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SERUM CONSTITUENTS OF THE MALAYSIAN PRAWN *(Macrobrachium rosenbergii)* AND PINK SHRIMP *(Penaeus marginatus)**

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Baseline serum values of newly captured Malaysian prawns *(Macrobrachium rosenbergii)* and pink shrimp *(Penaeus marginatus)* were determined by Sequential Multiple AutoAnalysis. Both of these species have considerable potential for commercial captive culture.

Sex differences in serum constituent levels were found within species. Female pink shrimp had higher serum glucose levels than the males. Malaysian prawn males had higher cholesterol levels than females, and the latter had higher levels or activities of urea nitrogen, creatinine and lactic dehydrogenase.

Pink shrimp held under laboratory conditions for 10 days had higher levels or activities of serum glucose and alkaline phosphatase and lower levels or activities of serum inorganic phosphorus, total protein, lactic dehydrogenase and glumatic-oxaloacetic transaminase than pink shrimp sampled immediately after capture.

INTRODUCTION

Few data are available on the composition of sera from shrimps or prawns which are candidates for commercial aquaculture. Studies have not been conducted which quantify the major serum constituents of either the fresh water Malaysian prawn *(Macrobrachium rosenbergii)* or the marine pink shrimp *(Penaeus marginatus)*, two crustaceans that can command a high market value and are commercially acceptable throughout most of the world. With the development of expertise to mass culture the Malaysian prawn (Fujimura and Okamoto, 1970), this species has become

one of the most promising aquatic animals for captive culture (Shang, 1972). Although complete, controlled reproduction in captivity has not yet been achieved for any Penaeidae, much research is being directed toward farming members of this family.

Mass rearing of these two species could be aided by a definition of normal baseline physiological parameters. Such data will be valuable in detecting and correcting for environmental, nutritional or pathological stresses.

This study was therefore designed to determine the normal concentrations and activities of the major biochemical constituents found in the hemolymph of freshly captured Malaysian prawns and pink shrimp. In addition, an experiment was conducted to determine the effects of the laboratory environment on these serum parameters in pink shrimp.

MATERIALS AND METHODS

Two separate experiments were conducted. The experimental procedures for each are listed separately.

Experiment 1: Specimens of *Penaeus marginatus* were trawl collected between 01 00 and 03 00 hours during the month of July, 1972, at a depth of 110 m approximately 4 km off the north shore of Oahu in the Hawaiian Islands. Each bottom trawl lasted no more than 20 min in order to minimize damage that might occur to animals caught in the net. Hemolymph samples were extracted from 51 animals (14 male, 37 female) immediately after capture. Sea water samples were collected with a modified Van Dorn sampler (Gundersen, 1972) during the same night from approximately 1.5 m off the ocean bottom in the area where the trawls were made. Water temperature was recorded immediately as the samples were brought to the surface.

Thirteen freshly seined *Macrobrachium rosenbergii* (3 male, 10 female) were also obtained during July, 1972, from a 0.3-ha freshwater pond (average depth 1.5 m) located in the Punaluu district of Oahu. Hemolymph was sampled from each animal immediately after capture. Pondwater samples were collected from 0.5 m beneath the surface and the temperatures were recorded. This pond had been constructed for a pilot study of the commercial rearing of *Macrobrachium rosenbergii.*

Hemolymph collections were made from the pericardial cavity of each species using either 1-cc or 2.5-cc disposable syringes (Plastipak, Rutherford, N.Y.) fitted with 22 G, 3.8-cm needles. External water present on each animal was removed prior to sampling. The needle was inserted through the intersegmental membrane between the cephalothorax and the

abdominal segment to reach the pericardial region and as much fluid as possible was withdrawn from each individual. Care was taken not to extract tissue particles with the hemolymph. Animals were weighed, measured and sex was determined after hemolymph extraction. Total body volume was estimated by water displacement. Dry matter content was determined after drying at 80°C in a forced air oven for 48 h.

Hemolymph samples were allowed to clot at ambient temperature (27°C) for 4 h after extraction. Serum was obtained by ultracentrifuging at 12 500 x *g* and 2 °C for 10 min and then at 20 000 x *g* and 2°C for 5 min. Water samples were treated in the same manner. After centrifuging, the clear, bluish supernate was pipetted into individual vials and stored at −10°C until analyzed.

Serum chloride, calcium, inoraganic phosphorus, glucose, cholesterol, urea nitrogen, total bilirubin, total protein, creatinine, lactic dehydrogenase, alkaline phosphatase and glutamic-oxaloacetic transaminase were determined using a modified Sequential Multiple AutoAnalyzer, SMA-12/30 (Tumbleson, 1969). The amount of serum obtained from individuals varied from 0.25 to 2.00 ml. Since at least 2.00 ml was needed for a complete analyses, samples containing less than this amount were pooled within sexes of each species to obtain a sufficient quantity. Centrifuged water samples were subjected to the same analyses.

Experiment 2: The second study was conducted to evaluate the effects of maintaining captured pink shrimp under laboratory conditions. A random sample of 24 *Penaeus marginatus* obtained at the same sample location and time as Experiment 1 were transferred to the laboratory at the Hawaii Institute of Marine Biology and held in 730-litre sea water aquaria for 10 days before hemolymph samples were collected. The shrimp were fed a compounded artificial diet (Table I) supplemented with fresh-frozen chopped squid (*Loligo* sp). The animals were fed 5% of their body weight twice daily. Uneaten foor was removed prior to each feeding.

TABLE I

Composition of compounded artificial diet fed to laboratory held *Penaeus marginatus*

Ingredient	Percent by weight
Shrimp meal	35
Tuna meal	30
Soybean meal	16
Brewer's yeast	5
Blood meal	7
Agar-agar (binder)	6
Vitamin-trace mineral mix	1

Water samples were collected from the laboratory sea water system for analyses. Temperature of the water was recorded daily. All serum and water samples collected were prepared and analyzed as detailed in Experiment 1.

RESULTS AND DISCUSSION

Experiment 1: Mean levels and ranges of pooled serum samples for newly captured *Penaeus* and *Macrobrachium*, including water samples and temperatures, are listed in Table II. Mean levels and ranges for the same measurements of each species by sex are shown in Table III. Pooling the serum resulted in a total of 11 *Penaeus* (4 male, 7 female) and 8 *Macrobrachium* (2 male, 6 female) samples for analyses. Mean levels were adjusted for the number of animals represented by each pooled sample.

Serum chloride was greater for *Penaeus* than for *Macrobrachium*. This may be related to the greater concentration of chloride in its sea water environment. Chloride level in serum from *Macrobrachium* (160 mEq/l) was higher than that found by Schlatter (1941) in the fresh water *Cambarus* (117 mEq/l).

Mean serum calcium in *Macrobrachium* was greater than in the penaeid shrimp even though the level in the sea water was more than 18 times greater than in the pond water. In both species, serum calcium was maintained at a higher level than in the water of the natural habitat. Similar results with marine shrimp *(Metapenaeus mastersii)* were found by Dall (1964) and in *Penaeus duorarum* by Bursey and Lane (1971). The level of serum calcium was higher in both *Macrobrachium* and *Penaeus* than that reported by Schlatter (1941) for fresh water crayfishes, *Astacus* (42–47 mg/100ml) and *Cambarus* (40–49 mg/100ml). The level for the marine lobster *Panulirus* (78 mg/100 ml) was higher than that found for *Penaeus*.

Serum inorganic phosphorus of both *Penaeus* (4.6 mg/100 ml) and *Macrobrachium* (2.0 mg/100ml) were higher than that found in *Panulirus* serum by Travis (1955), who reported an inorganic phosphorus level of 0.7 mg/100 ml.

There was a considerable range in the serum glucose levels for both species. Blood glucose levels found in marine crabs *(Callinectes, Uca, Hepatus, Libinia,* and *Panapeus*) ranged from 15.9 to 37.6 mg/100ml (Dean and Vernberg, 1965). Florkin (1960) listed serum glucose values for a number of freshly captured crustaceans from 3 to 182 mg/100ml.

Cholesterol was not determined for the freshly captured pink shrimp due to analytical difficulties. According to Jeuniaux (1971) the only data for serum cholesterol in crustaceans are for three species of crabs. A range of 12–49 mg/100ml was reported for these crabs by Damboviceanu (1932).

TABLE II

Means levels and ranges of serum constituents of freshly captured *Penaeus marginatus* (sea water, 19°C) and *Macrobrachium rosenbergii* (fresh water, 26°C)

Parameter	Species means and range					
	Penaeus marginatus		Sea water	*Macrobrachium rosenbergii*		Pond water
	Mean	Range		Mean	Range	
Chloride (mEq/l)	420	360 –460	300	160	130 –232	9
Calcium (mg/100 ml)	63	53 – 73	36	85	66 –112	2
Inorganic phosphorus (mg/100 ml)	4.6	3.6– 9.0	ND[1]	2.0	1.8– 2.4	ND
Glucose (mg/100 ml)	26	10 – 68	ND	83	44 –110	ND
Cholesterol (mg/100 ml)	AD[2]	–	ND	35	18 – 96	ND
Urea nitrogen (mg/100 ml)	5.2	2 – 8	ND	3.1	2 – 12	ND
Total bilirubin (mg/100 ml)	1.4	1.2– 1.6	ND	1.8	1.4– 2.8	ND
Total protein (g/100 ml)	9.4	7.6– 13.8	ND	12.6	10.4– 14.4	ND
Creatinine (mg/100 ml)	0.6	0.2– 1.2	0.2	2.0	1.0– 3.4	ND
Lactic dehydrogenase (W.U.)[3]	520	100 –960	ND	26	0 –144	ND
Alkaline phosphatase (K.-A.U.)[4]	4.6	4– 6	ND	7.8	6 – 10	ND
Glutamic-oxaloacetic transaminase (K.U.)[5]	199	30–315	ND	153.4	110 –236	ND

[1] ND = Not Detectable
[2] AD = Analytical difficulty in determination
[3] = Wacker Units
[4] = King-Armstrong Units
[5] = Karmen Units

TABLE III

Mean levels and ranges of serum constituents of freshly captured *Penaeus marginatus* and *Macrobrachium rosenbergii* by sex

Parameter	*Penaeus marginatus*				*Macrobrachium rosenbergii*			
	Male	Range	Female	Range	Male	Range	Female	Range
Chloride (mEq/l)	399	360 –430	441	416 –460	155	130 –167	164	144 –232
Calcium (mg/100 ml)	61	53 – 82	65	53 – 74	81	70 – 87	88	66 –112
Inorganic phosphorus (mg/100 ml)	4.0	3.6– 5.4	5.2	4.2– 9.0	1.9	1.8– 2.0	2.0	1.8– 2.4
Glucose (mg/100 ml)	13	10 – 27	38	10 – 68	75	44 – 90	91	62 –110
Cholesterol (mg/100 ml)	AD[2]		AD		47	22 – 96	24	18 – 32
Urea nitrogen (mg/100 ml)	5.2	3 – 6	5.3	2 – 8	2.0	2.0	4.2	2 – 12
Total bilirubin (mg/100 ml)	IS[6]		1.4	1.2– 1.6	1.8	1.8	1.7	1.4– 2.8
Total protein (g/100 ml)	8.8	7.6– 13.8	9.9	8.6– 11.0	13.3	12.1– 13.8	12.0	10.4– 14.4
Creatinine (mg/100 ml)	0.5	0.4– 1.2	0.6	0.2– 0.8	1.3	1.0– 2.0	2.6	1.8– 3.4
Lactic dehydrogenase (W.U.)[3]	497	290 –765	543	100 –960	ND[1]		51	40 –144
Alkaline phosphatase (K-A.U.)[4]	4.4	4 – 6	4.9	4 – 6	7.3	6 – 8	8.4	6 – 10
Glutamic-oxaloacetic transaminase (K.U.)[5]	205	130 –315	192	30 –250	147	124 –186	160	110 –236

[1] ND = Not detectable
[2] AD = Analytical difficulty in determination
[3] = Wacker Units
[4] = King-Armstrong Units
[5] = Karmen Units
[6] = Insufficient sample quantity

The mean cholesterol concentration of 35 mg/100 ml determined in the present study for *Macrobrachium* was within this range.

No apparent differences existed for urea nitrogen concentrations in the two species investigated. Florkin (1960) reported a range of 0.1–11 mg/100ml for serum urea nitrogen in several species of marine and freshwater crustaceans.

Bilirubin or a bilirubin-like compound was found in the serum of both species. Other workers have not reported this constituent in the serum of crustaceans.

Levels of total protein in the serum were similar in the two species examined. Total protein in Decapoda, according to data of Florkin (1960), varied from 0.7 to 8.8 g/100ml. Concentrations of 2.2–10.2 g/100ml have been given for the marine American lobster, *Homarus americanus* (Leone, 1953). In the same study, values for serum protein in the marine blue crab (*Callinectes sapidus*) and the king crab *(Limulus polyphemus)* ranged from 1.8 to 12.0 g/100ml and from 0.8 to 13.4 g/100ml respectively. Lynch and Webb (1973) reported a range of 0.2–2.3 g/100ml for total serum protein in *Callinectes.*

Information on the serum creatinine levels in crustaceans is limited. In this study creatinine was higher in *Macrobrachium* than in *Penaeus.*

Few data are available on the activity or presence of the enzymes examined in this study in Arthropoda. Jeuniaux (1971) makes reference to a lactate dehydrogenase having been identified in certain insects *(Tenebrio, Hyalophora* and *Samia).* Activity of serum lactic dehydrogenase in the present study was considerably higher in *Penaeus* than in *Macrobrachium* (520 vs 26 W.U.), while alkaline phosphatase activity was higher in *Penaeus.* There was little difference between species in the serum glutamic-oxaloacetic transaminase activity. Roch and Latreille (1934) identified a phosphatase in crab serum, however no values were reported.

Mean levels and ranges for the sera of the freshly captured animals of each species by sex (Table III) indicated that differences existed between sexes of *Penaeus* for serum glucose, with a greater level present in females. Lynch and Webb (1972), however, found no difference for serum glucose levels in male or female marine crabs, *Callinectes sapidus.*

Differences existed between sexes for levels of serum cholesterol, urea nitrogen and creatinine in *Macrobrachium.* Males had a greater cholesterol concentration (47 mg/100ml) than females (24 mg/100ml), but the latter had higher levels of serum urea nitrogen (4.2 vs 2.0 mg/100ml) and creatinine (2.6 vs 1.3 mg/100ml). No lactic dehydrogenase was found in the male *Macrobrachium* serum samples. No other differences in serum constituents were apparent between sexes.

Mean values with standard deviations for body measurements of the

TABLE IV

Mean body measurements of *Penaeus marginatus* and *Macrobrachium rosenbergii* after hemolymph extraction

Species and sex	Number of specimens	Weight (g)	Total length (cm)	Carapace length (cm)	Volume (cc)	% Dry matter
EXPERIMENT 1:						
Penaeus marginatus	51	24.8 ± 11.4	15.0 ± 2.3	5.6 ± 1.0	22.9 ± 10.6	25.8 ± 2.1
male	14	21.9 ± 8.8	14.6 ± 1.8	5.4 ± 0.7	20.1 ± 8.2	26.1 ± 2.5
female	37	26.1 ± 12.3	15.2 ± 2.5	5.7 ± 1.1	24.3 ± 11.5	25.7 ± 1.9
Macrobrachium rosenbergii	13	54.9 ± 14.8	17.1 ± 1.2	8.3 ± 0.7	51.4 ± 14.2	30.9 ± 2.8
male	3	77.5 ± 3.9	18.6 ± 1.0	9.3 ± 0.3	72.7 ± 4.6	32.0 ± 0.9
female	10	49.1 ± 1.1	16.6 ± 0.8	8.1 ± 0.5	45.0 ± 8.2	30.4 ± 3.1
EXPERIMENT 2:						
Penaeus marginatus	14	13.2 ± 4.4	12.4 ± 1.2	4.6 ± 0.5	12.1 ± 4.1	23.5 ± 1.3
male	1	12.7	12.2	4.8	12.0	22.2
female	13	13.3 ± 4.6	12.4 ± 1.2	4.7 ± 0.5	12.1 ± 4.3	23.6 ± 1.3

freshly captured *Penaeus* and *Macrobrachium* taken after hemolymph extraction are listed in Table IV. *Macrobrachium* were greater in weight, length and volume than *Penaeus*. Female *Penaeus* trawled in the Hawaiian Islands are generally heavier and longer than males (Balazs, 1972, unpublished data). In contrast, female *Macrobrachium* were smaller in both total weight and carapace length than the males. The absence of large chelae and a lighter exoskeleton in *Penaeus* was responsible for the lower dry matter content levels compared with that of *Macrobrachium*.

Experiment 2: Twenty-four freshly trawled pink shrimp were transferred to the laboratory. After the 10-day experimental period, only 13 of the original 15 females and only 1 of the original 9 males remained alive. All mortality occurred on days 6, 7 or 8. No problems were apparent with feed consumption and the probable cause of death was not determined. No pathological changes were apparent after post-mortem examination. Death may have been caused by the higher water temperature in the laboratory (25°C) compared with the normal environmental temperature (19°C) at the trawl depth of 110 m.

The serum constituent values for the surviving laboratory-held pink shrimp are presented in Table V. Since the mean levels were composed

TABLE V

Mean levels and ranges for serum constituents of *Penaeus marginatus* held in the laboratory (10 days at 25°C)

Parameter	*Penaeus marginatus*		
	Mean	Range	Laboratory water
Chloride (mEq/l)	422	345 –460	300
Calcium (mg/100 ml)	63	58 – 66	36
Inorganic phosphorus (mg/100 ml)	1.9	1.8– 2.1	ND[1]
Glucose (mg/100 ml)	57	30 – 70	ND
Cholesterol (mg/100 ml)	38	38	ND
Urea nitrogen (mg/100 ml)	3.7	3 – 4	ND
Total bilirubin (mg/100 ml)	1.3	1.2– 1.4	ND
Total protein (g/100 ml)	6.8	5.7– 7.4	ND
Creatinine (mg/100 ml)	0.8	0.8– 0.9	0.3
Lactic dehydrogenase (W.U.)[2]	48	45 – 50	ND
Alkaline phosphatase (K-A.U.)[3]	7.3	6 – 8	ND
Glutamic-oxaloacetic transaminase (K.U.)[4]	74	66 – 70	ND

[1] ND = Not detectable
[2] = Wacker Units
[3] = King-Armstrong Units
[4] = Karmen Units

almost entirely of female shrimp serum data, it was more valid to restrict a comparison of the values to those of the freshly captured *Penaeus* females. When only these values were compared, differences were detected for levels of serum inorganic phosphorus, glucose, total protein, lactic dehydrogenase, alkaline phosphatase, and glutamic-oxaloacetic transaminase. Glucose and alkaline phosphatase were present at a higher level in the laboratory held shrimp. Levels of inorganic phosphorus, total protein, lactic dehydrogenase and glutamic-oxaloacetic transaminase were lower in laboratory maintained shrimp.

The fact that relatively rapid changes occurred in the concentrations of these constituents in animals held in captivity for a short period demonstrated the importance of stating environmental and physiological conditions when reporting serum values for crustaceans.

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REFERENCES

Bursey, C.R. and Lane C.E. (1971) Ionic and protein concentration changes during the molt cycle of *Penaeus duorarum*. *Comp. Biochem. Physiol.*, 40A, 155–162.

Dall, W. (1964) Studies on the Physiology of a shrimp, *Metapenaeus mastersii* (Haswell) (Crustacea:Decapoda:Penaeidae) 1. Blood constituents. *Aust. J. Mar. Freshw. Res.*, 15, 145–161.

Damboviceanu, A. (1932) Composition chimique et physico-chimique du liquide cavitaire chez les Crustaces Decapodes (Physiologie de la calcification). *Arch. Roumaines Pathol. Exptl. Microbiol.*, 5, 239–309.

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

Florkin, M. (1960) Blood chemistry. In: T.H. Waterman (Editor) *The Physiology of Crustacea* Academic Press, New York, N.Y., p.141–159.

Fujimura, T. and Okamoto, H. (1970) Notes on progress made in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. *Proceedings of the 14th Session Indo-Pacific Fisheries Council, FAO, Rome*, 14 pp.

Gundersen, K., Mountain, C.W. Taylor, D. Ohye, R. and Shen J. (1972) Some chemical and microbiological observations in the Pacific Ocean off the Hawaiian Islands. *Limnology and Oceanography*, 17(4), 524–531.

Jeuniaux, C., (1971) Hemolymph-Arthropoda. In: M. Florkin and B.T. Scheer (Editors), *Chemical Zoology, Vol. 6, Arthropoda, Part B.* Academic Press, New York, N.Y., p.63–118.

Leone, C.A. (1953) Preliminary observations on intraspecific variation of the level of total protein in the sera of some decapod crustacea. *Science*, 118, 295–296.

Lynch, M.P. and Webb, K.L. (1973) Variations in serum constituents of the blue crab, *Callinectes sapidus:* Glucose. *Comp. Biochem. Physiol.*, 45A, 127–139.

Roche, J. and Latreille M. (1934) Sur les phosphatases su sang et de l'hemolymphic. *Compt. Rend. Soc. Biol.*, 116, 1033.

Schlatter, M.J. (1941) Analyses of the blood serum of *Cambarus clarkii, Pachygrapus crassipes* and *Panulirus interruptus. J. Cell. Comp. Physiol.*, 17(2), 259–261.

Shang, Y.C. (1972) Economic feasibility of fresh water prawn farming in Hawaii. *Economic Research Center, Univ. Hawaii, Honolulu*, 48 pp.

Travis, D.F. (1955) The molting cycle of the spiny lobster, *Panulirus argus* Latreille. III. Physiological changes which occur in the blood and urine during the normal molting cycle. *Biol. Bull.*, 109, 484–503.

Tumbleson, M.E. (1969) Modification of the Sequential Multiple AutoAnalyzer (SMA-12/30) for use in animal research studies. *Clin. Biochem.*, 2, 357–367.