

Biochemical Changes in Rock Lobster *Panulirus homarus* During Live Transportation Process

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Abstract: The present study aims to find out the effect of hybernational and starvational stress during live lobster (*Panulirus homarus*) transportation process on biochemical changes in tissues and haemolymph samples. Healthy lobsters were selected and packed in thermocool boxes in live condition. In every 2h interval up to 14h, tissues and haemolymph samples were taken for analysis. There was 100% survival up to 10th h. The protein content in the muscle, hepatopancreas and gill tissues was between 12.60 to 21.60% in control lobsters of holding tanks, whereas it decreased considerably from 21.60 to 19.00%, 19.00 to 18.00% and 12.60 to 11.60%, respectively in the same tissue samples of experimental lobsters. Similarly, the carbohydrate and lipid contents of the tissue samples were decreased gradually in experimental lobsters. Haemolymph protein content was 4.20% in control lobsters, but it reduced to 2.80% in the experimental lobsters during 14th h. Likewise, the haemolymph glucose level in control lobster was 46.0 mg/ml, whereas it increased gradually and reached a maximum of 65.0 mg/ml during 6th h, further it reduced and reached to 47.0 mg/ml at 14th h. Lactate content in haemolymph and muscle tissue samples of experimental lobsters elevated with subsequent increase in incubation period, but the lactate content at the end of experiment in haemolymph was higher (6.73 mmol/l) than the lactate content of muscle (14.94 μ mol/g).

Key words: Rock lobster • *Panulirus homarus* • Starvation • Biochemical changes

INTRODUCTION

Living systems have to incessantly interact with an environment, which will not be favor to them always like many of the poikilotherms, fishes also capable of withstanding relatively longer periods of food deprivation, during which body resource are utilized for maintenance of routine body functions [1]. The nature of response to food deprivation however differs from species to species, in the type of resource utilized and tissue from which these are drawn [2]. During starvation of crustaceans, at all life stages including larvae; these are distinct faces of biomass degradation [3]. Initially, energy rich lipid reserves are preferably mobilized resulted in depletion of lipid: protein ratios, which is typical of short term food deprivation [4].

Stress response occurs when a regulated physiological system is pushed beyond normal external factors or “stressors” acting upon it [5]. During the past 10 years, an ever-increasing number of studies have focused on the biological significance of the stress response, particularly on possible function of stress proteins. Thermal (Low and high temperature), osmotic, oxidative, hibernation and starvation and salinity stresses are among the conditions that biological systems encounter as part of their normal life cycle. Organismal level adaptations to such stresses have evolved in many species [6].

Generally food (vegetables and animals) processing industries are fulfills the need for the variety of foods across the world. Especially in aqua food processing industries magnetize great economics in which especially

the market need for live shipment of lobsters is increasing day by day. The first pioneering study of live lobster transport was done by McLeese [7] using air-freight method through train or truck, but this method had the drawback that it affects the physicochemical property of water used in this practice by the animals transported. Hence he developed the live dry transport technique for lobsters and simultaneously investigated the European original method of packing lobsters in dry wood shavings was followed by local dealers. The aim of this method was primarily to increase the insulation in box, in order to maintain a low temperature reached by both pre-packing chilling procedure and by the addition of containers in ice within the carton. Survival rate of lobsters in this practice could be greatly improved by maintaining the carton at low temperature ($<10^{\circ}\text{C}$) and by providing perforations in cartons for gaseous exchange.

The development of processing and handling techniques currently used by processors has been determined through empirical approaches [8]. It is only in recent years, the ongoing growth of lucrative marketing of live crustaceans has prompted the need for the processing industry to assess and develop techniques contributing to an improved quality in harvesting, storage and transport of live products [9]. As a result, applied studies have been conducted to investigate the impact of existing processing methods on the products [10, 11] and its metabolism in the face of environmental changes [12]. The major difficulty faced was physiological and biochemical changes of transported animals. Most particularly, crustaceans demonstrate an important seasonal and thermal variability, a size and gender dependence to environmental effects and responsiveness to a range of environmental factors such as temperature, salinity etc. [12], but the outcome of such studies being species specific, the transfer of knowledge from one species to another is limited [11]. Studies related to biochemical changes occurred during live lobster transportation process are still stand as lacunae. Hence the present study was undertaken with the biochemical changes in body tissues as well as haemolymph in live rock lobster *Panulirus homarus* at different hour's intervals of transportation process.

MATERIAL AND METHODS

The experimental animal, live rock lobster *Panulirus homarus* were collected from Chinnamuttom fisheries harbour, Kanyakumari, India. They were brought to the

laboratory and acclimatized to ambient laboratory condition (Temperature $27-30^{\circ}\text{C}$, salinity 35 ppt dissolved oxygen 5mg/l) for 10 days. Healthy and active lobsters at inter moult/early moult stage; weighing about $150 \pm 5.0\text{g}$ sizes were segregated and maintained at low temperature in a small cooling tank with sea water before packing.

Pre-Packing and Chilling of Lobsters: One of the most important steps in the transportation process of live lobsters is cooling the lobsters in holding tank with sea water. The water temperature of cooling tank along with lobsters was gradually brought up to 12°C to 15°C (depending upon the traveling time) and the process aided by ice cubes containing polythene bags. Care was taken to avoid the contact of animals with ice bags and the water was continuously aerated to facilitate uniform cooling and O_2 supply. Processors believe the distinct objective of this process is to reduce the physiological and metabolic activity of animals for extended survival in live transport.

Preparation of Packages: Sterilized dry sawdust, straw and pieces of gunny bags were aseptically pre-cooled in a freezer (-20°C) along with 0.5 l capacity plastic bottles filled with water. Then a layer of cooled sawdust was spread on the bottom of a thermo cool box ($40 \times 30 \times 15\text{ cm}$), over which a layer of cooled straw was placed. Then two frozen ice bottles were swapped with filter paper were placed at the sides of the box. The lobsters were placed on the head of straw (10 lobsters each/box) by gently folding the antennae and abdomen to bring them close to their body so as to uniformly accommodate. Finally the lobsters were covered with a piece of pre-cooled gunny bag and then the box was closed with its lid and sealed with broad adhesive tape. Simultaneously seven such boxes were maintained for the experiment and once in 2 h intervals up to 14 h, one box was opened and immediately survival of the lobster was noticed. Then the tissue samples (hepatopancreas, gill and muscle) were aseptically collected from the lobsters and packed in labeled containers individually. Similarly the haemolymph samples from the experimental lobsters were also collected individually by using syringe from the appendages and immediately EDTA (1mg/ml) was added mainly for preventing from clotting. Before packing, the tissue and haemolymph samples were also drawn from the lobsters and it was treated as control (0 h). The collected samples were stored in a deep freezer at -40°C for the biochemical analysis.

Biochemical Analysis: Protein, carbohydrate and lipid content of the tissue samples and haemolymph samples were analyzed by the method of Lowry *et al.* [13], Roe [14] and Folch *et al.* [15], respectively. The glucose and lactate contents in haemolymph samples were also analyzed by the method described by Tietz [16] and Gutman and Wahlefeld [17], respectively.

Statistical Analysis: The results of experimental samples were compared with control samples (unpacked lobsters), the data were expressed as Mean±SD and were analyzed statistically by ANOVA at 5% significant level using a computer software Statistica 6.0 (Statsoft,UK).

RESULTS

Survival of Lobsters: Every two-hour interval, the survival of lobsters was determined and it showed 100% survival up to 10th h, beyond this time, 10% mortality was observed till the end of the experiment (Figure 1).

Tissue Protein Content: The protein content in muscle, hepatopancreas and gill tissues registered incubation time dependent variation and it varied from 12.60 to 21.60% in the control lobsters. But lobsters in packed condition, during transportation process at different time interval, the protein content of all the tissue samples was varied considerably. In muscle tissue, the protein content reduced from 20.20 to 19.0% during 2nd to 6th h of incubation period. Whereas, the protein content increased further in subsequent time of incubation i.e. from 20.53 to 21.3%, respectively during 8th to 14th h. In hepatopancreas tissue, the protein content started declining from the initial value of 19.00 to 18.0% during 6th h. Further increase

in incubation time, a marginal increase in protein content was observed (18.45 to 18.90% during 8th to 14th h). But in gill tissue, a linear reduction in protein content was noticed with respect to increase in incubation time from 12.40% at 2nd h to 11.60% at 14th h of incubation (Table 1). The statistical analysis (Two-Way ANOVA) revealed that the variation among the protein content between various tissue samples was statistically high significant (P < 0.0001); whereas the variation between the time intervals was statistically not significant (P > 0.05).

Tissue Carbohydrate Content: The carbohydrate content recorded in the sampled tissues of control lobsters varied between 2.60 and 4.50%; whereas it showed much variation in experimental lobsters. For instance, in muscle tissue, the carbohydrate content decreased from the initial value of 3.30 to 2.80% during 2nd h, further decreased to 2.0% during 14th h of incubation. The carbohydrate content in the hepatopancreas tissue also showed a declining trend. During 2nd h, it was 4.20%, it remained at the same level up to 4th h and further it decreased to 3.10% at 14th h. In gill tissue, it was 2.20% at 2nd h and then it gradually decreased to 1.60% during 14th h (Table 1). The statistical analysis by two-way ANOVA revealed that the variation between the carbohydrate content in different tissue samples as well as between the time intervals were statistically high significant (P<0.001).

Tissue Lipid Content: The lipid content in the tested tissue samples of control lobsters was ranged from 3.0 to 14.06%. Likewise, the lipid content of various tissue samples in experimental lobsters at different incubation time intervals was varied much. For example during 2nd h interval, the lipid content was remaining unchanged

Table 1: Protein, carbohydrate and lipid content in different tissues of live rock lobsters (*P. homarus*) at different time intervals during transportation process. (Each value is a Mean ± SD ; n = 3)

Time intervals (h)	Protein content (%)			Carbohydrate content (%)			Lipid content (%)		
	M	H	G	M	H	G	M	H	G
0	21.6 ± 0.20	19 ± 0.20	12.6 ± 0.10	3.3 ± 0.30	4.5 ± 0.26	2.6 ± 0.12	3 ± 0.15	14.06 ± 0.30	3.4 ± 0.20
2	20.2 ± 0.10	18.43 ± 0.20	12.4 ± 0.26	2.8 ± 0.20	4.2 ± 0.19	2.2 ± 0.20	3 ± 0.10	13.2 ± 0.26	3 ± 0.26
4	19.46 ± 0.20	18.13 ± 0.23	12.2 ± 0.20	2.6 ± 0.20	4.2 ± 0.13	2.1 ± 0.36	2.7 ± 0.13	12.5 ± 0.10	3 ± 0.26
6	19 ± 0.20	18 ± 0.34	12 ± 0.26	2.3 ± 0.26	3.6 ± 0.14	2 ± 0.04	2.6 ± 0.13	12.5 ± 0.20	2.8 ± 0.26
8	20.53 ± 0.25	18.45 ± 0.23	12 ± 0.30	2.3 ± 0.36	3.4 ± 0.10	2 ± 0.12	2.4 ± 0.14	13.06 ± 0.30	2.8 ± 0.10
10	20.8 ± 0.10	18.6 ± 0.20	11.8 ± 0.26	2.2 ± 0.26	3.4 ± 0.15	1.8 ± 0.05	2.4 ± 0.20	13 ± 0.20	2.73 ± 0.20
12	21 ± 0.26	18.6 ± 0.26	11.6 ± 0.26	2 ± 0.20	3.2 ± 0.19	1.8 ± 0.03	2.2 ± 0.17	12.5 ± 0.20	2.7 ± 0.10
14	21.3 ± 0.26	18.9 ± 0.26	11.6 ± 0.20	2 ± 0.30	3.1 ± 0.14	1.6 ± 0.18	2.2 ± 0.21	12 ± 0.20	2.6 ± 0.20

M = Muscle; H = Hepatopancreas; G = Gill

Data were analyzed by Two- way ANOVA (P< 0.05)

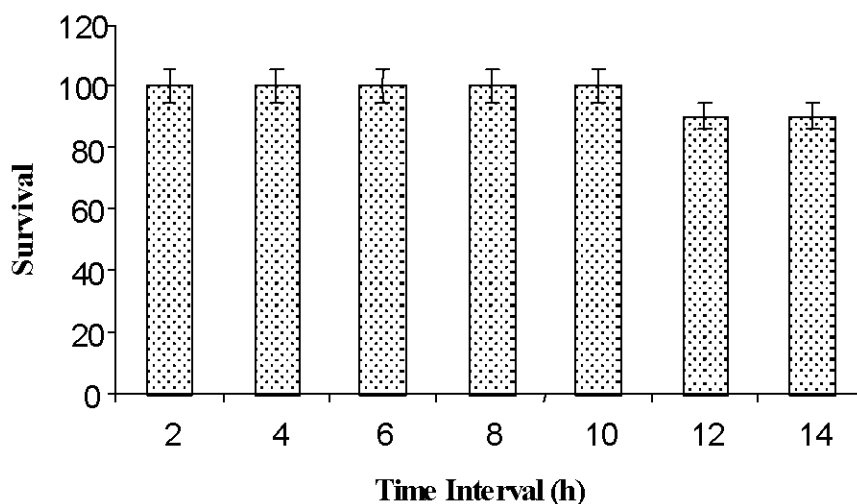


Fig. 1: Survival (%) of live rock lobsters (*P. homarus*) at different time intervals during transportation process (n =10 numbers in each package)

(3.0%) in muscle tissue. Further in incubation duration of 4 to 8 h resulted in decrease of muscle lipid content (2.7 to 2.4%). Later in extending duration of incubation period (12th and 14th h) there was no change in muscle lipid content (2.20% each). Similarly the lipid content of hepatopancreas tissue of experimental lobsters during 2nd h showed 13.2%; whereas this level was further decreased (12.5%) during 4th h. Invariably, the lipid level rose to 13.06% during 8th h of incubation and again it decreased to 12.50 and 12.0% during 12th and 14th h of incubation, respectively (Table 1). More or less a linear decrease in lipid level was reciprocated with the increase in incubation time was noticed in the gill sample of experimental lobsters and the decreasing level was from 3.0 to 2.6% from 2nd to 14th h, respectively (Table 1). The Two-way ANOVA on changes in lipid level showed that the variation between tissue samples was statistically high significant ($P < 0.0001$) than the variation due to incubation time ($P < 0.01$).

Tissue Lactate Content: Lactate content in muscle tissue of experimental lobsters revealed an increase with subsequent increase in incubation time. It increased gradually from 2nd (6.32 $\mu\text{mol/g}$) to 6th h (7.94 $\mu\text{mol/g}$) and it reached a steep increase of 11.05 $\mu\text{mol/g}$ during 8th h of experimentation. Then it increased constantly and reached a maximum of 14.94 $\mu\text{mol/g}$ during 14th h of incubation (Figure 2). One-way ANOVA for the data on lactate content in the muscle tissue of lobsters as a function of variation between incubation periods was statistically high significant ($P < 0.0001$).

Haemolymph Protein Content: The protein content in the haemolymph sample of control lobster was 4.20%; whereas, this level was increased marginally in the haemolymph sample of experimental lobsters i.e. 4.40 to 5.66% during 2nd to 6th h of incubation period. Further, the haemolymph protein started declining from 8th h onwards and reached 2.80 % during 14th h of incubation (Table 2).

Table 2: Protein (%) and glucose (mg/ml) contents in the haemolymph of live rock lobsters (*P. homarus*) at different time intervals during transportation process (Each value is a Mean \pm SD; n = 3)

Time intervals (h)	Haemolymph protein (%)	Haemolymph glucose (mg ml ⁻¹)
0	4.20 \pm 0.20	46.00 \pm 2.00
2	4.40 \pm 0.10	52.66 \pm 2.51
4	4.83 \pm 0.15	56.0 \pm 2.64
6	5.66 \pm 0.25	65.00 \pm 3.00
8	4.30 \pm 0.20	62.33 \pm 2.08
10	3.50 \pm 0.30	54.00 \pm 3.60
12	3.16 \pm 0.35	51.66 \pm 3.05
14	2.80 \pm 0.20	47.00 \pm 3.60

Data were analyzed by One-way ANOVA ($P < 0.05$)

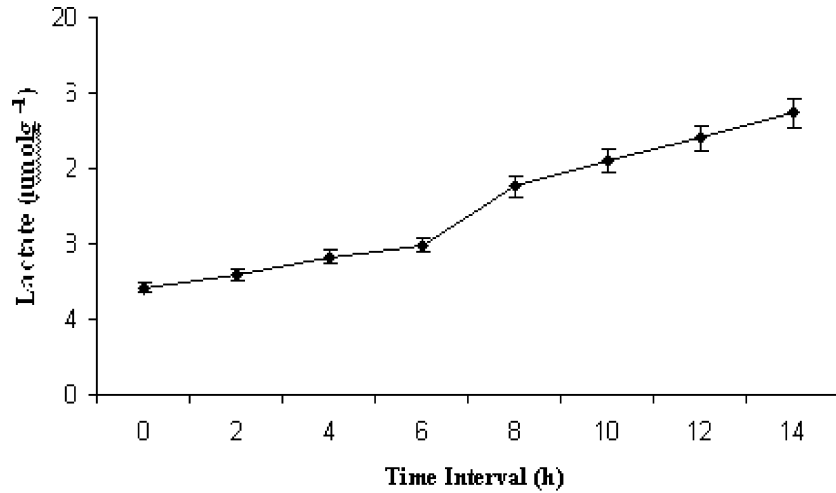


Fig. 2: Lactate content ($\mu\text{mol g}^{-1}$) in the muscle of live rock lobsters (*P. homarus*) during transportation process (values are Mean \pm SD; n = 3; data were analyzed by one-way ANOVA; $P < 0.05$).

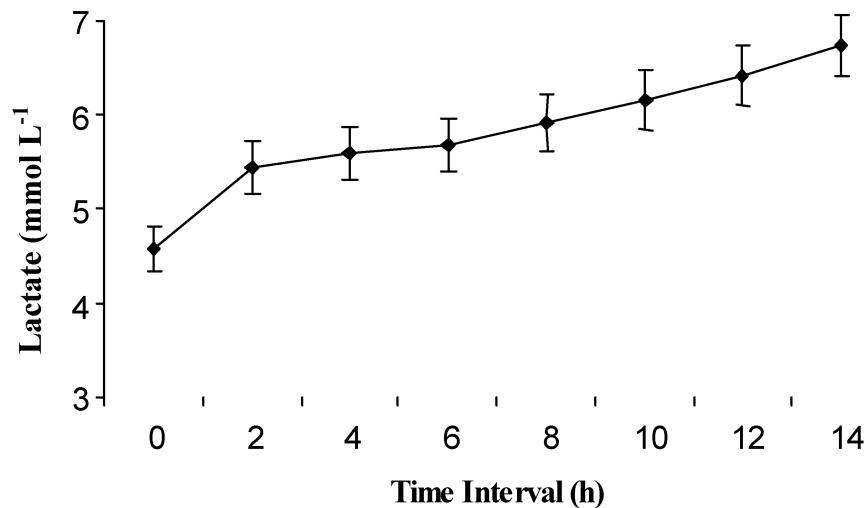


Fig. 3: Lactate content (mmol L^{-1}) in the haemolymph of live rock lobsters (*P. homarus*) during transportation process (values are Mean \pm SD; n = 3; data were analyzed by one-way ANOVA; $P < 0.05$).

The statistical analysis (One-way ANOVA) revealed that the change in haemolymph protein level at different time of incubation period was statistically high significant ($P < 0.001$).

Haemolymph Glucose Content: The haemolymph glucose level of control lobster was 46.0 mg ml^{-1} . But in the experimental lobsters, the haemolymph glucose level increased from 52.66 to 65.0 mg/ml during 2nd to 6th h of incubation period. Further, it decreased gradually and reached to 47.0 mg/ml during 14th h (Table 2). The statistical analysis by one way ANOVA on changes in glucose level in the haemolymph samples as a function of

incubation time revealed that it was statistically high significant ($P < 0.001$).

Haemolymph Lactate Content: The lactate content in the haemolymph sample of control lobster was 4.57 mmol/l . But it was found to be increased with increase in time of incubation. For instance during 2nd h, the lactate content was 5.43 mmol/l , whereas it increased gradually from 5.59 to 6.73 mmol/l respectively during 4th to 12th h (Figure 3). One-way ANOVA for the data on lactate content in the haemolymph samples of lobsters as a function of variation between incubation periods was statistically significant ($P < 0.001$).

DISCUSSION

Though the live transportation of lobsters and other crustaceans have noteworthy economics across the world, the stresses while transporting the animals deserve adverse effects. In the present study, a hibernation technique was adopted and the rock lobsters, *P. homarus* were treated with low temperature for live transport. During the experiment, changes in survival and biochemical constituents of lobsters were assessed at every 2h interval up to 14h.

The survival of live lobsters in packed condition for transportation process showed 100% upto 10th h, beyond this time 10% mortality was observed up to 14th h. Generally the survival rate of animals is varied after this type of transportation process. Similar process was done for kuruma prawns transported to Japan and formed 98% survival rate after 24 h of air travel [18]. Also the same technology adopted in other commercially important shrimp species like black tiger shrimp (*Penaeus monodon*), the white leg shrimp (*P. vannamei*) and the giant fresh water prawn (*Macrobrachium rosenbergii*) showed a varying degrees of success [18].

The variations in biochemical constituents in hibernated lobsters were compared with that of those control individuals (without starvation and hibernation), even though in the shorter duration, marked variations were noticed. Since starvation involves calorific deficiency [19]. The pattern of replenishment may be different during early and prolonged period of starvation. The catabolism of tissue is presumably under the control of adrenocortisol hormones and among this cortisol appears to be the most important proteolysis, fat metabolism and hepatic gluconeogenesis [20]. Therefore in most species studied, fat appears to be the most important storage materials and protein depletion occurs only after the majority of the fat reserve have been utilized [21, 22]. In decapod crustaceans, the major depletion of lipid resource in the starved shore crab *Carcinus manas* was in contrast with predominant uses of protein resource in *Hemigrapsus* species [23].

Although, the lipid was considered as the primary reserve for the cray fish [24] and protein was metabolized predominantly in *O. virilis* [25]. Whereas in *O. limosus*, the protein and lipid were utilized during progressive starvation [26]. Shrimp *Crangon crangon* starved for 4 weeks consumed mainly carbohydrate than lipid; finally protein reserves [27], lipid and protein were considered as the principal source of energy [28]. Yet no change in metabolic rate was evident in starved *P. japonicus* [29] or

in *P. esculentus* [30]. Composition of hepatopancreas of pink shrimp *P. duraramum* starved for 12 days suggested initial use of lipid and finally carbohydrate resources [31].

In the present study, the actual protein, lipid and carbohydrate constituents were at the range between 12.60 and 21.60%, 3.0 and 14% and 2.60 and 4.50% respectively. When the lobsters hibernated during transportation process for 14 h, the same constituents were altered to 11.60 and 21.30%, 2.20 and 12.0%, 1.60 and 3.10% respectively in muscle, hepatopancreas and gill tissues. The protein depletion among the tissues showed, gill registered maximum depletion followed by muscle and hepatopancreas. Similarly the lipid depletion among the tissues showed in the order of hepatopancreas > gill > muscle. The carbohydrate depletion was in the order of hepatopancreas > muscle > gill. The critical analysis of the data on percentage depletion of tested biochemical constituents of *P. homarus* in the present study revealed that, in the tested tissues starvation and hibernation induced oxidation reserve carbohydrate first, followed by lipid and proteins.

The present results find support from the findings of Cuzon and Cessaldi [27] for the shrimp *Crangon crangon* and also Uma Devi [32] for the lobster *P. homarus*. They found that the starvation of these two organisms mainly oxidised carbohydrate than lipid and finally protein. Biochemical changes in starved *P. japonicus* also revealed the reserve utilization of biochemical constituents in the order of carbohydrate, lipid and finally protein [33].

Also the present work revealed, some irregular trend in the level of protein was observed in the haemolymph samples of experimental animals. In the initial stage, the haemolymph protein level was 4.20%, further this was increased up to 5.66% during the 6th h. Then this level decreased to certain extent (2.8% during 14th h). Similar trend was observed in haemolymph glucose level also, here in the control lobsters, the haemolymph glucose was 46.0 mg/ml and further it increased to 65.0 mg/ml during 6th h of transportation process. But after 6th h, this level suddenly decreased and occurred more or less saturated level during 14th h (47.00%). A rise in glucose level was a classical response of crustaceans to disturbance [34] and the animals mobilized their glucose in order to sustain vital metabolic activities. The present study also agrees with the above findings since an increase in the haemolymph glucose level was observed up to 6 - 8 h of live lobster transportation process. Then a gradual decrease of haemolymph glucose occurred, which might

be due to the increased uptake and utilization of glucose in different tissues. Glucose concentration in crustacean haemolymph generally rises in response to a number of stressors such as handling, emersion and disease to a certain extent [34, 35].

As far as the lactate is concerned, high concentrations of this metabolite in haemolymph and muscle tissue have been extensively described as being associated with conditions of aerial exposure, locomotor activity and disturbance. When comparing the values recorded in the present study for haemolymph (4.57 ± 0.31 to 6.73 ± 0.43 mmol/l) and muscle (5.68 ± 0.16 to 14.94 ± 0.16 μ mol/g) were in consonance with earlier reports. This increase in lactate content is likely to occur when a period of more than two hours aerial exposure was experienced [36, 37]. It suggests that these animals were subjected to either longer periods of aerial exposure or to additional disturbance. Comparably, Whiteley and Taylor [38] reported the remarkable increase in lactate content of lobsters (*Hommarus gammarus*) subjected to the aerial exposure. In agreement with the above, Paterson and Spanoghe [39] was also noticed the increase in lactate content during live transport of *Panulirus cygnus*. Further, changes in the haemolymph protein profile of *P. homarus* are being under investigation in our laboratory.

CONCLUSION

The results obtained in the present study revealed that, conditioned live transport of lobster, *P. homarus* extensively exhibited variation in biochemical components of haemolymph and tissue samples. In such a way, it provides a baseline data to amend the methods of transportation in order to achieve high quality live shipments.

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