e-ISSN:2347-7830
p-ISSN:2347-7822

Research & Reviews : Journal of Ecology and Environmental Sciences

Effects of Temperature and Salinity on the Growth of Microalga *Tetraselmis* sp. and Tilapia *Oreochromis* sp. in Culture Pond, Tamil Nadu, India

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Research Article

Received date: 10/07/ 2015
Accepted date: 17/08/ 2015
Published date: 21/08/2015

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Keywords: Temperature, Salinity, *Tetraselmis* sp., *Oreochromis* sp., Climatic change.

ABSTRACT

The aquaculture industry has been hampered due to the lack of virulent and saline tolerable fish strain in marine food sector. The natural climatic changes were increased the water temperature and salinity in grow out culture system. This study dealt the effects of surface water temperature and salinity on the growth of microalga *Tetraselmis* sp. and tilapia *Oreochromis* sp. The study revealed that the microalgae and tilapia can survive in the wide range of temperature and salinity between 22.3 and 36.4oC and 45 and 92.5 ppt respectively. During the study period of 2013 and 2014, the physico-chemical and biological changes were hardly occurred where the density of *Tetraselmis* sp. declined from 368000 cells/ml to 750 cells/ml. Likewise, tilapia fish survived up to the optimum level of temperature and salinity. However it could not tolerate the salinity above 90 ppt. The length and weight of tilapia reached from 7.6 to 25.6 cm and 80 to 250 g respectively. This result inferred that the *Tetraselmis* sp. and *Oreochromis* sp. could be cultured in hyper saline water against of climatic changes.

INTRODUCTION

The world water environment contains various kinds of living organisms with various ecosystems. The plankton plays a significant role in aquatic environment. These organisms provide energy to higher trophic consumers and their tissue development. In recent years, raising climatic changes affecting living organisms via food chain and food web. Microalgae play an important role in the water environment especially for marine ecosystem as a primary producer which contribute to maintain the secondary production and eventually to enhance the fishery production. Some species of microalgae are used as live feed for finfish, shellfish and other invertebrates in aquaculture farms [1-3]. Some species are also used for industrial purpose to eliminate organic matters in waste disposal through gas exchange [4]. The physiology of microalgae is affected by physico-chemical factors such as water temperature, salinity, pH, light intensity and nutrient concentration.

Tilapia has been referred to as the 'aquatic chicken'. An *Oreochromis* sp. could be easily identified by dark bands of strips found on their body which is most prominent in mature forms. Nile tilapia *Oreochromis* sp. (Family: Cichlidae) having vital importance to fisheries. Tilapias are one of the most economically important groups of aquaculture species because they serve as major sources of protein in most countries. They are versatile species of fish, which is found in almost all type of tropical aquaculture systems ranging from traditional to highly intensive production systems. They withstand wide range of environmental conditions and perform well regardless of the water salinity and temperatures to which they are exposed to some species are even able to thrive and breed in full strength seawater. Among the tilapia species, the Nile tilapia, *Oreochromis* sp. is the preferred species for culture as this fish dominates production in freshwater and brackishwater ponds and cages [5]. However, it has low tolerance to high salinity levels.

On the other hand, the Mozambique tilapia, *Oreochromis mossambicus* is a euryhaline species and is one of the best studied tilapias in terms of elucidating the mechanisms involved in euryhalinity among fishes [6]. One of the constraints in the

e-ISSN:2347-7830
p-ISSN:2347-7822

culture of tilapias in high saline environments is its sensitivity to handling and susceptibility to secondary infections [7]. Hence the tilapia living in natural environment like ponds, lakes, pools etc. is depending natural herbs for that feeds. The natural climatic changes are affecting growth of micro algae and fishes, especially traditional fish of Tilapia. Because of these problems, there have been intensive research efforts made on improving the salinity tolerance of tilapias either through modifications in the culture techniques or stock improvement. The culture of tilapias in saline water is well-documented based on numerous research studies done in the past years. The limited space for freshwater aquaculture and pressures on providing the food demands of the population, tilapias are now being cultured in brackishwater ponds and even in marine cages. This scenario will further intensify in the years to come in order to cope with food requirements of the increasing human population. This article focuses on the effect of temperature and salinity on the growth of cultured microalgae and tilapia fish in pond system.

MATERIAL and METHODS

Microalgae Culture

Microalga *Tetraselmis* sp. collected from the Bay of Bengal, Tuticorin, Tamil Nadu, India, and the species were isolated and cultured at the laboratory in the cell density between 5 and 10 million cells/ml using Conway's culture medium [8] for maintaining indoor culture of *Tetraselmis* sp. while commercial grades of fertilizers (Urea, NPK, silicate) were used for outdoor culture of *Tetraselmis* sp. where cell density reached 0.5-3 million cells/ml. Microalgae were maintained at optimum level of physico-chemical parameters. The physico-chemical parameters were analysed according to Cho [9].

Tilapia Culture

Nile tilapia (*Oreochromis* sp.) seeds (2500 numbers and initial size of 3 to 5 cm) were collected from Tuticorin backwater and released in to 1500 m^3 earthen pond. The temperature and salinity were maintained in the range of 23-25°C and 33-50 ppt respectively. Microalgae and tilapia were maintained in the same culture pond and determined the effect of temperature and salinity on the growth of microalgae and tilapia.

Length-Weight Relationship

Monthly sampling of tilapia fish was made. The total length and weight of the fishes were noted to the nearest 1.0 mm and 1.0 gram respectively. The study was based on the length and weight data of 2500 specimen (length 7.6 to 25.6 cm and Weight 80 gram to 250 gram) collected during the study period 2013 to 2014. The method suggested by Le Cren [10] was followed to compute the length and weight relationship. Accordingly, the length-weight relationship can be expressed as:

$$W = {}_{a}L^{b}$$

Where W and L are weight (g) and length (cm) of the fish respectively and 'a' and 'b' are two constants (initial growth index and regression constants respectively). When expressed logarithmically be above equation becomes a straight line of the formula: Log W= Log a+b Log L Where, a= intercept, y=log W; x=log L and b=slope.

RESULTS

Analyses of Physico-Chemical Parameters

The physico-chemical parameters were observed in the culture ponds and it was fluctuated based on the atmospheric temperature. During 24 months observation, the minimum temperature of 22.3 and 23.1°C was observed in the month of October, 2013 and 2014 respectively while the maximum temperature of 36.2 and 36.4 was recorded during June 2013 and 2014. The minimum salinity (45 ppt and 50 ppt) was reported during October (2013) and (2014) while the maximum salinity (89 ppt and 92.5 ppt) observed during September (2013) and (2014). The detailed statistical calculation was shown in **Tables 1 and 2** and **Figures 1 and 2**.

Table 1. Univariate statistical value of water Temperature for 2013 and 2014.

Temperature	October	November	December	January	February	March	April	May	June	July	August	September
N	2	2	2	2	2	2	2	2	2	2	2	2
Min	22.3	23.4	23.8	24.8	26.3	29.3	32.5	34.6	36.2	32.1	27.3	24.1
Max	23.1	23.9	24.2	25.3	27.3	30.1	33.4	36.1	36.4	35.4	28.3	24.5
Mean	22.7	23.65	24	25.05	26.8	29.7	32.95	35.35	36.3	33.75	27.8	24.3
Std.error	0.4	0.25	0.2	0.25	0.5	0.4	0.45	0.75	0.1	1.65	0.5	0.2
Variance	0.32	0.125	0.08	0.125	0.5	0.32	0.405	1.125	0.02	5.445	0.5	0.08
Stand.dev	0.565	0.3535	0.253	0.3535	0.707	0.566	0.636	1.0606	0.141	2.334	0.707	0.283

e-ISSN:2347-7830
p-ISSN:2347-7822

Median	22.7	23.65	24	25.05	26.8	29.7	32.95	35.35	36.3	33.75	27.8	24.3
25 prcntil	16.725	17.55	17.85	18.6	19.725	21.975	24.375	25.95	27.15	24.075	20.475	18.075
75 prcntil	17.325	17.925	18.15	18.975	20.475	22.575	25.05	27.075	27.3	26.55	21.225	18.375
Skewness	0	0	0	0	0	0	0	0	0	0	0	0
Kurtosis	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75
Geom. mean	22.696	23.649	23.99	25.048	26.795	32.697	32.947	35.342	36.299	33.709	27.795	24.2992
Coeff.var	2.492	1.495	1.179	1.4113	2.634	1.905	1.931	3.004	0.389	6.914	2.544	1.164

Table 2. Univariate statistical value of water salinity for 2013 and 2014.

Salinity	**October**	**November**	**December**	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**
N	2	2	2	2	2	2	2	2	2	2	2	2
Min	45	48	52	59	61	64	68	72	76	79	84	89
Max	50	53	53.9	61.3	62.7	64.5	71.3	73.6	78.4	81.5	84.5	92.5
Mean	47.5	50.5	52.95	60.15	61.85	64.25	69.65	72.8	77.2	80.25	84.25	90.75
Std.error	2.5	2.5	0.95	1.15	0.85	0.25	1.65	0.8	1.2	1.25	0.25	1.75
Variance	12.5	12.5	1.805	2.626	1.445	0.125	5.445	1.28	2.88	3.125	0.125	6.125
Stand.dev	3.54	3.53	1.343	1.626	1.202	0.354	2.334	1.131	1.697	1.767	0.353	2.475
Median	47.5	50.5	52.95	60.15	61.85	64.25	69.65	72.8	77.2	80.25	84.25	90.75
25 prcntil	33.75	36	39	44.25	45.75	48	51	54	57	59.25	63	66.75
75 prcntil	37.5	39.75	40.43	45.98	47.025	48.375	53.475	55.2	58.8	61.125	63.375	69.375
Skewness	0	0	0	0	0	0	0	0	0	0	0	0
Kurtosis	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75
Geom.mean	47.434	50.44	52.94	60.139	61.844	64.25	69.63	72.796	77.19	80.24	84.249	90.733
Coeff.var	7.443	7	2.54	2.704	1.944	0.55	3.35	1.554	2.198	2.203	0.419	2.727

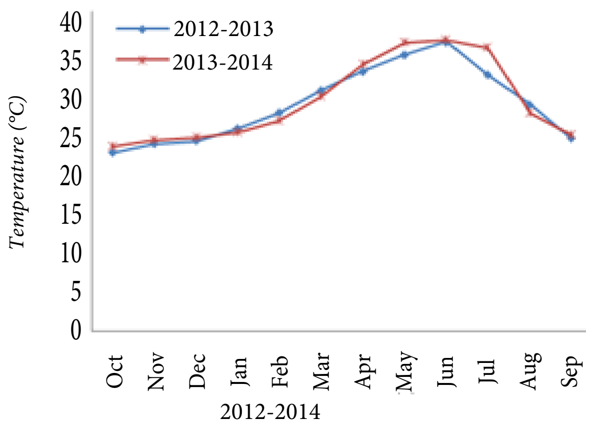

Figure 1. Temperature variations between 2013 and 2014.

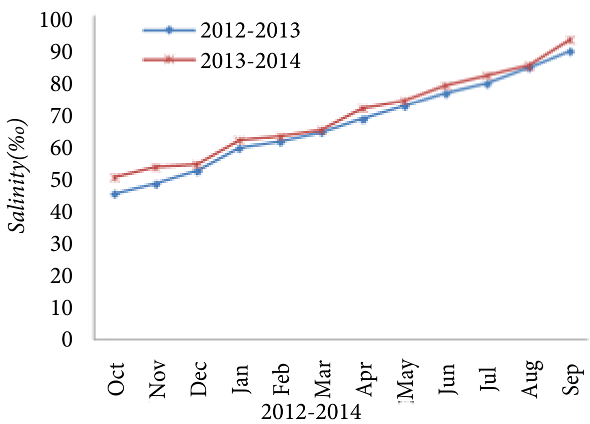

Figure 2. Salinity variations between 2013 and 2014.

Variation in density of *Tetraselmis* sp.

The minimum microalgae density of 800 and 750 cells/ml were recorded during the month of September 2013 and 2014 respectively. The maximum microalgae density of 365000 and 368000 cells/ml was recorded during December 2013 and 2014 respectively. The statistical variation shown in **Table 3** and **Figure 3**.

Table3. Univariate statistical value of Algae density for 2013 and 2014.

Algae	October	November	December	January	February	March	April	May	Jun	July	August	September
N	2	2	2	2	2	2	2	2	2	2	2	2
Min	350000	359000	365000	325000	245000	201000	153000	117000	85000	21000	3000	750
Max	355000	360000	368000	335000	269000	213000	163000	124000	105000	41000	5000	800
Mean	352500	359500	366500	330000	257000	207000	158000	120500	95000	31000	4000	775
Std.error	2500	500	1500	5000	12000	6000	5000	3500	10000	10000	1000	25
Variance	1.25	500000	4500000	5.00E	2.88E	7.20E	5.00E	2.45E	2.00	2.00E	2000000	1250
Stand.dev	3535.5	707.10	2121.3	7071.06	16970.5	8485.28	7071.06	4949.74	14142.1	14142.1	1414.2	35.35
Median	352500	359500	3666500	330000	257000	207000	158000	120500	95000	31000	4000	775
25 prcntil	262500	269250	273750	243750	183750	150750	114750	87750	63750	15750	2250	562.5
75 prcntil	266250	270000	276000	251250	201750	159750	122250	93000	78750	30750	3750	600
Skewness	0	0	0	0	0	0	0	0	0	0	0	0
Kurtosis	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75	-2.75
Geom.mean	352491.1	359499.7	366496.9	256719.7	256719.7	206913	157920.9	120449.2	94472.22	29342.8	3872.983	774.59
Coeff.var	1.0029	0.1966	0.5788	6.60333	6.60333	4.09917	4.47535	4.10767	14.8864	45.6197	35.355	4.562

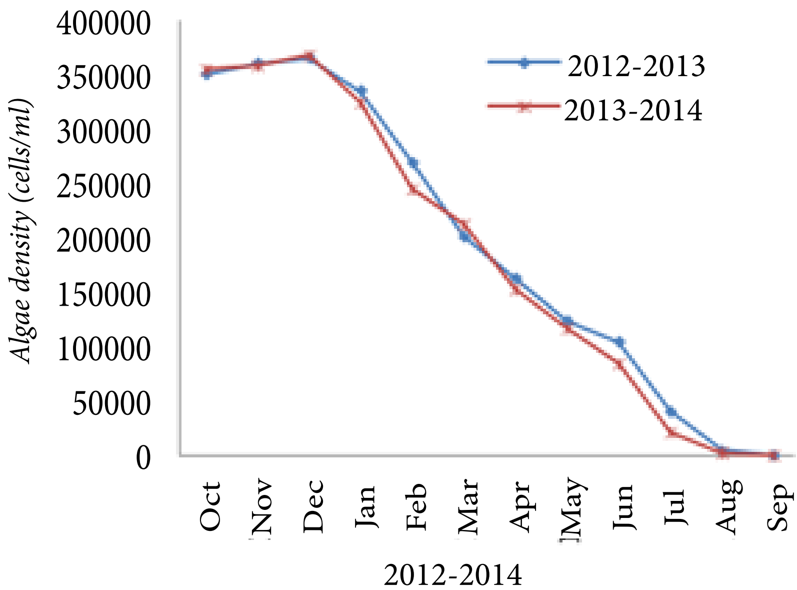

Figure 3. Algae density between 2013 and 2014.

Growth (Length-weight) variations of Tilapia fish (Plate-1)

The length-weight of tilapia fish were examined and minimum size of 7.6 and 8.1 cm noticed during the month of October 2013 and 2014 respectively. Maximum length of 25.6 and 25.5 cm were recorded during the month of May 2013 and 2014 respectively. The least weight (89 and 80gm) was observed during October 2013 and 2014 while the highest weight was noticed during August 2013 (247 grams) and June 2014 (250 grams). The Linear regression was shown in **Table 4** and **Figures 4-5.**

Table 4. Regression parameters for length-weight relationship of Tilapia fish.

Linear regression	Regression equation (Weight)	Regression equation (Length)	R^2
2013	Log (w)=0.037x+1.988	Log (L) = 0.052x+0.905	Weight = 0.942 Length= 0.886
2014	Log (w)=0.039x+1.990	Log (L) = 0.046x+0.952	Weight = 0.855 Length= 0.807

DISCUSSION

The fluxion of climatic changes are affecting natural aquatic environment through increasing of atmospheric temperature. In past few years, world aquatic ecosystem are suffering by several natural disasters, these natural sick significantly affects the aquatic organisms especially micro and macro algae, micro and macro faunas and fishes etc. Present investigation determined the changes of micro algae and tilapia fish in aquaculture ponds due to increased water temperature and salinity through rising of atmospheric temperature. The physico-chemical and biological parameters were examined in culture pond and observed vast changes in microalgae density and growth of tilapia fishes. The present study inferred that the increasing temperature and salinity were adversely affects the growth of microalgae and tilapia fish. In the present investigation, the microalgae showed potential growth at 45 to 50 ppt of salinity and further increase in salinity from 50 ppt can leads to the declined growth. In our study tilapia can survived up to 80 ppt salinity and more than this salinity range the fish shown mortality. However the earlier worker stated that the tilapia fish tolerates maximum of 50 ppt salinity [11].

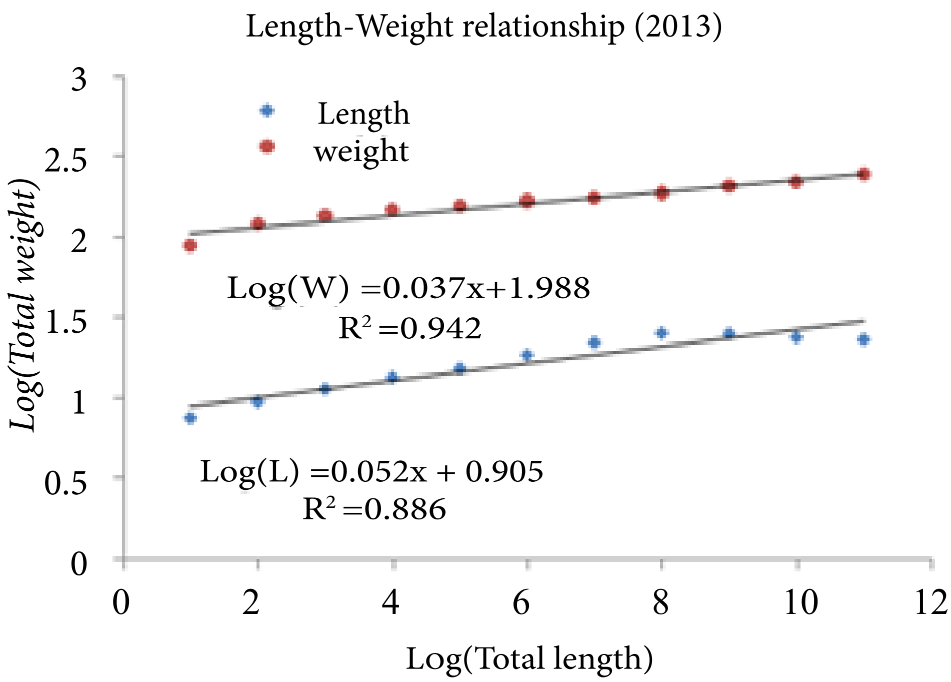

Figure 4. Length – weight relationship of Tilapia fish (2013).

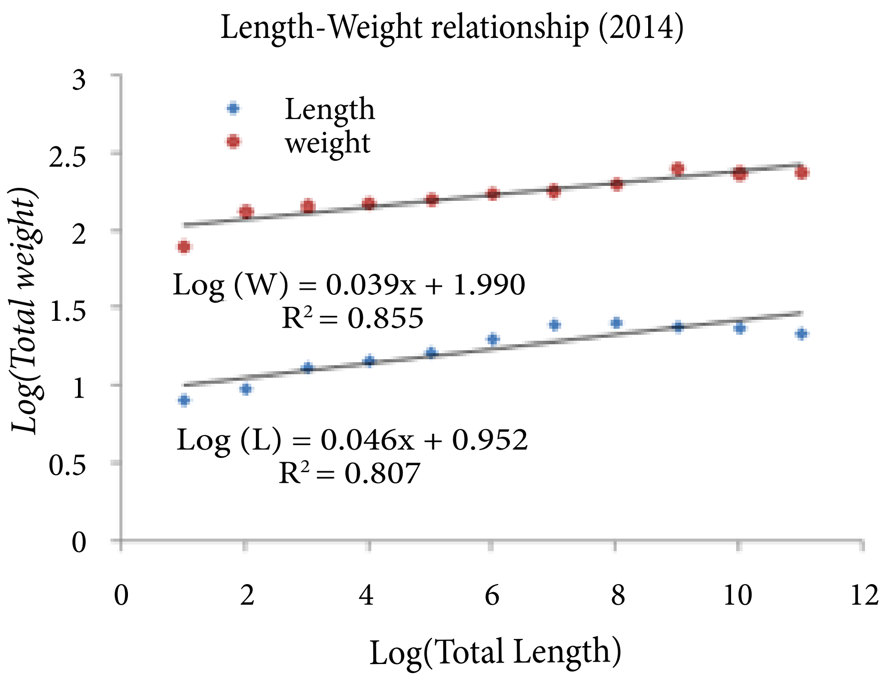

Figure 5. Length – weight relationship of Tilapia fish (2014).

However, it is noticed that when there was an abrupt change in salinity to a maximum increase of 5 ppt per day, the fish were sluggish for a few minutes after exposure but recovered within one hour after and resumed their normal feeding activity. We have also observed that when the salinity level reached at least above 50 ppt the fish had dark pigmentation, showed erratic swimming behavior and stopped feeding but resumed to normal conditions within a few hours after they adjusted to the salinity conditions. In the course of the salinity tolerance test, we maintained the optimum water quality parameters in the containers. One important factor that we considered was water temperature, which was noticed in the range of 25-32ºC. This optimum water temperature is crucial in maintaining a steady state plasma osmolity in the fish during exposure to salinity changes [12] and could also be partly responsible in preventing mortalities in the fish.

The physico-chemical observations were made earlier by Goldman and Ryther and the culture depth by Persoone [13]. However, the growth of microalgae is affected and influenced by the culture conditions such as light intensity, nutrient limitation, temperature, pH, and salinity [9]. The presently obtained growth on tilapia (*Oreochromis* sp). culture technique with different physico-chemical parameters was positively correlated with Thongprajukaew [14]. Effects of different salinities on the growth and proximate composition of *Nannochloropsis* sp. and *Tetraselmis* sp. isolated from South China Sea was earlier studied by [3].

Healthy Tilapia fish (Salinity <50%)

Affected Tilapia fish (Salinity > 60%)

Tilapia fish mortality (Salinity > 90%)

Dead Tilapia fish

Plate 1. Growth view of Tilapia fish (*Oreochromis* sp.)

In aquaculture, only microalgae with valuable properties were used and the composition of the algal biomass in regards to lipid, carbohydrate and protein determines its overall economic potential [15]. *Nannochloropsis* sp. and *Tetraselmis* sp. are common microalgae species that have promising potential especially in aquaculture industry application [16]. A good alternative is a commercial-scale production of microalgae biomass because this could reduce the cost and ecological impact of intensive fish farming [17]. The increasing in the prices for these fish-based products leads to the search of alternatives to these sources [18].

Decreasing salinity is a unique way to change the biochemical composition of marine microalgae although the changeable role of salinity on starch metabolism indicates it's species-specific and cultivation condition-dependent nature [19]. Beyond initial survival in brackish and seawater salinities debate continues as to whether tilapia could survive external factors (predators, current, temperatures, disease) they might encounter. Most notable of these factors is temperature. There is little information available on the interactive effects of temperature and salinity tolerance in tilapia [20] and further examination is warranted.

Tilapias are widely cultured throughout the world, and many tilapia species have been evaluated for culture purposes. Several recent research initiatives in tilapia aquaculture involve genetic improvement of these stocks described [21,22]. *Oreochromis aureus* and *O. mossambicus* both have higher tolerances for salinity with experimental production of blue tilapia occurring at 44 ppt [23]. Tilapia surviving exposure to 64 ppt salinity. Nile tilapia, *O. niloticus*; blue tilapia, *O. aureus*; and mossambique tilapia, *O. mossambicus* these three species have differing salinity tolerances. The Nile tilapia exhibits a moderate tolerance to salinity with 60 ppt fish surviving direct transfer up to 25 ppt, but its highest growth was achieved at 0-10 ppt [24].

This paper defined the measurement of a tilapia's ability to survive elevated salt concentrations. Tolerance of Nile tilapia in moderate salinity (20 ppt) and high salt tolerance (>35 ppt) in blue tilapia have been documented and were determined to be suitable for comparisons of salinity tolerance with the two hybrid-based varieties. The Nile tilapia will reportedly thrive in any aquatic habitat except for torrential river systems and the major factors limiting its distribution are salinity and temperature [25]. Results suggest that several notable characteristics among varieties of tilapia expressed the greatest tolerance for elevated salinity levels, with 96% survival at 20 ppt and 30 ppt salinities. As blue tilapia were exposed to 35 ppt salinity, survival decreased abruptly to 49%. Blue tilapia has been recognized to have high salt tolerance, in excess of 35 ppt in prior studies with survival at as high as 53 ppt after acclimation [23].

The survival of blue tilapia at 35ppt was noticed and when the salinity increased the animal might be died. However previous studies have indicated that while direct transfer to seawater can result in almost complete mortality, graduated acclimatization is sufficient to acclimate salt tolerant in tilapia successfully [26,27]. Nile and Mississippi commercial tilapias withstood rapid acclimatization up to 20 ppt salinity. A more gradual acclimation of tilapia might increase survival, but may not properly model an accidental release of tilapia. Studies have also indicated that early exposure to salinity while in the egg or larval stage increases salinity tolerance significantly [28-30].

Depending on the food source, they will feed either via suspension filtering or surface grazing (GISD 2012), trapping plankton in a plankton rich bolus using mucus excreted from their gills [31]. Nile tilapia are known to feed on phytoplankton, periphyton, aquatic plants, invertebrates, benthic fauna, detritus, bacterial films [32] and even other fish and fish eggs [33]. Aid organizations promoted aquaculture as a means of improving food security with low grain to feed conversion rates, and minimal environmental impacts [34]. Today, tilapia is often farmed with multiple species in the same pond, such as shrimp and milkfish. Nile tilapia can live longer than 10 years (GISD 2012). Food availability and water temperature appear to be the limiting factors to growth for *O. niloticus* [35]. Optimal growth was achieved at 28-36ºC and declines with decreasing and increasing temperature [32,36]. This study was aimed to determine the optimum temperature and salinity that can result in higher growth of *Tetraselmis* sp. and tilapia fish. These results can be applied by farmers and industry in culturing microalgae and tilapia with a targeted growth and achieved under certain culture conditions.

CONCLUSION

The results presented in this paper have demonstrated that the climatic changes affecting physico-chemical and biological properties in aquaculture industry. The raising atmospheric temperature can adversely affects the water temperature, salinity, microalgae and fishes in aquaculture pond. During the study period, salinity was gradually increased and same time density of microalgae (*Tetraselmis* sp.) was declined up to 80 ppt of salinity, beyond this range the algae cell attained reverse-osmosis and slowly the algae cell was attained lethal in nature. The change in microalga growth caused by elevated water temperature and salinity can concomitantly affects the growth of cultured fish (*Oreochromis* sp.). Present study concluded that the maximum temperature and salinity tolerable range of microalga *Tetraselmis* sp. are 25-32ºC and 40-50 ppt. Likewise tilapia *Oreochromis* sp. can tolerate the temperature and salinity range of 22.3-36.4ºC and 45-92.5 ppt respectively.

ACKNOWLEDGEMENTS

The authors are thankful to The Head, Department of Marine Science and authorities of Bharathidasan University, Tiruchirappalli-24, Tamil Nadu, India, for providing necessary facilities. One of the author (TV) thank the University Grants Commission, Government of India, New Delhi for Post-Doctoral Fellowship (Ref.No.F./PDFSS-2014-15-SC-TAM-8547; dated, 05.02.2015).

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