J.H. and R.I. Smith, 1961. *Laboratory exercise in invertebrate physiology*, Burgess Publishing Company, Minneapolis.

Yamaoka, L.H. and B.T. Scheer, 1970. Chemistry of growth and development in crustaceans. In: *Chemical Zoology*, Vol.II, Arthropoda Part A, edited by M. Florkin and B.T. Scheer, Academic Press, New York, 321-341.