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Probiotics (*Bacillus subtilis* and *B. cereus*) Performances as anti-stress on catfish (*Clarias anguilaris*) fingerlings during transportation and post-transportation conditions

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Abstract
The study investigated the performances of *B. subtilis* and *B. cereus* on catfish (*C. anguillaris*) fingerlings as potential anti-stress administered at the rate of 30 mgl^{-1}. Although, there were fluctuations of the physico-chemical parameter of the water during the transportation; there was no significant difference ($p \geq 0.05$) throughout. There were significant differences ($p \leq 0.05$) in the survival of fingerlings treated with both *B. subtilis* (97.39±1.0%) and *B. cereus* (98±0.73%) during the transportation. The cumulative survival of fingerlings treated with *B. subtilis*, before, during and post-transportation was lower (93.92%) than the control**,** but growth gain was highest (1.6g) with Specific Growth Rate of 0.27g. Fingerlings treated with *B. cereus*, recorded 98% cumulative survival, having weight and length gain of 0.9g and 0.9 cm and specific growth of 0.15% higher than the control group. So, *B. cereus* performed better in survivability, while, *B. subtilis* did better in the growth performance. The pH and EC in water treated with both isolates, the T ^{O}C and DO for *B. subtilis* and TDS for *B. cereus* increased steadily with significant differences ($p \leq 0.05$), while the rest recorded higher values at the middle of transportation, but, all were within the optimum values for raising catfish.

Keywords: Fingerlings, transportation, probiotics, anti-stress, performance

Introduction

Aquaculture is described as the farming of aquatic organisms (GAA, 2019) [17] with fish as the principal form (FAO, 2016) [15] which implied some sort of intervention in the rearing process to enhance production processes such as regular stocking, feeding, and protection from diseases and predators (FAO, 2017) [16]. The value or contribution of fish to the globe cannot be over emphasized in terms of enhancing human nutrition such as provision of essential nutrients including vitamin D, iodine and calcium, animal protein (Mahboub, *et al.*, 2020) [26], provision of essential fatty acids such as omega-3 and -6 fatty acids needed by humans for good health, immunity enhancement and prevention of cancer and heart diseases (FAO, 2017) [16] to mention, but few. Packing and transportation activities in fish production business is inevitable, because, the fry, fingerlings, juvenile and broodstock must be moved from one region or farm to another, either within the same area, state, country, or even across a country to another (Omeji, *et al.*, 2019) [34] for multiplication resulting in stressful conditions that may cause great loss. Proper handling and transporting are important to the success of a producer. In the cause of transportation of aquatic animals in general, the major concern are the management of handling stress, mechanical shock, heat stress and the quality of the transport water (Luca, *et al.* 2015) [25]. Transportation has been considered as one of the main causes of stress (Adenkola and Ayo, 2010) [5] which affects the productivity of animals adversely.

Many factors affect the survival of fish during transportation, such as the depletion of dissolved oxygen for respiration, increase of temperature, accumulation of ammonia-N, changing of acidity beyond the optimal levels. Some water quality parameter of utmost importance during transportation include dissolved oxygen (DO), temperature (T ^{O}C) changes, pH, hardness, carbon dioxide, ammonia-N, salt imbalance of the fish's blood (NAERLS, 2013, Nayla, 2020) [30, 32].

In the course of transportation, fish may be so stressful coupled with harsh environmental factors which exacerbate stress as the journey may involve travelling across different geographical zones (Dhanasiri, *et al.*, 2013) [13]. The goal of transportation is to deliver at the destination no matter the distance, healthy active alive fish of any age without injury (NAERLS, 2015) [31].

Fish transportation involves counting, sorting, loading and unloading, shaking of the vehicle especially travelling along the rough road before a final destination affects the health or welfare of the fish. NAERLS (2013) [30] reported that any of this procedure is stressful to fish and they exhibit most, if not all, of the physiological and biochemical responses to stressors which are seen in mammals. Stress responses are generally considered adaptive in natural situation and total lack of stress would be an impossible goal. Metabolic disturbances, enzymatic dysfunctions and many other malfunctions in fish may occur as a result of physiological changes caused by handling (Leonardo, *et al.*, 2011) [24] and/or transportation. Physical and psychic exertion that occurs during transportation of food-producing animals disrupts their homeostasis and metabolism stress increase activities of enzymes and hormones that destabilize and cause mortality (Muruganandam, 2012) [29].

Some microorganisms called probiotics have been used to ameliorate stress factors in both terrestrial and aquatic animals on different occasions including transportation (Daniel *et al.* 2016) [12]. Due to the peculiarity of the aquaculture environment, Zorriehzahra *et al.* (2016) [48] defined probiotic in aquaculture as a live microbial supplement that is administered via feed or directly into rearing water which provides benefits to the animal. Recent studies use probiotics for improving water quality (Hassan and Banerjee, 2020) [19], enhancing nutrient utilization (Al Mamun *et al.* 2019) [7] health status (Ringoe *et al.* 2020) [38], stress response (Valcarce *et al.* 2020) [44], disease resistance and performance (Abdelfatah and Mahboub, 2018) [3] which is in part achieved by optimizing the microbial balance within the animal and water environment.

Several agents such as salt (Omoike, *et al.*, 2012) [35], palm oil (Christian and Jin-Liang, 2014) [11], anesthetics/additives (Mohammed and Dapens, 2018, Ugwemorubong and Ojo, 2011) [27, 43], herbal anesthetics (Ali and Morteza, 2019) [6] and conventional anti-stress (Rafael, 2012) [37] have been used intensively during transportation to alleviate several stress-related problems. These agents are added to reduce stress thereby improving the catfish condition by increasing the ability to resist stress. The effects can also reduce the level of physical injury or lower the extent of mortality. There are naturally occurring microorganisms which play a key role in aquatic environments, as they can fulfill a wide range of roles, including recycling nutrients, degrading organic matter and protecting fish against infections and reduce stress due to handling, and or transportation.

A probiotic named as Efino®L, Bentoli, Inc., USA, which is a product of microbial cultures and selected nutrients have been used for lowering the stress response during transportation of live fish fry and fingerlings (Luca, *et al.*, 2015) [25], which are products combined for use in shrimps, fish and reptilian hatcheries. The administration of *Bacillus subtilis,* as water additives in rearing water of olive flounder (*Paralichthy folivacious*) significantly reduced ammonia level and fish mortality (Sunitha and Padmavathi, 2013) [41]. Recently, different kinds of probiotics have proven effective on ammonia nitrogen degradation (Zhenzhen, *et al.*, 2016) [46] which are regarded as eco-friendly (Addo, 2013) [4] or bio-friendly (George, *et al.*, 2016) [18] to control toxic compounds to improve water quality (Sultan, *et al.*, 2016) [40] making the environment conducive for culture organisms. These products have been scientifically shown to counteract stressful conditions of hatcheries and improve the health and survival of the aquatic animals (Nwogu, *et al.*, 2011) [33].

Although, investigation on the application of probiotics and products on freshwater fish in the tropics are limited, series of trials to test the effects of probiotics on survival of fry and fingerlings during transportation of aquatic animals (Omeji, *et al.*, 2017**,** Luca, *et al.*, 2015, Andrea, *et al.*, 2019) [34, 25, 8] have been conducted. Their trials reported that probiotic application prior to packing stress improved the survival and post-transportation growth of fish fry.

The aim of this experiment was to determine the stress resistance of fingerlings caused by transportation in terms of survival and to evaluate the growth performance of the fingerlings in water treated with probiotic bacteria before, during**,** and post-transportation conditions and to compare the performances of the fingerlings that did not receive any form of probiotics or only treated during post-transportation conditions with the probiotics.

Materials and Methods

This study was carried out in March 2020 at the Federal University of Agriculture, Makurdi, Benue State, Nigeria. Makurdi town is located between latitude 7°38'N - 7°50'N, and longitude 8°24'E - 8°38'E with an elevation of 104m above sea level (Abah, 2013) [1]. The town is traversed by the second largest river in the country (River Benue), which is the main drainage channel traversing the town truncating it into the North and South Banks. Benue state is bounded by Nasarawa, Kogi, Enugu, Cross River, and Taraba states and Cameroun Republic. This experiment was conducted between 2:00PM and 8:00PM as one of the hottest period of the day in northern and central part of Nigeria to serve as one source of stress to the fingerlings.

Experimental Fish and management

Three thousand six hundred (3600) *C. anguillaris* fingerlings with a mean length of 7.4±1.1 cm and mean body weight of 3.1±1.2 g were purchased from a homestead fish farm in Makurdi metropolis and transported to the experimental site (FUAM), where the fingerings were acclimatized for 10 days in round-bottomed plastic basins of 100 liter capacity. During this period of acclimatization, the fingerlings were fed by 7:00 AM and 5:00 PM to satiety using commercial pelleted (Coppens®, Thailand) feed size 1.5 mm. There was no provision of aeration, so the water was changed 100% every other day.

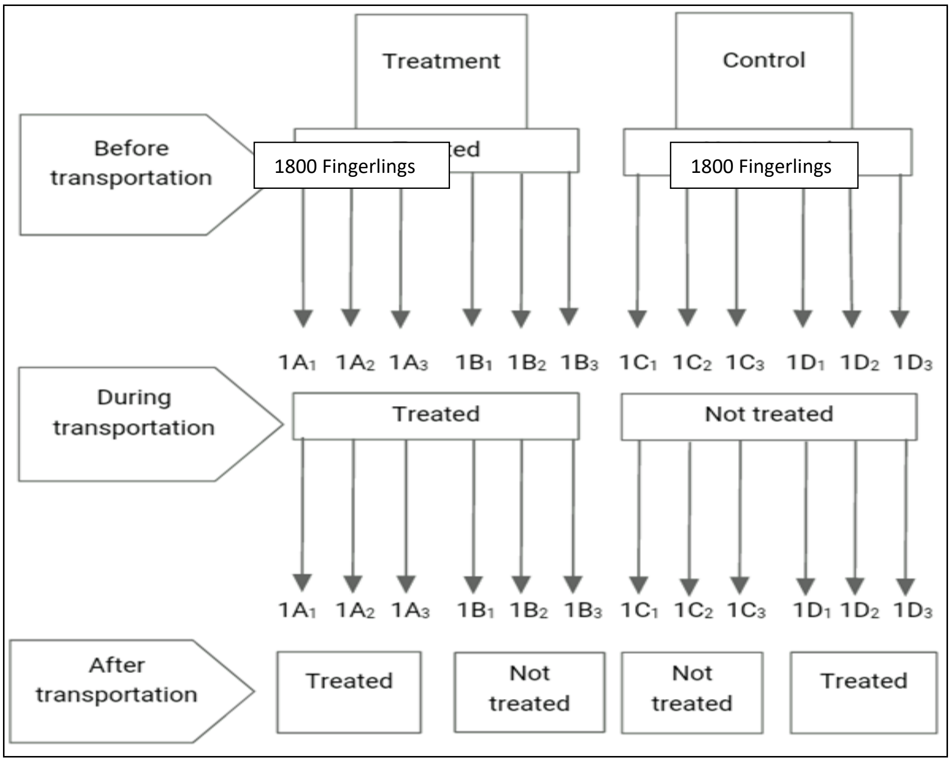
Water quality parameters

Six physico-chemical parameters including Temperature (T ^{O}C), pH, Total dissolved solids (TDS), electrical conductivity (EC), dissolved oxygen (DO) concentration, and ammonium-**N** or un-ionized ammonia (UIA) concentration were measured during this study. Water temperature and dissolved oxygen were measured using handheld Hanna Multi-probe meter (model Hi-9142, Hanna Instruments Inc., USA) while pH was measured using handheld Hanna meter (model Hi-98127, Hanna Instruments Inc., USA). Ammonium-N (un-ionized ammonia) concentration was also measured using a portable Hanna ammonia medium-range meter (model Hi-96715C, Hanna Instruments Inc., USA). The value of ammonia-N (un-ionized ammonia) was calculated (According to Raphael and Evoy, (2009) and Edward, *et al.* (2010) method. While total dissolved solids, electrical conductivity was measured using the Hanna Portable Photometer. These parameters were recorded prior to, during and immediately after transportation of the fingerlings.

Experimental Design

Experimental block design was used in this study with 3600 Fingerlings. Two potential probiotics (*B. subtilis and B. cereus*) represented 2 separate experiments (1 and 2) were tested on the *C. anguillaris* fingerlings. Experiment 1 was treated with *B. subtilis*, and Experiment 2 with *B. cereus* at the rate of 30mgl^{-1}

The experimental design for the transportation and post-transportation growth trial was conducted in the following manner for probiotic1 (*B. subtilis*) as seen below in Fig 1.



After reaching the destination, the Jeri-cans were off-loaded from the vehicle and left packed without removing the fingerlings or changing their water. The fingerlings were unpacked after 13 h and mortality was recorded immediately after unpacking. Fingerlings from each Jeri-can were transferred into labeled shallow plastic basins of 60-liter capacity with 9 liters of fresh water. The post-transportation growth trial was conducted for 6 days treated with probiotic 1 (*B. subtilis*) as seen in figure 1 above.

The same approach was performed for Experiment 2 treated with *B. cereus*. All the treatments were done in replicates as in Experiment 1. Probiotic was added at the rate of 30mgl^{-1} per day after water exchange, but before first feeding. The fingerlings were fed ad *libitum* two times (07:00 AM and 5:00 PM) per day with commercial pelleted feed (Coppens ®, Thailand). Growth and survival were monitored for six days of post-transportation. During this period mortalities were picked every day.

Data Analysis

Data were subjected to Student t-test to compare the survival between the treated and Control fingerlings, while one-way analysis of variance (ANOVA) was used to assess the significant difference of the physico-chemical parameters of the water using IBM®SPSS® statistics version 21. Duncan Multiple Range Test was used to separate the significant differences at $p \leq 0.05$.

Results

Table 1 presents the survival rate (%) of the *C. anguillaris* fingerlings treated with *B. subtilis* prior to packing and during transportation exercise. The result showed a higher survival rate immediately after the journey. Fingerlings in the water treated with probiotic 1 (*B. subtilis*) had an average survival of 97.39±1.0%, compared to the control (96.57±1.0%). There was significant difference ($p \geq 0.05$) in the survival of the fingerlings.

Table 1: Survival % of *C. anguillaris* fingerlings treated with a probiotic (*B. subtilis*) after 13 h of packing and transportation stress

Probiotics (Treated)		Control	
ID Number	% Survivability	ID Number	% Survivability
$1A_1$	96.08	$1C_1$	97.06
$1A_2$	97.06	$1C_2$	96.08
$1A_3$	97.06	$1C_3$	97.06
$1B_1$	99.02	$1D_1$	98.04
$1B_2$	97.06	$1D_2$	95.10
$1B_3$	98.04	$1D_3$	96.10
Mean ± SD	97.39±1.0^a	Mean ± SD	96.57±1.0

Key: superscript a is significantly different ($p \leq 0.05$)

Likewise, the result of the survival of fingerlings treated with *B. cereus* prior to packing and during transportation presented on Table 2 showed higher survival rate immediately after transportation with significant difference ($p \leq 0.05$). In water treated with *B. cereus* had an average survival of 98 ±0.73%, and that of Control had an average of 96±0.80% (Table 2).

Table 2: Survival % of *C. anguillaris* fingerlings treated with probiotic a (*B. cereus*) after 13 h of packing and transportation

Probiotics (Treated)		Control	
ID Number	**% Survivability**	**ID Number**	**% Survivability**
$1A_1$	97.10	$1C_1$	97.06
$1A_2$	98.04	$1C_2$	96.08
$1A_3$	98.04	$1C_3$	96.08
$1B_1$	98.04	$1D_1$	95.10
$1B_2$	99.02	$1D_2$	97.06
$1B_3$	97.06	$1D_3$	97.06
Mean ± SD	98.00 ± 0.73^{a}	Mean ± SD	96.00±0.80

Key: Superscript a is significantly different ($p \leq 0.05$)

Water Quality Parameters

The results of water quality parameters are presented in Table 3. The physico-chemical parameters for pH were in the range of 6.0±0.01-6.7±0.01 for water Treated with *B. subtilis* and 6.1±0.02-6.8±0.01 for the Control. Similar results were obtained in water Treated with *B. cereus*. The temperature rose from 32±0.05 - 35±0.15 ºC and dropped down to 28±0.10 ºC by the end of the transportation (evening) in Treated and Control water and for both *B. subtilis* and *B. cereus*. Dissolved oxygen was in the range of 9.1±0.10 - 12±0.10 mgL^{-1}; total dissolved solid 138±1.0 - 385±2.0 mg L^{-1}, electrical conductivity 280±2.0 - 763±0.53 μSm^{-1}, ammonium-N 0.002±0.001 - 0.036±0.003 mgL^{-1}, throughout the transportation exercise for both *B. subtilis* and *B. cereus* (Table 3).

The water temperature in this study was recorded highest during the transportation and dropped drastically at the end of the 200 km distance was covered by 20 h of the day. The pattern of dissolved oxygen was found to be maintained more or less at uniform level of 11.0 mgL^{-1} average throughout the transportation, except the treated group with *B. subtilis* which recorded less than 110.mgL^{-1}. The TDS and EC increased steadily with significant values ($p \leq 0.05$) at the end of the transportation. Although the ammonium-N which is the most toxic water quality parameters were seen to increase from the beginning of the transportation and slightly reduced towards the end of the exercise with significant difference in both of the treatments the values were within optimal range for fish 0.05 mgL^{-1} (Francis-Floyd *et al.*, 2015) [49]. The general low value of ammonia-N reported in this study could be attributed to the high level of dissolved oxygen. This result agreed with the general principles that dissolved oxygen and ammonia-N are inversely proportional to each other (Francis-Floyd, *et al.*, 2015) [49]. The maximum ammonia-N recorded throughout was 0.03 mgL^{-1}

Table 3: Phyisco-chemical parameters of the transportation water.

Parameters	Activity	Bag label	*Probiotic 1 (B. subtilis)*			Bag label 2	*Probiotic 2 (B. cereus)*		
			Before	**During**	**After**		**Before**	**During**	**After**
pH	Treated	1A	6.0 ± 0.01^{c}	6.1 ± 0.01^{b}	6.7 ± 0.01^{a}	2A	6.0 ± 0.01^{c}	6.3 ± 0.02^{b}	6.7 ± 0.03^{a}
		1B	6.0 ± 0.01^{c}	6.2 ± 0.03^{b}	6.7 ± 0.01^{a}	2B	6.0 ± 0.01^{c}	6.2 ± 0.01^{b}	6.7 ± 0.02^{a}
	Control	1C	6.1 ± 0.01^{c}	6.3 ± 0.01^{b}	6.7 ± 0.01^{a}	2C	6.1 ± 0.01^{c}	6.3 ± 0.01^{b}	6.7 ± 0.01^{a}
		1D	6.1 ± 0.02^{c}	6.3 ± 0.01^{b}	6.8 ± 0.01^{a}	2D	6.1 ± 0.01^{c}	6.3 ± 0.01^{b}	6.8 ± 0.01^{a}
Temperature (ºC)	Treated	1A	32 ± 0.05^{b}	35 ± 0.10^{a}	28 ± 0.20^{c}	2A	33 ± 0.45^{b}	34 ± 0.20^{a}	28 ± 0.05^{c}
		1B	32 ± 0.05^{b}	35 ± 0.15^{a}	27 ± 0.15^{c}	2B	33 ± 0.55^{b}	34 ± 0.05^{a}	28 ± 0.10^{c}
	Control	1C	32 ± 0.00^{b}	35 ± 0.45^{a}	28 ± 0.10^{c}	2C	32 ± 0.05^{b}	35 ± 0.45^{a}	28 ± 0.10^{c}
		1D	33 ± 0.15^{a}	33 ± 0.10^{a}	28 ± 0.25^{b}	2D	33 ± 0.10^{b}	34 ± 0.10^{a}	28 ± 0.10^{c}
Total Dissolved Solids (mg/L)	Treated	1A	144 ± 1.0^{c}	182 ± 1.0^{b}	381 ± 1.0^{a}	2A	142 ± 1.0^{c}	176 ± 3.5^{b}	353 ± 15.0^{a}
		1B	147 ± 3.0^{c}	181 ± 5.5^{b}	344 ± 9.0^{a}	2B	144 ± 3.0^{c}	181 ± 3.5^{b}	327 ± 11.0^{a}
	Control	1C	142 ± 0.6^{c}	174 ± 2.0^{b}	359 ± 7.5^{a}	2C	142 ± 0.6^{c}	174 ± 2.0^{b}	359 ± 7.5^{a}
		1D	144 ± 1.5^{c}	176 ± 1.0^{b}	373 ± 1.5^{a}	2D	144 ± 1.5^{c}	176 ± 1.0^{b}	373 ± 1.5^{a}
Electrical Conductivity (μS/m)	Treated	1A	287 ± 1.0^{c}	368 ± 5.0^{b}	763 ± 0.6^{a}	2A	284 ± 2.5^{c}	351 ± 6.5^{b}	706 ± 28^{a}
		1B	295 ± 6.5^{c}	367 ± 7.0^{b}	688 ± 19^{a}	2B	289 ± 5.5^{c}	353 ± 13^{b}	660 ± 25^{a}
	Control	1C	284 ± 1.0^{c}	348 ± 0.6^{b}	716 ± 14^{a}	2C	284 ± 1.0^{c}	348 ± 0.6^{b}	716 ± 14^{a}
		1D	290 ± 1.0^{c}	361 ± 5.5^{b}	729 ± 25^{a}	2D	287 ± 4.0^{c}	354 ± 2.0^{b}	742 ± 3.0^{a}
Dissolved Oxygen (mg/L)	Treated	1A	10 ± 0.65^{a}	09 ± 0.10^{b}	10 ± 0.35^{a}	2A	11 ± 0.25^{a}	11 ± 0.00^{a}	11 ± 0.00^{a}
		1B	11 ± 0.15^{a}	10 ± 0.35^{b}	11 ± 0.25^{a}	2B	12 ± 0.00^{a}	11 ± 0.10^{ab}	11 ± 0.60^{b}
	Control	1C	11 ± 0.25^{a}	11 ± 0.20^{a}	11 ± 0.15^{a}	2C	11 ± 0.25^{ab}	11 ± 0.20^{b}	11 ± 0.15^{a}
			11 ± 0.35^{a}	11 ± 0.10^{a}	11 ± 0.15^{a}	2D	11 ± 0.10^{a}	11 ± 0.30^{a}	11 ± 0.5^{a}
Ammonium-N (mg/L)	Treated	1A	0.002 ± 0.00^{b}	0.033 ± 0.01^{a}	0.030 ± 0.00^{a}	2A	0.002 ± 0.00^{b}	0.035 ± 0.01^{a}	0.030 ± 0.00^{a}
		1B	0.002 ± 0.00^{c}	0.030 ± 0.01^{a}	0.023 ± 0.00^{b}	2B	0.002 ± 0.00^{c}	0.026 ± 0.01^{b}	0.028 ± 0.00^{a}
	Control	1C	0.004 ± 0.00^{c}	0.036 ± 0.00^{a}	0.031 ± 0.00^{b}	2C	0.004 ± 0.00^{c}	0.030 ± 0.01^{b}	0.031 ± 0.00^{a}
		1D	0.002 ± 0.00^{c}	0.032 ± 0.01^{a}	0.030 ± 0.00^{b}	2D	0.002 ± 0.01^{c}	0.033 ± 0.01^{a}	0.030 ± 0.00^{b}

Key: ^{abc} Mean ± SD with different subscript within a row differ significantly ($p \leq 0.05$)

After continuing probiotic treatment for six days post-treatment conditions, the results of the cumulative survival and growth performances of the fingerlings are presented in Table 4. The cumulative survival rate of Control group ($1C_{1-3}$) which did not receive probiotic throughout the experiment recorded the least (92.27%), followed by the group ($1A_{1-3}$) which were treated with probiotics before and during transportation as well as during post-transportation trials (93.92%), but recorded the highest final weight (4.7g), growth gain (1.6g) and Specific Growth Rate (0.27g) and the length measurement followed suit compared to all the treatment. Although, group $1D_{1-3}$ which were treated with probiotic only during the 6 days post-transportation exercise recorded highest cumulative survival rate (96.28%), but recorded the least growth gain, followed by $1B_{1-3}$ group treated with probiotic prior to and during transportation, but was not receive probiotic during the 6 day trials (94.00%). In this experiment, fingerlings treated with *B. subtilis* before, during and after, did better in terms of growth performance, while those fingerlings that received *B. subtilis* only during post-transportation trials survived better and the Control group recorded the least.

Table 4: Cumulative survival (%) and growth performances of *C. anguillaris* fingerlings during six days post- transportation conditions treated with *B. subtilis*

Treatment	Survival rate (%)	Measurement	Final	Initial	Growth gain	Specific growth rate
$1A_{1-3}$	93.92	Weight (g)	4.7	3.1	1.6	0.27
		Total length (cm)	8.5	7.4	1.1	0.18
$1B_{1-3}$	94.00	Weight (g)	4.2	3.1	1.1	0.18
		Total length (cm)	8.3	7.4	0.9	0.15
$1C_{1-3}$	92.27	Weight (g)	5.5	3.1	1.4	0.23
		Total length (cm)	8.4	7.4	1.0	0.17
$1D_{1-3}$	96.28	Weight (g)	3.7	3.1	0.6	0.10
		Total length (cm)	8.0	7.4	0.6	0.10

Key: $1C_{1-3}$ = Control which did not receive probiotics throughout the experiment

The performance of *C. anguilaris* fingerlings treated with probiotics (*B. cereus*) is presented in Table 5 below. The cumulative survival of fingerlings in water treated with *B. cereus* before, during and post-transportation ($2A_{1-3}$) recorded 98% higher than all other treatment. The growth performance obtained from weight and length gain was at the same unit of 0.9g and 0.9cm respectively resulting to a specific growth rate of 0.15g and 0.15cm per day. The group that was treated before and during the transportation, but not treated during the post-transportation (($2B_{1-3}$) also survived well (97%) with growth gain of 1.0 similar to the total length which was the second highest cumulative survival rate. However, fingerlings in Control group ($2C_{1-3}$) that were not treated with probiotics throughout performed least in terms of cumulative survival (95.93%), but growth performances in terms of weight gain and specific growth rate was good (0.9g and 0.9cm) and similar to the group that received probiotic throughout the exercise ($2A_{1-3}$). The lowest growth performance was seen from the control group $2D_{1-3}$ that did not receive probiotic before and during transportation, but treated during post-transportation with growth performance of 0.7g body weight and 0.4 cm total length gain within the 6 days trials (Table 5). In this experiment, fingerlings treated with *B. cereus* before, during and after, did better in terms of cumulative survival and growth performance, while those fingerlings that received group *B. cereus* before and during transportation, but not treated during the post-transportation followed next, and the performance was recorded in the control group was least.

Table 5: Cumulative survival (%) and growth performances of *C. anguillaris* fingerlings during six days post-transportation conditions treated with *B. cereus*

Treatment	Survival rate (%)	Measurement	Final	Initial	Growth gain	Specific Growth rate
$1A_{1-3}$	98.00	Weight (g)	4.0	3.1	0.9	0.15
		Total length (cm)	8.3	7.4	0.9	0.15
$1B_{1-3}$	97.00	Weight (g)	4.1	3.1	1.0	0.17
		Total length (cm)	8.4	7.4	1.0	0.17
$1C_{1-3}$	95.93	Weight (g)	4.0	3.1	0.9	0.15
		Total length (cm)	8.3	7.4	0.9	0.15
$1D_{1-3}$	96.27	Weight (g)	3.8	3.1	0.7	0.12
		Total length (cm)	7.8	7.4	0.4	0.07

Key: $1C_{1-3}$ = Control which did not receive probiotics throughout the experiment

The growth performances were found higher in experiment treated with *B. subtilis* than in those treated with *B. cereus*. Surprisingly the survival rate was higher in the water treated with *B. cereus*. Survival percentage showed a significant variation in the probiotic treated groups to the Control group with *B. cereus*. There was no significant difference ($p \geq 0.05$) in the survival of fingerling between the treated and Control with *B. subtilis*, while, there was a significant difference ($p \leq 0.05$) in the survival of fingerling in the treated with *B. cereus* and Control.

Discussion

Effects of probiotics (*B. subtilis* and *B. cereus*) on the quality of transporting water and the fingerlings during the transportation period

Although, the water quality parameters fluctuated significantly during the transportation there the survival of the fingerlings was statistically significant ($p \leq 0.05$) to the Control, since all the physico-chemical parameters were found within the optimal condition of rearing catfish according to Keremah, *et al.* (2014) [20] who listed the optimum water quality parameters which include: Temperature 20-30 °C, pH 6.5-9, Dissolved Oxygen 8-10 mgl^{-1}, Total Dissolved Solids 500 mgl^{-1}, Electrical Conductivity 30-1500uScm^{-1} and ionized ammonia 2.5-4.0 mgl^{-1}). In this study, dissolved oxygen was significantly high ($p \leq 0.05$) which was good for the fingerlings and the ammonium-N level was below minimum level, which could be attributed to the effect of both probiotics (*B. subtilis* and *B. cereus*) in moderating water parameters and improving the responses of the fingerling to transportation stress (Luca, *et al.* 2015, Zokaeifar *et al.*, 2012) [25, 47]. Most of the above-mentioned water quality parameters (except water temperature and ammonia-N in *B. subtilis* treated water) during the entire transportation period were found to increase significantly, though, are in the optimum range for catfish rearing (Mohapatra, *et al.*, 2011) [28]. The activities of probiotic bacteria in improving water quality in this experiment agreed with several workers (Padmavathi, *et al.*, 2012, Lakshmi *et al.*, 2015) [36, 23].

The improvement of water quality in this study by these two probiotic bacteria is not farfetched from the reports of other workers that, some probiotic bacteria directly uptake or decompose the organic matter or toxic material in the water, thus improving the water quality (Lakshmi *et al.*, 2015, Zorriehzahra *et al.*, 2016) [23, 48], through excretion of exo-enzymes as a natural by-product of their metabolic activities (Addo, 2013) [4]. These enzyme excretions infuse and spread throughout the pond or receptacle medium, changing its chemistry and destroying bad bacteria (Sunitha and Padmavathi, 2013) [41] causing more performance in terms of survival and growth as demonstrated in this experiment.

Survival % of *C. anguillaris* fingerlings treated with probiotics (*B. subtilis* and *B. cereus*) during the 13 h of unpacked and transportation stress.

The significant difference ($p \leq 0.05$) in the survival of the fingerlings treated with *B. subtilis* and *B. cereus* signified that there was influence from the probiotic bacteria on the fingerlings compared to those in Control, which resulted to higher survival rate of fingerlings after the transportation, which could be attributed to the effect of Bacillus probiotic bacteria in regulating the water quality parameters most especially these Bacillus species used in this study. This result generally corroborates with the work of Vivian, *et al.* (2020) [45] who reported high fry survival of *Catla catla* fry in water containing probiotics immediately after transportation but no significant difference. They however, evaluated the performance of probiotic on *Catla catla* fry during packing, transportation stress and post-transportation conditions and their results showed that probiotic application prior to, during transportation and after transportation condition increased fry survival compared to the rest of the treatments with control group recorded highest mortality. The result of this study also agreed only with the isolated *B. cereus* in survival rate, and corroborates in growth performance with both isolated probiotic bacteria (*B. subtilis* and *B. cereus*). The performance of *B. subtilis* in this study on the survival of fingerling in post-transportation condition did not much agree with their result, because they recorded the highest survival rate and growth performance in fry treated with probiotics before, during, and post-transportation conditions, but in terms of growth performances this study corroborates with them.

The survival of fingerlings in water treated with *B. subtilis* and *B. cereus* before and during transportation, were higher than those in water that never received treatment at any point in time (Table 1 and 2). This is true with effects of most Bacillus probiotic bacteria in response of animals to stress. Fish response to stressors in aquaculture vary according to the source, effects, environment and characteristics of stressors (Carl and LIuis, 2016) [9] and is an integrated reaction with behavioral, neural, hormonal, and physiological elements all combined together to provide fish with the best possible chance of survival. This can be supported by the results in Table 4 and 5 which show that the isolated probiotic bacteria played a significant role in maintaining good water quality parameters that promotes the high survival and growth rate of the fingerlings.

Cumulative survival (%) and growth performances of *C. anguillaris* fingerlings during six days post-transportation conditions treated with *B. subtilis* and *B. cereus*

The average body weight of the fingerlings differ significantly ($p \leq 0.05$) in the probiotic treated water throughout the experimental period which showed an increase in the fish body weight and length (Table 3 and 4) that could be attributed to the effect of the probiotic bacteria (*B. subtilis*) in modeling the environment (physico-chemical parameters of the water) to be conducive and performed better (Zokaeifar, *et al.*, 2012) [47]. The effects of 2 probiotic products (Pondplus® and Aquaphoto®) and sand filtration were studied on growth performances of Tilapia (*O.niloticus*) and Pangas (*Pangasianodon hypophthalmus*) in earthen ponds by Sultan, *et al.* (2016) [40] reported that the pond treated with either probiotics or with sand filtration resulted in having high O_2 content, high water transparency, less ammonium-N, and fewer *Cyanobacteria* after rearing the fish for 7 months more than un-treated pond. These good results were attributed to the activities of the probiotic bacteria products in the treated ponds.

The success of application of probiotics in aquaculture in terms of disease prevention, promoting growth, improving digestibility, boosting immunity and improving water quality for cultured organisms have been reported (Abareethan and Amsath, 2015, Zorriehzahra, *et al.*, 2016) [2, 48]. The probiotic bacteria such as *Bacillus* spp are associated with the improvement of water quality, reduction of pathogenic population in culture environment, enhancement of survival and growth rate and better health condition of the aquatic organism which was common with the isolated *B. subtilis* in this study. On the other hand, the growth performance of fingerlings in water treated before, during and after, performed better than all treatments.

The control ($1C_{1\text{-}3}$) recorded the least growth performance with *B. subtilis*. However, the survival, growth performance of fingerling was recorded better with fingerlings in water treated with *B. cereus*, before, during, and after the journey, followed by those in water treated before and during, but no treatment during the 6 days post-transportation condition. This experiment corroborated with the work of Sayed, *et al.* (2011) [39] on growth performance of *Oreochromis niloticus* using 3 probiotic products. The performances of these probiotic bacteria in this study differed from each other in terms of survival and growth performance, but it is not a categorical statement probably depending on rearing condition, the species strain, the dosage, or the duration of application.

The target of this work was to assess whether *C. anguillaris* fingerlings in water treated with probiotics either *B. subtilis* or *B. cereus* will survive better than those not treated during transportation. Also to evaluate whether the fingerings will perform better in terms of stress resistance, survivability, and or growth performance when treated with probiotic bacteria, before packing, during transportation, but no treatment after the transportation, no treatment with probiotics before packing, nor during the journey, but treated during a 6 days post-transportation, or no probiotic treatment at any point throughout, neither before, during the transportation or during the 6 days post-transportation conditions. Both probiotic played a significant role in regulating the water quality parameters to maintain within the optimal level of rearing catfish, which is similar to the works of Krishinan, (2014) [22] and Tarnecki *et al.* (2019) [42] on the potential of *Bacillus species* on regulating water quality and improve the growth performances of catfish and larval fish survival and transportation stress resistance. This result also agreed with

Zhenzhen, *et al*. (2016) [46] who reported the ability of *Lactobacillus spp.* JK-8 and JK-11 simultaneously removed nitrogen and pathogens from contaminated shrimp culture water, while Chauhan and Singh (2018) [10] and Kolndadacha, *et al*. (2011) [21] reported the effects of *Bacillus sp*. to improve water quality consequently increased the survivability and growth rate of fish reared. A probiotic containing multiple strains of bacteria like *Bacillus acidophilus, B. subtilis, B. lechiniformis, Nitrobacter, Nitrosomonas Aerobacter species* and *Saccharomycetes cerevisiae* have been applied to fish culture water with great success to control water quality according to Edun and Akinrotimi (2008) [14] which agrees with the use of *B. subtilis* and *B. cereus* in this study.

Conclusion

This study revealed that, while the isolated *B. subtilis* was good in improving growth performance, *B. cereus* was good in reducing the effect of stress in the fingerling with evidence of a high survival rate. Both isolated probiotics (*B. subtilis* and *B. cereus)* showed positive potentials as growth promoters in catfish production. During transportation of fingerlings, *B. cereus* could be administered in water a few hours before and during the journey to reduce the effects of transportation stress to arrive safely with minimal mortality. After transportation, *B. subtilis* could be administered to improve the growth performance of the fish. The performance of these organisms on improving water quality and transportation of fish larvae and fry are gaps that need to be evaluated.

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