# International Journal of Biosciences | IJB | ISSN: 2220-6655 (Print), 2222-5234 (Online) http://www.innspub.net Vol. 17, No. 6, p. 40-53, 2020

## **OPEN ACCESS**

Impact of high stocking density on growth parameters, amino acid profile and fatty acid profile of different fish species: a review

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Key words: Stocking density, Growth, Growth parameters, Amino acid profile, Fatty acid profile.

http://dx.doi.org/10.12692/ijb/17.6.40-53

Article published on December 12, 2020

## Abstract

Stocking density of fish depicts the number of fish that are stocked supplied at first per unit area. It is one of the most significant factors in determining the production of a fish farm. The stocking fish at low density results in high production costs, low economic gain and inadequate utilization of space. Increasing stocking density may activate stress response which negatively affects growth parameters, survival, behavior, health, feeding of fish. Different metabolic pathways related to lipid, carbohydrate and protein metabolism are also affected by this stress. So, for good yield fish should be stocked at optimum level. Optimum stocking density is the level where the maximum yields are reached. Fish are considered as a potential source of animal protein and essential nutrients in human diet. Amino acids, the building blocks of protein, act as a precursor of many enzymes, hormones, neurotransmitters, nucleic acids and other molecules essential for life. High stocking density affects amino acid profile of fish. The enzymatic activities and synthetic rates of functional proteins that is associated with responses of stress, HSD that is used as a stressor may influence amino acid requirements. High stocking density significantly affects water quality, growth parameters and amino acid profile of fish and it may induce stress in fish and may have negative impact on fatty acid profile if environmental conditions are not suitable for fish.

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#### Introduction

Aquaculture is the farming of aquatic organisms such as fish, mollusks, shellfish, and plants. Farmers have been associated with various types of aquaculture for many years, with early reported proof going back similarly as 500 BC in China (Allen et al., 2011). Aquaculture has two overall objectives, increase in production and increase in growth rate (Naylor et al., 2000). Aquaculture is a quickest developing industry with an overall extension in recent years and it appears that development is set to proceed. Due to this overexpansion welfare of farmed fish receive more attention. Welfare of farmed fish is not only important for product acceptance, personal perception, marketing but also important for product quality, its quantity as well as product efficiency. The world absolute interest for fish and fishery items is around 50 million tons to 183 million tons by 2015, and it is normal that out of this expansion, 73% will originate from aquaculture, representing 39% of worldwide fish production (FAO, 2004).

### Stocking density

The stocking density of fish describes the number of fish that are stocked initially per unit area. It is one of the most important factors in determining the production of a fish farm (Sayed, 2006). Increasing the biomass of fish per unit area result in increase overall production yield. However, stocking density has direct effect on fish survival, growth, behavior, health, water quality as well as feeding and production. Therefore, high biomass of fish tends to decrease production yield. Increasing number of fish per unit area can increase competition among fish for space and access to feed and it may reduce fish growth (Quiros, 1999). Furthermore, Water quality in fishponds is also affected due to high biomass which ultimately reduces fish growth. Optimum stocking density is the level where the yield reach to its maximum (Herrera, 2015) as shown in Fig. 1.

The selection of stocking densities of fish depends on two factors the first one is in economic importance and second is market demands of particular fish species. Increasing number of fishes may also reduce the average weight of fish. Therefore, a farmer suggests to stock fish with optimum range for yield to produce large fish. There are different factors affected by high stocking density as shown in Fig. 2. The biomass of fish is an important factor that determined the economic viability of the production system (Aksungu and Aksungur, 2007). Fishes stocked in pond with optimum density can be affected by different in environmental conditions and production system management such as temperature, dissolve oxygen (DO), quality of fish feed, species as well as sex of fish (Pompa and Green, 1990). Different strains of Nile Tilapia such as Chitralada, GIFT, GET-EXCEL, FaST Geno Mar and Supreme show different growth performance, production vield, mortality and resistance for environmental changes. (Dos Santos et al., 2007; Ponzonia et al., 2008).

#### Impact of high stocking on growth parameters

Stocking density is one of most important factor considered in aquaculture that effect fish growth. Several studies conducted to check effect of different rearing densities on growth parameters and metabolism in cultured fish species (Montero *et al.*, 1999; Alvarellos *et al.*, 2005; Herrera *et al.*, 2009; Li *et al.*, 2012). Increasing number of fishes per unit area may reduce fish body length (cm) and body weight (g) (Gomes *et al.*, 2000). Harbi and Siddiqui (2000) considered the impacts of feed and stocking density of hybrid tilapia (*Oreochromis niloticus x O. aureus*) on growth and water quality of fish. There were no noteworthy impacts of stocking densities and feed input either on nitrate nitrogen fixations or pH of the water.

High biomass of fish could activate stress among fishes due to high competition of feed and space tend to low metabolic activities such as decarboxylation of lipids, carbohydrate and protein (Costas *et al.*, 2008; Carrión *et al.*, 2012). While on the other hand high biomass resulted in higher yield per unit of production cost than low stocking biomass which consume high surface area and more cost comparatively HSD (Rahman *et al.*, 2006). Water quality could deteriorate with high (i.e., increase in

concentration of ammonium or nitrite) compromising the growth parameters of fish species (Deane and Woo, 2007; Dosdat *et al.*, 2003; Ferreira *et al.*, 2013; Foss *et al.*, 2009; Sinha *et al.*, 2012; Heras *et al.*, 2015). The best individual growth of *Heteropneustes fossilis* stocked with low number of fish show best growth performance (Narejo *et al.*, 2005). High biomass of fish could reduce its growth with an increase in the feed consumption rate (Aijun *et al.*, 2006). Dover sole fish show specific growth rate (SGR) significantly decreased with increasing biomass. Chances of mortality increase with increasing number of fish per unit area (Schram *et al.*, 2006).

Rahma et al. (2006) observed at harvesting time, the average weight gain of fish inversely affects stocking biomass. Bakeer et al. (2006) observed that gross and net yield production were significantly different. Stocking densities show direct relation with growth rate but specific growth rate, survival rate and feed conversion rate were unaffected. (Bakeer et al., 2006) Increasing number of fish the resulted in high yield production but it has inverse relation with growth parameters (Schram et al., 2006). Direct relation was observed between productivity and biomass of fish. However, Argyrosomus japonicus and Argyrosomus regius show positive relationship between growth rates and stocking densities (Cubillo et al., 2011; Pirozzi et al., 2009). Hosfeld et al. (2009) observed that growth of Atlantic salmon had no inverse effect with high biomass as long as circulation of water, water quality parameters and food rations were monitored equally. Check and balance of fish diet is an important parameter that can affect fish growth at high density. So, to avoid negative growth sufficient food should supply to get maximum yield and better growth (Hosfeld et al., 2009). Whitened fish show no negative impact of high biomass (Aijun et al., 2006).

Merino *et al.* (2007) examined that *Paralichthys californicus* can be culture in shallow tanks and raceways at a generally high stocking biomass without fundamentally affecting growth rate and survivability. Imsland *et al.* (2009) through his work proposed that profitability of fish expanded straightly with

increasing number of fish per area. Loading number of fish and improvement of culture system show inverse association through his work suggested (Ouattara et al., 2003; Gibtan et al., 2008; Chakraborty et al., 2010). No huge contrasts of high biomass (P > 0.05) in day by day weight increase, explicit development rate, mean weight gain, relative development rate (Osofero et al., 2009). No clear impact of large number of fish was seen on the survival rate and development of adolescent rabbit fish (Saoud et al., 2007). Fish growth rate diminished might be due to less feed and high stocking biomass (Ellis et al., 2002; Naderi et al., 2017). Feed conversion ratio (FCR) expanded with increasing number of fishes and it is recommended that high stocking biomass may affect and reduce fish development through decreasing food conversion efficiency (Li et al., 2011).

There is negative impact of High stocking biomass on fish growth rate. Also, the survival was lowest with high biomass (Ronald *et al.*, 2014. Ali *et al.* (2018) reported that growth parameters of *Rita rita* show negative impact on high biomass. Best survival rate and production rate of fish can be achieved when fish culture at low stocking density with suitable environmental conditions and maintained water quality parameters. At high biomass serum concentrations of glucose, triglyceride, and total cholesterol significantly increase as stocking density increased. High stocking density led to the decline in serum levels of thyroid hormones (Refaey *et al.*, 2018).

Zahedi *et al.* (2018) studied that final weight gain and condition factor was significantly decreased and feed conversion ratio was increased in high biomass as compared to low density biomass. Lemos *et al.* (2018) observed that circumneutral circumneutral pH and medium stocking biomass important parameters for culture of Nile tilapia. Overcrowding is detrimental for the scallop which cannot just lead to high mortality and moderate development, yet additionally aim more powerless against pathogenic microscopic organisms (Liu *et al.*, 2019). Medium number of fish stocked per unit area exhibited a higher muscle quality and healthier condition than those cultures with high biomass that could increase competition of feed among fish as well induce crowding stress (Zhao *et al.*, 2019). Fish culture with high density show negative impact on growth rate, stress, and immune responses of fish (Long *et al.*, 2019). High biomass of fish led to increase serum cortisol levels and suppress thyroxin levels. Overall, fishes stocked with high biomass induce chronic stress. Negatively affected the growth and feed conversion ratio of fish was a chronic stressor in this experiment and had a negative effect on the growth, feed conversion ratio (Yang *et al.*, 2020).

Mane *et al.* (2019) found out that the specific growth rate (SGR) values were statistically similar among all the treatments which indicated that higher stocking densities did not affect the instantaneous growth rate. Highest length gain and weight gain were obtained in the lower stocking densities which may be due to the less competition for space and food among fishes. The survival percentage for Rohu was highest in lower stocking densities after a culture period of 115 days. Survival rate in higher stocking densities was lower than the less stocking density, probably due to competition for food and space.

Sharma and Chakrabarti (2003) found out that FCR showed a direct relationship with stocking density. Food was more efficiently utilized in the lower stocking density, as indicated by a significantly lower (P < 0.05) value of FCR, compared to the high stocking density. Gomes et al. (2000) observed significantly higher value of SGR at low stocking density during the second week. This showed that space began to limit growth at the high stocking density. Wallace et al. (1988) observed that growth rate in Arctic charr, Salvelinusal pinus, declined after a near maxima, whereas in Atlantic cod, Gadus morhua, SGR was not influenced by stocking density (Baskerville-Bridges and Kling, 2000). Shireman et al. (1977) reported an increased growth for grass carp with decrease in density. Reduced growth of our fish at high stocking density as probably due to individuals disturbing each other during feeding and during normal activity. At low stocking density such disturbances might be absent resulting in increased growth and uniform sized fish.

Yousefi et al. (2016) found that most ordinary way to enhance production of fish in aquaculture sector is increasing stocking density per space unit. But increased fish stocking density can lead to crowding stress and deterioration of water quality. Persistent fish growth may slow down at high stocking density, expand disorder vulnerability, and may cause pressure responses (Sadhu et al., 2014). (Zhao et al. (2019) described that high and low stocking density may lead towards the anti-oxidative inhibition, lowest expressions of immune elements, infiltration of muscle and fats, thereby obtained better fat deposition and decrease resistance against disease. In short, Ctenopharyngodon idella at was cultured at medium stocking rate showed a good quality of fish muscle and more healthy state as compared to which are nourished in crowding strain or at utmost low stocking rate.

Borlongan and Satoh (2002) observed decrease in growth rate at HSD Probably due to higher energy expenses, intense voluntary suppression of appetite, antagonistic behavioral interchange (Duan *et al.*, 2011), food competition and competition for living space, and increased level of stress (Ouattara *et al.*, 2003). A number of authors have postulated that there exists a negative correlation between stocking density and growth of fish (Abdel-Tawwab 2012; Ayyat *et al.*, 2011; El-Sayed 2002; Ridha 2006).

# Impact of high stocking density on amino acid profile

Fish are considered as a likely potential source of animal protein and fundamental supplements in human weight control plans. Other than being nutritious, fish are good in taste and can effectively be processed. It is assessed that around 60% of individuals in many developing nations rely upon fish for over 30% of their animal protein supplies (Osibona *et al.*, 2009); thus, fish plays an important role in food security. Amino acids, the building blocks of protein, act as a precursor of many enzymes, hormones, neurotransmitters, nucleic acids and other molecules essential for life. They are classically divided into three categories: essential, non-essential and conditional essential amino acids. Amino acids play important role in cell signaling and act as regulators of gene expression and protein phosphorylation cascade, nutrient transport and metabolism in animal cells and innate and cellmediated immune responses (Mohanty *et al.*, 2014).

Roles of essential amino acids and nonessential amino acids in physiological functions and metabolism of aquatic animals given in Table 1 and Table 2.

Table 1. Roles of essential amino acids in physiological functions and metabolism of aquatic animals.

			Non-essential amino acids		
#	Amino acids	Metabolite	Function	Species	Reference
1	Cysteine	Glutathione	Antioxidant and cell signaling	All animal	Wu <i>et al.</i> , 2004
2	Aspartic Acid	Nucleotides	Regulates the secretion of important hormones	Tropical anchovy	Wu, 2010
3	Asparagine	Nucleotides	Genetic information storage and expression,	Various fishes	Li and Gatlin, 2006
			biosynthesis, immunity and reproduction. Used to		
			balance nitrogen in fish nutrition		
4	Serine	Directly	Used for treatment of schizophreni	Tropical anchovy	Wu, 2010
5	Glutamic Acid	GABA	Role in transamination reactions	Mackerel	Hou and Zhao, 2011
		Directly	Synthesis of key molecule such as glutathione	Atlantic Salmon	Sathivel <i>et al.</i> , 2005
6	Glutamine	GABA	Affecting secretion of pituitary hormones	Rainbow trout	Mañanos et al., 1999
		Direct	Improve intestinal structure and activate intestinal	Red drum	Cheng <i>et al.</i> , 2011
			enzymes	Channel catfish	Pohlenz et al., 2012
			Improve immune response Protect against oxidative		
			damage		
7	Glycine	Directly	Role in metabolic regulation, preventing tissue injury,	Catfishes	Mischoulon and Fava,
			enhancing antioxidant activity		2002
8	Alanine	Directly	Appetite	Many fishes	Shamushaki <i>et al.</i> , 2007
9	Proline	P5C	Enhance growth	Salmon	Aksnes et al., 2008
		Hydroxyproline	Collagen function		
10	Tyrosine	Epinephrine	Neurotransmitters that modulate stress responses	Flounder	Damasceno-Oliveira et
		norepinephrine			al., 2004

Among all basic amino acids, the extent of leucine and Arginine was high in Tilapia. Leucine concentration was high because leucine is limiting amino acid and solely utilized for body protein. Exclusively used for body protein synthesis. Leucine stimulates and catalase body protein and has significant remedial role in therapeutic conditions like injury and sepsis. Leucine has been found to slow the degeneration of muscle tissue by expanding the blend of muscle proteins (Mohanty *et al.*, 2014).

The lysine to arginine proportion may be assessed in fish diets to maintain a strategic distance from threats, in light of the fact that impeded proportions of lysine or arginine can lessen fish growth and feed proficiency (Wu, 2013). Amino acids significantly affect fish growth as given in Table 3. Zhao (2019) observed that in muscle tissues the concentration of both essential amino acids (EAAs) and nonessential amino acids (NEAAs) were observed in middle stocking density (MSD) and HSD (P < .05). While no significance difference were observed between MSD and HSD, the concentrations of histidine (His), arginine (Arg), valine (Val), isoleucine (Ile), phenylalanine (Phe), and leucine (Leu) in low stocking density (LSD) were significantly lower excluding for Leu. At the same time, at HSD in C. idellus muscles, the maximum level of EAAs was observed followed by middle and high stocking samples, respectively. In NEAAs, the concentrations of aspartic acid (Asp), serine (Ser), glutamate (Glu), and alanine (Ala) only found to have significant differences (P < .05), among Low and High stocking density groups whereas, no difference significantly

was observed when compared it to MSD. Overall, in ultra-upper density treatment group the higher level of NEAAs were mostly noted. However, in MSD Glycine (Gly) and alanine (Ala) were found greatest. Furthermore, by affecting the enzymatic activities and synthetic rates of functional proteins that are associated with responses of stress, high stocking density (HSD) that is used as a stressor may influence amino acid requirements (Costas *et al.*, 2007). Costas *et al.* (2008) indicated that significantly lower Trp concentrations are observed when fish is held at HSD. Although non-essential amino acids are de-novo synthesized in body but they play important role in nutritional mechanisms such as gene regulation, blood flow, nutrient transportation, development of adipose tissues, antioxidant responses, in immunity and cell signaling. Aspartic acid (FAA) and serine were found to be high throughout the study period and significantly showed differences (P < 0.05). Glutamic acid is the precursor of essential amino acids such as methionine, threonine, isoleucine, and lysine and regulates the secretion of important hormones. Also, serine is the forerunner of glycine, cysteine, and tryptophan and assumes numerous significant roles in cell flagging. Serine is additionally being utilized for treatment of schizophrenia.

Fable 2. Roles	of non-essentia	l aminc	o acids in p	hysio	logical	functions	and r	netab	olism o	f aquatic ar	nimals	•
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3	Asparagine	Nucleotides	Genetic information storage and expression, biosynthesis,	Various fishes	Li and Gatlin, 2006
			immunity and reproduction. Used to balance nitrogen in fish		
			nutrition		
4	Serine	Directly	Used for treatment of schizophreni	Tropical anchovy	Wu, 2010
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		Hydroxyproline	Collagen function		
10	Tyrosine	Epinephrine	Neurotransmitters that modulate stress responses	Flounder	Damasceno-Oliveira et al.,
		norepinephrine			2004

# Effect of high stocking density on fatty acid profile of fish

Lipids are the vital constituent of the food, as an essential fatty acids and energy source that fish requirement for elementary functions, such as development, reproduction and the conservation of well tissues (Porpoura and Alexis, 2001). The main lipid storage site in fish differ according to the species, they are sited mainly in the subcutaneous tissues, the belly fold, the muscular tissue, the liver, the mesenteric tissue and head (Ackman, 1994). The saturated fatty acids are dominated in fish lipids by myristic (C14:0) and palmitic (C16: 0) acids followed via stearic, while the main monounsaturated fatty acids are the oleic acids and palmitoleic acids (Kolakowska et al., 2003). Fish oils have been considered the significant sources of omega-3 fatty acids (Gbogouri et al., 2006), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that diminish risk of coronary heart disease, both DHA and EPA lower blood pressure and prevent hypertension development (Frenoux et al., 2001). Aidos et al. (2019) evaluated raising density consequence on development and growth of muscle in larvae of Siberian sturgeon (Acipenser baerrii). The three various stocking densities were experienced: high (HD, 150 larvae/1), mid (MD, 80 larvae/1) and low (LD, 30 larvae/1) at a recirculating aquaculture system. Among densities no difference was exposed in fatty acid (FA) profile, throughout development.

Table 3. Continued

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References

Daudpota and Kalhoro (2014)

Diana et al. (1994)

Garcia (2009)

Oliveira (2010)

Osofero and Otubusin (2009)

Ammar (2009)

Garcia et al. (2013)

Survival

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Yield

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#	References	Densities(fish/m <sup>2</sup> )	Mean Weight Gain	Survival	FCR (%)	Yield
1	Kapinga <i>et al</i> . (2014)	3, 13	$\downarrow$	$\downarrow$	$\downarrow$	1
2	Chakraborty and Banerjee (2010)	0.5,1, 1.5 ,2, 2.5 ,3	$\downarrow\uparrow$	$\downarrow$	$\downarrow$	$\downarrow\uparrow$
3	Ribeiro and Garcia (2009)	2, 4, 6, 8	$\downarrow$	$\downarrow$	1	1
4	Ronald <i>et al</i> . (2014)	1000, 1330, 2000,	$\downarrow$	$\downarrow$	1	$\downarrow$
		2670, 4000 and 5330 fry/m				
5	Klanian and Adame (2013)	400, 500, 600 fish/m	$\downarrow$	$\downarrow$	$\downarrow$	$\downarrow$
6	Chakraborty and Banerjee (2012)	1, 5, 10, 15, 25, 50, 75, 100fish/m <sup>3</sup>	$\downarrow\uparrow$	$\downarrow$	$\downarrow$	$\downarrow$

Densities(fish/m<sup>2</sup>)

200, 250. 300

fish/hapa

3,6,9 fish/ m<sup>3</sup>

2. 4. 6. 8 fish/ m<sup>3</sup>

90, 120, 150 fish/m

50, 100, 150, 200fish/m

1.2,1.6, 2.1 fish/m

133, 333, 416, 500

fish/m

Table 3. Summarized results of studies on the effect of different fish stocking density on growth parameters.

Tolussi et al. (2010) determined stocking density
effect on fatty acid and growth of metabolism of
Barycon insignis. In eight ponds fingerlings (360)
were distributed by 2 stocking densities (210 and 105
g/m <sup>3</sup> ). Muscle and plasma lipid content were not
influenced via stocking density, The plasma and
muscle lipid content were not affected by stocking
density, while fish raised in lesser stocking offered
elevated mass of lipid, by no variations in the
hepatosomatic index ethics.

The profile of fatty acid in liver and muscle neutral portion were not exaggerated through stocking density, while fatty acid in polar portions varied among 2 stocking densities. At maximum stocking densities, omega-3 polyunsaturated fatty acid (PUFA) and total PUFA improved in liver, generally because of improved in the docosahexaenoic acid. Montero *et al.* (1999) investigated effect of various stocking densities on colour uniqueness, lipid content/fatty acid content as well as protein content/amino acid content constitution of fillet of rainbow trout. Selected stocking density was five (group A), fifteen (Group B), twenty-five (Group C) kg fish /m<sup>3</sup>. The fish in group C had maximum level of PUFA, particularly omega 3, eicosapentaenoic acid and docosahexaenoic acid compared to group A and B. The oleic acid (18;1n-9) reduced at total lipids of liver in fish kept in higher stocking density and omega-3 high unsaturated fatty acid as well as arachidonic acid were decreased in polar lipids of liver in those fish. Monetro et al. (2001) studied to find out collective consequence of stress and essential fatty acid deficit on a number of biochemical and organic parameters. Common deficiency symptoms were shown in fish that eaten essential fatty acid deficient diet in less stocking density. The fish that eaten essential fatty acid deficient diet in high stocking density showed a maximum level of essential fatty acid and insufficiency signs top to give rise to death, deposition of liver lipid, decreased muscle lipid as well as omega-3 high unsaturated fatty acid (HUFA) fulfilled, that mainly eicosapentaenoic acid (EPA) effected other than docosahexaenoic acid (DHA), showed a unique maintenance of last fatty acid, particularly in phosphoglycerides division.

Mean Weight Gain

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Fig. 1. Optimum stocking density of fish per unit area.

Brown and Shahidi (1995) determined the lipid FA constitution of *Archtic charr*, stocked in 40, 75 and 50 kg/m<sup>3</sup>. In fish fillets lipid fulfillment improved throughout the rearing period of twenty-four weeks, while not affected through stocking density (r = -0.7030). Lipid FA constitution in fillets of *Archtic charr* persist comparatively not changed from eight week of feeding, consequently showing a return time

of lower than 8 weeks to intramuscular inclusion in dietary lipids. Between density groups, individual FA contents in fish muscle were analogous. The monounsaturated as well as saturated fatty acids contents were oppositely associated at stocking density (r = 0.9963 and -0.9914, respectively), while those PUFA were directly associated at stocking density (r = .9984).



Fig. 2. Factors affected by stocking density.

Aidos *et al.* (2018) investigated fatty acid profile as well as muscle development and growth of Siberian sturgeon free embryos at three various raising densities. The larvae were raised in 18°C, by three different stocking densities: high (HD, 150 larvae/1), mid (MD, 80 larvae/1) and low (LD, 30 larvae/1. Statistical difference was not observed among various stocking densities in regarding fatty acids. But at the time of development in spite of rearing density, It was a general pattern: alpha-linolenic acid and linoleic acids, significantly reduced their comparative content, while docosahexaenoic acid and arachidonic acid were significantly improved. It was concluded that mid density may be well appropriate for that species at this development stage.

### Conclusion

According to the current literature the stocking density of fish should be optimum with best water quality parameters and optimum feed management. In the fish farm it is not be possible to reach high yield through the increase in density without the application of mechanical aeration combined with water exchange, to increase water quality. Moreover, improving feed quality or ensuring stable supply of extruded food stuff may lead to decrease in production cost and cultivation can be done more efficiently.

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