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Effect of different salinity levels on egg hatching and survival rate of different metamorphosis larval stages of Kuruma shrimp, *Penaeus japonicus* (Bate, 1888)

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ABSTRACT

The effect of six different salinity levels was studied on egg hatching percentage and survival rate of different larval stages of the Kuruma shrimp, *Penaeus japonica.* Eggs of the Kuruma shrimp and different larval stages included nauplius, zoea, mysis, and postlarvae were stocked at experimental glass vessels (2 L) in replicates for each treatment at a density of 50 per replicate. The different stages were evaluated under different water salinity levels included 20, 25, 28, 30, 35 and 38 ppt. All vessels were supplied with continuous aeration. Kuruma shrimp stages were fed during the experimental period with micro-algae, rotifers, and Artemia on a live food program. Some physico-chemical parameters included temperature, salinity, pH, Turbidity, TAN, NH_3, and DO were determined during the experimental period (23 days). The data of the eggs hatching percentage and survival rate of the different larval stages of Kuruma shrimp reared in different tested salinity levels showed that all stages reared in 20 ‰ salinity level gave the lowest survival rate in compared to those reared in 38 ‰ and the other tested salinity levels without significant differences ($P < 0.05$). Increasing the water salinity levels in the culture water over 20 ‰ resulted in an improvement in survival rate in all tested larval stages especially on 38 ‰, it was the best survival rate between all tested salinity levels in the Nauplius stages (N_1- N_6), Zoea stages (Z_1- Z_3) with significantly differences ($P < 0.05$). Also, the best survival rate was in 38‰ with significant differences ($P < 0.05$) for Mysis stages (M_1- M_3), Data showed that the highest tested salinity level (38 ‰) gave the higher survival rate to post larvae of the kuruma shrimp included 94%, 85%, and 80%, for PL_1, PL_6, and PL_{12}, respectively. The results showed that the survival rate for all larval stages was obtained in 38 ‰ salinity levels. Therefore, it could be recommended for applying and extending out these results under Egyptian conditions.

INTRODUCTION

Mariculture is one of the most important producing sector in the aquaculture field **(Eissa *et al.*, 2021a)** Kuruma shrimp, *Marsupenaeus japonicus* Bate (1888) is one of the most important marine shrimp species **(Eissa *et al.*, 2021b)** .it's a source of needed animal protein with high nutritional value, employment, income for farmers all over the world **(Troell *et al*., 2006; Gjedrem *et al*., 2012; Ottinger *et al*., 2016 and Eissa *et al*.,2021b).** Shrimp farming is one of the most profitable projects of the mariculture industry **(Troell *et al*., 2006).**The commercial farming of marine shrimp of the genus *Penaeus* has received much attention during the past decades and has become in recent years a very important industry worldwide **(Gjedrem *et al*., 2012).**

Increased demand for marine shrimp in the world markets has encouraged many farmers to enter into shrimp farming sector **(Swapan & Gavin, 2011),**reasons for this are the favorable demand of shrimp in the world market, the high cost of fishery shrimp harvested from the wild, and the high technology development in the field of hatching, nutrition, breeding, disease control, and water quality management **(Rosenberry, 1990; Tacon *et al*., 2002 and Swapan & Gavin, 2011).**

Kuruma shrimp, *Penaeus japonicus* (Bate, 1888) is one of the important marine shrimp species **(Eissa *et al*., 2021a).** its production technology began in japan and transferred to China, Southeast Asia, India and Latin America **(Thakur & Lin, 2003 and Tsoi *et al*., 2007)**. Salinity is a variable which strongly influences shrimp production via metabolism, physiology, and behavior effects **(Chang *et al*., 1998 and Benzie, 2009).**

Some minerals in salinity water act as strong catalysts, especially against stress **(Ren, *et al*., 2020).**Also The well-known red color change plays a significant role in consumer acceptability of crustacean species **(Pan *et al*., 2020).** The term larval quality is widely used to refer to the physiological condition, performance during culture (survival and growth) and resistance to stress tests “e.g., manipulation stress, changes in environmental conditions, resistance to pathogens” **(Racotta *et al*., 2003 and Mohapatra *et al*., 2013).**

The search and establishment of universal criteria to assess larval quality is a major concern at both research and production levels **(Racotta *et al*., 2003)**. Egg and naupliar quality depends principally on the condition of broodstock, but also on environmental conditions prevailing in spawning and hatching tanks **(Smith & Ritar, 2008).** Larval and postlarval quality is based on criteria that include development from zoea to postlarval stages and depend principally on larval culture conditions, although maternal effects may still have some influence **(Muller-Feuga *et al*., 2003).**

The major objective of this article is to evaluate the effects of six different salinity levels (20, 25, 28, 30, 35 and 38 ppt) on egg hatching percentage and survival rate of different larval stages of the Kuruma shrimp, *Penaeus japonicus.*

MATERIALS AND METHODS

1. Experimental facilities:

Experimental glass vessels (2 L) supplied with a continuous aeration were used in the present experiment. Vessels were filled up with experimental water. There were six tested water salinity levels in triplicate included 20, 25, 28, 30, 35 and 38 ‰ by adding filtered marine water (38 ‰) and freshwater (0.5 ‰). All experimental vessels were aerated using air blower to maintain DO levels in the suitable range using PVC pipes and tube.

2. Broodstock and experimental shrimp stages:

Matured Kuruma shrimp broodstock captured in Abo-Kir port, Alexandria, Egypt. Broodstock were transported in polythene bags under aerated conditions and spawned in Mariculture Research Center (MRC), Faculty of Environmental Agricultural Sciences, Arish University, Arish, North Sinai, Egypt.

Different shrimp stages included eggs, nauplius, zoea, mysis, and post larvae were stocked at experimental vessels in triplicates for each treatment at a density of 50 stages per replicate (150 stages / treatment). The different stages were evaluated under six different water salinity levels included 20, 25, 28, 30, 35 and 38 ‰ during 23 days.

3. Feeding and sampling collection:

Kuruma shrimp stages were fed on live food program during the experimental period with micro-algae (Diatoms - Tetraselms sp. with concentration (1000 cells ml^{-1}), freshly hatched rotifers and Artemia with concentration (animal ml^{-1}), by using a haemocytometer for counting (**Table, 1**). Eggs hatching percentage, survival rate of development stages (nauplius, zoea, mysis, and post larvae) were estimated for each experimental treatment and used as indicator to the suitable salinity levels.

4. Water quality parameters:

During the experimental period (23 days) dissolved oxygen and temperature were recorded twice a day (06:00 am and 16:00 pm) by using Oxygen meter (WTW Model, 315 i, Germany). pH was measured once a day (16:00 pm) by using pH meter (WTW Model 315i, Germany), Total ammonia nitrogen (TAN) was recorded using Photometer PF-11. Unionized ammonia (NH_3) was measured according to **(Van Wyk *et al*., 1999)**, turbidity was measured by using turbidity meter, Cole Parmer, Model 8391 - 45, USA, (**Table 2**).

Table (1): Live food program of Kuruma shrimp *P. japonicas* reared in different water salinity levels.

Day	Stage	Food type			
		Algae (1000 cells /ml)		Rotifer	Artemia
		Diatoms	*Tetraselms sp*	(animal/ml)	(animal/ml)
1	N1	-	-	-	-
2	N6	-	-	-	-
3	Z1	30	-	-	-
4	Z2	50	5	-	-
5	Z3	80	10	5	0.5
6	Z3 - M1	90	10	5	0.5
7	M1	100	10	10	0.5
8	M2	100	5	5	1
9	M3	100	2	-	1
10	PL1	50	2	-	2
11	PL2	30	1	-	2
12	PL3	20	-	-	3
13	PL4	15	-	-	3
14	PL5	15	-	-	4
15	PL6	10	-	-	4

5. Measurement of hatching percentage and survival rate

Eggs hatching percentage and survival rate of each shrimp stages (nauplius, zoea, mysis, and post larvae stages) were calculated according to the following equations:

Egg hatching (%) = (Number of hatching eggs /Total eggs number) x100

Survival rate (%) = (Number at final / Number at start) x100

Table (2): Average of water quality parameters of the tested eggs and different larval kuruma shrimp *Penaeus japonicas* reared in different water salinity levels.

Item	**Water salinity levels (‰)**						**SE**
	38	**35**	**30**	**28**	**25**	**20**	
Temperature (^{o}C)	25.0	24.8	25.3	24.0	25.0	25.0	0.26
pH	8.17	8.10	8.17	8.10	8.07	7.93	0.03
Turbidity (NTU)	11.23	11.57	11.33	11.27	11.27	11.23	0.06
TAN (mg/l)	0.01	0.01	0.01	0.01	0.01	0.01	0.00
NH3 (mg/l)	0.001	0.001	0.001	0.001	0.001	0.005	0.00
D O (mg/l)	5.50	5.40	5.30	5.43	5.53	5.55	0.04

* Values in the same row having a common superscript letter are not significantly different ($P > 0.05$)

6. Statistical analysis:

Statistical analysis was carried out using a completely randomized design (CRD) according to **(Snedecor & Cochran, 1982)**. All analyses were performed using the SPSS program (SPSS 16.0, 2007). Differences were subjected to Duncan's multiple range test **(Duncan, 1955)** at 0.05 significance level.

RESULTS

The averages of the water quality parameters during the experimental period are presented in **(Table, 2).** No significantly differences ($P > 0.05$) were observed between all tested water quality parameters during the experimental period included temperature, salinity, pH, Turbidity, TAN, NH_3, and DO. All water quality parameters were in the acceptable range to all larval stages of the kuruma shrimp *Penaeus japonicus*.

Data of the effect of different salinity levels on the eggs hatching (%) and survival rate of the eggs hatched of kuruma shrimp, *Penaeus japonicus* reared in different tested salinity levels are presented in **(Figs. 1 & 2)**.

Results showed that all nauplius stages reared in 20 ‰ salinity level gave the lowest survival rate in compared to those reared in 38 ‰ and the other tested salinity levels without significantly differences ($P > 0.05$). Increasing the water salinity levels in the culture water over 20 ‰ resulted in an improvement in survival rate in all tested larval stages. On 38 ‰ salinity level gave the best survival rate between all tested salinity levels in the nauplius stages (N_1- N_6) was shown in **Table (3).**

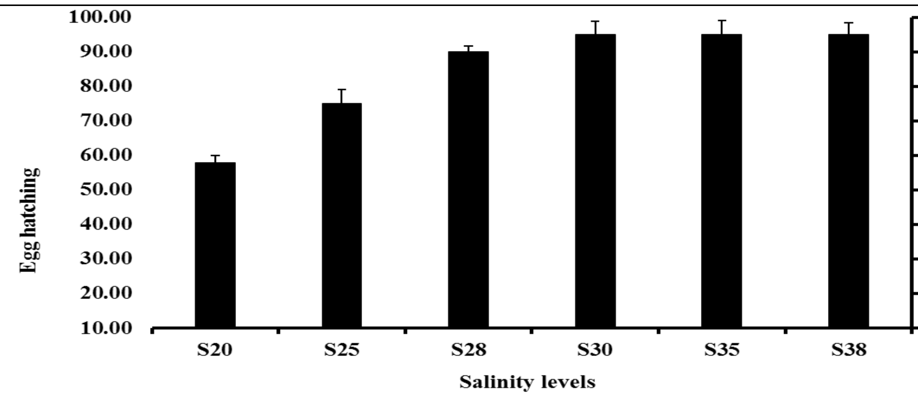

Fig. (1). Effect of different salinity levels on eggs hatching percentage of the kuruma shrimp *Penaeus japonicas*

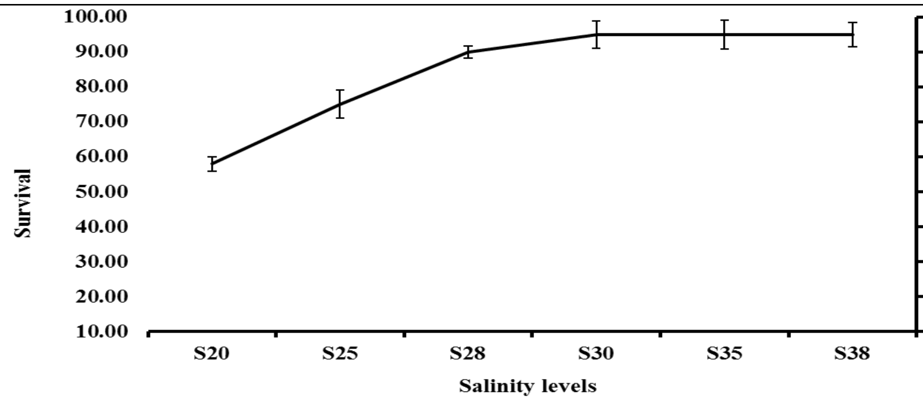

Fig. (2). Effect of different salinity levels on survival of eggs hatched of the kuruma shrimp *Penaeus japonicas*

Table (3): Effect of different salinity levels on survival rate of nauplius stages of the kuruma shrimp, *Penaeus japonicas*.

nauplius stage 1 (N1)						
Item	Salinity levels (‰)					
	38	35	30	28	25	20
Initial number	50.0	50.0	50.0	50.0	50.0	50.0
Final number	46.5	47.5	45.0	45.0	42.5	21.5
Survival (%)	95.0^{a}	93.0^{ab}	90.0^{b}	90.0^{b}	85.0^{c}	43.0^{d}
nauplius stage 2 (N2)						
Item	Salinity levels (‰)					
	38	35	30	28	25	20
Initial number	50.0	50.0	50.0	50.0	50.0	50.0
Final number	46.0	47.5	45.5	45.0	41.5	22.5
Survival (%)	92.0^{a}	93.0^{a}	91.0^{a}	90.0^{a}	83.0^{b}	45.0^{c}
nauplius stage 3 (N3)						
Item	Salinity levels (‰)					
	38	35	30	28	25	20
Initial number	50.0	50.0	50.0	50.0	50.0	50.0
Final number	47.0	46.0	41.0	41.0	31.0	26.0
Survival (%)	94.0^{a}	92.0^{a}	82.0^{b}	82.0^{b}	62.0^{c}	52.0^{d}

Nauplius stage 4 (N4)						
Item	**Salinity levels (‰)**					
	38	**35**	**30**	**28**	**25**	**20**
Initial number	50.0	50.0	50.0	50.0	50.0	50.0
Final number	45.0	40.0	39.5	29.0	27.0	15.5
Survival (%)	90.0[a]	80.0[a]	79.0[a]	58.0[b]	54.0[b]	31.0[c]

nauplius stage 5 (N5)						
Item	**Salinity levels (‰)**					
	38	**35**	**30**	**28**	**25**	**20**
Initial number	50.0	50.0	50.0	50.0	50.0	50.0
Final number	45.0	42.5	35.0	30.0	28.5	13.0
Survival (%)	90.0[a]	85.0[a]	70.0[b]	60.0[c]	57.0[c]	26.0[d]
nauplius stage 6 (N6)						
Item	**Salinity levels (‰)**					
	38	**35**	**30**	**28**	**25**	**20**
Initial number	50.0	50.0	50.0	50.0	50.0	50.0
Final number	46.0	39.5	39.0	32.0	31.0	26.5
Survival (%)	92.0[a]	79.0 [b]	78.0[b]	64.0[c]	62.0[c]	53.0[d]

* Values in the same row having a common superscript letter are not significantly different (P > 0.05)

The same trend was observed in the tested, Zoea stages (Z1- Z3) were shown in **(Figs. 3, 4 & 5)** with significantly differences (P > 0.05). Also there were significantly differences (P > 0.05) in survival rate between all tested salinity levels reared Mysis stages (M1- M3), the best survival rate was in 38 ‰ was shown in **(Fig. 6)**.

Data of the Post larvae experiment are presented in (**Fig. 7).** Data showed that the highest tested salinity level (38 ‰) gave the higher survival rate to post larvae of the kuruma shrimp *Penaeus japonicus* included 94%, 85%, and 80%, for PL1, PL6, and PL12, respectively. The lowest tested salinity level (20 ‰) produced high mortalities throughout the experiment reached 23%, 25%, and 31% for PL1, PL2, and PL3, respectively.

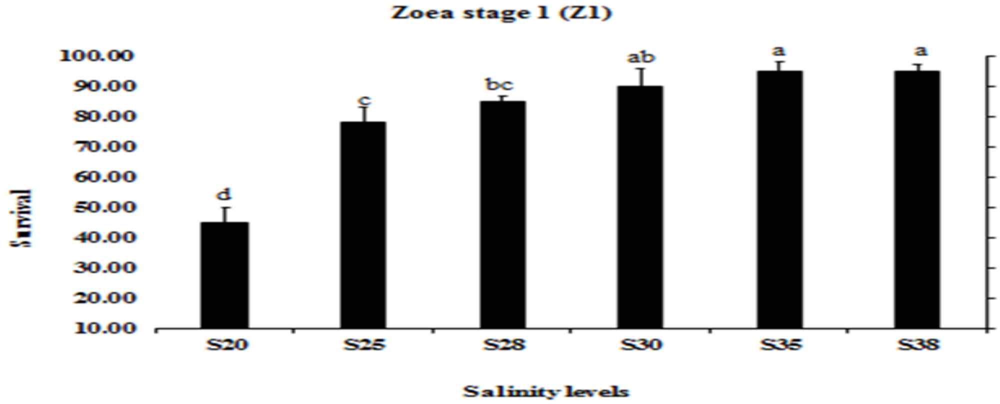

Fig. (3). Effect of different salinity levels on survival % of zoea stage1 of the kuruma shrimp, *Penaeus japonicas.*

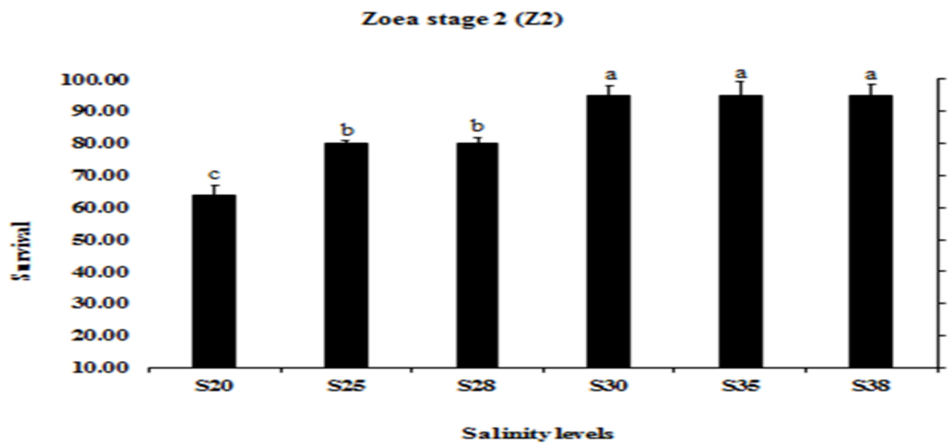

Fig. (4). Effect of different salinity levels on survival % of zoea stage2 of the kuruma shrimp, *Penaeus japonicas*

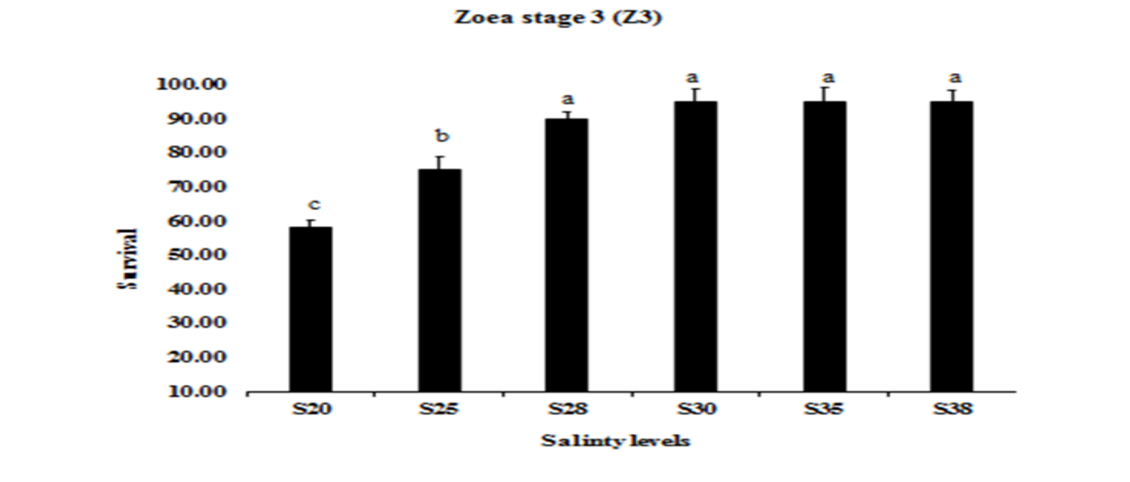

Fig. (5). Effect of different salinity levels on survival % of zoea stage 3 of the kuruma shrimp, *Penaeus japonicas*

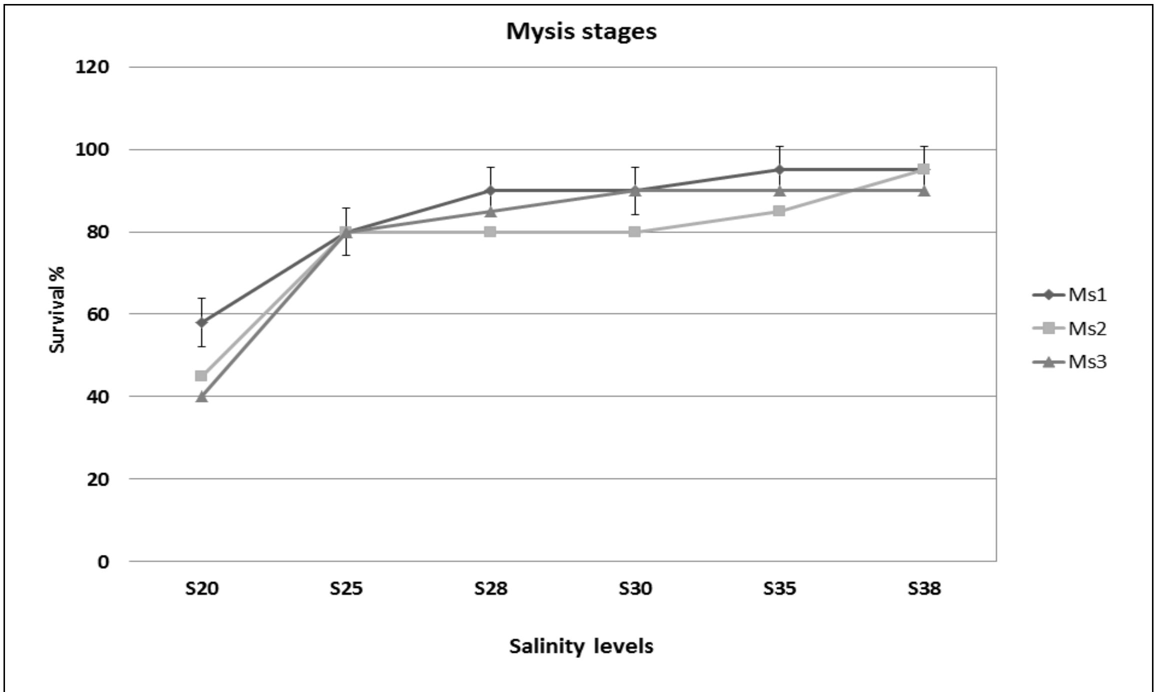

Fig. (6). Effect of different salinity levels on survival % of mysis stages of the kuruma shrimp, *Penaeus japonicas*

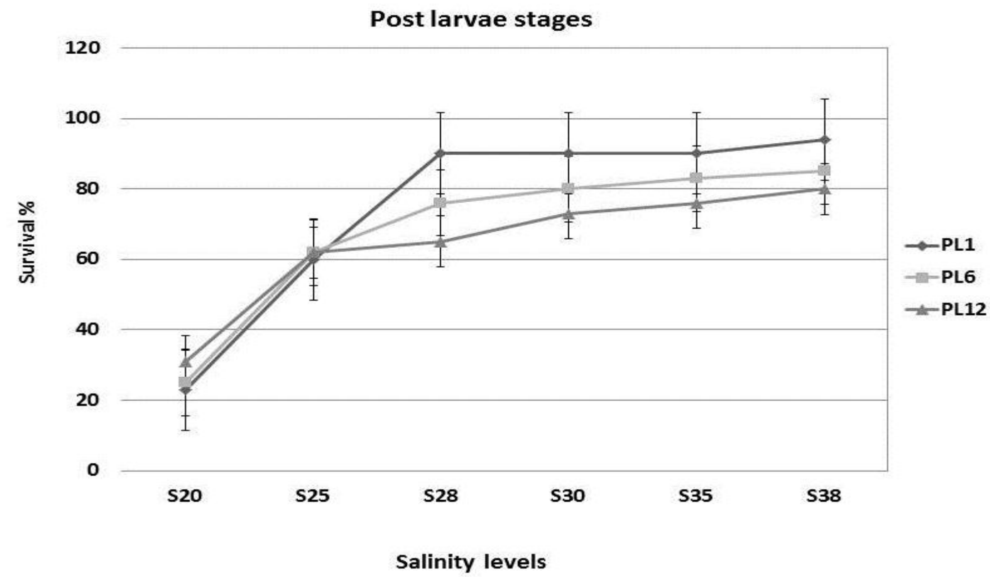

Fig. (7). Effect of different salinity levels on survival % of post larvae stages of the kuruma shrimp, *Penaeus japonicas*

DISCUSSION

The aim of the present study was to determine the optimal culture salinity level that sustains the greatest on egg hatching percentage and survival rate of different larval stages of the Kuruma shrimp, *Penaeus japonicas* **(Romano & Zeng, 2006).**

A considerable number of studies have been conducted to determine the effects of salinity during the nursery culture of various commercially important penaeid species, such as *P. monodon* **(Kumlu *et al*., 2001)**, *P. aztecus* **(Kumlu *et al*., 2001)**, *P. setiferus* **(Soyel & Kumlu, 2003)**, *P. vannamei*, *P. stylirostris*, *P. californiensis*, and *P. brevirostris* **(Mair, 1981)**, *P. merguiensis*, *P. esculentus*, *Metapenaeus bennettae* **(Rothlisberg & Jackson, 1987)**, P. japonicus and P. chinensis **(Ochwada-Doyle *et al*., 2011)** and *P.* indicus **(Ravi *et al*., 2009)**.

Studies with larval stages of several shrimp species have revealed that optimal culture salinity is species-specific **(Zacharia & Kakati, 2004)**, therefore, it is important to determine the optimum salinity level for each commercial shrimp species in intensive nursery systems in which the salinity can be altered according to the optimum requirements of a particular species **(Silva, 2014)**.

Kuruma shrimp, *Penaeus japonicus* is one of the important commercial species in different parts of the world. The optimal larval culture salinity has been reported to lie between 30 and 35 ‰ for this species **(Kumlu & Eroldoğan, 2000)**, while **Zacharia &**

Kakati (2004) found that 32 ‰ found to be optimal for the same species during the larval stages. In addition, **Harpaz & Karplus (1991)** reported that 36 ‰ for survival were the best salinity levels during the nursery culture of this shrimp species.

Post larvae of the kuruma shrimp, *Penaeus japonicus* gave low survival rate (23-31 %) at low salinity levels (20 ‰). However, mortality rate decreased with increasing the salinity levels (30-38 ‰), confirming that low salinities were stressful for the PLs. One may argue that the PLs might have needed a slower acclimation rate than that applied in the experiment. However, high mortalities observed at all the experiments at low salinities at different larval stages indicated that some other reasons might have contributed to the high mortality in this study.

Considerable losses may occur in penaeid shrimps during early nursery culture when the PLs are forced to shift from a natural diet to an artificial diet **(Kumlu *et al*., 2001).** In addition, most penaeid shrimps are known to be euryhaline species growing in a wide range of salinities, at least during their nursery stages. In the current work, egg hatching percentage and all larval stages had consistently better survival rate between 35 and 38 ‰ than at low (20-25 ‰) salinities, indicating that their optimal culture salinity lies above 35 ‰ **(Zacharia & Kakati, 2004)**.

Salinity optima for the larval culture of the same species inhabiting the north-eastern Mediterranean were also reported to be between 30 and 35 ‰ **(Kumlu & Eroldoğan, 2000)**. The lowest and highest critical salinity levels were 23 and 55 ‰, respectively, at an acclimation rate of 4-5 ‰ h^{-1} and that the preference was towards high rather than low salinities during the larval culture.

In a study by **(Harpaz & Karplus, 1991)**, 40-day-old PLs of the same species had highest survival rate at 36 ‰ and slow growth and low survival at 9 ppt. Hence, it appears that kuruma shrimp is not a good candidate for culture in waters of low salinity. **Kumlu & Jones (1995)** found poor growth at 5 ‰ or 45 ‰, and obtained 100% mortality at 55 ‰, whereas **(Harpaz & Karplus, 1991)** had 100% survival even at 38 ppt. Based on these results, it appears that kuruma shrimp prefer higher saline media even during their nursery stages whilst populations of the kuruma shrimp regions can grow and survive at lower salinity levels. This might be due to inherent differences in the salinity tolerance between these shrimp populations, as also suggested by **(Harpaz & Karplus, 1991). Kumlu & Jones (1995)** have already demonstrated different salinity tolerances of *P. indicus* shrimp populations inhabiting various geographical regions of the world. In view of these results, low salinity might have negative effects on shrimp. as also suggested by **Racotta *et al*. (2003)**, It is well know that response to these environmental parameters is species-specific and salinity effect on growth and survival in penaeid shrimps.

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