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Growth, survival and byssal attachment of the blacklip pearl oyster *Pinctada margaritifera* (Linnaeus 1758) spat exposed to different salinities

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ABSTRACT

The influence of salinity on growth, survival and byssal fibre attachment of the blacklip pearl oyster, *Pinctada margaritifera* spat was assessed. *P. margaritifera* enjoys a coastal distribution in the intertidal reef flats of Andaman and Nicobar Islands, with maximum density at 5 -10 m depth, where drastic changes in environmental parameters such as salinity, temperature and turbidity occur. Spat showed comparatively good growth in terms of instantaneous growth rates (IGRs) of dorso-ventral measurement (DVM), antero-posterior measurement (APM) and total weight (TWT) in a wide range of test salinities from 19 to 37 psu; IGRs were drastically decreased in spat reared at lower and higher salinities outside of this range. The highlight of the present study was better growth performance and 100% survival of *P. margaritifera* spat at lower salinity levels (22-28 psu) than in the ambient salinity (31 psu). The study also showed that sudden exposure to a higher salinity (above 40 psu) will be helpful for detachment of *P. margaritifera* spat from settlement tanks in the hatchery to facilitate stress-free spat transfer to sea farming systems. This will be useful in adopting the Japanese technique on post-operative culture for producing high quality pearls from *P. margaritifera*.

Keywords: Blacklip pearl oyster, Byssal attachment, Growth, *Pinctada margaritifera*, Salinity tolerance, Spat, Survival

Introduction

Salinity is one of the most important environmental factors affecting growth, survival, development and distribution of aquatic organisms. The growth rates of bivalves are primarily regulated by food supply with temperature being a secondary factor (Brown, 1988). Growth is also highly site-specific depending on food supply and salinity conditions. Lower salinities generally have a depressing effect on filtration rate of marine bivalves (Cole and Hepper, 1954). Alagarswami and Victor (1976) reported that the rate of filtration in Indian pearl oyster *Pinctada fucata* was generally higher at high salinities in laboratory experiments. In their experiment, mortality was total in 13.90 and 56.96 psu after 48 h and they observed 60% mortality at 19.90 psu. Jeyabaskaran *et al*. (1983) reported reduced growth of *P. fucata* at higher salinities within the range of 29-34 psu in pearl farms in the Gulf of Mannar region. Similarly, Dholakia *et al*. (1997), studying the limits of salinity tolerance and tolerance to abrupt salinity changes in Indian pearl oyster from the Gulf of Kutch, observed an increase in daily mortality rate at extreme salinities. However, Al Sayed *et al*. (1997) reported the occurrence of Akoya pearl oyster, *Pinctada radiata* in hypersaline (60 psu) areas of the Arabian Gulf.

Salinity greatly impacts survival, growth rate, clearance rate and byssal attachment in marine sedentary bivalves (Numaguchi and Tanaka, 1986; Kimani and Mavuti, 2002; Laing, 2002; Rupp and Parsons, 2004). The silver-lip pearl oyster, *Pinctada maxima* spat could reattach with 100% survival after a 24 h post-detachment exposure in hypersaline (40-45 psu) or sub-ambient salinities (25-30 psu) (Taylor *et al*., 1997). Recently, Welladsen *et al*. (2011) showed that the byssal threads produced by oysters (*P. fucata*) in pH 7.6 treatments were significantly thinner than those produced by oysters in the control (pH 8.1-8.2) group, indicating that environmental factors play a key role in the physiology of marine bivalves. In Japan, some pearl oyster farms are located in areas which are subject to the influence of freshwater discharge from rivers and this reportedly improves pearl quality (Gervis and Sims, 1992). This was again confirmed by Atsumi *et al*. (2011) in *P. fucata* where production of high quality pearls was better at low salinity (25 psu) than at normal salinity (33 psu) after pearl oyster surgery.

There is not much information available on the influence of salinity on growth rate and byssal fibres secretion of spat and adults of *Pinctada margaritifera* (Linnaeus 1758). Therefore, the present experiments were designed to find out the salinity tolerance of spat and the influence of different salinity gradients on the growth rate and byssal fibre secretion of spat in laboratory conditions. This can help in hatchery management of spat stock and also help in decisions on farm growout of spat. The study also focused on the possibility of detachment of *P. margaritifera* spat from settlement tanks through salinity alteration to facilitate spat transfer to grow-out farms. Physical detachment of spat from hatchery tanks results in heightened stress to the spat in the new location, leading to additional mortality.

Materials and methods

Hatchery produced *P. margaritifera* spat (45 days old) measuring 3.5 mm DVM (dorso-ventral measurement) were selected and reared at different salinities *viz*., 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43 and 46 psu. The experiment was carried out in 1 l acrylic containers for a period of 30 days with five replicates. In each container, 5 spats were stocked in 500 ml filtered seawater at different salinities as mentioned above and gentle aeration was provided. Spat maintained at ambient salinity (31 psu) was considered as control group. Higher salinities were manipulated by adding natural sea salt to seawater and lower salinities made up by adding freshwater to normal seawater. Salinity values were checked with a 0.1 psu precision refractometer. Complete water exchange was done daily using filtered seawater of the same salinity. Parameters such as room temperature, water temperature, and pH were recorded daily. Temperature was measured using a thermometer (0.1^{o}C accuracy) and other water quality parameters were measured using PioNneer 65 portable multiparameter instrument. Initially, spat were measured for APM (antero-posterior measurement) and DVM individually using a Leica L2 microscope and total wet weight (TWT) of all spat in each container was taken as a whole using a Shimadzu digital balance with an accuracy of 0.1mg. At five days intervals, spat measurements were taken during the experimental period of 30 days. Mortality and byssal fibres attachment were recorded every day during water exchange. Spat were transferred abruptly from seawater into the test salinities and degree of initial stress was shown by rate of byssal reattachment. Spat were detached after every 5 days of culture (a cluster) for length and weight measurements during the experimental period (30 days = 6 clusters). Thereafter they were checked for reattachment at equal intervals of time, *i.e.*, 24 h, 48 h, 72 h, 96 h and 120 h for each cluster.

Microalgae *Pavlova salina* (Pav), *Isochrysis galbana* (Iso) and *Chaetoceros calcitrans* (Cha) were used as live feed for spat during the experimental days. Axenic cultures of these microalgae were maintained indoor using Walne's medium with a photoperiod of 12: 12 (light /dark) in ambient conditions. Cultures were harvested during exponential growth phase for feeding. The feeding plan adopted was with these three microalgae (1 Pav:1 Iso:1 Cha) in equal proportion. Initial ration was 50,000 cells ml^{-1} day^{-1} and it was increased at the rate of 5000 cells every third day. The mean APM, DVM and TWT were used for estimating instantaneous growth rate (IGR) based on the formula of Hopkins (1992):

$$\text{IGR} = \frac{Ln_t - Ln_i}{t}$$

where, Ln_t = natural log of the length at time t and Ln_i = natural log of the initial length. IGR values of DVM, APM and TWT were used to assess the variations in growth rate of spat reared in different tested salinities. The variations in mean values of DVM, APM, TWT and survival in each treatment were compared using two-way ANOVA.

Results and discussion

The mean values of spat dimensions and corresponding wet weight recorded in different treatments is shown in Fig. 1. Growth was observed in *P. margaritifera* spat reared at all salinities except those reared at higher (46 psu) and lower (13 psu) salinities. The rate of growth differed in each salinity treatment. Spat showed comparatively good growth in test salinities ranging from 19 to 37 psu with two peaks. Spat reared above and below this range of salinity showed either slow growth rates or zero growth during the experiment. The IGR values of DVM, APM and TWT clearly indicated (Fig. 2) comparatively high growth rates in spat reared in these salinities.

Results of the 2-way ANOVA carried out to understand the variations in DVM, APM, TWT and survival rate of *P. margaritifera* in various days of culture (DOC) clusters (1 - 6 clusters) and also in different rearing salinities are shown in Table 1.

Spat showed highly significant variations in all the variables in different salinities ($p<0.001$). In the case of increase in DOC, only spat TWT and survival rate showed significant differences ($p<0.001$) from 0 DOC to 30 DOC, whereas, DVM and APM did not show any significant difference ($p>0.05$) with increase in DOC. Poor growth performance and high mortality rates were noticed in *P. margaritifera* spat reared in higher (46 psu) and lower (13 psu) salinity. The reduction in growth could be due to the relationship between clearance rate (CR) or pumping rate and salinity gradients. In *P. margaritifera,* the clearance rates show progressive increase with increase in salinity up to the ambient salinity and then decreases with further increase in salinity (Libini *et al*., 2017).

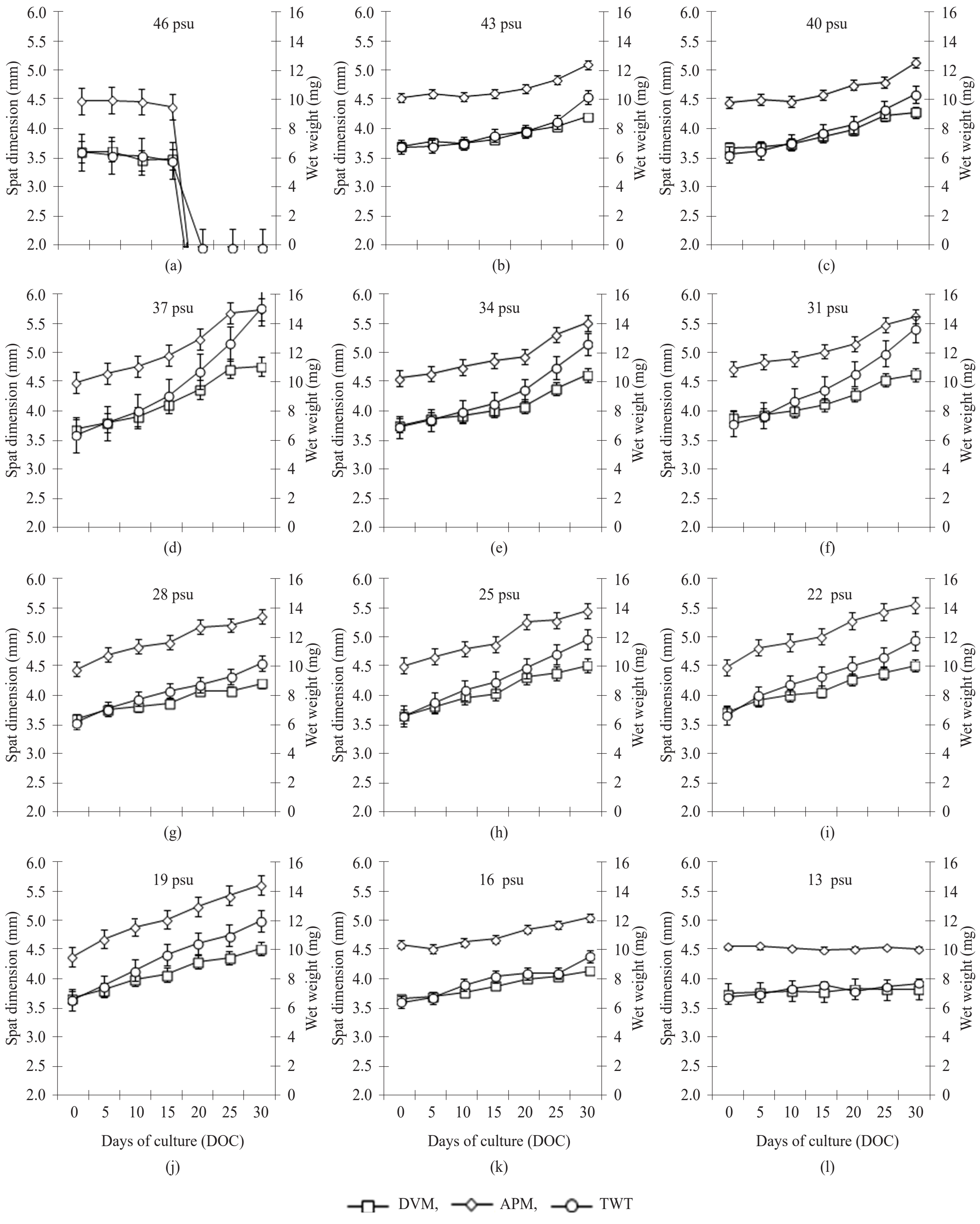

Fig. 1. Mean values of dimensions and wet weight of *P. margaritifera* spat reared in various salinities. Vertical lines indicate standard deviation from mean

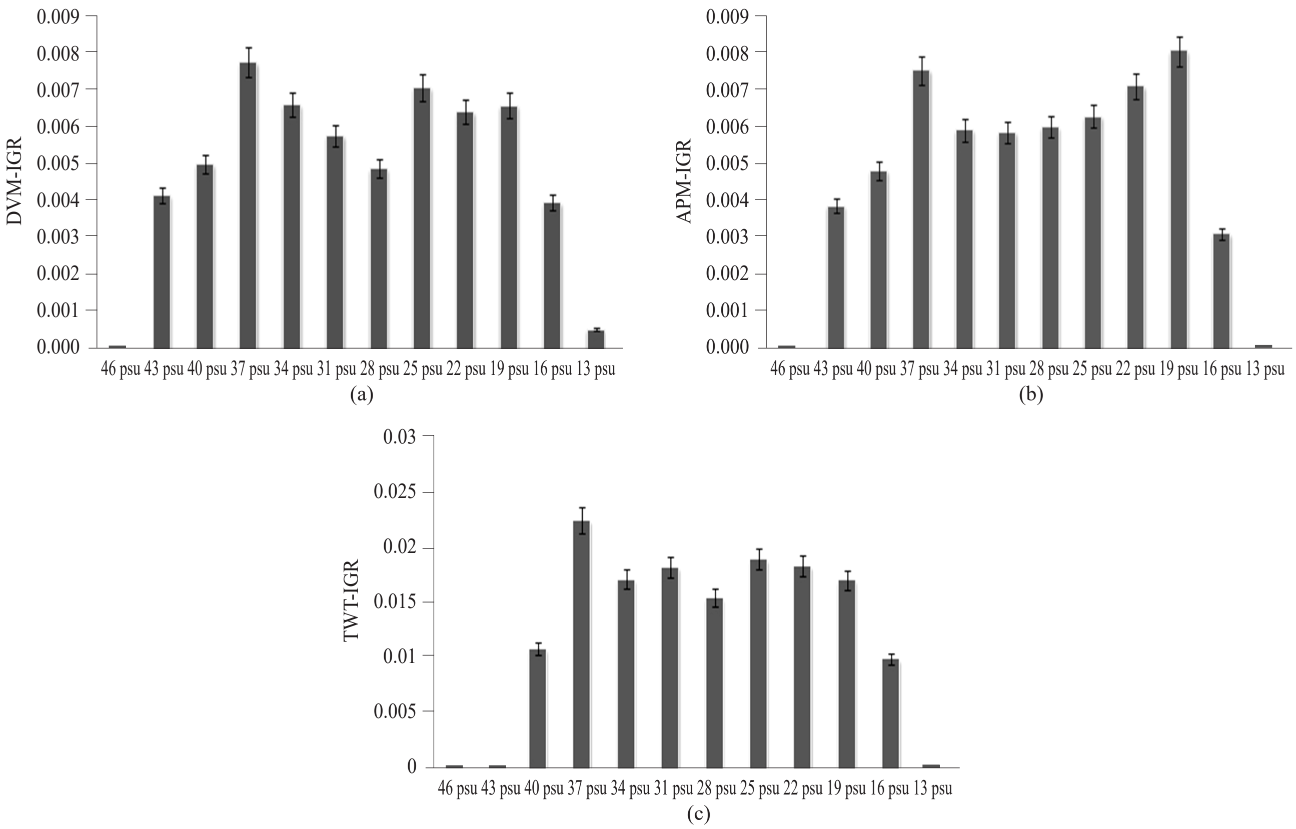

Fig. 2. Mean instantaneous growth rates (IGRs) in DVM, APM and TWT of *P. margaritifera* spat reared in different salinities. Vertical lines indicate standard deviation from mean

Table 1. Variations of DVM, APM, TWT and survival rate of *P. margaritifera* spat reared in different salinities and days of culture

Variable	Source of Variation	SS	df	MS	F	Significance level
DVM	DOC clusters	0.816	6	0.136	0.346	p>0.05
	Salinities	26.162	11	2.378	6.053	p < 0.001
APM	DOC clusters	1.090	6	0.182	0.298	p>0.05
	Salinities	37.156	11	3.378	5.537	p < 0.001
TWT	DOC clusters	118.605	6	19.768	7.836	p < 0.001
	Salinities	222.603	11	20.237	8.022	p < 0.001
Survival rate	DOC clusters	11.076	6	1.846	3.538	P < 0.001
	Salinities	48.904	11	4.446	8.522	P < 0.001

In Akoya oysters, Alagarswami and Victor (1976) noticed an increasing CR with increasing salinity with a peak at 34 psu and reducing thereafter with increasing salinity. Oysters are osmoconformers (Dharmaraj *et al*., 1987) and can tolerate salinity variations within certain limits through 'buffering'. This helps them adapt to wide salinity variations as observed in the present study too. In extreme salinities (<19 psu and >37 psu) there is decline in dissolved oxygen levels in the mantle cavity water, hindering the buffering efficiency by physiologically forcing them to gape, which subsequently leads to mortality. Taylor *et al*. (2004) also noticed that *P. maxima* juveniles can tolerate wide range of salinities (25-45 psu) during an exposure of 20 days without adversely affecting the survival rate.

P. margaritifera usually retains its byssus throughout its life. Salinity plays a key role in byssal fibre formation and attachment to substrata by pearl oysters. However, they retain the ability to move away from unfavourable conditions by severing the byssus and crawling to a more favourable site for reattachment (Taylor *et al*., 1997). In the present study, when the spat were transferred abruptly from ambient salinity to the test salinities, the degree of initial stress was indicated by the time period required for byssal fibre reattachment. The observations revealed that faster (24 h) as well as firm byssal attachments were achieved in salinities from 25 to 34 psu in all the six clusters (Table 2). Spat which were reared in salinities 40, 37, 22 and 19 psu formed only weak and partial attachments in initial clusters. They also took a longer period of time for the byssal fibre formation when compared with the optimum salinity range (25-34 psu). Numaguchi and Tanaka (1986) also witnessed similar fast reattachment in Akoya oyster spat, at 26.5-38 psu. However, in scallop, Rupp and Parsons (2004) observed maximum byssal fibre attachment at salinity above 29 psu. In the present study, at higher salinities (46 and 43 psu) and lower salinities (16 and 13 psu) spat did not show any sign of byssal fibres attachment during the entire experiment. In addition, no mortality was observed up to 10 DOC in all the tested salinities.

The percentage survival of *P. margaritifera* spat reared in different salinities is shown in Fig. 3. All the spat showed good survival rate (above 90%) except the spat reared at salinity 46, 43 and 13 psu. Spat showed very high survival rate upto 10 DOC in all the salinities, after which a decreasing trend in survival was observed in 40, 43, 46, 13, 16 and 19 psu. The percentage survival in 43 psu salinity and 13 psu salinity was 52% and 64% respectively at the end of the experiment.

The highlight of the present study was better growth performance and 100% survival of *P. margaritifera* spat in lower salinity levels (22-28 psu) than the ambient (31 psu). In the case of larval development of these oysters, Doroudi *et al*. (1999) reported high survival rate in salinity range between 25-32 psu. In Kenya, Kimani and Mavuti (2002) also recorded the occurrence of *P. margaritifera*

Table 2. Observations on time taken for byssal fibre attachment of *P. margaritifera* spat reared in different salinities

Salinity (psu)	pH	Mortality (%)	Period taken for byssal fibre attachment and no. of clusters					
			24 h	48 h	72 h	96 h	120 h	Remarks
46	8.0	96	-	-	-	-	-	No attachment
43	8.1	48	-	-	-	-	-	No attachment
40	8.1	12	-	-	-	4	4	Partially attached
37	8.0	8	-	-	5	6	6	Partially attached
34	8.0	4	6	6	6	6	6	Firmly attached
31	8.1	4	6	6	6	6	6	Firmly attached
28	7.9	0	6	6	6	6	6	Firmly attached
25	8.1	0	6	6	6	6	6	Firmly attached
22	7.8	0	-	5	5	6	6	Partially attached
19	7.6	4	-	-	-	5	5	Partially attached
16	7.6	8	-	-	-	-	-	No attachment
13	7.5	36	-	-	-	-	-	No attachment

Cluster 1 (0-5), 2 (5-10), 3 (10-15), 4 (15-20), 5 (20-25), 6 (25-30 days)

Therefore, sudden exposure to a higher salinity (above 40 psu) will be helpful for detachment of *P. margaritifera* spat from settlement tanks in the hatchery to facilitate stress-free spat transfer to sea farming system. This can prevent stress to the spat due to physical removal from its attachment in the hatchery tank, which ultimately leads to high mortality in grow-out systems. The pH also showed a decreasing trend from higher salinity (46 psu) to lower salinity (13 psu). No mortality was observed in spat that were reared in the salinity range between 22 and 28 psu. The mortality percentage of spat was extremely high (96%) in higher salinity (46 psu) than that in lowest salinity (13 psu). Welladsen *et al*. (2011) reported that pH of seawater adversely affects the quality of the byssus thread formation in *P. fucata*. They have experienced a significantly thinner byssus production in 7.6 pH treatment. However, pH manipulation is difficult in hatchery tanks, whereas, salinity manipulation is easier.

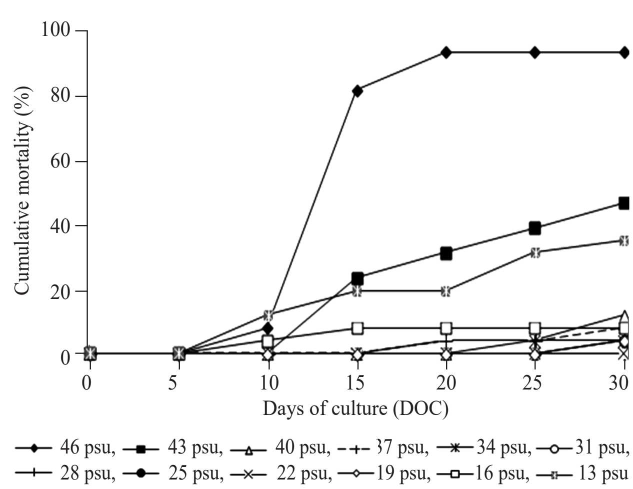

Fig. 3. Percentage survival of *P. margaritifera* spat reared in different salinities

oysters in abundance in Gazy Bay in salinity range of 26-37 psu in all the seasons. Similarly, in scallops *Pecten maximus* and *Nodipecten nodosus,* high survival, growth and clearance rate were recorded in salinity ranging between 28 and 30 psu (Laing, 2002; Rupp and Parsons, 2004). In *P. maxima*, juveniles can tolerate a wide range of salinity (25-45 psu) (Taylor *et al*., 2004). However, an improved growth rate was noticed at a reduced salinity (30 psu) than the normal salinity (34 psu). Jeyabaskaran *et al*. (1983) also observed improved growth of *P. fucata,* in lower salinities in the range 29-34 psu in farms in the Gulf of Mannar. Numaguchi and Tanaka (1986) suggested that the optimum salinity for *P. fucata* spat is about 26.5 psu and that they should be cultured at salinities greater than 23 psu.

The capacity of an organism to survive in its environment is restricted by its tolerance limits towards various abiotic factors including salinity. This study has mainly focused on the influence of salinity on the growth, survival and byssal attachment of *P. margaritifera* spat. *P. margaritifera* is typically associated with coral reefs and reef lagoons and occurs in less turbid waters (Yukihira *et al*., 1998). This study revealed that *P. margaritifera* in the Andaman waters can tolerate a wide range of salinity. In nature they are distributed in shallow sub-tidal areas (2-5 m) where rain water inflow is frequent (Alagarswami, 1983).

Akoya pearl industries in Japan prefer to culture oysters in areas with freshwater influx as it helps to produce good nacre quality (Gervis and Sims, 1992). Through a practical experiment with ten pearl farmers, Atsumi *et al*. (2011) found that low salinity (25 psu) treatment of oysters during the post-operative period could be an effective technique to increase the formation of pearls of high quality. This finding highlights the possibility of adopting the Japanese technique on post-operative culture for producing high quality pearls from *P. margaritifera*.

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