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Effects of culture salinity on growth and reproduction of the polychaete *Dendronereis chipolini*

Vu Ngoc Ut*, and Tran Trung Giang
College of Aquaculture and Fisheries, Can Tho University, Viet Nam
**Corresponding author (vnut@ctu.edu.vn)*

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ABSTRACT

Dendronereis chipolini is an important brackish water polychaete and has been wildly used as fattening feed for marine shrimp broodstocks. Investigation into the effects of salinity on performance of this worm species is fundamental for the development of mass culture procedure to produce feed for fattening shrimp broodstock and reduce pressure on wild catch. The study was conducted in a tank system with an area of 0.25 m^2 in which the young worms (0.9±0.2 cm in length and 0.007±0.003 g in body weight) were stocked at 100 inds/tank. Four salinity treatments, including 15, 20, 25, 30 ppt, were designed with three replicates each. The worms were fed once a day at 9 am with commercial shrimp feed at a rate of 3% body weight. After 150 days of rearing, the fastest growth of worms was recorded in 20 ppt. The survival rate was also higher in 20 ppt but no significant difference was found ($p>0.05$) between treatments. Higher absolute fecundity (103,890±17,389 and 112,740±22,328 eggs/female) was recorded at 20 and 25 ppt, respectively. The males in 20 ppt also produced a higher number of sperms. Overall, the polychaete D. chipolini reared at 20 ppt performed higher growth, survival and reproduction rates.

1. INTRODUCTION

Polychaete worms of the family Nereididae contain high levels of proteins and n-3 long-chain polyunsaturated fatty acids, which are essential ingredients in aquafeeds (Narciso & Fonseca, 2000; Brown & Eddy., 2011; Pajand et al., 2020). High levels of protein and fatty acids in the polychaetes are considered essential elements for the development of gonads in fish and shrimp broodstocks (Costa et al., 2006; Meunpol et al., 2005). Long-chain fatty acids such as prostaglandins, and bromophenols are found in the polychaetes. The bromophenol is importantly retained in fish and crustacean flesh and given the product the typical seafood flavor (Oddsen, 2014). Bischoff et al. (2009) stated that fishmeal can be partially replaced by polychaete meal and amino acid composition of new feed formulas can be increased by adding polychaete meals. In addition, Mandario (2018) reported that the endocrine-active compounds present in polychaete worms are responsible for the ovarian maturation of penaeid shrimp and enhance the quality and viability of offspring. Leelatanawit et al. (2014) demonstrated that male shrimps (*Penaeus monodon*) only fed with the polychaete *Perinereis nuntia* had higher survival, growth and sperm performance than those fed with commercially broodstock diets. *Dendronereis chipolini* is a new species of polychaetes found in brackish water aquaculture ponds near the estuary Kaoping, Kaohsiung, Taiwan, and in brackish water aquaculture ponds near Chigu lagoon (Hsueh, 2019). Recently, *D. chipolini* has been commonly found in the mangrove areas of the Mekong Delta, such as Ca

Mau and Tra Vinh province, Vietnam. They have been recently used as potential live feeds for shrimp broodstock fattening in the local shrimp hatcheries. However, the main source of these polychaetes used in the hatcheries is mainly the wild catch, which has been reduced because of overexploitation. Study on the effects of environmental factors on growth and reproduction performance of this polychaete species is an essential step toward the biomass culture procedure to provide food sources for shrimp broodstock and conserve the wild populations. Salinity was one of the first factors to test in the present study to find suitable ranges for growth and reproduction of the polychaete, *D. chipolini*.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Polychaete source

The worm broodstock was collected from an integrated mangrove - shrimp pond in Tan An commune, Ngoc Hien district, Ca Mau province (8°39'39.3"N 105°00'05.0"E). After being collected, they were placed in the 3 L aerated plastic bags and transported to the wet-lab of College of Aquaculture and Fisheries, Can Tho University, for fattening and breeding. The worms were stocked in the composite tanks for fattening with shrimp pellets (CP feed, size 0) until they reached maturity and spawned. After 45 days of spawning, the young worms reached a size of 0.9±0.2 cm, they were used for the experiment. The ones selected for the experiment were mostly uniform in size, natural in color, intact, and active.

2.1.2. Substrate, water source and monitoring

Substrate used for the worms in the experimental tanks was mud and collected from Bun Xang canal in Can Tho City (10°01'56.9"N 105°45'51.7"E). The mud was sieved through a net of 100 µm mesh size to remove all rubbish, coarse sediments and benthic organisms and soaked in 20 ppt seawater for a week.

Desired salinities were diluted from brine (80‰) with tap water. The seawater was treated with chlorine at a concentration of 20 ppm and neutralized with sodium thiosulfate before use.

Water parameters were measured once a week in the morning to guarantee good water quality for the worms and no difference between treatments. Monitoring was conducted by sampling and analyzing in the Water Quality Laboratory of the Faculty of Aquatic Biology and Environment Science, College of Aquaculture and Fisheries, Can Tho University. As the results, temperature, pH and DO remained around 27.8±0.4°C, 7.9±0.2 and 5.0±0.6 ppm, respectively. TAN and NO_2^- were also maintained at lower concentrations, with 0.061 ppm and 0.038 ppm, respectively.

2.2. Experimental designs

The experiment was designed in 12 plastic tanks with a bottom area of 0.25 m^2 covered by a layer of 5 cm mud and filled with a 30 cm water. Four treatments of salinity were set up including 15, 20, 25 and 30‰. The worm juveniles were stocked at 100 inds/tank. They were fed with commercial white leg shrimp feed (43% protein) at a rate of 3% of body weight. Aeration was maintained continuously throughout the culture period and water was renewed 30% every 3 days after siphoning. The duration of the experiment was 150 days.

2.3. Data collection and analysis

2.3.1. Growth performance

Every 30 days, the growth performance of polychaete worms in terms of length and weight was measured. Additionally, the maturity status was observed by randomly sampling 10 worms in each tank (30 inds/treatment). The formula below was used to calculate the growth rate.

Length Gain (LG, cm): $LG = L_t - L_i$

Daily Length Gain (DLG, cm day^{-1}): $DLG = (L_t - L_i)/(T_{day})$

Specific Growth Rate for Length, (SGR_L, % day^{-1}): $SRG_L = [(\ln L_t - \ln L_i)/(T_{day})]x100$

Weight Gain (WG, g): $WG = W_t - W_i$

Daily Weight Gain (DWG, g day^{-1}): $DWG = (W_t - W_i)/(T_{day})$

Specific Growth Rate for Weight (SGR_W, % day^{-1}): $SRG_L = [(\ln W_t - \ln W_i)/(T_{day})]x100$

Where: L_i/W_i is initial body length (cm)/initial weight (g); L_t/W_t is the final body length (cm)/Weight (g); T_{day} is experiment duration (days).

2.3.2. Survival rate

The worms were counted at day 60, 120 and 150 to determine the survival rates following the formula:

Survival Rate (SR, %): $SR = (N_t/N_i)x100$

Where: N_i is the initial number; N_t is the final number.

2.3.3. Reproduction performance

The reproductive characteristics of polychaetes that were determined included:

Maturation rate: determined by the numbers of worms that contained eggs or sperms in body cavity (mature gonad) observed under a binocular and calculated as:

Maturation rate = The number of worms with mature gonad/total worms observed

Absolute fecundity:

AF_M (male) = Weight of testis/males

AF_F (female) = Number of eggs/females.

Time of starting reproduction

The time when worms reproduced was observed and recorded during the experiment. When being mature and ready to reproduce, the worms often swim to the water surface to release eggs and sperm. In this experiment, it was recorded that on day 135, the worms began to come out of the mud, swim to the surface, and take part in spawning. A number of worms taking part in spawning was collected and recorded to count for the proportion of worms that took part in reproduction and also their fecundity from day 135 until day 150 when the experiment was terminated.

2.4. Data analysis

Data are shown as mean and standard deviation (SD). One-way ANOVA was used to identify differences among treatments, followed by a DUNCAN multiple-comparison test to examine significant differences among treatments. Mean values were considered significantly different at $p<0.05$. All analyses were performed using SAS computer software version 9.1 (SAS Institute, Cary, NC, USA).

3. RESULTS AND DISCUSSIONS

3.1. Growth performance

3.1.1. Growth in length

There was no statistically significant difference in length between treatments from stocking to 60 days ($p>0.05$). However, from day 90 to the end of the experiment, the body length was found significantly different between treatments ($p<0.05$). On day 90, the length of the worms was longest in 20 ppt with an average of 3.6±0.5 cm. In other treatments, the average length was 2.9-3.0 cm. Similarly, at the end of the experiment, the body length of worms was also significantly different between the treatments. Higher body length was obtained in 15 ppt (5.2±0.8 cm) and 20 ppt (5.1±0.7 cm) differed significantly from the 25 ppt (3.5±1.0 cm) and 30 ppt (3.3±0.8 cm) ($p<0,05$). These results showed that salinity affected the growth of the polychaete *D. chipolini*. The suitable salinity for length growth of this polychaete species is 15 to 20 ppt. When salinity is higher from 25 to 30 ppt, growth can be negatively affected (Table 1).

Table 1. The body length (cm) of *D. chipolini* in different salinity

Day	Treatments			
	15 ppt	20 ppt	25 ppt	30 ppt
1	0.9±0.2^{a}	0.9±0.2^{a}	0.9±0.2^{a}	0.9±0.2^{a}
30	1.7±0.4^{a}	1.6±0.4^{a}	1.6±0.5^{a}	1.6±0.4^{a}
60	2.7±0.5^{a}	2.5±0.3^{a}	2.4±0.4^{a}	2.4±0.3^{a}
90	3.0±0.5^{ab}	3.6±0.5^{b}	3.0±0.5^{ab}	2.9±0.4^{a}
120	4.4±1.1^{c}	3.9±1.1^{bc}	3.2±1.0^{ab}	2.6±0.9^{a}
150	5.2±0.8^{b}	5.1±0.7^{b}	3.5±1.0^{a}	3.3±0.8^{a}

Mean values followed by the same letter within each row are not significantly different ($p>0.05$)

Length gain (LG, cm), daily length gain (DLG, cm day^{-1}), and specific growth rate (SGRL, % day-1) of length were also high in 15 ppt, 20 ppt and significantly differed from those in 25 ppt and 30 ppt ($p<0.05$). After 150 days, the highest body length gain (4.3±0.0 cm) was recorded in 15 ppt treatment, and the lowest (2.3±0.4 cm) was observed in 30 ppt (Table 2).

Table 2. LG, DLG and SGR_L of *D. chipolini* in different salinity

Treatments	Body length of *D. chipolini* after 150 day		
	LG (cm)	**DLG** (cm day^{-1})	**SGR_L** (% day^{-1})
15 ppt	4.3±0.0[b]	0.029±0.000[b]	1.15±0.03[b]
20 ppt	4.1±1.0[b]	0.027±0.006[b]	1.12±0.12[b]
25 ppt	2.6±0.2[a]	0.017±0.001[a]	0.88±0.04[a]
30 ppt	2.3±0.4[a]	0.016±0.002[a]	0.83±0.07[a]

Mean values followed by the same letter within each column are not significantly different at p>0.05

3.1.2. Growth in weight

The weight of worms increased gradually during the experiment duration. In the first 60 days, there was no significant difference in weight between treatments. However, from day 90 to the end of the experiment, a significant difference in weight was observed ($p<0.05$). Similar to length, at day 90, the weight of the worm was higher in 15 and 20 ppt treatments, whereas lower weight was noticed in 25 and 30 ppt. At day 150, weights of the worms recorded in the 15, 20, 25, 30 ppt were 0.380±0.122, 0.415±0.072, 0.216±0.086, 0.167±0.075 g, and weight gains (WG) were 0.37±0.06, 0.41±0.11, 0.21±0.01, 0.16±0.02 g, respectively (Table 3).

Daily weight gain (DWG) of the worms after 150 days in 15, 20, 25, and 30 ppt were 2.5±0.4, 2.7±0.8, 1.4±0.1, 1.1±0.2 mg day^{-1}, respectively. DWG of the worms in 15 and 20 ppt were significantly higher than those in 25 and 30 ppt ($p<0.05$). Similarly, SGR_W (2.64±0.10, 2.69±0.18 % day^{-1}) in the lower salinity (15 and 20 ppt) were higher compared to those in 25 and 30 ppt (2.27±0.04, 2.09±0.09 % day^{-1}).

Table 3. The body weight (g) of *D. chipolini* in different salinity

Day	Treatments			
	15 ppt	**20 ppt**	**25 ppt**	**30 ppt**
1	0.007±0.003[a]	0.007±0.003[a]	0.007±0.003[a]	0.007±0.003[a]
30	0.020±0.009[a]	0.016±0.007[a]	0.018±0.011[a]	0.015±0.006[a]
60	0.063±0.030[a]	0.061±0.023[a]	0.058±0.026[a]	0.050±0.019[a]
90	0.184±0.039[ab]	0.240±0.070[b]	0.151±0.039[a]	0.117±0.024[a]
120	0.299±0.105[a]	0.244±0.120[a]	0.187±0.075[a]	0.111±0.079[a]
150	0.380±0.122[ab]	0.415±0.072[b]	0.216±0.086[a]	0.167±0.075[a]

Mean values followed by the same letter within each row are not significantly different (p>0.05).

Table 4. WG, DWG and SGR_W of *D. chipolini* in different salinity

Treatments	Body weight of *D. chipolini* after 150 day		
	WG (g)	**DWG** (mg day^{-1})	**SGR_W** (% day^{-1})
15 ppt	0.37±0.06[c]	2.5±0.4[c]	2.64±0.10[c]
20 ppt	0.41±0.11[c]	2.7±0.8[c]	2.69±0.18[c]
25 ppt	0.21±0.01[b]	1.4±0.1[b]	2.27±0.04[b]
30 ppt	0.16±0.02[a]	1.1±0.2[a]	2.09±0.09[a]

Mean values followed by the same letter within each row are not significantly different (p>0.05)

3.2. Survival rate

Survival rates of worms after 60 days in 15, 20, 25, and 30 ppt were 59.7±9.5%, 75.0±6.0%, 61.7±2.1%, 63.3±6.1%, respectively (Fig. 1). Survival rate of the worms in 20 ppt treatment was significantly higher than those in the others ($p<0.05$). However, no significant differences in survival rates were observed at 120 and 150 days ($p>0.05$). At day 150, survival rates in 15, 20, 25, and 30 ppt were 42.8±6.0%, 49.8±6.9%, 44.7±2.1%, and 43.3±3.0%, respectively. On average, the survival rate of the worms was highest in 20 ppt although no significant difference at the end of the experiment. The results showed that salinity may have little effect on the survival rate of the worms reared in 15 to 30 ppt.

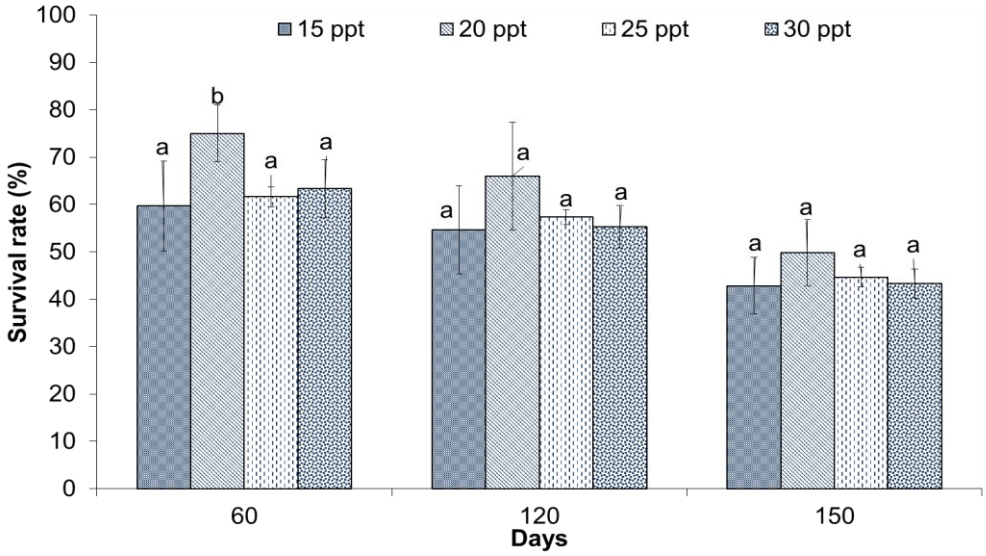

Figure 1. Survival rate of *D. chipolini* reared at different salinity

3.3. Reproductive performance

3.3.1. Maturation rate

The worms were mature and started to reproduce from day 135 as described in the methods. The maturation percentage was highest in 15 ppt and 20 ppt. The percentage of mature worms in 15, 20, 25, and 30 ppt were 36.0±8.7, 38.0±3.7, 20.0±3.6, and 8.0±2.0%, respectively, at day 150. The highest number of mature worms was recorded in 15 and 20 ppt and significant differences among treatments ($p<0.05$). The lowest value was found in 30 ppt (8.0±2.0%). The smallest sizes of maturation were around 2.3 cm with a body weight of 0.2 g.

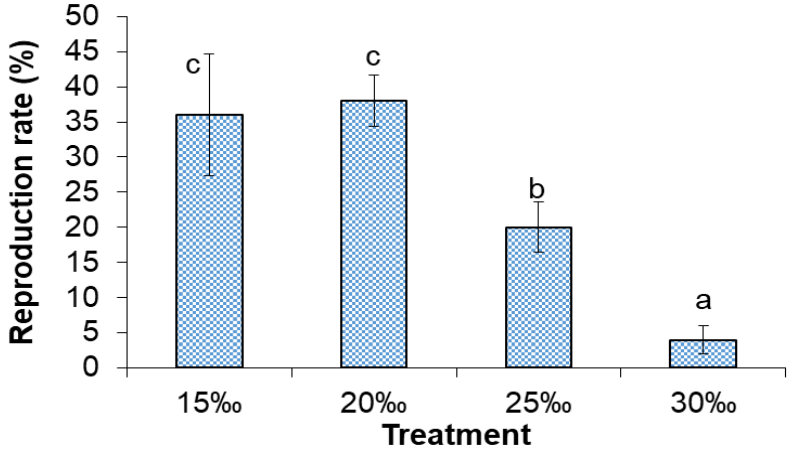

Figure 2. Maturation rate of the worms at different salinity

3.3.2. Fecundity

As the gonad is developed along the body of the worm, in all segments except the final two segments of the head and the tail, the absolute fecundity of males and females is proportional to their body size/weight. The worms with larger sizes produce higher absolute fecundity. The testis weight of the worms reared in different salinities ranged from 0.089 to 0.229 g/male and the highest was recorded in 20 ppt. No significant differences in absolute fecundity of males between treatments were observed ($p>0.05$). However, there was a significant difference in the numbers of eggs between treatments ($p<0.05$). Higher egg numbers were recorded in 20 ppt and 25 ppt with 103,890±17,389 and 112,740±22,328 eggs/female, respectively.

Lower numbers were found in 15 ppt and 30 ppt with 63,696±11,167 and 28,267±11,623 eggs/female, respectively (Figure 3). Absolute fecundities of the polychaete *D. chipolini* in 20 ppt and 25 ppt were significantly higher than those in 15 ppt and 30 ppt ($p<0.05$). The results showed that this worm species reproduces better at the salinity of 20-25 ppt in this experiment.

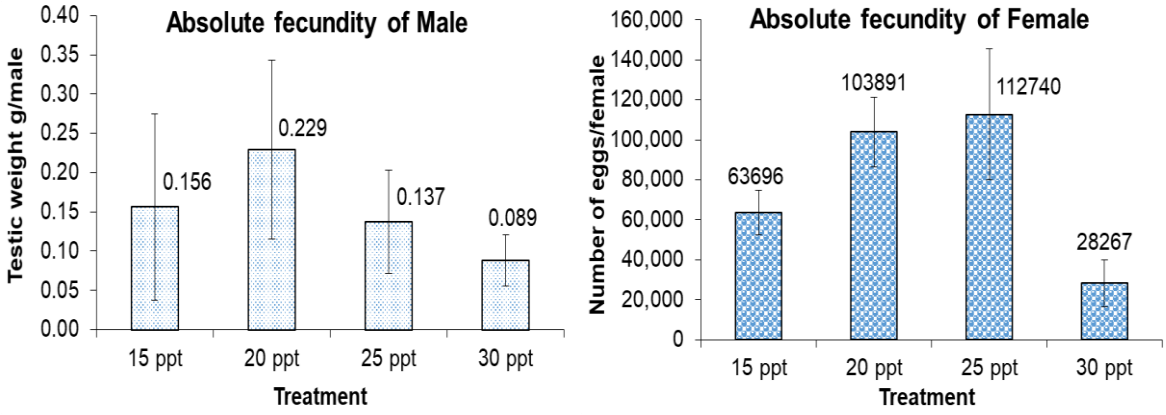

Figure 3. Fecundity of *D. chipolini* in different salinity

4. DISCUSSION

The polychaete species *D. chipolini* is commonly found along the coasts, especially in the mangrove areas of the Mekong Delta. There is not much information published other than morphological description for this species at the present time. The morphological characteristics of *D. chipolini* were first described by Hsueh (2019) who found this species in the brackish ponds near the coasts of southern Taiwan. In the current study, the effects of salinity on the growth and reproductive performance of this species were determined. As in numerous invertebrates, the growth and reproduction of this polychaete species may be also controlled by both environmental and endocrine factors. Rosas et al. (1999) and Pechenik et al. (2007) stated that salinity can reduce the growth and survival rate of polychaetes and also can reduce their rate of fecundity. The results of the current study show that *D. chipolini* species performed better growth and reproduction in salinity of 20 ppt compared to those of 15, 20, 25, and 30 ppt.

Effects of salinity on the growth of the rockworm *Marphysa sanguinea* were also determined in 15, 20, 25, 30 and 35 ppt by Em et al. (2019). The authors found that after three months of culture, the weight gain of the worms was found to be significantly higher in 25 ppt compared to other salinity and lowest weight gain was in 15 ppt.

A study conducted by Dung (2021) on the polychaete *Perinereis nuntia* var. *brevicirris* showed that the worms performed better in salinity of greater than 25 ppt with faster growth than in lower salinity (15 ppt and 20 ppt). Similarly, the growth rate of *Perinereis rullieri* was lower in low salinity than those in high salinity (Prevedelli et al., 1991). In contrast, the polychaete *Nereis diversicolor* displayed a faster growth rate in low salinity while *N. succinea* was in an opposite trend (Neuhoff, 1979). This shows that different polychaete species have different capacities of salinity tolerance and adaptation, resulting in different performances.

Daniela & Renata (1997) reported that the growth rate of the polychaetes *P. rullieri* was influenced significantly by salinity. In 10 ppt, the growth rate was very slow and for a given time the sizes obtained were approximately one-half of those in higher salinity. It has known that under laboratory conditions, a few aquatic invertebrates attain maximum growth rates in salinity, which are lower or higher than those of natural waters (Kinne, 1971).

In previous studies, salinity was not only influencing the growth of the polychaetes but also their survival. Em et al. (2019) investigated the effects of salinity on the survival of the polychaete, *M. sanguinea* and noted that the worms obtained the highest survival rate (85%) in 25 ppt. They concluded that salinity significantly affects not only the growth but also the survival of *M. sanguinea* adults. Bergamino et al. (2009) also confirmed that the polychaete abundance and diversity severely decreased with decreasing salinity because of the increase in rainfall. In the current study, *D. chipolini* distributes in a wide range of salinity. They can

thrive in different salinity ranges from 15-30 ppt but the best growth rate was at 20 ppt. No significant difference in survival rates was observed at the end of the experiment, 150 days. This has shown that salinity has little effect on the survival rate of the polychaete *D. chipolini* under a salinity range of 15-30 ppt.

A study by Thao et al. (2012) on the effects of salinity on larvae development of the species *Tylorrhynchus heterochaetus* showed that the rate of artificial insemination of this worm reached 78.9-79.1%, absolute fertility in September reached 46,207 eggs/ female. Embryo development of the worms occurs normally in salinity of 15 ppt. In another study, Tuan et al. (2018) found that the highest number of the worm *T. heterochaetus* participated in reproduction at a salinity of 10 ppt (82.11±1.67%), followed by those in 12 and 14 ppt (75.33±1.67% and 61.11±0.96%, respectively). These results were also in agreement with the data found by Chuong (2008) who reported that marine worms could produce gametes in water salinity of above 5 ppt. However, no reproductive activity was observed in salinity above 18 ppt.

In this study, higher salinity (15-20 ppt) may induce the reproductive process of the worms showed by large numbers of worms participating in reproduction and higher fecundity. The results also showed that this worm species displayed high absolute fertility in both males and females. The highest testis weight (0.229 g/male) and sperm production were recorded in 20 ppt, and higher egg numbers (135,068 eggs/female) were recorded in 25 ppt. Results of this study have confirmed the suitable salinity for reproduction of this polychaete species is 20–25 ppt. Artificial breeding of this species is therefore feasible, especially when the worms are totally mature (full sperm and eggs). Different levels of salinity in the present study may affect the metabolism and feeding behavior of the polychaetes. This resulted in varying growth and reproductive performance of the polychaete *D. chipolini*.

5. CONCLUSION

The suitable salinity for the growth and survival of the polychaete *D. chipolini* is 20 ppt. Starting by day 135 of rearing, the worms began reproducing at big sizes both in length and body weight. Their highest fecundity was also obtained in 20-25 ppt. The worms can spawn naturally in experimental tanks under this range of salinity, especially when they are fully mature. The findings of optimum salinity ranges can support the mass production of this polychaete to provide live feed for aquaculture species.

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