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# Influence of sediment characteristics on shrimp physiology: pH as principal effect

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**Abstract:**
Penaeid shrimp reared in earthen ponds are exposed to sediment, which can, in some instances, induce a stress. In seawater, the osmoregulatory capacity (hyporegulation) is a useful tool to compare the physiological condition of shrimp exposed to various stressors. By keeping some shrimp in cages at different locations of a single pond heterogeneous in terms of sediment quality, it was possible, using osmotic pressure (OP), to identify some locations where the stress was maximum and some others where it was minimum. Simultaneously, sediment samples were taken and analysed in order to evaluate some physico-chemical parameters that could be related to the stress observed in the shrimps kept in the cages. This approach allowed to show a significant positive correlation between the pH of the sediment surface and the shrimp osmotic pressure. This result was confirmed in a study carried out in experimental 70-l tanks, where osmotic pressure decreased significantly as water pH decreased from 7.0 to 6.5. The methodology developed in this study may be useful to evaluate the stress caused by sediment in shrimp farmsRésumé

**Keywords:** Shrimp physiology; Osmotic pressure; Stress; Sediments; pH

## 1. Introduction

Bottom soil quality has long been recognised as a factor influencing water quality and aquatic animal production (Hussenot and Feuillet-Girard, 1988; Boyd, 1990; 1992; Munsiri et al., 1996). Pond bottom conditions are particularly critical for shrimp as they spend most of their time on the bottom, burrow into the soil and ingest some of it (Boyd, 1989; Chien, 1989). During grow-out, nutrients and organic residues tend to accumulate on the bottom and an excessive accumulation may result in the deterioration of the pond system. However, deposition is not uniform across the pond (Avnimelech, 1995; Smith, 1996; Lemonnier et al., 2002). Avimelech (1995) estimated that the area covered by sludge in typical shrimp ponds in Thailand reaches nearly 50% of the pond area, and suggested that shrimp growth, activity and health may be negatively affected in this zone. In intensive system, Delgado et al. (2003) found that shrimp abundance was significantly lower in the central sediment enriched zone, suggesting conditions may have been less suitable for shrimp growth and health. Avnimelech and Ritvo (2003) in a review reported that accumulated sediments in shrimp ponds are highly reduced, enriched in organic matter and enriched in nitrogen and phosphorus. It is possible that toxic anaerobic products originating from the sediment may influence stress in shrimps, reducing vitality and disease resistance. Reduced feeding, slower growth (Ram et al., 1982; Avnimelech and Zohar, 1986), low survival (Hopkins et al., 1994; Lemonnier and Brizard, 2001) and possibly higher sensitivity to disease are reported in relation with deterioration of the pond bottom (Avnimelech and Ritvo, 2003). The lack of knowledge on this important topic points out the need for more research (Avnimelech and Ritvo, 2003). In fact, different methodologies were developed to analyze sediment quality (Boyd, 1995; Hussenot and Martin, 1995), and to test the toxicity of sediment on shrimps survival or weight gain in field laboratory (Moraes et al., 2000; Ritvo et al., 1998a,b, 2000). However little has been done to study the effect of sediment on shrimp physiology in either field or laboratory experiments.

Variation in osmotic regulation can be used as a non-specific indicator for detecting physiological stresses in invertebrates including in *L. stylirostris* (Giesy et al., 1988; Mayer et al., 1992; Lagadic et al., 1994). The blue shrimp *L. stylirostris* is a hypo-osmoregulator in seawater. Haemolymph osmotic pressure (OP) has been proposed as a parameter for the evaluation of the physiological state of peneids (Charmantier et al., 1989) as it is influenced by a large variety of environmental parameters (Lignot et al., 2000). In previous studies, osmotic hyporegulation has been demonstrated to be sensitive to ammonia (Lin et al., 1991; Young-Lai et al., 1991; Lin et al., 1993, Mugnier and Justou, in press), oxygen depletion (Charmantier et al., 1994), temperature (Charmantier, 1975, 1987; Lemaire et al., 2002) heavy metals (Bambang et al., 1995a,b) and fenitrothion exposure (Lignot et al., 1997).

In a preliminary study (Cochard et al., 1997), OP appeared to be a convenient tool to detect areas of the rearing pond where sediments are degraded and potentially harmful to shrimps. The objective of the present study was to evaluate the stress caused by sediment on shrimps held in bottom cages at different points of the pond and correlate the level of stress with sediment parameters.

## 2. Material and methods

### *2.1. Field experiment*

#### *2.1.1 Experimental design*

*Litopenaeus stylirostris* (Stimpson, 1874) were reared in a 1-Ha earthen pond at the Laboratoire d'Aquaculture de Calédonie at less than 20 animals/m$^2$ according to the methods described by Aquacop (1984). Shrimps were caught in the pond and transferred into cages as fast as possible. Salinity and Secchi of the pond water were respectively 34 ‰ and 50 cm. The wire mesh cages were 100x50x15cm, pore size was 8 mm. The mean weight of the shrimp was 13.2g ±1.9. Twenty to thirty shrimp were placed in each cage. The depth was measured at each point. These cages were carefully placed directly in contact with the sediment at 12 stations across the pond (stations 1 to 12), without sediment perturbation. After 24 hours of exposure to the sediment, the shrimps were taken out of the cages. Cages were placed in, and taken out of the water in a random order. Setting all cages in the water did not last more than 2 h. Cages were lifted up after around 24 h at a rate of one per 45 min. Therefore, shrimps stayed in the cages randomly from 22 to 28 h according to the stations studied. The experimental design used was the "randomized complete blocks design" (Sokal and Rohlf, 1981). The experiment was repeated four times (blocks A, B, C, D) over a 2-week period. Each time, two groups of shrimps (controls) were sampled in the same pond using a cast net, once in the morning and once in the afternoon.

### *2.1.2. Stress measurement*

Shrimps were transferred from the cages to aerated 30-l containers and immediately brought to the laboratory for haemolymph sampling. Molting stage was assessed by stereo-microscope examination of the uropod according to the method of Drach and Tchernifovtzeff (1967). Since haemolymph concentrations fluctuate with molt stage (Charmantier et al., 1994, Lignot et al., 1999), only shrimps in C and D0 molting stages were sampled to measure haemolymph osmotic pressure. The haemolymph was withdrawn from the ventro-lateral sinus of the abdomen into a 1.0-ml syringe. The OP was measured using 10 µl of haemolymph in a WESCOR 5500 vapor pressure osmometer and was expressed in $mOsm.kg^{-1}$.

### *2.1.3. Sediment characteristics*

The sediment parameters were studied at each station respectively at the beginning of the first experiment and of the third experiment. The redox potential (Eh) was assessed *in situ* on the first centimetre using a specific electrode (Cofralab PT5700A) according to the method described by Hussenot and Martin (1995). In a same way, pH of pore water was measured directly in the first centimetre with a pH meter (Hussenot and Martin, 1995). The first centimetre layer of sediment was collected at each station using a 40-cm-long PVC tube of 10 cm diameter. Samples were removed manually in polypropylene sampling tubes. Twenty millilitres of each sediment sample was weighed before and after drying for 5 days at 60°C to calculate water content. The loss of weight by ignition of the dry sediment was determined at 550°C for 5 h. One hundred grams of each sediment sample (100 g) were centrifuged at 2000xg for 30 min. The supernatant (pore water) was immediately filtered through 1.2-µm nominal pore size Whatman GF/C glass fibre filters before colorimetric analysis for total ammonia nitrogen TAN ($N\text{-}NH_3\text{-}N$ + $N\text{-}NH_4^+\text{-}N$) (Koroleff, 1976) using a Technicon Autoanalyser (Treguer and Le Corre, 1975). $N\text{-}NH_3$ fraction was calculated according to the equation of Bower and Bidwell (1978) taking into account temperature, salinity and pH. The results show the means calculated from the two samplings.

## *2.2. Laboratory experiments*

Shrimps (*L. stylirostris*, Stimpson, 1874) used in these experiments were reared in ponds according to the methods described by Aquacop (1984). The shrimps were captured using a cast net. The average weight of shrimps was 19 g. Only shrimps in C and D0 molting stages were sampled and were acclimated for 24 h in 70- l experimental tank with aeration. The experiment was carried out twice in six closed tanks of 70-l capacity. Eight shrimps were placed in each tank for 24 h. Five pH levels between 6.0 to 8.0 were tested. At the beginning of the experiment, pH was adjusted with $NaH_2PO_4$ and $Na_2HPO_4$ solutions. The pH of the control group was 8.2 (marine water). Each tank was aerated. Temperature and salinity were respectively 26°C and 36-37‰. During experiments, pH was controlled independently every hour and adjusted with NaOH (1N) or hydrochloric acid (HCl 0,1N) solutions. TAN concentration was measured after 6, 12, 18 and 24 h. After 24 h exposure to different pH level, haemolymph of shrimps were sampled for osmotic pressure measurements. Tanks were sampled randomly.

*2.3. Statistics*

Statistical comparisons of experimental data were performed by Two-ways ANOVA followed by a Protected Least Significant Difference Test (PLSD) and regression analysis using Statview 4.02 ®(Abacus Concepts) and ANOVA as described by Scherrer (1984). Results are presented as means ± SD. Difference was recorded as ** ($p<0.01$) when highly significant and as ns when not significant ($P>0.05$).

## 3. Results

*3.1. Field experiment*

*Physiological aspects*

Mean survival was 79 % (SD = ± 12%), and was not significantly different between cages ($F_{11/36} = 0.66$, $p = 0.77$). Results of OP measurements are presented in table 1. In seawater, an increase of OP indicates a dysfunction in osmoregulation associated with various stress. The two-ways ANOVA showed a highly significant effect for sampling day ($p<0.001$), cage ($p<0.001$) and a highly significant interaction ($p<0.001$). Captive shrimps showed an increased of OP in relation with the station zone. Comparison of the means by PLSD (Table 2) showed a significantly higher OP for all captive groups with some cages inducing a significant increase compare to others. Comparison of controls for the four series of experiments showed a variation with time ($p<0.001$). However, there was no significant difference between morning and afternoon controls. Their mean was used to assess the effect of the cage on the OP. Fig. 1 illustrates the effect level of cages location in the pond. There is a gradient in the increase of OP between point 2 and 3 and point 12.

*Sediment characteristics*

Figure 2 shows a schematic presentation of parameters studied in pond. Mean Depth of the pond was 1.7 m. Stations 2, 3, 4 and 8 were the deepest stations. The loss by ignition was most important in stations 1, 2 and 4, which were characterised by fluid sediment (higher water content). TAN concentration was maximum in the centre of the pond. Variations of Eh and pH were concomitant with depth. The lowest redox and highest pH were found at the deepest point of the pond.

Correlation analysis (table 3) showed that depth was correlated with some sediment characteristics. Both pH, Eh and N-$NH_3$ concentration were highly correlated to depth; with low depth related to more acid and oxygenated sediments. pH was positively correlated to N-$NH_3$ and negatively to TAN and redox. The loss by ignition was only correlated to the water content (table 3) with the highest value recorded on stations 2 and 4, located on a previous mangrove zone.

*Correlation study between osmotic pressure and sediment characteristics*

The correlation matrix in table 3 shows significant negative correlations between the shrimp mean OP and station depth, sediment pH and level of N-$NH_3$ in pore water. Graphs of the relationship between these media/environmental parameters and OP are shown in Fig. 3 (graphs a, b, c, d). It can be noted that the set of points in the pH/OP graph is made of two sub-sets with similar slopes. Stations 2, 5 and 7 seem to be different from the other stations.

Shrimps sampled at these stations seem to be less stressed as their OP is lower. In both cases, we note a negative linear correlation between pH and OP with dots following two almost parallel straight lines. In an exploratory approach, when data from those stations are excluded from the analysis (Fig. 3, graphs e, f, g, h), the significant correlations described before are reinforced. Even Redox Eh becomes significantly correlated to shrimp OP. These correlations become very high as $R^2$ coefficients reach - 0.94 for the pH, - 0.80 for depth, +0.65 for redox Eh and -0.60 for correlation with N-$NH_3$.

*3.2. Laboratory experiment*

Results are shown Fig. 4. The two-ways ANOVA shows a pH effect on shrimp OP ($p<0.001$) but no sampling day effect ($p=0.21$). Mortality was observed in the first experiment in shrimps exposed to the lowest pH values (6 out of 8 at pH 6.0 and 2 out of 8 at pH 6.5) but not in the second set of experiments. The mortality was related to an increase in the shrimp OP which induced the significant relationship shown by the ANOVA. N-$NH_3$ levels which ranged from 0.35 to 0.75 mg/l were not significantly related to pH but the highest level was recorded in the controls.

## 4. Discussion

Although the experimental design was powerful enough to show the effect of captivity on shrimps osmoregulation, it did not allow to distinguish this stress from the one induced by the sediment. For future experiments, the effect of cage only on shrimp physiology will be taking into account by avoiding contact with sediment. However, results in the present study show the effect of different sediment quality on the stress of shrimp kept captive in bottom cages. Despite the significant variation in the mean OP of the control shrimps, it was possible to assess the most stressful zones.

Three correlations were found between shrimp haemolymph OP and, respectively, N-$NH_3$ in pore water, pH and depth. Three more significant correlations were found between depth and respectively redox, N-$NH_3$ in pore water and pH. In New Caledonia, shrimp farms are built on the intertidal zone between mangroves and agricultural land, with ponds integrated as much as possible into the natural landscape. Soil quality varies within these marshes with an increase in the amount of clay, organic matter and pH from the upper side (agricultural land side) towards the lower side (mangrove side) (Lemonnier et al., 2002). Therefore, pond topography and as a consequence water depth is strongly related to soil quality (texture, organic matter, pH). The depth of the pond ranged between 125 and 170 cm and this difference (maximum 45 cm) is not likely to have an effect on shrimp physiology. Therefore, the negative correlation found between depth and OP should not be seen as a direct relationship between the two but rather be explained as resulting from the effect of sediment quality on shrimp hyporegulation. The negative relationship between N-$NH_3$ and OP is mainly due to the important role of pH in the $NH_3$-$NH_4$ equilibrium. Moreover, N-$NH_3$ concentrations in pore water were low, less than 0.018 mg/L and below the N-$NH_3$ concentration safety levels defined for *P. monodon* and *P. vannamei* (respectively 0.08 and 0.16 mg/L) (Chen et al., 1990; Lin and Chen, 2001). The most significant correlation was between sediment pH and osmotic pressure of the captive shrimps. Post-experiment laboratory checks showed that low pH lead to an increase of osmotic pressure. This increase seems to be correlated to a pH range between 6.0 and 7.0 which fits what was seen in the field study. Osmotic pressure of captive shrimps is directly related to the acidity of sediment.

Conventionally, pH determinations are made on 1:1 dry soil/distilled water paste. An empirical relationship between this method and pH *in situ* has been found by Ritvo et al. (2003) and can be used to evaluate the *in situ* pH. The pH measured directly by inserting the pH electrode directly into the sample is the same as that of the pore water (Masuda and Boyd, 1994). An important effect of soil flooding is to depress the pH of sodic and calcareous soils due to the accumulation of $CO_2$ and to increase of the pH of acid soils due to the consumption of $H^+$ ions by different reduction processes (Ponnamperuma, 1972). Soil pH in pore water in aquaculture ponds built on Clayey Ultisols could be apparently controlled by the equilibrium: $FeCO_3(s) + H^+ = Fe^{2+} + HCO_3^-$ (Massuda and Boyd, 1994).

The negative effects of acid sulphate soils and pond acidity on shrimp yields are well known (Boyd, 1990; Binh et al., 1997) and pH water was previously described as a stress factor for crustacean. In a study conducted by Allan and Maguire (1992), low pH water (5.9) has been reported to cause decreased growth in *P. monodon.* The effect of low pH on hyporegulation was worse, compared with hyperregulation at low salinity and high pH and there was no significant interaction. In the present study, haemolymph osmoregulation decreased significantly as pH water decreased from 7 and 6.5. Wickins (1984) showed that decreasing pH from 7.9 to 6.7 induced carapace weight loss, increased magnesium content and decreased strontium content. The minimum acceptable pH levels in *Macrobrachium rosenbergii* were 6.2 and 7.4 based on growth and feeding, respectively (Chen and Chen, 2003). The effect of pH on crustacean physiology has been seldom studied and mainly on freshwater species. Low pH disturbed ion regulation in crayfish and acid-base imbalance in crayfish and freshwater shrimp (Morgan and McMahon, 1982; Chen and Lee, 1997). The exposure of crayfish to low pH results in a net loss of $Cl^-$ and $Na^+$ (Zanotto and Wheatly, 1993). The $Cl^-/HCO_3^-$ exchange regulates haemolymph $Cl^-$ transport and $HCO_3^-$ influence $CO_2$ haemolymph partial pressure.

The linear correlation study between pH and OP shown in this paper suggested the effect of several other factors on OP: Points 2, 5 and 7 are on a straight line parallel to the one made of the nine other points. These latter points might be under the influence of another unmeasured stress factor whose effect could be added up to the first one at the same time. Several parameters may play a role such as dissolved oxygen (Le Moullac et al., 1998, Allan and Maguire, 1991), ammonia (Chen et al., 1990; Chen and Kou, 1992), hydrogen sulfide (Bagarinao and Vetter, 1992; Ritvo et al., 2000), carbon dioxide (Hall and Van Ham, 1998), nitrites (Chen and Lee, 1997), each of these parameters having been described as potentially stressful and even toxic to farmed animals.

Sedimentation dynamics and recycling of organic mater accumulated in the pond varies greatly along the grow-out period, leading to a variability of sediment quality with time. Increased concentrations of organic matter in soil or larger input of feed to ponds favour greater microbial activity in soils and higher concentrations of TAN in pore water (Masuda and Boyd, 1994). Thus, the water-sediment interface (first centimetre) in intensive ponds commonly showed higher ammonia concentration, lower *in situ* pH, and lower redox than in semi-intensive ponds at the end of the rearing (Lemonnier et al., 2004). Suplee and Cotner (1996) showed an increase of sediment oxygen demand, sulphate reduction rates, sulphate reducing bacteria abundances over the growing season. In fish ponds, components such as $NH_4^+$ or $H_2S$ therefore do accumulate in the sediment continuously (Avnimelech and Lacher, 1979; Ram et al., 1981). However, few works have been conducted on the evolution of pond bottom quality during rearing, especially on pH. In fact, most studies on pond bottom have been carried out at the end of the rearing or during the dry-out period (Morales et al., 1991; Ayud et al., 1993; Boyd et al., 1994a, b, Munsiri et al., 1995, 1996; Ritvo et al, 1998c, 1999; Sonnenholzner and Boyd, 2000; Avnimelech et al., 2001; Lemonnier et al., 2004) or in laboratory experiment (Boyd and Munsiri, 1997; Blackburn et al., 1988). It might be interesting to study the evolution of sediment quality during the grow-out with a tighter time lag, as potentially stressful parameters may vary during this period. This study showed a spatial variability in the animals physiology related to the cages location. Generally speaking, uneaten feed, faeces, plankton and debris can settle in a certain pattern throughout the pond bottom. This settling pattern can be affected by pond depth and water circulation (Boyd, 1992; Smith, 1996). These factors introduce a high degree of spatial variability into soil parameters (Ritvo et al., 1998d) which is seldom considered in soil quality studies. In the same way, the original soil variability is also little considered. Indeed, samples within a pond are usually pooled. The resulting mean for one parameter does not allow to know the differences within one pond and thus the limiting values susceptible to stress the crop. The results obtained in this work raise the need for renewed thought, development and assessment of strategies to analyse sediment quality taking account its temporal and spatial variability. The methodology developed in this study may be applicable to evaluate the stress caused by sediment for shrimps in pond environment and may be used to assess pond bottom conditions which will be dangerous for shrimps.

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Table 1. Average and standard deviation of osmotic pressure of shrimps *Litopenaeus stylirostris* (mOsm/kg) kept for 24h in cages at different locations on the bottom of the pond and in the pond (control).

| **Day Cage** | **Experiment A** | | **Experiment B** | | **Experiment C** | | **Experiment D** | |
|---|---|---|---|---|---|---|---|---|
| | Mean ± SD | n. | Mean ± SD | n. | Mean ± SD | n. | Mean ± SD | n. |
| **1** | 846 ± 24 | 15 | 849 ± 21 | 14 | 882 ± 26 | 10 | 847 ± 26 | 13 |
| **2** | 815 ± 20 | 14 | 839 ± 28 | 11 | 853 ± 25 | 13 | 855 ± 16 | 14 |
| **3** | 848 ± 22 | 11 | 810 ± 17 | 14 | 852 ± 23 | 12 | 847 ± 30 | 13 |
| **4** | 849 ± 23 | 8 | 852 ± 19 | 14 | 832 ± 24 | 9 | 862 ± 13 | 9 |
| **5** | 844 ± 21 | 12 | 832 ± 30 | 7 | 848 ± 18 | 6 | 853 ± 20 | 7 |
| **6** | 836 ± 32 | 14 | 862 ± 16 | 8 | 872 ± 27 | 9 | 875 ± 21 | 7 |
| **7** | 837 ± 19 | 12 | 870 ± 21 | 12 | 848 ± 13 | 9 | 859 ± 21 | 13 |
| **8** | 843 ± 13 | 13 | 857 ± 31 | 15 | 858 ± 24 | 13 | 853 ± 26 | 11 |
| **9** | 827 ± 14 | 6 | 870 ± 16 | 13 | 869 ± 25 | 5 | 863 ± 31 | 10 |
| **10** | 849 ± 20 | 10 | 868 ± 10 | 4 | 867 ± 30 | 10 | 882 ± 18 | 9 |
| **11** | 883 ± 23 | 10 | 870 ± 21 | 10 | 868 ± 17 | 9 | 848 ± 20 | 10 |
| **12** | 875 ± 27 | 14 | 867 ± 20 | 14 | 879 ± 22 | 11 | 868 ± 17 | 12 |
| **Control AM** | 799 ± 16 | 15 | 812 ± 30 | 13 | 824 ± 19 | 15 | 816 ± 14 | 15 |
| **Control PM** | 813 ± 18 | 12 | 813 ± 17 | 15 | 831 ± 14 | 13 | 811 ± 14 | 13 |
| **Cage average** | 846 | | 853 | | 861 | | 858 | |
| **Control average** | 805 | | 813 | | 827 | | 814 | |

Table 2. Comparison between osmotic pressure in cage, between control and between cages and controls by Protected Least Significant Difference analysis.

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **Control 1** | **Control 2** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | - | | | | | | | | | | | | | |
| **2** | **0.002** | - | | | | | | | | | | | | |
| **3** | **<0.001** | 0.567 | - | | | | | | | | | | | |
| **4** | 0.281 | 0.059 | **0.016** | - | | | | | | | | | | |
| **5** | **0.044** | 0.444 | 0.208 | 0.342 | - | | | | | | | | | |
| **6** | 0.496 | **<0.001** | **<0.001** | 0.101 | **0.013** | - | | | | | | | | |
| **7** | 0.998 | **0.002** | **<0.001** | 0.293 | **0.050** | 0.509 | - | | | | | | | |
| **8** | 0.816 | **0.003** | **0.001** | 0.389 | 0.071 | 0.371 | 0.820 | - | | | | | | |
| **9** | 0.201 | **<0.001** | **<0.001** | **0.029** | **0.003** | 0.562 | 0.213 | 0.138 | - | | | | | |
| **10** | **0.019** | **<0.001** | **<0.001** | **0.002** | **<0.001** | 0.114 | **0.023** | **0.011** | 0.327 | - | | | | |
| **11** | **0.005** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.047** | **0.006** | **0.002** | 0.176 | 0.741 | - | | | |
| **12** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.002** | **<0.001** | **<0.001** | **0.016** | 0.189 | 0.311 | - | | |
| **Control 1** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | - | |
| **Control 2** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.344 | - |

Probabilities in boldface are significant at P<0.05.

Table 3. Correlation matrix for regression among chemical variables in sediment, water depth and osmotic pressure

| | OP | Depth | pH | Redox Eh | Loss by ignition | Water content | TAN | $N\text{-}NH_3$ |
|---|---|---|---|---|---|---|---|---|
| OP | - | **-0.78** | **-0.74** | 0.44 | -0.38 | -0.29 | 0.42 | **-0.66** |
| Depth | **0.002** | - | **0.85** | **-0.70** | 0.54 | 0.52 | -0.40 | **0.83** |
| pH | **0.004** | **<0.001** | - | **-0.81** | 0.26 | 0.33 | **-0.72** | **0.80** |
| Redox Eh | 0.162 | **0.009** | **0.001** | - | -0.13 | -0.27 | 0.49 | **-0.76** |
| Loss by ignition | 0.224 | 0.070 | 0.422 | 0.697 | - | **0.92** | -0.01 | 0.40 |
| Water content | 0.377 | 0.084 | 0.305 | 0.398 | **<0.001** | - | -0.04 | 0.52 |
| TAN | 0.182 | 0.203 | **0.007** | 0.108 | 0.956 | 0.907 | - | -0.19 |
| $N\text{-}NH_3$ | **0.017** | **0.000** | **0.001** | **0.003** | 0.205 | 0.086 | 0.563 | - |

The right part and the left part of the table show respectively correlation coefficients and probabilities. Correlation coefficients in boldface are significant at probability level of 5% (OP: osmotic pressure; TAN: total ammonia nitrogen).

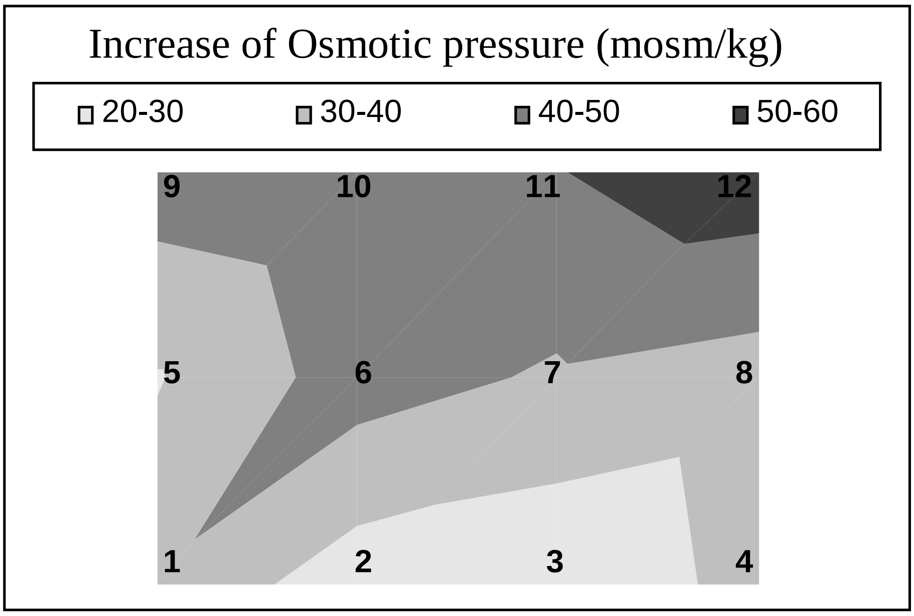


Fig. 1. Effect of level of cages on haemolymph osmotic pressure in pond. Values correspond to the difference between osmotic pressure in cage and osmotic pressure mean of the controls.

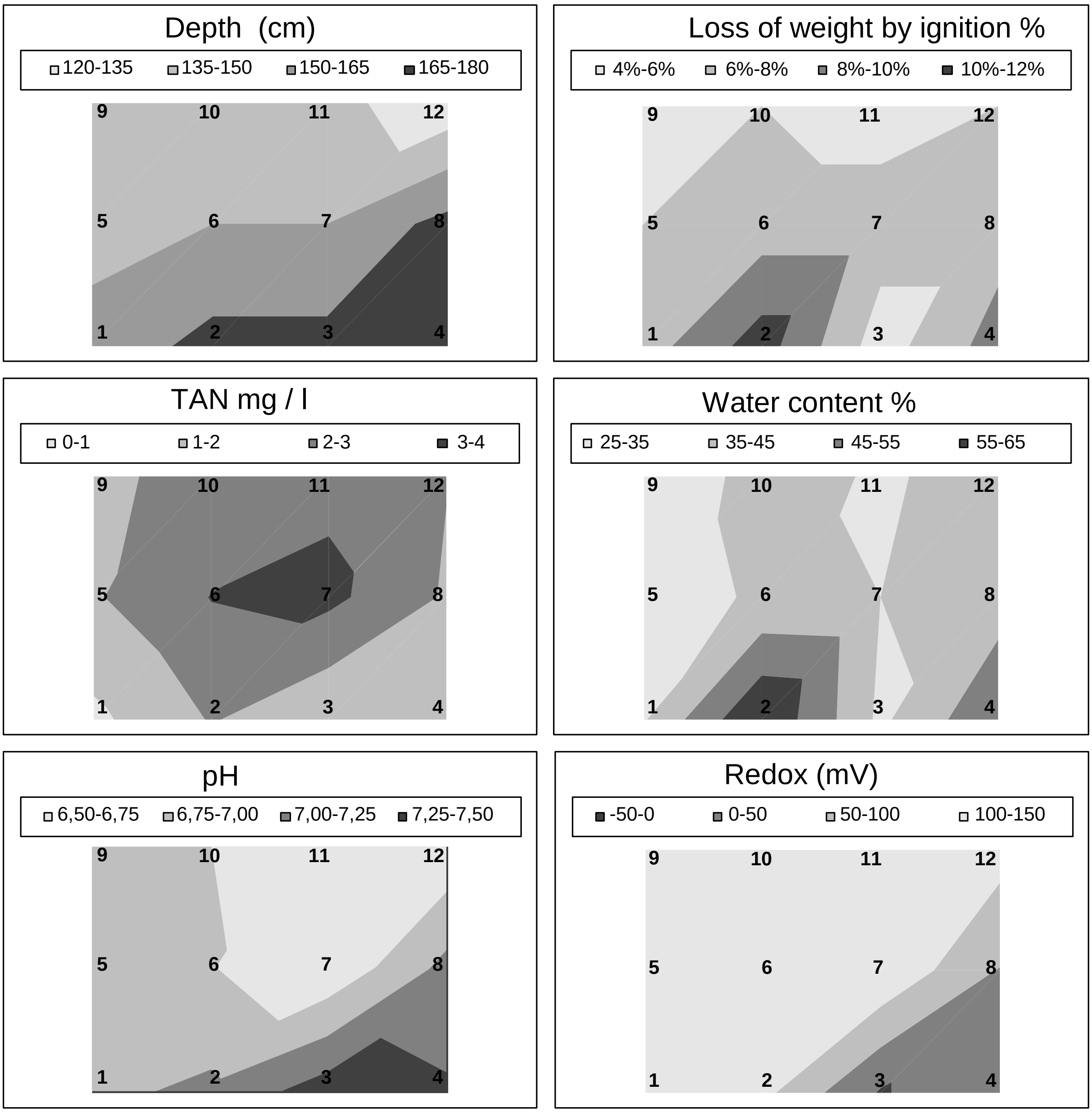


Fig. 2. Schematic representation of physico-chemical variables of pond sediment. (TAN: total ammonia nitrogen)

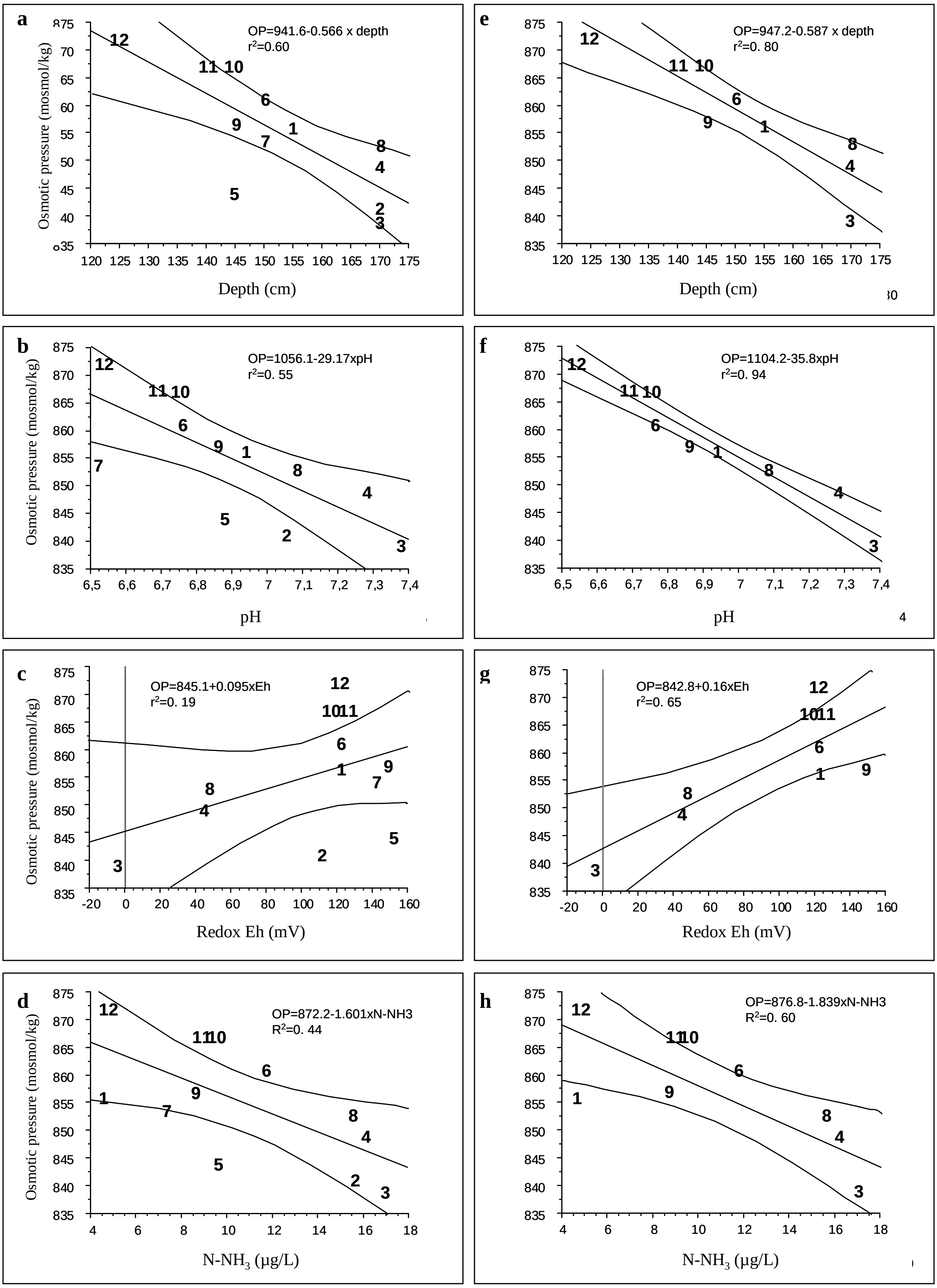


Fig. 3. Influence of depth, pH, redox Eh and N-$NH_3$ in sediment on osmotic pressure. In graphs e, f, g and h, points 2, 5 and 7 were excluded as part of an exploratory approach of the data.

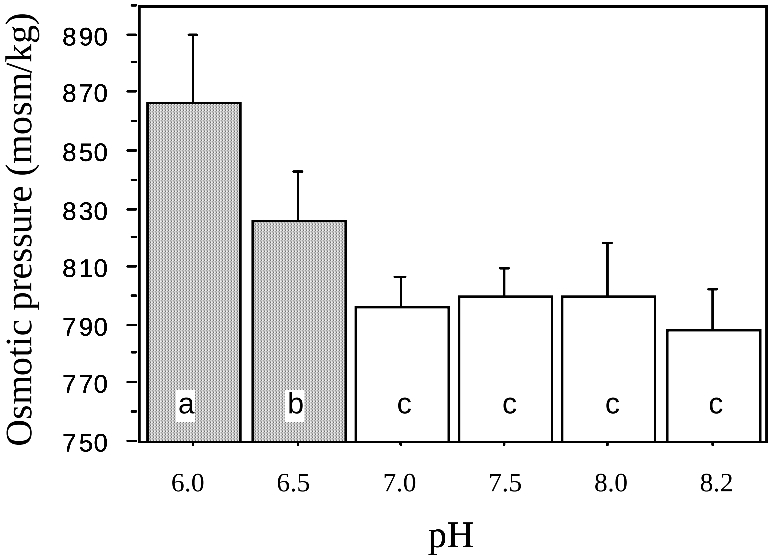


Fig. 4. Effect of pH on shrimp osmotic pressure in tank experiments. Means indicated by the same letter do not differ at p = 0.01. Bars represent standard deviation.