

Accepted Manuscript

Effect of tank colour on foraging capacity, growth and survival of milkfish (*Chanos chanos*) larvae

Aritra Bera, M. Kailasam, Babita Mandal, Krishna Sukumaran, M. Makesh, Tanveer Hussain, T. Sivaramakrishnan, R. Subburaj, G. Thiagarajan, K.K. Vijayan



PII: S0044-8486(19)30744-6
DOI: <https://doi.org/10.1016/j.aquaculture.2019.734347>
Article Number: 734347
Reference: AQUA 734347
To appear in: *aquaculture*
Received date: 29 March 2019
Revised date: 27 June 2019
Accepted date: 27 July 2019

Please cite this article as: A. Bera, M. Kailasam, B. Mandal, et al., Effect of tank colour on foraging capacity, growth and survival of milkfish (*Chanos chanos*) larvae, *aquaculture*, <https://doi.org/10.1016/j.aquaculture.2019.734347>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Effect of tank colour on foraging capacity, growth and survival of milkfish**(*Chanos chanos*) larvae****Aritra Bera^{*}, M.Kailasam, Babita Mandal, Krishna Sukumaran, M. Makesh, Tanveer Hussain, T. Sivaramakrishnan, R. Subburaj, G.Thiagarajan, K.K.Vijayan**ICAR-Central Institute of Brackishwater Aquaculture, 75-Santhome High Road, RA Puram,
Chennai-28, Tamil Nadu, India.

*Author for Correspondence: Present address: Scientist, ICAR-Central Institute of Brackishwater Aquaculture, 75, Santhome High Road, Chennai, Tamil Nadu – 600 028, India. Telephone: +91 44 24618817 (Office); Fax: +91 44 24610311.

E-mail address: bera.aritra@gmail.com; aritra@ciba.res.in

Abstract

Larviculture of milkfish (*Chanos chanos*) associated with issues like larval deformity, mass mortalities which contribute in variable seed production. Effect of abiotic factors as rearing tank colour and illumination on larvae foraging behaviour, prey localization and ingestion were investigated to improvise milkfish larval rearing system. Reinforced Cement Concrete (RCC) tanks (capacity, 8 t; water salinity, 32 ppt) with three different background colors, white, blue and yellow were divided in five (05) treatment groups with triplicates, i.e. indoor white (T1/C), blue (T2), yellow (T3) color tanks with artificial illumination and semi outdoor blue (T4), yellow (T5) color tanks with solar illumination. Newly hatched milkfish larvae (tl, 3.4 mm) were stocked in the experimental tanks @ 2.5 no Γ^{-1} and apart from background colour and source of illumination uniform water quality and feeding regime were maintained in all treatments. Phytoplankton, *Chlorella salina* @ $10^3 - 10^4$ cells ml^{-1} were maintained from 2 dph to 20 dph; *Chlorella* grown rotifers, *Brachionus plicatilis* (enriched with *Nannochloropsis oculata* paste) were provided @ 20-30 no ml^{-1} from 3 dph to 14 dph

depending on the larval density. *Artemia* nauplii @ 0.5– 1.0 no ml⁻¹ was introduced from 15 dph. At the end of the experiment- 20 dph, highest ($p < 0.05$) larval survival (45 ± 5.63 %) was achieved in tanks providing yellow background colour (T5) compared to control and other treatments. Larval growth (tl., 17.1 ± 1.37 mm) was also found to be highest ($p < 0.05$) in T5. Increased survival and growth of milkfish in T5 was synchronized with significantly higher ($p < 0.05$) specific growth rate (SGR), larval gut content relative to other treatments. Milkfish larvae being a day feeder did maximum foraging during 0700h to 1600h evident from decreasing prey abundance during that period and as a result positive correlation found between larval standard length and gut content. Larval visibility enhancement in solar illuminated yellow tank act synergistically to perform necessary foraging to acquire nutritional energy for metamorphosis to fry. Above phenomenon may not have occurred in other treatments except T5 and partially in T3. Solar illuminated yellow colour tanks significantly contribute towards mass scale seed production of milkfish.

Key words: Milkfish, larval rearing, prey visibility, foraging capacity, yellow tank

1. Introduction:

Indoor finfish larval rearing system is completely different from natural environment where marine fish larvae have to hunt for food in order to survive harsh environment. Improvisation in indoor larval rearing technology increases growth and survival. Abiotic factors such as light and colour plays active role in larval visual field, retinal development, prey selection, foraging and survival (Shand et al., 2008 and Cobcroft et al., 2012). It is important to induce the foraging behaviour of early larvae through the provision of abiotic factors such as light and tank background colour for efficient live feed preying. Indoor larval rearing simultaneously modifies the larval natural feeding behaviour (Butts, 2016). Under rearing conditions marine larvae do not utilize their full capacity of highly adaptive sensory systems

to detect and locate prey, but rather, they are able to obtain food using a limited range of their sensory potential primarily visual capacity (Ullmann et al., 2011). Larval visual ability is dependent on its spectral sensitivity of retina, visual capabilities, ambient light environment, photoperiod and most importantly tank background colour. Tank background colour may play a critical factorial role as it can change visual field of larvae. Early stage larvae having primitive visual field can identify live prey against tank background if higher contrast is provided (Shand et al., 2008).

There are of reports where effect of different tank background colour and light spectrum on larval survival and growth has been studied in different finfish species but not sufficient to understand how tank background colour affects foraging behaviour, visual field and prey localization in tank. Effect of tank background colour in finfish larvae of Eurasian perch, *Perca fluviatilis* (Tamazouzt et al., 2000), juvenile rainbow trout, *Oncorhynchus mykiss* (Luchiari and Pirhonen, 2008) , yellow perch, *Perca flavescens* (Hinshaw, 1985 & Jentoft et al., 2006), mahi mahi, *Coryphaena hippurus* (Ostrowski, 1989), striped bass, *Morone saxatilis* (Martin- Robichaud and Peterson, 1998), grouper, *Epinephelus suillus* (Duray et al., 1996), haddock, *Melanogrammus aeglefinus* (Downing and Litvak, 1999), guppies, *Poecilia reticulata* (Ruchin, 2004) and Black bream (Shand et al., 2008), *Acanthopagrus butcheri* were studied. Varied background colour develops different frequency of shorter or longer-wavelength sensitive cones in retina which helped larvae to enhance its visual field for prey localization (Shand et al., 2008). In depth research into these factors will help to improve feeding rates and therefore improve efficiency in the aquaculture industry.

Milkfish (*Chanos chanos*) is an acclaimed food fish widely cultured in countries such as Philippines, Indonesia and Taiwan (Gapasin et al., 1998). During 2016 milkfish farming contributed 1188 thousand t production which is 2% of total finfish produced globally from

aquaculture (FAO, 2018). Indian milkfish aquaculture is traditional way of farming and predominately dependent on wild caught seeds. In India prevalence of milkfish seed from wild has been reported during two season viz., March – May and Sept – Oct from coastal Andhra Pradesh and Rameswaram or Pamban areas of Tamil Nadu. The wild caught seeds are not adequate and are often mixed with predatory species (Silas et al., 1982). Therefore, research towards developing milkfish captive breeding and seed production technology is the need of the hour to meet the seed demand for the farming community. To overcome this problem ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA), Chennai, India, has standardized comprehensive technology of induced breeding of milkfish during June 2015 and since then severe need was felt to improve larval survival. In spite of milkfish breeding (Lee et al., 1986) and seed production technology developed by Aquaculture Department of Southeast Asian Fisheries Development Centre issues such as larval deformity, mass mortalities, and variable production still exists. Reports are scanty on use of different tank colour and strict feeding regime on milkfish larvae survival. Milkfish being transparent larvae gives us an opportunity to understand its feeding preferences and capacity in a vivid way. Vision in fish larvae is a neural process which starts with absorption of light by retinal photoreceptors. Hence larval ability to detect a light stimulus is dependent on spectral absorption properties of opsin proteins in photoreceptors of retina. It has been found that in vertebrates there are four opsin classes in retinal cone photoreceptors i.e. short wavelength-sensitive 1 (SWS1) pigments having peak sensitivities (λ_{max}) in the UV–violet region of the spectrum, short wavelength sensitive 2 (SWS2) pigments with λ_{max} in the blue region, middle wavelength-sensitive rod-like (Rh2) pigments with λ_{max} in the green region, and long wavelength-sensitive (LWS) pigments with λ_{max} in the yellow–red region (Shand et al., 2008). In this study we selected three different tank colours i.e. white, blue and yellow from entire range of spectral sensitivity to understand how reflection type, scattering

phenomenon influences spectral perceiving power of larvae ultimately influencing preying capacity growth and survival. The objective of this experiment was to study the effect of tank background colour viz. white, blue and yellow on the foraging capacity, growth and survival of milkfish larvae.

2. Materials and methods

2.1. Milkfish Larvae

Fertilized eggs of milkfish (*Chanos chanos*) were obtained from hormone pellet implanted more than 10 years old broodstocks (Total 16 numbers, 4.4 – 7.2 kg) maintained in 144 t capacity open RCC tank at Muttukadu Experimental Station of ICAR-CIBA, Chennai, India. Eggs (1.24 mm dia) were collected from egg collection tank during early morning (6 am) and incubated in 500 l capacity conical FRP tanks with mild flow through (1.75 l/min) of filtered seawater (Salinity 32 ppt, Temperature 27° C - 29° C) and constant aeration to facilitate movement and floatation of eggs to hatch out within 25 h – 26 h after fertilization (Lee et al., 1986a & 1986b).

2.2. Ethics Statement

The research undertaken complies with the current animal welfare laws in India. Even though fish larvae have been used in the present study, no animals have been stressed or sacrificed for the same. However, care and treatment of brood stock used in this study for procurement of eggs and larvae, were in accordance with the guidelines of the CPCSEA [Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment & Forests (Animal Welfare Division), Govt. of India] on care and use of animals in scientific research. The study was undertaken with approval of statutory authorities of the Central

Institute of Brackishwater Aquaculture, Chennai, India. As the experimental fish, *Chanos chanos* is not an endangered fish, the provisions of the Govt. of India's Wildlife Protection Act of 1972 are not applicable for experiments on this fish.

2.3. Experimental design and larval rearing

Fifteen RCC tanks (each capacity, 8 t; water salinity, 32 ppt) with three different background colours, white, blue and yellow were divided in five (05) treatments groups i.e. indoor white (T1/C), blue (T2), and yellow (T3) background color tanks with artificial illumination and semi outdoor blue (T4), and yellow (T5) background color tanks with solar illumination in triplicates. Tanks were painted with epoxy color having code yellow (7861), Blue (9206) and white (L152) from Asian Paints, India following manufactures instruction. Treatments such as T1, T2 and T3 were illuminated with non-heating white florescent light provided with timer regulated 12 h: 12 h: L: D photoperiod. Treatments such as T4 and T5 were also maintained 12 h: 12 h: L: D photoperiod by using collapsible black sheet during 18.00hr to 06.00 hr for 12 h interval over tank (Average day length was 12.38 hr). White background tanks under solar illumination was prone to infest with algae on wall and lost white background very fast and was not considered for experiment. Apart from background colour and source of illumination other factors such as water quality, water exchange rate, feeding frequency etc. were uniformly maintained in the larval rearing tank (LRT) in all the treatments. Newly hatched milkfish larvae having a large yolk sac with initial mean total length (TL) of 3.4 ± 0.06 mm were collected from incubation tank and stocked in RCC tanks at the rate of 2.5 larvae L^{-1} . Total of 0.9 million newly hatched larvae of three cohorts (each 0.3 million larvae from three different spawning) were equally distributed in triplicate in 15 RCC tanks @ 20000 numbers/tank over three larval rearing cycles (March-May 2017) of each 21 days.

Phytoplankton, *Chlorella salina* with mass culture cell density of 0.4-0.5 million cells mL^{-1} were introduced in LRTs from 2 dph to 20 dph to maintain the green water with a cell density of 10^3 to 10^4 cells mL^{-1} . Mass cultured *Brachionus plicatilis* (140 – 210 μm) were collected and enriched with frozen green algae paste *Nannochloropsis oculata* (Nanno 3600 TM, 68 billion cells/ml) (Thépot, 2016) and fresh *Chlorella salina* (1:1 ratio) in a 100 L volume container overnight and were supplied as initial feed to the larvae @ 20-30 numbers mL^{-1} from 3 dph to 14 dph. Larvae were co-fed with rotifers until 15 dph during the rotifer–*Artemia* transition (Woolley et al., 2012). Newly hatched *Artemia* nauplii @ 0.5– 1.0 numbers mL^{-1} was introduced from 15 dph till 20 dph following initiation of weaning to artificial feed (200-300 μm) from 21 dph. Water exchange was initiated from 6 dph, initially at 10 % once a day, and increased to 50 % once a day by 20 dph. Rotifer and *Artemia* nauplii were added two times a day only (0700 h & 1600 h) without adding extra prey in-between during study period. Prey was counted every 3 h till 20 dph and any reduction recorded in the prey density was adjusted by adding the required feed in the experimental tanks following the above feeding schedule. Sufficient aeration was provided in LRTs to ensure homogenous distribution of algae and live feeds throughout the water column. Sand filtered seawater at 28° C - 29° C was used for entire study period and important water quality parameters were always in optimum range (APHA 2005; Bagarinao, 1986; Kailasam et al., 2002 & 2007; Sorgeloos et al., 1986; Gapasin and Marte 1990).

2.4. Larval growth and survival rate analysis

A total of 30 larvae from each triplicate of all the treatments were sampled during 0, 5, 10, 14 and 20 dph for Specific Growth Rate (SGR), length and weight analysis and survival rate estimation. Milkfish larvae were categorized into pre-flexion larvae (TL 5.0-6.2 mm), flexion

larvae (TL 5.4-10 mm), post flexion larvae (TL 10-17 mm) and metamorphosed larvae (TL 15-20 mm) during sampling according to Bagarinao, T.U., 1999. Samples of larvae were anaesthetized in 0.6% 2-phenoxyethanol (Himedia, India) solution (Cobcroft et al., 2012) and morphometric characteristics were measured in Motic BA210 trinocular microscope using software to measure morphometric parameters. Weight was taken (pooled data of 30 larvae) using an electronic digital balance after blotting the larvae with water absorbent paper (Biswas et al., 2010)

Following parameters were calculated using the formula given bellow

$$1. \text{ Specific Growth Rate (SGR)} = \frac{\text{Log (Final Weight)} - \text{Log (Initial Weight)}}{\text{Rearing duration (days)}} \times 100$$

Weight recorded in milligram (mg) after hatching and 20 dph.

2. The mathematical relationship between length and weight was calculated at 20 dph using the conventional formula, $W = aL^b$, by regression after log transformation (Pauly, 1993; Le Cren, 1951). Where, W=Weight of fish (mg), L is observed total length (mm), 'a' is the regression intercept and 'b' is the regression slope. The logarithmic transformation of the above formula is-

$$\text{Log } W = \text{Log } a + b \text{ Log } L$$

Final number of larvae

$$3. \text{ Survival rate (\%)} = \frac{\text{Final number of larvae}}{\text{Initial number of larvae}} \times 100$$

Survival rate was estimated using above equation in representative 30 l nylon cage (300 μ m mesh size) with 50 larvae inside respective LRTs and other larval rearing condition was similar.

2.5. Estimation of feed consumption and gut content analysis

Enriched rotifers were harvested from bin by syphoning the rotifer culture over a sub-merged 55 μ m screen to ensure survival. Harvested rotifers were washed with UV treated, filtered seawater and fed to the larvae. To understand the diurnal abundance of rotifers in different treatments, systematic alternate day sampling were done during 3 dph – 14 dph (before introduction of artemia) and 15 dph – 18 dph (after introduction of artemia) phase at 0600 h, 0700 h (feeding point), 1000 h, 1300 h, 1600 h (feeding point), and 1900 h. Number of rotifers was estimated by counting 1 ml samples ($n = 4$) using a Sedgwick rafter counter and a Motic BA210 trinocular microscope. *Artemia* sp count was also estimated in similar procedure only during 15dph – 21 dph (Thépot et al., 2016). Estimation of larval gut content procedure was modified from Butt (2016) and Blanco et al. (2017). Milkfish larvae are transparent and gut content is clearly visible under light microscope. Ingested rotifers can be identified and counted by presence of lorica under microscope within 1 h of feeding. Artemia is visible inside gut due to contrast in colour and shape. Gut rotifer content was estimated between 5 dph and 14 dph in all the treatments while gut artemia content was estimated between 15 dph and 21 dph. Light microscope pictures of 5 dph and 10 dph larvae were analyzed for qualitative assessment of gut content in different treatments. Prey was counted in entire length of gut in larvae and expressed as number/gut ($n = 30$). Gut content was denoted as EG: empty gut, RT: rotifer, RT⁺: more rotifer, RT/ EG⁺: less rotifer- more empty gut, RT⁺/ EG⁻: more rotifer – less empty gut (Fig.4C-4L). Correlation study was made with gut rotifer content and larval standard length at 14 dph to understand interdependency of both the factors.

2.6. Statistical analysis

Statistical significance of different parameters was analysed using one-way analysis of variance (ANOVA) and Student's paired t test via SPSS 19.0 for Windows. Tukey method was used for post hoc comparison of mean ($P < 0.05$) between different tank background colour. All data presented in the text, figures and tables are means \pm standard error and statistical significance for all statistical tests were set at $P < 0.05$. Asterisks were used to indicate significant differences between two different treatments while comparing at same time point and within same treatment at different time point (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

3.Result

3.1. Development, growth and survival response of milkfish larvae

The growth of milkfish was determined by increase in standard length (SL), SGR, L-W relationship i.e. b value and overall survival. Maximum ($p < 0.05$) larval SL (17.1 ± 1.37 mm) was recorded in T5 at 20 dph with complete metamorphosis (Fig. 1B). Concomitant with this result it was observed that solar illuminated yellow tank (T5) had improved ($p < 0.01$) SL in larvae compared to artificially illuminated yellow tank (T3) as well as control (T1) ($p < 0.001$) with 12.1 ± 0.86 mm and 9.5 ± 0.50 mm length respectively. In all the treatments significant ($p < 0.05$) increase in SL was noticed during 10 dph to 20 dph but T1 larvae reached only flexion stage (SL: 5.4 -10 mm) whereas larvae in T2, T3 and T4 were able to reach post flexion (SL: 10 -17 mm) stages at 20 dph. Flexion larvae were transparent and without forked caudal fin where as in of post flexion stage larvae were showing pigment deposition with characteristic forked caudal fin. After 20 day of rearing specific growth rate of larvae also followed the similar trend and maximum ($p < 0.05$) SGR was recorded in T5 with 3.2 ± 0.7 %/day compared to C, T2, T3, T4 with SGR of 1.21 ± 0.14 %/day, $1.64 \pm$

0.03 %/day, $2.7 \pm 0.02\%$ /day and 2.48 ± 0.06 %/day, respectively. Length weight relationship of larvae in different tank background clearly indicates allometric growth pattern with $b \neq 3$. Larvae grown in T5 showed allometric growth with b value of 3.66 near to 3 with r^2 value around 0.93 (Table 1). Survival of milkfish larvae gradually decreased ($p < 0.05$) from 5 dph to 20 dph in all the treatments and significantly ($p < 0.05$) high survival was achieved in T5 with 45 ± 5.63 % compared to T4 and C with survival of $32 \pm 3.2\%$ and $10 \pm 0.98\%$ respectively. It was evident that in all the treatment group significant reduction in survival happened during 5 dph – 10 dph. Survival during 10 dph – 20 dph was stable in all the treatments. (Fig. 1A)

3.2. Consumption rate and abundance of rotifer and artemia in larval rearing tank

There is a clear variation of rotifer and artemia in all the larval rearing tanks of different treatments. Prey abundance was checked in LRTs every 3 hours and prey was introduced two times during 0700 h and 1600 h. Relative abundance of live prey in tank was proportionate with rate of prey ingestion by milkfish larvae. During 3 dph to 14 dph rotifer abundance pattern in all the groups except C, indicates lowest ($p < 0.05$) prey abundance during 1600 h before feeding and subsequently increase at night during 1900 h with a reduction at morning 0600 h. Rotifer abundance pattern rest of the day also followed similar trend with a peak at 0700 hr after feeding, with gradual decrease at 1000 h, 1300 h and 1600 h. In spite of following general abundance pattern absolute rotifer density in tanks varied significantly ($p < 0.05$) within treatments at different time points, except control. Rotifer abundance was found lowest at T5 in all sampling points showing minimum (9.00 ± 0.72 number/ml) at 1600 h compared to control with 21.00 ± 2.45 number/ml at same time from initial average prey density of 20.00 ± 0.01 number/ml and 29.00 ± 2.50 number/ml respectively. It is remarkable to note that rotifer preying rate in control was very slow compared to T5 (Table 2) throughout

the day. Similar trend of rotifer abundance is true during 15 dph to 18 dph of larval rearing when rotifer co-feeding is in practice with artemia. Post 14 dph of larval rearing lowest rotifer abundance during 1600h in T5 and control was found to be 02.00 ± 0.14 number/ml and 14.00 ± 1.51 number/ml respectively. Residual rotifer contributing to relative abundance was always high in T2, T3 and T4 during 3 dph to 14 dph as well as during 15 dph to 18 dph. Artemia abundance since 15 dph was recorded highest ($p < 0.05$) in control with maximum and minimum values of 1.52 ± 0.09 number/ml and 1.10 ± 0.10 no/ml during 0700 h and 1600 h respectively whereas with significant reduction ($p < 0.05$) at same time point in T5 maximum and minimum artemia abundance were recorded as 0.50 ± 0.03 number/ml and 0.30 ± 0.04 numbers/ml respectively. Residual artemia contributing to relative abundance was always high in T2, T3 and T4 compared to T5 during 15 dph to 21 dph (Fig 2. A-C).

3.3. Gut rotifer and artemia content

Relative prey abundance and gut content of larvae in same tank is inversely proportional i.e. higher the relative abundance of rotifer or artemia in tank, lesser the count inside the gut and vice versa. It was found that gut rotifer content in milkfish larvae was significantly higher ($p < 0.01$) in T5 both at 5 dph and 14 dph with 10.5 ± 0.04 number/gut and 14.78 ± 0.13 numbers/gut respectively, compared to control having 4.11 ± 0.04 numbers/gut and 6.66 ± 0.01 numbers/gut during same point of time (Fig. 3A). Gut rotifer content in 5 dph larvae (Fig 3.C-G) were analysed and T5 is showing gut full of rotifer (RT^+ / EG^-) but T1/C, T2, T3 have substantially empty gut (EG) containing unidentified masses and sporadically occurred rotifers. Similar trend was found during 10 dph where T1, T2 and T3 showed comparatively higher occurrence of rotifer although higher percentage of empty gut (EG^+) prevailed as larvae has grown in five days but larvae in T5 were found with gut full of rotifers (RT^+ / EG^-) (Fig 3. H-L). As higher gut content has positive bearing on larval growth, it was found that

during 14 dph larval length is positively correlated with gut content with a r^2 value of 0.97 (Fig.2D). Similar to gut rotifer content artemia was also found to be significantly higher ($p < 0.001$) in T5 with 4.12 ± 0.01 number/gut and 7.23 ± 0.06 number/gut during 15 dph and 21 dph respectively, compared to control having 2.9 ± 0.00 numbers/gut and 4.22 ± 0.01 numbers/gut during same point of time (Fig. 3B).

4. Discussion

Larval nutrition is one of the key factors in growth and survival during initial phase of larval rearing in most of the marine finfish hatchery. It was found that milkfish larval nutrition is dependent on larvae's ability to prey upon live feeds. Tank background colour/visual environment has profound effect on larval growth, survival, malformation, prey ingestion capacity etc. Background of tank colour significantly affects the feeding intensity when other abiotic and biotic factors are uniform. Proper larval nutrition gives balanced amount of dietary micronutrients, HUFA, phospholipids etc. which are physiological requirements for metamorphosis (Koven et al., 2018).

4.1. Semi-outdoor tank with yellow colour background improves growth and survival

The reflection of incident light from larval rearing tank can be roughly categorized into two types of reflection: specular reflection where light reflected from surface at a definite angle, and diffuse reflection, where light reflects in all directions. Reflection type, intensity of spectrums of incident light from tank wall is dependent on background colour and associated with phototactic responses of larvae (Cobcroft et al., 2012). It is reported that white tanks reflects more light than other tank background and induces a strong phototactic response of larvae towards the wall causing larval injury and distraction from prey. It is documented that larvae do maximum walling behaviour in white tanks and induce early appearance of jaw malformation (Cobcroft et al., 2009 and 2012). Scattering is a form of diffuse reflection of

light and higher the scattering higher is the contrast of prey within tank. Tank other than white and blue colour increases the scattering phenomenon and eventually prey visibility. Larval nutrition must have compromised in T1, T2 and T4 where lower prey intake during rotifer and artemia feeding phase due to poor scattering phenomenon within tank which further lowered the prey visibility to milkfish larvae. In artificially illuminated tanks (T3) with yellow background have higher light scattering than T1, T2, T4 but lower than T5, as it was illuminated by sunlight. As solar illuminated yellow colour tanks have maximum scattering (Shand et al., 2008) along with light scattered by algal cells or other suspended particles in the culture water significantly reduced specular reflection from tank walls compared to white and blue tanks and encouraged a uniform distribution of milkfish larvae across the tank. Milkfish larvae in nature lives as part of small zooplankton community in spectrally poor turbid environment such as estuaries, backwaters, lagoons. Planktonic larvae must feed continuously during day time, survive by avoiding predators and travel to preferred location for larval development. Fish larval vision is very important factor in daytime feeding unlike shrimp larvae which depends mainly on chemoreception for night time feeding. Colour vision requires at least two types of photoreceptors with different spectral sensitivities in blue, green and yellow spectral regions. Different colours have different contrasts against background colour and influence the efficiency of detecting and catching the prey or feeds by sight. A high contrast leads to higher visibility and more prey ingestion. In nature water turbidity can affect preying rate by increasing or decreasing contrast between prey and background due to the scattering of incident light (Kawamura, G et al., 2016). Similarly in our experiment tank background colour played role same as turbidity by increasing or decreasing contrast between prey and background due to scattering of light. As yellow colour tank did maximum scattering must have given better contrast for rotifer and artemia for milkfish larvae and contributed in potentially high prey ingestion and survival as high as

45±5.63 %. Consistent growth in T5 may be due to initial significant growth contributed in increased visual field with age and development till 20 dph. Lower prey contrast in white and blue tank due to lesser contrast contributed in lower survival due to insufficient feeding. In terms of tank background colour, higher standard length, specific growth rate and survival in T5 can be explained by a potentially higher prey contrast vision of larvae to rotifers and artemia against a yellow background compared with the white and blue wall background (Browman and Marcotte, 1987; Ostrowski, 1989; Utne-Palm, 1999).

4.2. Scattering in yellow background tank increases prey visibility

Yellow environment provides best contrast for food which is brown (rotifer) in colour. Best growth in barramundi was found in red and yellow environment (Ullman et al., 2011). In trichromatic human eye perceived yellow as most bright colour as it stimulates both green and red photoreceptors. Different tank backgrounds generate differing spectral irradiance in tank. Milkfish being a pelagic teleost tend to possess wide range of visual pigments inside photoreceptor in retina as they lived in a varied spectral irradiance in varied salinities during evolution in nature since their appearance 40-50 billion years ago (Bagarinao et al., 1999). As milkfish larvae find it easy to prey upon in yellow background tank, it may be due to the retina having long wavelength sensitive (LWS) pigments in opsin protein mosaic showing peak sensitivity (λ_{\max}) in the yellow – red region of spectra which is bouncing back from yellow wall (Shand et al., 2008). These pigments are vitamin A derived and algae enriched rotifers are good source of Vit A for larvae. As larval nutrition improves in T5 it further contributes adaption during metamorphosis for improved survival. Rearing of black bream, *Acanthopagrus butcheri* in yellow environment significantly increased LWS pigments in retina. Under yellow environment milkfish larvae also must have adopted above strategy. In our experiment tanks were illuminated with artificial fluorescent light or sunlight both having

entire range of spectral wavelengths. It is well documented that lower the wavelength higher is the scattering event. As source light contains blue spectra having shorter wavelength, it must have contributed to the required scattering (blue light causes maximum scattering) phenomenon while bouncing back from yellow background tanks, in contrary which must have absorbed by blue colour tanks. Majority of teleost larvae naturally have shorter wavelength sensitivity towards blue spectrum and it may be concluded that naturally present SWS pigments helped milkfish to prey with less effort in T5 as scattering from yellow wall act as an advantage for milkfish larvae with a vision evolutionarily biased to use it for prey locationing (Shand et al., 2008, Ullmann et al., 2011). Additional expression of LWS pigments in retina helped to perceive yellow background which is otherwise absent in natural environment. In solar illuminated yellow colour tank (T5) (Fig. 4) milkfish larvae enhanced their preying capacity with enhanced vision of prey which were uniformly scattering from yellow background and perceived by LWS pigment in retina.

4.3. Milkfish larvae is a daytime feeder

Taking clue from above discussion it may be explained that milkfish is a sight feeder i.e. preying capacity is external light dependent. Benitez, L. V. et al. (1989) already found that intestinal amylase activity consistently reached the peak at about noon when milkfish gut was full. This confirms that milkfish is a daytime feeder. Our experiment also shows similar result as residual rotifer and artemia in larval tank starts decreasing since 1000 h and found minimum during 1600 h. As discussed earlier T5 improved larval capacity to prey upon live feeds, it is also encouraging to notice that different degree of preying capacity has developed a diurnal pattern of rotifer and artemia abundance inside tank. Larvae with enhanced vision in T5 and T3 has showed increased preying capacity with less residual prey in every time point whereas in T1, T2 , T4 larvae with inadequate vision to prey upon live rotifers or artemia

significantly increased residual numbers per milliliter of water compared to T5 and T4. Carnivorous fish like Asian seabass (*Lates calcarifer*) larvae contains abundant rods and large cone cells as well as lipid tapetum lucidum at back of the eye which reflects light back into the retina causing the pupil to glow in the dark and ultimately gives ability to see in the dark (Iigo et al., 1997). Unlike seabass, milkfish don't have night vision and mainly feeds on day time. During initial 21 days of larval rearing milkfish need to metamorphose majorly from daytime feeding unlike seabass which have the luxury to prey upon during night. This explains why milkfish larvae in experiment needed more than 16-20 numbers (Fig. 3L) of rotifer/ml of water compared to species like Asian seabass, snapper and rabbitfish where below 20 numbers of rotifer/ml of water is sufficient during day time (Marte et al. 2003). Comparatively high abundance of residual prey in all the treatment groups after 1900 h can be explained from above phenomenon. Gut rotifer and artemia content is inversely proportional to the amount of residual prey per millilitre of water. Larvae from T5 and T3 showed higher rotifer and artemia gut content which is marker of enhanced foraging capacity. Maximum gut rotifer content in larvae from solar illuminated yellow tank (T5) post yolk absorption since 5 dph to 10 dph (Fig. 3G & 3L) easily explains required reduction of rotifer in tank water compared to control and this sequential feeding strategy is highly correlated (Fig. 2D) with final larval growth (Koven et al., 2018)

5. Conclusion

Milkfish (*Chanos chanos*) larval rearing technology was not available for Indian condition. As the fish breed under captive conditions first time in India during June 2015 at ICAR-CIBA muttukadu experimental station, need has been felt to fine tune the existing milkfish larval rearing technology available in literature. It has been observed that milkfish larval rearing is successful in yellow background tanks which are illuminated by solar or artificial

fluorescent light. We have understood that yellow background tank do maximum scattering of light from its wall which in turn helps in enhancing visibility and contrast of prey in yellow background for milkfish larvae. Milkfish larvae being a day feeder do maximum foraging during 0700 h to 1600 h and visibility enhancement during that period act synergistically to perform required foraging to harness nutritional energy for metamorphosis to fry in next 21 days. Yellow tank rearing method for mass milkfish seed production is giving significantly better result than other tank colours.

Acknowledgement

The authors thank Indian Council of Agricultural Research for providing fund support to carry out this research programme. We also appreciate fish hatchery staff at Muttukadu Experimental Station for their help in conducting the experiment.

References

American Public Health Association, APHA. 2005. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. American Public Health Association, Washington DC, 1220p.

Bagarinao, T., 1986. Yolk resorption, onset of feeding and survival potential of larvae of three tropical marine fish species reared in the hatchery. *Mar. Biol.* 91, 449–459.

Bagarinao, T.U., 1999. Ecology and farming of milkfish. *Ecology and farming of milkfish*. Tigbauan, Iloilo, Philippines: Southeast Asian Fisheries Development Centre, Aquaculture Department.

Benitez, L. V., 1989. Milkfish nutrition: a review. In R. D. Fortes, L. C. Darvin, & D. L. de Guzman (Eds.), Fish and crustacean feeds and nutrition: Proceedings of the seminar-workshop on fish and crustacean feeds and nutrition held on 25-26 February 1985 at UPV, Iloilo City, pp.31-34.

Biswas, G., Thirunavukkarasu, A.R., Sundaray, J.K. and Kailasam, M., 2010. Optimization of feeding frequency of Asian seabass (*Lates calcarifer*) fry reared in net cages under brackishwater environment. *Aquaculture*, 305(1-4), 26-31.

Blanco, E., Reglero, P., Ortega, A., de la Gándara, F., Fiksen, Ø. and Folkvord, A., 2017. The effects of light, darkness and intermittent feeding on the growth and survival of reared Atlantic bonito and Atlantic bluefin tuna larvae. *Aquaculture*, 479, 233-239.

Browman, H.I., Marcotte, B.M., 1987. Effects of prey color and background color on feeding by Atlantic salmon alevins. *Progressive Fish Culturist* 49, 141–143

Butts, I.A.E., Sørensen, S.R., Politis, S.N. and Tomkiewicz, J., 2016. First-feeding by European eel larvae: A step towards closing the life cycle in captivity. *Aquaculture*, 464, 451-458.

Cobcroft, J.M. and Battaglione, S.C., 2009. Jaw malformation in striped trumpeter *Latris lineata* larvae linked to walling behaviour and tank colour. *Aquaculture*, 289(3-4), 274-282.

Cobcroft, J.M., Shu-Chien, A.C., Kuah, M.K., Jaya-Ram, A. and Battaglione, S.C., 2012. The effects of tank colour, live food enrichment and greenwater on the early onset of jaw malformation in striped trumpeter larvae. *Aquaculture*, 356, 61-72.

Downing, G., Litvak, M.K., 1999. The effect of photoperiod, tank colour and light intensity on growth of larval haddock. *Aquaculture International* 7, 369–382.

Duray, M.N., Estudillo, C.B., Alpasan, L.G., 1996. The effect of background colour and rotifer density on rotifer intake, growth and survival of the grouper (*Epinephelus suillus*) larvae. *Aquaculture* 146, 217–224

FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome.

Gapasin, R. S. J., & Marte, C. L., 1990. Milkfish hatchery operations. Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department.

Gapasin, R.S.J., Bombeo, R., Lavens, P., Sorgeloos, P. and Nelis, H., 1998. Enrichment of live food with essential fatty acids and vitamin C: effects on milkfish (*Chanos chanos*) larval performance. *Aquaculture*, 162(3-4), 269-286.

Hinshaw, J.M., 1985. Effects of illumination and prey contrast on survival and growth of larval yellow perch *Perca flavescens*. *Transactions of the American Fisheries Society* 114, 540–545.

Iigo, M., Furukawa, K., Nishi, G., Tabata, M. and Aida, K., 2007. Ocular melatonin rhythms in teleost fish. *Brain, behavior and evolution*, 69(2), 114-121.

Jentoft, S., Øxnevad, S., Aastveit, A.H., Andersen, Ø., 2006. Effects of tank wall color and up-welling water flow on growth and survival of Eurasian perch larvae (*Perca fluviatilis*). *Journal of the World Aquaculture Society* 37, 313–317.

Kailasam, M., Arasu, A.R.T., Abraham, M., Chandra, P.K. and Subburaj, R., 2002. Influence of size variation and feeding on cannibalism of Asian sea bass *Lates calcarifier* (Bloch) during hatchery rearing. *Indian J. of Fish.*, 49(2): 107-113.

Kailasam, M., Thirunavukkarasu, A.R., Selvaraj, S. and Stalin, P., 2007. Effect of delayed initial feeding on growth and survival of Asian sea bass *Lates calcarifer* (Bloch) larvae. *Aquaculture*, 271(1-4), 298-306.

Kawamura, G., Bagarinao, T., Yong, A.S.K., Jeganathan, I.M.X. and Lim, L.S., 2016. Colour preference and colour vision of the larvae of the giant freshwater prawn *Macrobrachium rosenbergii*. *Journal of experimental marine biology and ecology*, 474, 67-72.

Koven, W., Nixon, O., Allon, G., Gaon, A., El Sadin, S., Falcon, J., Besseau, L., Escande, M., Agius, R.V., Gordin, H. and Tandler, A., 2018. The effect of dietary DHA and taurine on rotifer capture success, growth, survival and vision in the larvae of Atlantic bluefin tuna (*Thunnus thynnus*). *Aquaculture*, 482, 137-145.

Le Cren ED (1951) The length-weight relationships and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). J Anim Ecol 20:201–219.

Lee, C.S., Tamaru, C.S., Banno, J.E., Kelley, C.D., Bocek, A. and Wyban, J.A., 1986a. Induced maturation and spawning of milkfish, *Chanos chanos* Forssakal, by hormone implantation. Aquaculture, 52(3), 199-205.

Lee, C.S., Tamaru, C.S., Kelley, C.D. and Banno, J.E., 1986b. Induced spawning of milkfish, *Chanos chanos*, by a single application of LHRH-analogue. Aquaculture, 58(1), 87-98.

Luchiari, A.C., Pirhonen, J., 2008. Effects of ambient colour on colour preference and growth of juvenile rainbow trout *Oncorhynchus mykiss* (Walbaum). Journal of Fish Biology, 72, 1504–1514.

Marte, C.L., 2003. Larviculture of marine species in Southeast Asia: current research and industry prospects. Aquaculture, 227(1-4), pp.293-304.

Martin-Robichaud, D.J., Peterson, R.H., 1998. Effects of light intensity, tank colour and photoperiod on swimbladder inflation success in larval striped bass, *Morone saxatilis* (Walbaum). Aquaculture Research 29, 539–547

Ostrowski, A.C., 1989. Effect of rearing tank background color on early survival of dolphin larvae. Progressive Fish Culturist 51, 161–163.

Pauly, D. 1993. Editorial, Fish byte. NAGA, ICLARM Q.,16-26.

Ruchin, A.B., 2004. Influence of colored light on growth rate of juveniles of fish. *Fish Physiology and Biochemistry* 30, 175–178.

Shand, J., Davies, W.L., Thomas, N., Balmer, L., Cowing, J.A., Pointer, M., Carvalho, L.S., Trezise, A.E.O., Collin, S.P., Beazley, L.D., Hunt, D.M., 2008. The influence of ontogeny and light environment on the expression of visual pigment opsins in the retina of the black bream, *Acanthopagrus butcheri*. *Journal of Experimental Biology*, 211, 1495–1503.

Silas, E.G., Mohanraj, G., Gandhi, V. and Thirunavukkarasu, A.R., 1982. Spawning grounds of the milkfish and seasonal abundance of the fry along the east and southwest coasts of India. In: *Proceedings of the Symposium on Coastal Aquaculture, Part 3*; MBAI, 12-18 January 1980, Cochin.

Sorgeloos, P.; Lavens, P.; Leger, P.; Tackaert, W. and Versichele, D., 1986. -Manual for the culture and use of brine shrimp *Artemia* in aquaculture. State University of Ghent, Belgium, 319p

Tamazouzt, L., Chatain, B., Fontaine, P., 2000. Tank wall colour and light level affect growth and survival of Eurasian perch larvae (*Perca fluviatilis* L.). *Aquaculture* 182, 85–90.

Thépot, V., Mangott, A. and Pirozzi, I., 2016. Rotifers enriched with a mixed algal diet promote survival, growth and development of barramundi larvae, *Lates calcarifer* (Bloch). *Aquaculture Reports*, 3, pp.147-158.

Ullmann, J.F., Gallagher, T., Hart, N.S., Barnes, A.C., Smullen, R.P., Collin, S.P. and Temple, S.E., 2011. Tank color increases growth, and alters color preference and spectral sensitivity, in barramundi (*Lates calcarifer*). *Aquaculture*, 322, 235-240.

Utne-Palm, A.C., 1999. The effect of prey mobility, prey contrast, turbidity and spectral composition on the reaction distance of *Gobiusculus flavescens* to its planktonic prey. *Journal of Fish Biology* 54, 1244–1258.

Woolley, L.D., Partridge, G.J. and Qin, J.G., 2012. Mortality reduction in yellowtail kingfish (*Seriola lalandi*) larval rearing by optimising *Artemia* feeding regimes. *Aquaculture*, 344, 161-167.

Table 1: Growth responses of milkfish larvae

Treatments	SGR (%/day)	b	r ²	L-W Relationship (Log W = Log a + b Log L)
T1 (control)	1.21 ±0.14 ^a	1.0627	0.3535	Log W = 1.0627Log L - 1.017
T2	1.64 ±0.03 ^b	3.2889	0.7609	Log W = 3.2889Log L - 3.255
T3	2.71 ±0.02 ^c	4.7088	0.7442	Log W = 4.0788Log L - 4.9736
T4	2.48 ±0.06 ^c	1.1956	0.8871	Log W = 1.1956Log L - 1.1066
T5	3.21 ±0.07 ^d	3.6634	0.9364	Log W = 3.6634Log L - 4.3328
P < 0.05				

Different superscripts in the same column indicate significant difference (P<0.05) amongst different treatments (Tukey test, $\alpha = 0.05$). Values are expressed as mean \pm SE (n=30). Unit: Specific growth rate (%/day) , b = regression slope , r² = coefficient of determination, L= Length , W = Weight

Table 2: Hourly rotifer and artemia abundance in different larval rearing tank

Hours	Diurnal rotifer abundance (3 dph – 14 dph)					Diurnal rotifer abundance (15 dph – 18 dph)					Diurnal artemia abundance (15 dph – 21 dph)				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
0600	27.00 ± 2.17 ^a	24.00 ± 1.71 ^{ab}	16.00 ± .85 ^a	20.00 ± 1.25 ^{ab}	14.00 ± .62 ^{ab}	20.00 ± 1.44 ^{ab}	17.00 ± 1.07 ^{ab}	9.00 ± .40 ^{ab}	13.00 ± .70 ^{ab}	7.00 ± .25 ^b	1.08 ± .06 ^a	0.93 ± .04 ^{ab}	0.56 ± .01 ^b	0.70 ± .02 ^b	0.38 ± .02 ^{ab}
0700	29.00 ± 2.50 ^a	27.00 ± 2.09 ^{ab}	23.00 ± 1.37 ^{bc}	25.00 ± 1.71 ^{bc}	20.00 ± .01 ^{cd}	22.00 ± 1.71 ^{ab}	20.00 ± 1.38 ^{bc}	16.00 ± .82 ^{cd}	18.00 ± 1.08 ^{bc}	13.00 ± .54 ^e	1.52 ± .09 ^b	0.98 ± .05 ^b	0.70 ± .02 ^a	0.78 ± .03 ^b	0.50 ± .03 ^c
1000	26.00 ± 2.48 ^a	24.00 ± 2.07 ^{ab}	20.00 ± 1.37 ^{ab}	22.00 ± 1.70 ^{abc}	16.00 ± .95 ^{bc}	19.00 ± 1.65 ^{ab}	17.00 ± 1.32 ^{ab}	13.00 ± .78 ^{bc}	15.00 ± 1.03 ^{ab}	9.00 ± .46 ^b	1.34 ± .09 ^{ab}	0.85 ± .05 ^{ab}	0.62 ± .02 ^{ab}	0.69 ± .03 ^b	0.42 ± .03 ^{bc}
1300	22.00 ± 2.26 ^a	21.00 ± 1.97 ^{ab}	18.00 ± 1.36 ^{ab}	20.00 ± 1.69 ^{ab}	11.00 ± .73 ^{ab}	15.00 ± 1.41 ^a	14.00 ± 1.19 ^{ab}	11.00 ± .74 ^{ab}	13.00 ± .99 ^{ab}	4.00 ± .23 ^a	1.24 ± .09 ^{ab}	0.78 ± .05 ^{ab}	0.59 ± .03 ^{ab}	0.66 ± .04 ^{ab}	0.38 ± .04 ^{ab}
1600	21.00 ± 2.45 ^a	18.00 ± 1.94 ^a	14.00 ± 1.25 ^a	16.00 ± 1.58 ^a	9.00 ± .72 ^a	14.00 ± 1.51 ^a	11.00 ± 1.09 ^a	7.00 ± .57 ^a	9.00 ± .81 ^a	2.00 ± .14 ^a	1.10 ± .10 ^{ab}	0.71 ± .06 ^a	0.38 ± .02 ^a	0.49 ± .03 ^a	0.30 ± .04 ^a
1900	32.00 ± 4.06 ^a	31.00 ± 3.66 ^b	27.00 ± 2.70 ^c	29.00 ± 3.16 ^c	25.00 ± 2.28 ^d	25.00 ± 2.96 ^b	24.00 ± 2.63 ^c	20.00 ± 1.83 ^d	22.00 ± 2.21 ^d	18.00 ± 1.49 ^d	1.22 ± .12 ^{ab}	0.99 ± .09 ^b	0.60 ± .04 ^{ab}	0.82 ± .07 ^b	0.47 ± .07 ^c
<i>P</i>	> 0.05					< 0.05									

Different superscripts in the same column indicate significant difference ($P < 0.05$) amongst different treatments (Tukey test, $\alpha = 0.05$). Values are expressed as mean \pm SE ($n=6$). Unit: rotifer abundance (no/ml of water), artemia abundance (no/ml of water) dph = day post hatch

Figure 1: Survival (A) and standard length (B) of milkfish larvae in different tank background colour. Different superscripts indicate significant difference ($P < 0.05$) amongst different treatments (Tukey test, $\alpha = 0.05$). Values are expressed as mean \pm SEM [n=30(standard length), n = 50 (survival)]. Asterisks were used to indicate significant differences between T1, T3 and T5 (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure 2: Hourly rotifer and artemia abundance in different larval rearing tank (A-C). Unit: no/ml of water. Values are expressed in Table 1 as mean \pm SE (n=30). Correlation of SL and gut rotifer content (D).

Figure 3: Milkfish larvae gut rotifer (A) and artemia (B) content. Unit: numbers/gut. Asterisks were used to indicate significant differences between different rearing days and within the treatments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Values are expressed as mean \pm SEM (n=30). Gut content in different larval rearing tanks during 5 dph (C, D, E, F,G) and 10 dph (H, I, J, K, L). EG= empty gut, RT = rotifer, + = enhanced, - = decreased

Figure 4: Solar illuminated semi outdoor yellow colour tank .

Highlights of present study are as follows:

Milkfish (*Chanos chanos*) larval rearing technology was not available for Indian condition. As the fish bred under captive conditions first time in India during June 2015 at ICAR-CIBA fish hatchery, need has been felt to fine tune the existing milkfish larval rearing technology available in literature. It has been observed that milkfish larval rearing is successful in yellow background tanks which are illuminated by solar or artificial florescent light. First report on effect of tank colour and solar illumination on milkfish larval rearing.

1. *Rearing in semi-outdoor tank with yellow colour background improves milkfish larval growth and survival.*
2. *Prey ingestion rate of milkfish larvae is highest in yellow colour background tanks due to effective contrast against live feeds.*
3. *Milkfish larvae are daytime feeder and this feeding habit synergistically improves preying capacity when reared in yellow coloured tank.*

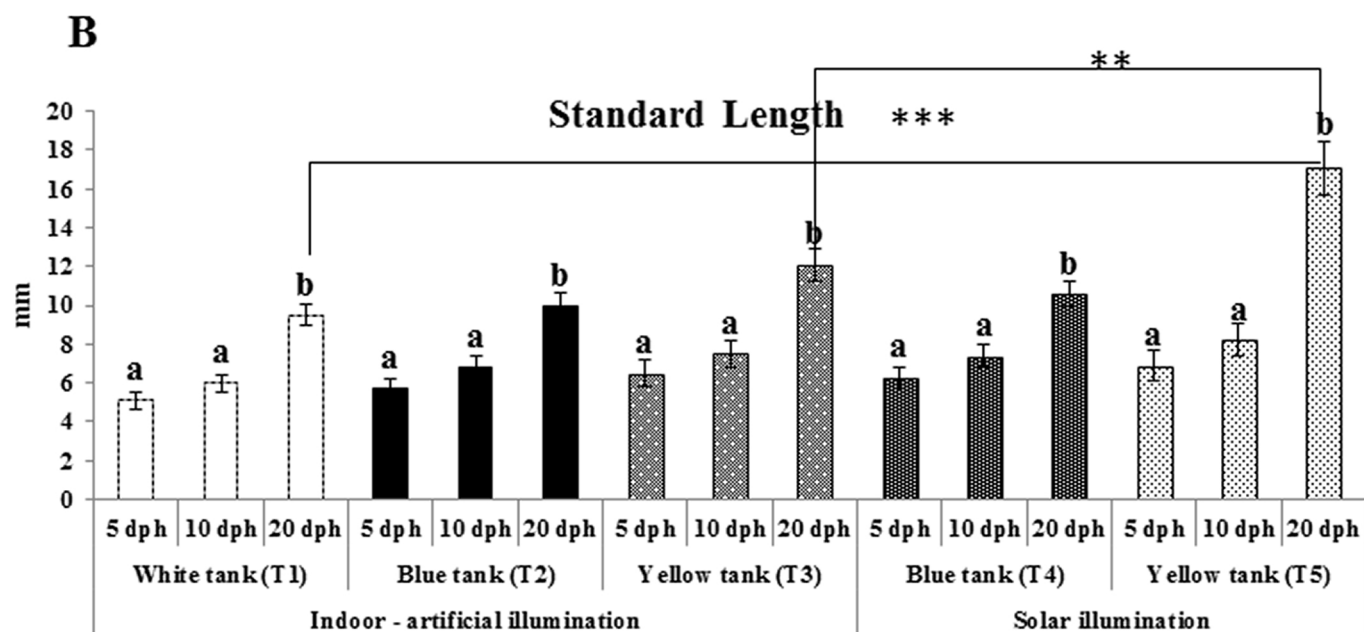
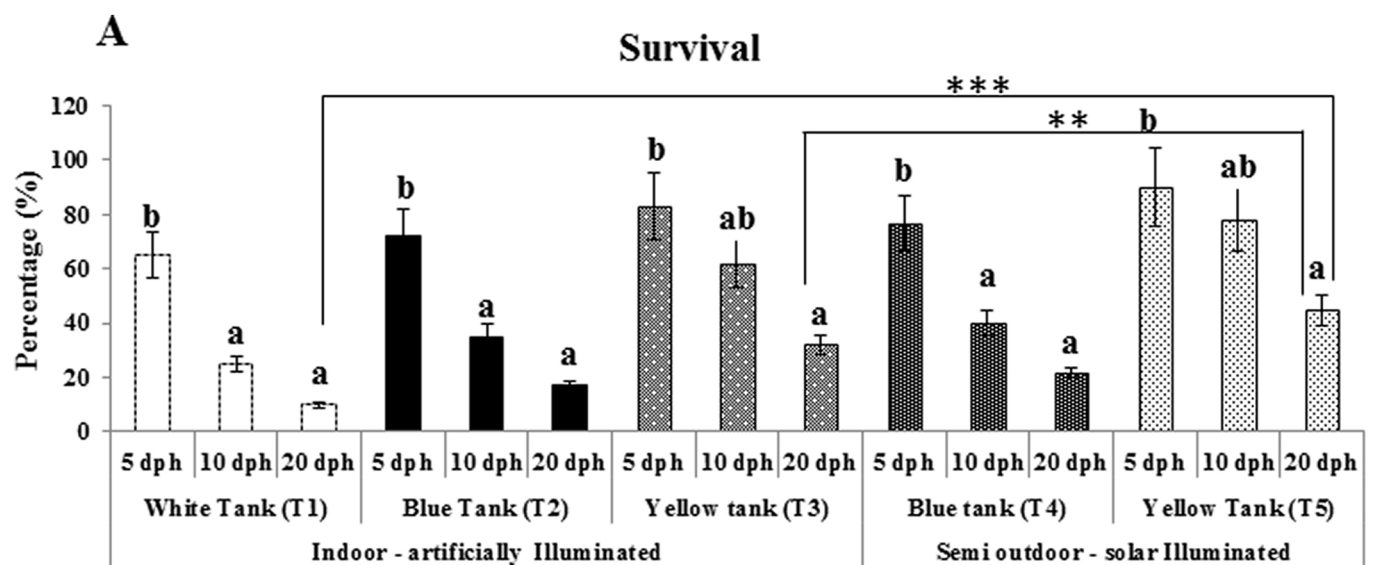


Figure 1

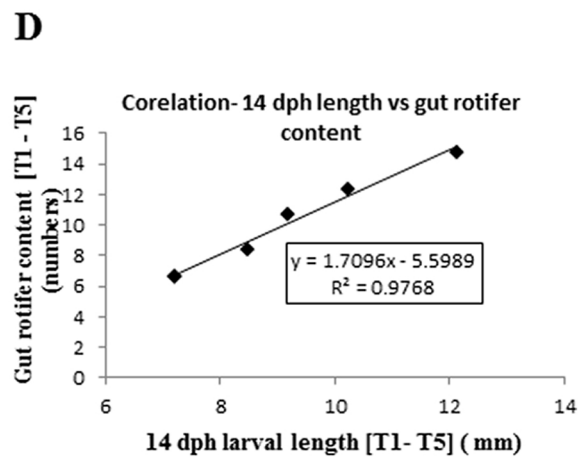
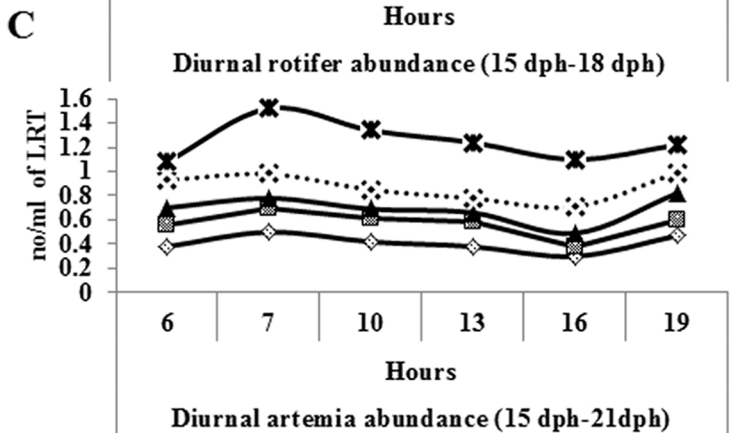
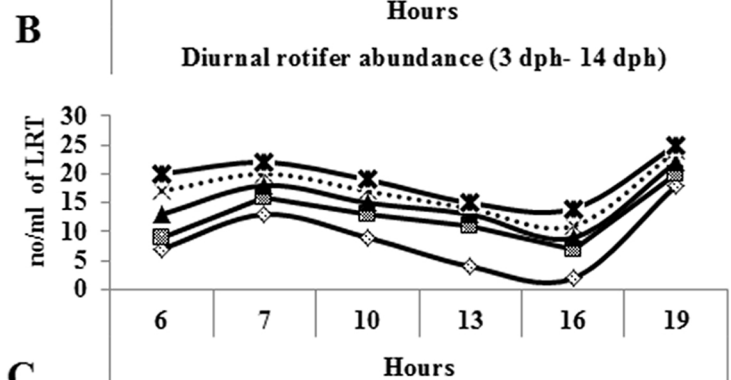
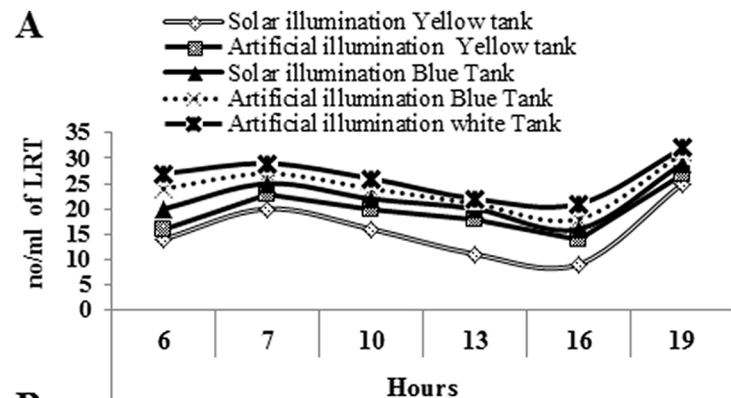


Figure 2

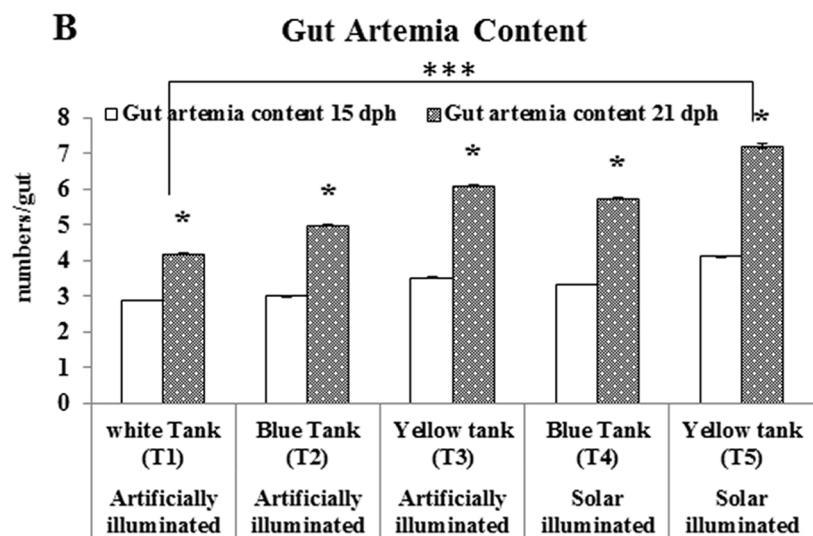
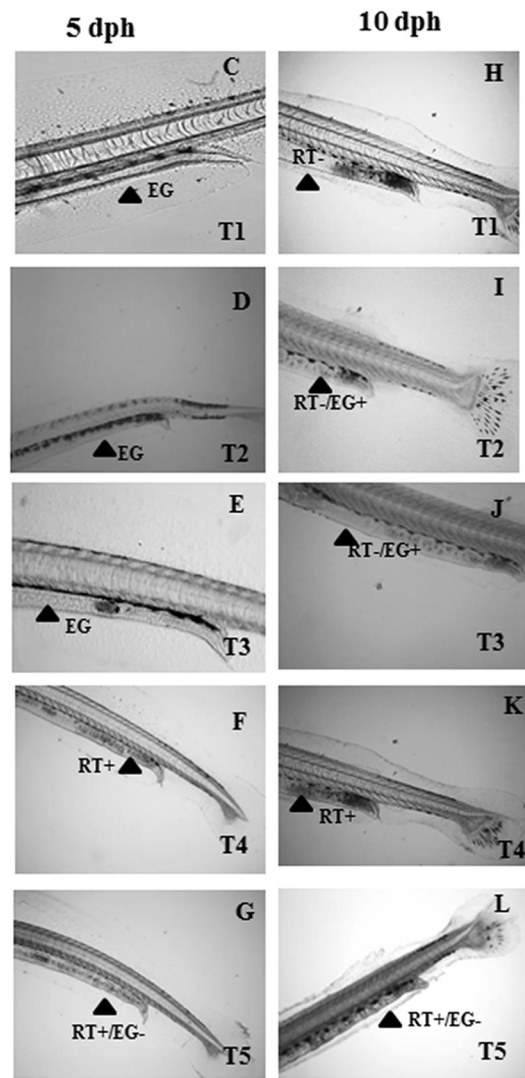
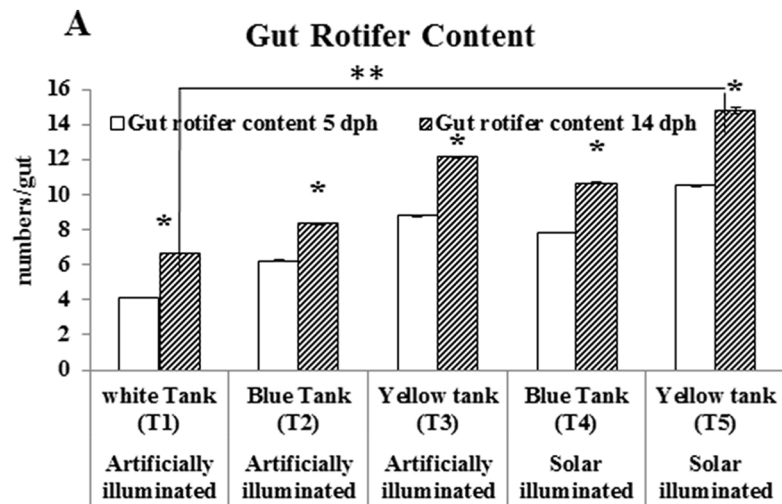


Figure 3



Figure 4