CAN AQUI-S HELP AS AN ANEASTHETIC IN LONG-DISTANCE LIVE TRANSPORTATION OF SPINY LOBSTERS *(PANULIRUS ORNATUS AND P. HOMARUS)?*

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ABSTRACT

In Southern Central Vietnam, there are approximately 40,000 spiny lobster cages producing 1,500-2,000 metric tonnes of product annually, worth around US$70 million. The major international markets for commercial-size lobsters are main-land China, Hongkong and Taiwan.This study includes three successive experiments including suitable AQUI-S dosage determining, dry transport and wet transport.The suitable AQUI-S dosage withdrawn from dosage trial was 50 mg/L which was suitable for local transport and handling procedures in 15 minutes. What we can see from the wet transport trial with AQUI-S were as follows: the (day 7) survival rate was 100% for both species P. ornatus and P. homarus. The health status of the survived lobster was normal. The possible transport density for P. ornatus and P. homarus was 200-400 individuals per cubic metre, respectively.In the dry transport method with AQUI-S, the survival rate of the ornate and homarus lobster at day 1 for 30 hr and 40 hr transport was 100% and 92%, and 91 and 73%respectively. The survival rate at day 7 for 30 hr and 40 hr transport of the ornate and homarus lobster was 88% and 79%, and 91 and 64%, respectively. The health status of the survived lobster of both species was normal, except for the homarus spawners (~50%) in 40 hr treatment. The possible transport density for P. ornatus and P. homarus was 530 and 720 individuals per cubic metre, respectively.

Keywords: Spiny lobster, Panulirus ornatus, Panulirus homarus, Live transportation, AQUI-S.

I. INTRODUCTION

In Vietnam, cagemariculture of spiny lobsters, primarilythe ornate spiny lobster (*Panulirus ornatus*) and scalloped spiny lobster (*P. homarus*), started in 1992. The industry has increasingly expanded and by 2006, there were approximately 40,000 cages producing 2,000 metric tonnes of productin the Central Vietnam. However, in late 2006, diseases significantly impacted on lobster aquaculture production, which declined to less than 1,000 metric tonnes in 2008/09 crop. The industry has recovered more recently with annual production of about 1,500 metric tonnes worth around US$70 million [3; 4]. In operations involving commercial lobster productionfacilities, it is not frequently necessary to sedate or anaesthetise, but a complete productioncycle, however, involves grading, handling, transport, and different disease treatments. All of these procedures are potential stressors that can provoke anunwanted stress reaction like in other species[1, 6, 7, 8, 9, 10, 11, 12, 13]. The major international markets for commercial-size lobsters are main-land China, Hongkong and Taiwan. Additionally, big cities in Vietnam such as Hanoi, Ho Chi Minh, Da Nang are a mongst important local markets [4]. For long distance transport of lobsters, can AQUI-S help as an effective aneasthetic, in terms of improving health and survival of lobsters compared with normal transport methods carried out by local people? This study includes three successive experiments including suitable AQUI-S dosage determining, dry transport and wet transport, with the objectives of the first trial are to determine at which AQUI-S® concentration the spiny lobster (*P. ornatus* and/ or *P. homarus*) will be suitable for transport and handling procedures and in how long. The suitable AQUI-S® concentration value will be used in the following experiments ('X value') and to determine whether all lobster make a full recovery showing normal behaviour and

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after how long of being placed in clean water; and the objectives of the second trial on dry transport are to access the health status and survival of lobsters after simulated transport 30 hrs with and without AQUI-S supplement and to access the health status and survival of lobsters after simulated transport 40 hrs and 40 h with AQUI-S supplement; and, finally the objective of the third trial on wet transport, is to access the health status and survival of lobsters after simulated transport 40 hrs with and without AQUI-S supplement.

II. MATERIALS AND METHOD

1. The dosage trial

1.1. Experimental lobster and procedures

Five animals including *Panulirus ornatus* of approximately 500-800g were transferred to tanks at 24-25ºC. Five concentrations of AQUI-S® (7.5, 20, 30, 40 and 60 mg L^{-1}) were then dispersed in separate baths and the animals will be exposed for up to 30 minutes. The animals were observed for the progression through sedation to surgical anaesthesia. The lobsters were removed from the AQUI-S® bath once they had lost the reflexive tail curl but still retained leg turgor.When performing the experiments, single lobster was quietly scooped and transferred from the acclimation tank and immersed to the anaesthetic solution bath. After reaching the final stage, surgical anaesthesia, stage 3b (Table 1) time was noted. At that time, the lobster was weighed before transferred to the resuscitation tank containing aerated sea water only. The maximum exposure time to each anaesthetic agent was 30 min. If no anaesthetic effect (Stage 3b) was observed during those 30 min, the concentration of anaesthetic was considered insufficient.

Table 1. Stages of anaesthesia modified from Burka et al. [2]

Stage	*Description*	*Behaviour sign*
0	Normal	Active swimming patterns; reactive to external stimuli; normal equilibrium; normal muscle tone.
1	Light sedation	Reduce swimming activities; slight or total loss of reactivity to visual and tactile stimuli.
2	Light necrosis	Slight loss of equilibrium.
3a	Deep necrosis	Total loss of equilibrium; decreased muscle tone; reactivity to strong tactile stimuli; decreased respiratory rate.
3b	Surgical anaesthesia	Total loss of activity; total loss of muscle tone; low respiratory rate; tail swinging stopped.
4	Medullary collapse	Respiration ceases; cardiac arrest; death ensure.

1.2. Data analysis

Descriptive statistics, especially trend graph was applied to analyze the progression to anaesthesia over time by AQUI-S concentration. The data were analyzed using Excel 2007.

2. The dry transport trial

Lobsters (*P. ornatus*) were used for this experiment with the weight of approximately 500-800 g each. The water temperature in the live holding (4 or 9 m^3) tank was gradually lowered over a 3-4 hour period. The final temperature was approximately 24ºC as same as what the locals applied. Four AQUI-S® bath tanks (50 L each) were set up next to the live holding tank and with 24ºC water before the experimental pack-out. The AQUI-S® was added to each bath. The final tested concentration in the bath was 50 mg L^{-1} which was withdrawn from the experiment 1. A group of three boxes (~55 L) without anaesthetic, but the other conditions kept as other boxes, were used as control. Each treatment was tested in triplicate. The lobsters were removed from the live holding tank and transfer to each AQUI-S® bath in bulk using scoop nets. The lobsters were removed from the AQUI-S® bath once they had lost the reflexive tail curl but still retained turgor. Sedated lobsters were then tightly packed in polystyrene boxes, with eight lobsters per box. Dry cut paper was added for insulation and to ensure a snug fit. Two ice bottles were added to the top of the box between the paper wool and the lid. Lobsters were then kept in an air-conditioned room with a temperature of 24ºC for a time period of 30 hrs for one group and 40 hours for another group. After 30 or 40-h, animals were transferred to recovery tanks (4-m³ each) and monitored for 7 days.

3. The wet transport trial

Lobsters (*P. ornatus*) were used for this experiment with the weight of approximately 600-700 g. Eight animals were placed into each of 150 L fibre-glass tank filled with aerated sea water and containing AQUI-S anaesthetic. The anaesthetic concentration tested was 7.5 mg L_{-}^{1} which was withdrawn from the dosage trial done in experiment 1. The transfer was done in triplicate. A group of three tanks without anaesthetic was used as control. Stock solutions of the anaesthetic agent were prepared fresh prior to the start of the experiment. After 40 h, surviving animals were transferred to recovery tanks (4-m³ each) and monitored for 7 days.

4. Other procedures and analyses

4.1. Anaesthetic agent: The anaesthetic selected for these studies is AQUI-S (New Zealand Ltd.).

4.2. Post-transport survival: After the 40 hr wet transfer the sedated and control lobsters were released into 4-m³ concrete tanks containing aerated filtered sea water and monitored. After the 30 hr, 40 hr and control transfer of the dry transport expetriment, lobsters were also released into separate 4-m³ concrete tanks containing aerated filtered sea water and monitored. Separate tanks were maintained for all experimental groups for 7 day observation post-transport for mortality. During this period, the lobsters were kept in tanks with continuous filtered sea water supply and subsistence feeding.

4.3. Preparation of sedatives solutions: The anaesthetic was expressed in mg L_1 in relation to the active substance. AQUI-S was measured in grams on scales to 2 decimal places. AQUI-S was prepared as a stock solution at a ratio of 1 part AQUI-S solution to 10 parts of water. Time for induction and recovery was recorded in seconds using electronic stopwatch.

4.4. Water temperature: it was recorded by a digital temperature meter (Singapore).

4.5. Parameters: Induction (lobsters lose reflexive tail curl but still retain turgor) time for each lobster species; Behavioural observations of lobsters under the simulated transport conditions after 30 or 40 h; Cumulative mortality (%) vs Post shipment days.

4.6. Statistical analyses: All results for means comparison were analysed by one-way analysis of variance (ANOVA), using SPSS (version 16) statistical software package (SPSS Inc., Chicago). Difference among treatments means were determined by using Duncan test. The differences were considered significant at the level of 5% ($P < 0.05$).

III. RESULTS AND DISCUSSION

1. The dosage trial

The results of the dosage trial were as follows (Fig. 1): At an AQUI-S® concentration between 20 and 60 mg/L, lobster were suitable for transport and handling procedures in 12 to 28 minutes.Lobster in AQUI-S® at 60 mg/L became handleable more than twice as fast as lobster in AQUI-S® at 20 ppm.All lobster made a full recovery showing normal behaviour within 10 minutes of being placed in clean water. To reduce the time to reach surgical anaesthesia of ornate spiny lobsters around 15 minutes which are suitable for transport and handling procedures in Vietnam's conditions, an interpolation was applied based on a regression equation. The suitable AQUI-S dosage value withdrawn from the interpolation was 50 mg/L (Fig. 2).

The western rock lobster or western crayfish, *Panulirus cygnus*, is a clawless marine crustacean found off the west coast of Australia and the southern rock lobster, *Jasus edwardsii*, is a also clawless marine crustacean found throughout coastal waters of New Zealand, Southern Australia and South Africa. A study which carried out by AQUI-S New Zealand Ltd showed that at an AQUI-S® concentration between 20 and 80 ppm (mL per 1000L of water) *Panulirus cygnus* lobsters were suitable for transport and handling procedures in 1 to 8 minutes [14].

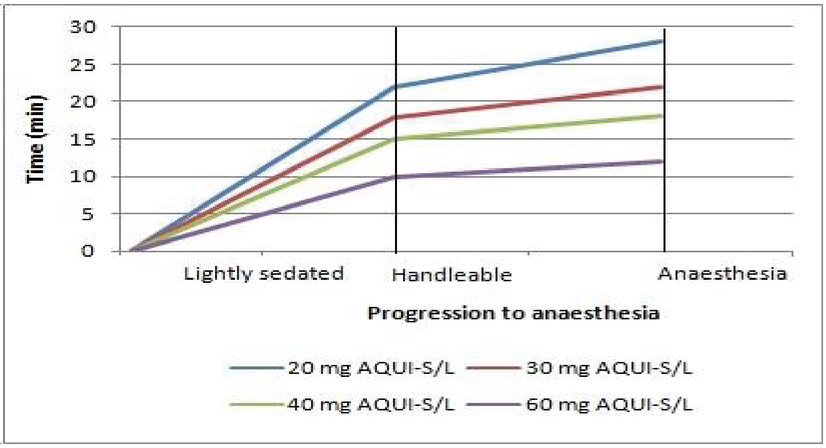

Figure 1. The trend graph showing progression to anaesthesia of ornate spiny lobster by various AQUI-S dosages

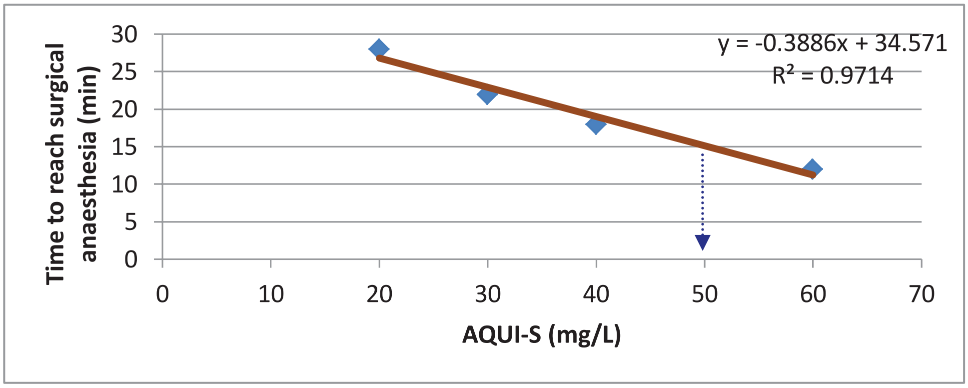

Figure 2. The relationship between AQUI-S dosage and the time to reach surgical anaesthesia

Another study which also carried out by AQUI-S New Zealand Ltd showed that at an AQUI-S® concentration between 17 and 68 ppm (mL per 1000L of water) *Jasus edwardsii* lobsters were suitable for transport and handling procedures in 1 to 6 minutes [15].

Table 2. The AQUI-S® efficacy with three spiny lobster species

Lobster species	*AQUI-S® concentration ($mL.L^{-1}$)*	*AQUI-S® concentration ($mg.L^{-1}$)*	*Efficacy time (min)*
Panulirus ornatus	18.3 – 55.0	20.0 – 60.0	12 – 28
Panulirus cygnus	20.0 – 80.0	21.8 – 87.2	1 – 8
Jasus edwardsii	17.0 – 68.0	18.5 – 74.1	1 – 6

With some modifications (AQUI-S: 1 mL = 1.09 mg; Nic Paton, Pers. Com., 2017), the results from three studies can be summarised in the Table 2 from which it can be seen clearly that it took longer time for *Panulirus ornatus* lobster to reach surgical anaesthesia compared with the other lobster species. It could be due to the fact that *P. ornatus* has a wide geographical range in the Indo-Pacific, from the Red Sea and KwaZulu-Natal in the west to Japan and Fiji in the east while the others are mainly temperate species [16]. A wider geographic range distribution seemed to help the ornate lobster have higher resistance to external factors.

2. The dry transport trial

2.1. The in-box temperature during the trial

The temperatures in boxes where the ornate lobsters were transported in dry condition were

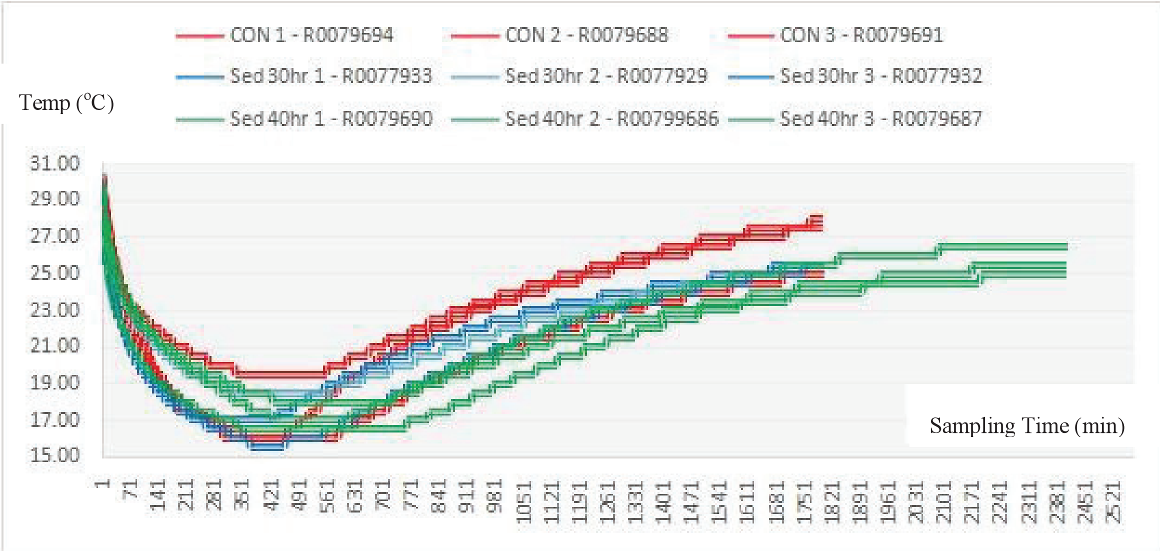

Figure 3. The in-box temperature during the dry transport trial

shown in the figure 3. The common pattern was as follows: the temperature dropped from 31ºC to around 16ºC – 19ºC within 7 – 8 hours after finishing packing, and then the temperature was raised from around 16ºC – 19ºC to 23ºC – 27ºC within 22 – 23 or 32 – 33 hours after reaching the lowest temperature for the boxes in 30 hr or 40 hr transport, respectively. The highest temperatures of the two extreme values almost occurred in the control boxes (19ºC for the lowest – 27ºC for the end).

2.2. The mortality occurence and final survival

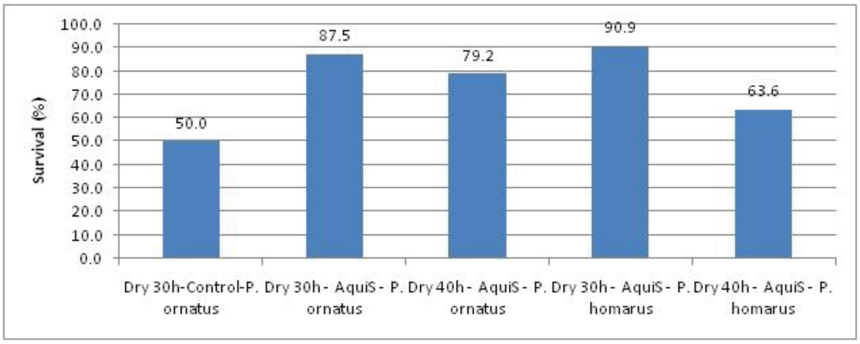

Figure 4. The survival of lobsters after the dry transport 7 days

With 30 hr dry transport, AQUI-S supplement improved the survival of the ornate spiny lobsters compared with those without AQUI-S supplement – the control treatment ($P<0.05$) (Fig. 4 and Table 3).

AQUI-S supplement could prolong the transport duration from 30 hr to 40 hr without statistically significant difference in survival of the ornate spiny lobsters ($P>0.05$) (Table 3). The post transport mortality occurred within

Table 3. The post dry transport survival of ornate spiny lobsters

Treatment	*Survival (%)*
Dry 30h - Control	50.0 ± 7.22^{a}
Dry 30h - AQUI-S	87.5 ± 7.22^{b}
Dry 40h - AQUI-S	79.2 ± 11.02^{ab}

the first three days, especially high in the first day of ornate lobsters in the treatment without AQUI-S supplement (37.5%) and scalloped lobsters with AQUI-S supplement and 40 hr transport (27.3%) (Fig. 5).

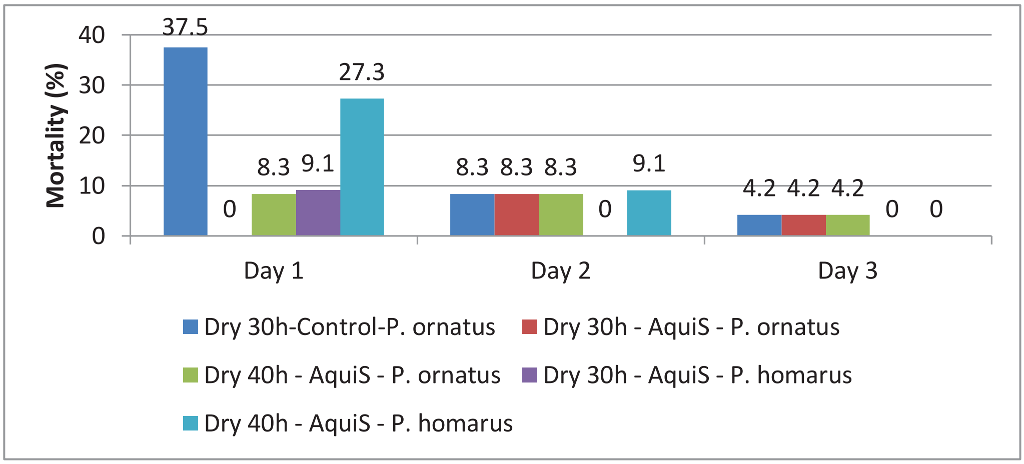

Figure 5. The post dry transport mortality of lobsters

2.3. The transport density and health status of survived lobsters post dry transport

It can be seen clearly from the Table 4 that the real transport densities of the ornate and scalloped lobsters were 175 and 180 individuals per cubic metre, respectively.

However, the space inside the box used for packing was only one third or one fourth for the ornate and scalloped lobsters, respectively. Therefore, the possible transport densities for these lobster species can be multiplied by 3 (*P. ornatus*) or by 4 (*P. homarus*) as in *Potential Density* column. With the control treatment (30 hrs, P. ornatus), the survial rate within 7 days after the trial transport was low (50%) and the survived lobsters were in a large range of health status from 'normal' (recovered completely within 1 days post transport) to 'weak' (recovered completely within 3 days post transport) and 'too weak' (recovered within 7 days post transport, but partially lost appetite and died at molting afterwards). These results could be associated with no anaesthetic treatment which made the lobsters unslept, lost more energy for motion. This was accelerated seriously by the highest temperatures of the two extreme values which occurred in the control boxes (19ºC for the lowest – 27ºC for the end) as mentioned previously. With the 40 h AQUI-S treatment for *P. homarus*, the health status of the lobsters can be explained by the presence more spawners (50%).

Table 4. The technical traits of the lobster dry transport

Box	*Box volume (m^3)*	*Box bottom Area (m^2)*	*Trial Density (Inds.m^{-3})*	*Potential Density (Inds.m^{-3})*	*Health status of survived lobsters post transport*
Dry 30h-Contr-P. ornatus	0.046	0.20	175	530	Normal -Weak-Too Weak
Dry 30h-AquiS-P. ornatus	0.046	0.20	175	530	Normal
Dry 40h-AquiS-P. ornatus	0.046	0.20	175	530	Normal
Dry 30h-AquiS- P. homarus	0.061	0.21	180	720	Normal
Dry 40h-AquiS-P. homarus	0.061	0.21	180	720	Normal-Weak-Too Weak

3. The wet transport trial

3.1. The temperature: The room temperature was kept around 24ºC during the trial.

3.2. The mortality occurence and final survival: With 40 h wet transport, AQUI-S supplement improved the survival of the ornate spiny lobsters compared with those without AQUI-S supplement – the control treatment. However, there was no significant difference in the survival (P>0.05) (Fig. 6).

With the scalloped lobsters, there was a similar trend as the ornate lobsters. Because there was no replication in the trial with this species, no statistical conclusion could be withdrawn from this exploratory trial (Fig. 6).The post transport mortality occurred within the first two days, especially high in the first day of both species in the treatments without AQUI-S supplement (Fig. 7).

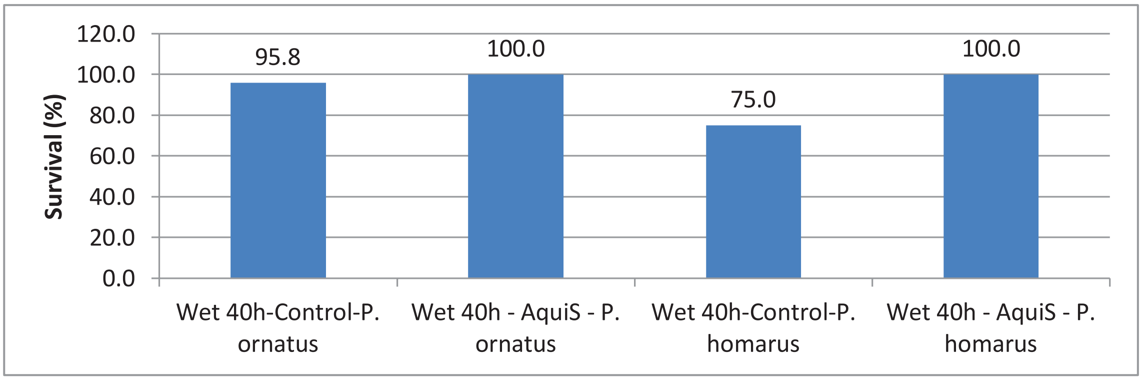

Figure 6. The survival of lobsters after the wet transport 7 days

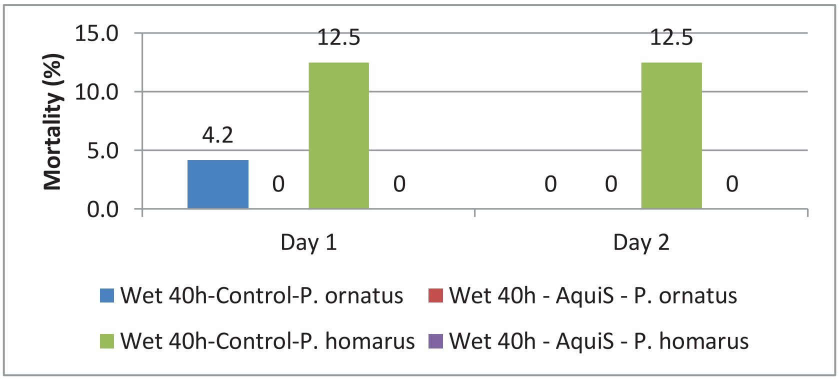

Figure 7. The post wet transport mortality of lobsters

3.3. The transport density and health status of survived lobsters post wet transport

From the Table 5, It is clear that the trial transport densities of the ornate and scalloped lobsters were 40 individuals per square metre. However, the total biomass of the ornate lobster was more than twice as big as that of the scalloped lobster. Additionally, in local

Table 5. The technical traits of the lobster wet transport

Box	*Box Bottom Area (m^2)*	*Trial Density (Inds/m^2)*	*Potential density (Inds/m^3)*	*Health status of survived lobsters post transport*
Wet 40h-Contr-P. ornatus	0.2	40	200	Normal-Weak
Wet 40h-AquiS P. ornatus	0.2	40	200	Normal
Wet 40h-Contr-P. homarus	0.2	40	400	Normal-Weak-Too Weak
Wet 40h-AquiS P. homarus	0.2	40	400	Normal

practices, there were five approximatley 1-m² baskets arranged vertically inside a one cubic metre tank [5]. Therefore, the possible transport densities for these lobster species can be multiplied by 5 and 10 for the ornate and the scalloped lobster, respectively as in *Potential Density* column. With the control treatments (40 hrs, *P. ornatus* and *P. homarus*), the health status of the lobsters was from 'normal' to 'weak' (P. ornatus) or 'too weak' (P. homarus). These results could be associated with no anaesthetic treatment which made the lobsters unslept, lost more energy for motion. With the 40h control treatment for *P. homarus*, the weaker health status of the lobsters can be also explained by the presence more spawners.

4. General discussion

Some major traits withdrawn from the results of our previous survey [5] and these trials are summarised in the Table 6 from which it is clear that with the same transport method for a lobster species, the potential transport density in trials was higher than that of the surveys. The wet transport with AQUI-S and without water change resulted in 100% survival rate and healthy lobster for two species after 40 hr transport. The wet transport without AQUI-S in trials could make the lobster weak, or even too weak like those in the survey.The dry method with AQUI-S could increase remarkably the transport density: the density increased by 1.5 times to 5.3 times compared with local dry transport and wet transport of ornate lobster without AQUI-S, respecively. However, the transport method for lobster spawners needs further investigation to avoid the low survival and so poor health status as shown in the case of the dry method for scalloped lobster with AQUI-S in 40 hours (50% of the stock was spawners).

Table 6. Comparison of transport methods with various lobster species and time duration

Transport method	*Source*	*Species*	*Density (Inds/m³)*	*Health status*	*Survival¹*	*Survival²*
Wet, ~30hrs, water change ~80-90%	*Survey*	*P. ornatus*	100	Normal – Weak – Too Weak	80%	na
Wet, ~30hrs, water change ~ 80-90%	*Survey*	*P. homarus*	200	Normal – Weak – Too Weak	80%	na
Wet, 40hrs, no AQUI-S, no water change	Trial	*P. ornatus*	200	Normal-Weak	96%	96%
Wet, 40hrs, AQUI-S, no water change	Trial	*P. ornatus*	200	Normal	100%	100%
Wet, 40hrs, no AQUI-S, no water change	Trial	*P. homarus*	400	Normal-Weak-Too Weak	88%	75%
Wet, 40hrs, AQUI-S, no water change	Trial	*P. homarus*	400	Normal	100%	100%
Dry, <30hrs	*Survey*	*P. ornatus*	360	Normal – Weak – Too Weak	75%	na
Dry, 30hrs, no AQUI-S	Trial	*P. ornatus*	530	Normal – Weak – Too Weak	63%	50%
Dry, 30hrs, AQUI-S	Trial	*P. ornatus*	530	Normal	100%	88%
Dry, 40hrs, AQUI-S	Trial	*P. ornatus*	530	Normal	92%	79%
Dry, 30hrs, AQUI-S	Trial	*P. homarus*	720	Normal	91%	91%
Dry, 40hrs, AQUI-S	Trial	*P. homarus*	720	Normal – Weak – Too Weak	73%	64%

¹ Survival at finishing the transport (Day 1 survival); 2Survival at the 7th day post transport; na – not available.

IV. CONCLUSION

1). At an AQUI-S® concentration between 20 and 60 mg/L, lobster were suitable for transport and handling procedures in 12 to 28 minutes. To reduce the time to reach surgical anaesthesia of ornate spiny lobsters around 15 minutes, the suitable AQUI-S dosage value withdrawn from the interpolation was 50 mg/L.

2). In the wet transport method with AQUI-S, the (day 7) survival rate was 100% for both species, *P. ornatus* and *P. homarus*. The health status of the survived lobster was normal. The possible transport density for *P. ornatus* and *P. homarus* was 200-400 individuals per cubic metre, respectively.

3). In the dry transport method with AQUI-S, the survival rate of the ornate and homarus lobster at day 1 for 30 hr and 40 hr transport was 100% and 92%, and 91 and 73%respectively. The survival rate at day 7 for 30 hr and 40 hr transport of the ornate and homarus lobster was 88% and 79%, and 91 and 64%, respectively. The health status of the survived lobster of both species was normal, except for the homarus spawners (~50%) in 40 hrs treatment. The possible transport density for *P. ornatus* and *P. homarus* was 530 and 720 individuals per cubic metre, respectively.

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