Growth and Survival of *Oreochromis niloticus* (Linnaeus, 1758) Reared in Earthen Ponds with Aquatic Macrophytes

Ledesma, Rene Geraldo Guerrero

Senior Science Research Specialist, National Fisheries Research and Development Institute, Aquaculture Research and Development Division, Fish Nutrition and Feed Development Section, 101 Mother Ignacia Street, Quezon City Philippines 1103

Abstract: ***A feeding trial was conducted to determine tilapia (O. niloticus Linn, 1758) survival and growth under a plant-based feeding regime of fresh aquatic macrophytes (two duckweed: Lemnaperpusilla (Torrey, 1843), Spirodelapolyrrhiza (L.) Schleid., and one aquatic fern: Azollapinnata (R.Br.). A diet of crushed snails (Pomaceacanaliculata) served as Control. After eight months of culture, tilapia fed with A. pinnata, S. polyrrhiza, L. perpusilla and Control exhibited survival rates of 59.20, 46.40, 39.60 and 34.20, respectively. Best tilapia growth rates were recorded with S. polyrrhiza and A. pinnatamacrophytes that had corresponding higher crude protein levels than L. perpusilla. Chi-square values at $x^2_{0.95,\ v=2}$ (5.9<34.6) and $x^2_{0.95,\ v=3}$ (7.81<70.43) indicated that tilapia could utilize aquatic macrophytes per se; however, poor growth (0.46 and 0.52g/day) and survival rates (34.20 – 59.20%) were observed due to varied crude nutrient profiles of the macrophytes. Tilapia fed with crushed snails in the Control showed highest mortality (65.80%) and smallest average wet weight value (112.28g/fish) when compared to those reared in the three macrophyte-stocked ponds.***

Keywords: aquatic macrophyte, earthen pond, tilapia

1. Introduction

This study evaluated the survival and growth of Nile tilapia fry fed duck weed (*L. perpusilla*, *S. polyrrhiza*), aquatic fern (*A. pinnata*) or crushed snails (*P. canaliculata* Lamarck) under extensive system. Experimental design and methodology were patterned after the single trial (no replicate) 150 day duckweed feeding experiment on tilapia juveniles done by El Shafai *et al.* (2014). El Shafai's fishponds used treated and untreated anaerobic sludge blanket reactor (UASB) sewage water as well as freshwater.

2. Materials and Methods

2.1 Experimental set-up

Four (4) 300m^2 x 1m rain-fed, earthen ponds were used for this study. No water replacement was undertaken, similar to the settled sewage pond El Shafai *et al.* used to grow duckweed and tilapia. Ponds were fertilized using processed chicken manure broadcast at a rate of 50Kg each, allowing gradual decomposition for one month. Three ponds were stocked with duckweeds *L. perpusilla*, *S. polyrrhiza* and aquatic fern *A. pinnata* at a rate of 250Kg each to serve as starter culture for macrophyte self-proliferation (daily infusion of fresh macrophyte into the ponds was not provided thereafter). The fourth pond serving as the control pond was devoid of any aquatic macrophyte species hence a daily input of crushed snails was provided*ad libitum* as tilapia food source.

Five hundred (500) pieces of size 14 (1.90g/pc) Nile tilapia were distributed to each pond. After eight culture-months tilapia were harvested, total wet biomass, average final wet weights, survival, mortality and growth rates were determined.

Crude nutrient profiles of dried *L. perpusilla* samples were compared with published profiles of *Lemna minor* (Chakrabarti *et al.*, 2018), *S. polyrrhiza* (Russof *et al.*, 1980) and *A. pinnata* (Roy et al., 2016). *L. perpusilla* biology and proximate composition were presumed to be similar to that of *L. minor* (www.cabi.org>isc>datasheet). Both are the smallest *Lemna*species (1.5 – 4.0mm leaf diameter) thriving in Southeast Asian rice fields.

2.2 Statistical design

Tilapia survivor values were subjected to chi-square tests to determine: H_o[1] if the percentage of survivors was similar in the three macrophyte compartments, and H_o[2] if the percentage of tilapia survivors was similar among the control and macrophyte compartments.

3. Results and Discussion

Tilapia biomass and survival

The four ponds combined yielded 107Kg Nile tilapia after eight (8) culture-months. Wet biomass (Kg) varied among the compartments: highest biomass values were measured from the *S. polyrrhiza* and *A. pinnata* compartments while lowest were obtained from the *L. perpusilla* and control compartments (Table 1). Tilapia cultivated in *S. polyrrhiza* and *A.pinnata* compartments were larger (8 *vs.*9 fish/Kg) and heavier (162.50 *vs.*142.50g) than tilapia in the *L. perpusilla* and Control ponds. Growth rates of tilapia in *S. polyrrhiza* and *A.pinnata* ponds were faster than those in the *L. perpusilla* and Control ponds.

Tilapia survivor numbers in the three macrophyte-infused compartments were distinct at $x^2_{0.95,\ v=2}$ (5.9<34.6).The largest tilapia cohort was obtained in the *S. polyrrhiza* compartment (294 individuals/500 initially stocked) while the lowest were in the *L. perpusilla* and Control compartments at 198 and 171individuals, respectively (Table 2).

Tilapia survival rates were highest for *S. polyrrhiza* followed by *A. pinnata*, *L. perpusilla* and Control (59.20, 46.40, 39.60 and 34.20%, respectively). Nonetheless, average survival rate in the four compartments was poor (44.85%). Significant chi-squarevalue at $x^2_{0.95,\ v=3}$ (7.81<70.43) indicated that macrophyte infusion in ponds served as a source of nutrients that boosted tilapia survival. Absence of aquatic vegetation and reliance of tilapia on natural pond food in the Control compartment resulted in the smallest biomass (19Kg) and size of tilapia (142.50g).

Tilapia consumption of predominant macrophyte diets in H_o[1] resulted in slow growth (Table 1) and an average mortality of 51.6% (Table 2). Tavares et al.(2008) observed impeded growth in tilapia that subsisted on pure duckweed diets *per se*. El Sayed (1992) noted that a sole diet of *A. pinnata* diminished tilapia growth and increased carcass moisture. Solomon and Okomoda (2012), Fasakin *et al.* (1999), Olayini and Oladunjoye (2012), Hassan and Edwards (1992) limited duckweed inclusion rates to 5, 10, 25 and 30% dry weight for tilapia diets, respectively. In contrast, Talukdar et al. (2012) recommended an inclusion of fresh duckweed at 50% total body weight of fish in ponds.

By consuming fresh macrophytes tilapia could not adequately meet protein and lipid levels at 37 and 7%, respectively (Tacon, 1987) (Table 3).

Table 1: Growth and survival of tilapia, this study

Compartment	Total Wet Biomass (Kg)	Average Initial Weight (g)	Average Final Weight (g)	Survival Rate[1] (%)	Absolute Growth Rate[2] (g/day)	Specific Growth Rate[3] (%)	Relative Growth Rate[4] (%)
[A] Control (crushed snail)	19.2	1.9	112.28	34.2	0.46	1.7	24.21
[B] *A. pinnata*	29.2	1.9	125.96	46.4	0.52	1.75	27.18
[C] *S. polyrrhiza*	37.5	1.9	126.69	59.2	0.52	1.75	27.37
[D] *L. perpusilla*	22.4	1.9	113.13	39.6	0.46	1.7	24.39

n.b.

[1]SR = $\frac{Nf}{Ni}$ x 100%   [2]AGR = $\frac{(Wf-Wi)}{days}$ [3]SGR = $\frac{(\ln Wf-\ln Wi)}{days}$ x 100%   [4]RGR = $\frac{Wf - Wi}{Wi \times days}$ x 100%

Table 2: Tilapia survival and mortalities, and chi-square comparisons among the three macrophyte and the control ponds

Compartments>	[A] Control	[B] *A. pinnata*	[C] *S. polyrrhiza*	[D] *L. perpusilla*	H_o [1] Total	H_o [1] (%)	H_o [2] Total	H_o [2] (%)
survivors	171	232	296	198	726	48.4	897	44.85
mortalities	329	268	204	302	774	51.6	1, 103	55.15
Totals>	500	500	500	500	1, 500		2, 000	

For H_o [1] x^2 = 34.6; $x^2_{0.95,\ v=2}$ (5.9<34.6), the percentage of tilapia survivors was not similar in the three macrophyte compartments.
For H_o [2] x^2 = 70.43; $x^2_{0.95,\ v=3}$ (7.81<70.43), the percentage of tilapia survivors was not similar among the control and macrophyte compartments.

Table 3: Proximate composition of macrophytes *versus O. niloticus* and *P. canaliculata*

Crude Nutrient (%)	*S. polyrrhiza*[1]	*A. pinnata*[2]	*L. perpusilla*[3]	*O. niloticus*[4]	*P. canaliculata*[5]
Protein	29.1	24.6	19.5	37	12.2
Ether extract	4.5	3.8	4.8	7	0.4
Fiber	8.8	9.3	14.3	3	3.2
Ash	15.2	15.9	15.4		
Nitrogen Free Extract	42.4	46.4	46	40	6.6
Dry Matter	5.1	8	13		

References:
[1]Rusoff et al. (1980)
[2]Roy et al. (2016)
[3]This study
[4]Tacon (1987) for *O. niloticus* juveniles
Nurjanah *et al.* (2019) for *P canaliculata* meat

Table 4: Eaa amino acid requirements of Nile tilapia as % of dietary protein and % in total diet (in brackets), along with eaa % values of macrophytes and *P.canaliculata* diets in this study.

Amino acid	*S. polyrrhiza*[1]	*A. pinnata*[2]	*L. minor*[5]	*O. niloticus*[3]	*P. canaliculata*[4]
Arg	5.3	5.9	3.06	4.2 [1.2]	9.1
His	2.2	2.1	0.89	1.7 [0.5]	1.6
Ile	3.8	4.5	2.04	3.1 [0.9]	4.1
Leu	6.9	8.4	4.13	3.4 [1.0]	8.2
Lys	4.3	4.7	2.68	5.1 [1.5]	6.8
Met + Cys	0.8	(Met, 1.4) (Cys, 1.6)	(Met, 0.86) (Cys, 0.38)	(Met, 2.7) [Met, 0.8] (Cys, 0.5)	(Met, 1) (Cys, 0.8)
Phe + Tyr	(Phe, 3.8) (Tyr, 3.1)	(Phe, 5.4) (Cys, 3.6)	(Phe, 2.57) (Tyr, 1.90)	(Phe, 5.5) [Phe, 1.1] (Tyr, 1.8)	(Phe, 4.3) (Tyr, 4.5)
Thr	3.5	4.7	1.92	3.8 [1.1]	4.7
Val	4.4	5.5	2.66	2.8 [0.8]	2.1

References:
[1]Rusoff et al. (1980)
[2]Roy et al. (2016)
[3]Santiago and Lovell (1988)
[4]Ghosh et al. (2018)
[5]Chakrabarti et al. (2018)

Due to low protein levels in fresh aquatic plants, tilapia needs to consume substantial amounts to satisfy their eaa and energy requirements. Fresh duckweed could yield 92% water (Leng *et al.*, 1995; Goopy and Murray, 2003), hence feeding would be a long process for tilapia. Blue tilapia could consume 117.87% of fish body weight in fresh *L. minor* within 48 hours to meet energy requirements (Heaton and Rodgers, Jr., 2017). A consumption rate of fresh *L. minor* per 50% total body weight of *O. niloticus* and other freshwater fish reported by Talukdar *et al.* (2012). The carrying capacity for *O. niloticus* stocked in a fertilized pond with abundant aquatic vegetation could amount to as much as 3 tons/hectare (Diana et al., 1992). Hence the 300m^{2}macrophyte ponds in this study could have yielded 90Kg of tilapia.

Most essential amino acid (eaa) levels of macrophytes and snail meat used in this study were above tilapia levels (Rusoff et al., 1980; Roy et al., 2017; Ghosh et al., 2018; Chakrabarti et al., 2018; Santiago and Lovell, 1988). However, lower lysine and methionine levels were observed in macrophyte diets (Okomoda et al. 2012) (Table 4).

Macrophyte digestibility by tilapia enzymes is also impeded by cellulose and crude fiber mingling in plant nutrients (Pinandoyo et al. 2019), anti-nutritional factors (ANFs) (*e.g.* phytates and anti-trypsin inhibitors) (El-Sayed, 2004) and non-palatable plant substances oxalic acids, phenolic compounds, tannins and saponins (Goopy and Murray 2003; Okomoda et al. 2012; Sonta et al. 2019). Non-palatability of duckweed by tilapia could be reduced by maintaining good water quality in recirculating systems (Utami et al., 2018). Phyto-remediative capacity of duckweeds could regulate phosphorus levels (Chismadha et al., 2019) or reduce harmful chemicals in sewage (El-Shafai et al.2004).

Preferential issues of different macrophytes by tilapia result in selective feeding behavior that influences growth rates and survival (Wee, 1991). Hassan and Edwards (1992) observed preferential feeding of Nile tilapia on *L. perpusilla*over *S. polyrrhiza*. Nile tilapia likewise manifested a high consumption rate for *Elodea Canadensis* and partial preference for *S.polyrrhiza* (Setlikovaa and Adamek 2004). Heaton and Rodgers, Jr. (2017) noted preference of blue tilapia for *L. minor* over other aquatic plants.

4. Conclusion

Overall, factors of low protein levels, ANFs, non-palatable plant substances, high water content of fresh macrophytes, and selective feeding behavior of tilapia towards duckweed and water fern species resulted in high mortality, small sizes and slow growth rates of tilapia observed in this study. Removal of macrophyte oxalic acids, phytates and anti-trypsin inhibitors through chemical treatment would not be feasible for small tilapia farmers. A mixed formulation consisting of commercial feed and at most 25% fresh macrophytes would improve tilapia growth and survival as opposed to a 100% macrophyte diet, consistent with the observations of Gaigher*et.al.* (1984).

5. Future Scope

This study provides an insight into the practice of organic fish farming. In organic tilapia farming, where feed inputs are derived from the natural pond ecosystem, restrictions (*e.g.* non-utilization of GMO substances, low stocking density, non-use of synthetic chemicals, low total protein input in the culture system at 25% maximum) (Dube and Chanu, 2012; Philippine National Standard, 2016) have lengthened tilapia culture to reach marketable size of 250 grams. Nonetheless, higher priced organic tilapia have led to the creation of a niche market accepted by health-conscious consumers.This study recommends conducting feasibility and cost-benefit studies to determine acceptable prices for organic tilapia.

References

[1] **CABI Invasive Species Compedium**; www.cabi.org>isc>datasheet.

[2] **Chakrabarti, R., ClarkW.D., SharmaJ.G., GoswamiR.K., Shrivastav, A.S. and Trocher, D.R.**; Mass production of *Lemna minor* and its amino acid and fatty acid profiles; Frontiers in Chem, October 2018, volume 6 article 479, 16 pages, www.frontiersin.org.

[3] **Chrismadha, T., Suryono, T., Magfiroh, T., Mardiati, Y. and E. Mulyana**; Phytoremediation of Maninjau lake water using minute duckweed (*LemnaperpusillaT*orr.); IOP Conf. Series: Earth and Environmental Science 308; (2019); 012021.

[4] **Diana, J.S., Lin, C.K., andSchneeberger, P.J.**; Relationships among nutrient inputs, water nutrient concentrations, primary production, and yield of *Oreochromis niloticus* in ponds; Aquaculture; (1991); 92: 323-341.

[5] **Dube, K. and Chanu, T.**; Organic aquaculture: way to sustainable production; Advances in Fish Research; (2012); Pages 219-229.

[6] **El-Sayed, A.F.M.**; Effects of substituting fish feed with *Azollapinnata* in practical diets for fingerling and adult Nile tilapia, *Oreochromis niloticus*(L.); Aquaculture Research Volume 23, Issue 2 March 1992 pages 167 – 173 (1992).

[7] **El-Sayed, A.F.M.**; Protein nutrition of farmed tilapia: searching for unconventional sources. Pp. 364-378. *In:* Bolivar, R.B., G.C. Mair and K. Fitzsimmons. 6th International Symposium on Tilapia in Aquaculture; (2004); Philippine International Convention Center, Philippines, September 12 – 16, 2004.

[8] **El-Shafai, S.A., El-Gohary, F.A., Nasr, F.A., van der Steen, N.P., and Gizen, H.J.**; Suitability of using duckweed as feed and treated sewage and water source in tilapia aquaculture. TESCE, Vol. 30, No. 2, December 2014.

[9] **Fasakin, E.A., Balogun, A.M. and Fasuru, B.E.**; Use of duckweed, *Spirodelapolyrrhiza* L. Schleiden, as a protein feedstuff in practical diets of tilapia, *Oreochromis niloticus* L. Aquaculture Research; (1999); Vol 30 no. 5 pp. 313 – 317.

[10] **Gaigher, I.G., Porath, D., and Granoth, G.**; Evaluation of duckweed (*Lemnagibba*) as feed for

tilapia (*Oreochromis niloticus x O. aureus*) in a recirculating unit. Aquaculture; (1984); 41: 235 – 244.
[11] **Ghosh, S., Jung, C., and Meyer-Rochow, V.B.**; Snail as mini-livestock: nutritional potential of farmed *Pomaceacanaliculata* (Ampullariidae).Agriculture and Natural Resources xxx ;(2018); 1-8.
[12] **Goopy, J.P. and Murray, P.J.**; A review on the role of duckweed in nutrient reclamation and as a source of animal feed. Asian-Aus. J. Anim. Sci.; (2003); Vol 16, No. 2: 297-305.
[13] **Hassan, M.S. and Edwards, P.**; Evaluation of duckweed (*Lemnaperpusilla* and *Spirodelapolyrrhiza*) as feed for Nile tilapia (*Oreochromis niloticus*). Aquaculture; (1992); 104: 315-326, issues 3-4, 15 June 1992.
[14] **Heaton, W.C. and Rodgers, J.H. Jr**.; Common duckweed (*Lemna minor*) consumption by blue tilapia (*Oreochromis aureus*) in feeding trials. J. Aquat. Plant Manage; (2017); 55: 123-125.
[15] **Leng, R.A., Stambolie, J.H.and Bell, R.**; Duckweed – a potential high-protein feed resource for domestic animals and fish. Livestock Research for Rural Development; (1995) vol 7 no. 1 October 1995.
[16] **Nurjanah, T.N., Hidayat, T.andAmeliawati, M.A.**; Profile of micro-mineral and carotenoids in *Pomaceacanaliculata. Curr. Res. Nutr Food Sci Jour.*; (2019);Vol 7(1), 287-294 (2019).
[17] **Okomoda, V.T., Solomon, S.G. and Ataguba, G.A.**; Potential uses of the Family Lemnaceae. Journal of Agriculture and Veterinary Sciences; (2012); Volume 4, June 2012.
[18] **Olaniyi, C.O. and Oladunjoye, I.O**.; Replacement value of duckweed (*Lemna minor*) in Nile tilapia (*Oreochromis niloticus*) diet. Transnational Journal of Science and Technology; (2012); October 2012 edition vol.2, No. 9.
[19] **Philippine National Standard** Organic Aquaculture; (2016); Bureau of Agriculture and Fisheries Standards 112:2016.
[20] **Pinandoyo, J.H., Darmanto, O.K. Radjasa and Herawati, V.E.**; Growth and nutrient value of tilapia (*Oreochromis niloticus*) fed with *Lemna minor* meal based on different fermentation time. AACL Bioflux; (2019); Volume 12, Issue 1.
[21] **Roy, D.C., Pakhira, M.C. and Roy, M.**; Estimation of amino acids, minerals and other chemical compositions of *Azolla*. Advances in Life Sciences 5(7); (2016); 2692-2696, 2016.
[22] **Rusoff, L.L., Blakney, E.W. and CulleyD.D.**; Duckweeds (Lemnaceae Family): a potential source of protein and amino acids. J. Agric. Food Chem.; (1980); 28: 848-50.
[23] **Santiago, C.B. and Lovell, R.T.**; Amino acid requirement for growth of Nile tilapia. J. Nutr.; (1988); 118: 1540-1546.
[24] **Setlikova, I. and Adamek, Z.**; Feeding selectivity and growth of Nile tilapia (*Oreochromis niloticus* L.) fed on temperate-zone aquatic macrophytes. Czech J Anim. Sci.; (2004); 49, 2004 (6): 271-278.
[25] **Solomon, S.G. and Okomoda, V.T.**; Growth performance of *Oreochromis niloticus* fed duckweed (*Lemna minor*) based diets in outdoor hapas. International Journal of Research in Fisheries and Aquaculture; (2012); 2(4): 61-65.
[26] **Sonta, M., Rekiel, A.and Batorska, M.**; Use of duckweed (*Lemna*L.) in sustainable livestock production and aquaculture – a review. Ann. Anim. Sci., Vol. 19, No. 2 (2019) 257-271.
[27] **Tacon, A.G.J.**; The nutrition and feeding of farmed fish and shrimp – a training manual. 1. The essential nutrients. Food and Agriculture Organization of the United Nations; (1987).
[28] **Talukdar, M.Z.H., Shahjahan, M. and Rahman, M.S.**; Suitability of duckweed (*Lemna minor*) as feed for fish in polyculture system. Int. J. Agril. Res. Innov. & Tech. 2(1): 42-46, June, 2012.
[29] **F.A. Tavares, Rodrigues, J.R., Fracalossi, D.M., Esquivel, J.and Roubach, R.**; Dried duckweed and commercial feed promote adequate growth of performance of tilapia fingerlings. Biotemas, 21(3): 91-97, setembro de 2008.
[30] **Utami, R.H., Nirmala, K., Rusmana, I., Djokosetiyanto, D.and Hastuti, Y.P.**; The application of phytoremediation *Lemnaperpusilla* to increase the production performance of Nile tilapia *Oreochromis niloticus* in a recirculation system. JurnalAkuakultur Indonesia 17 (1), 24-42 (2018).
[31] **Wee, K.L.**; Use of non-conventional feedstuff of plant origin as fish feeds – is it practical and economically feasible? Pp. 13 – 32. *In* De Silva, S.S. (ed.) Fish nutrition research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop; (1991); Asian Fish. Soc. Spec. Publ. 5, 205p. Asian Fisheries Society, Manila, Philippines.

Author Profile

Rene Geraldo G. Ledesma is a licensed fisheries technologist and senior science research specialist at the National Fisheries Research and Development Institute of the Philippines currently doing nutrition research on aquatic animals.