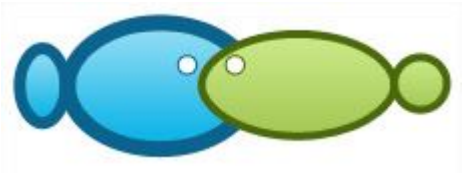

Effects of electroshock on climbing perch (*Anabas testudineus*) microbiological and physicochemical quality

Nurul U. Karim[1,2], Rohisyamuddin Othman[2], Abol M. A. Bolong[1], Marina Hassan[2]

[1] School of Fisheries and Aquaculture Science, Universiti Malaysia Terengganu, Terengganu, Malaysia; [2] Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Terengganu, Malaysia. Corresponding author: N. U. Karim, ulfah@umt.edu.my

Abstract. Electroshock had been widely used for scientific studies on riverine fish. The present study was carried out on the bacteriological and psychochemical quality of climbing perch (*Anabas testudineus*) that subjected to different voltages of electroshock (50, 70, 90 and 110 V) with controls left untreated. Electric charge at 70-90 V gives significant benefits to reduce ($p<0.05$) the bacteria accumulation in *A. testudineus* flesh. However, the total bacteria count were not significantly effects after treated at 50 and 110 V compared to the untreated fish. Electroshock were not significantly ($p>0.05$) giving an excessive accumulation on lactic acid that might alter the pH value in all samples. Furthermore, treatment at 90-110 V showed a significantly ($p<0.05$) lower amount of total colour compared to the untreated samples and give a pale looks and discoloration to the fish flesh. The suitable electric charge for fish sampling were suggested at 70-90 V, which significantly contributes to reduce microbial effects and minimal attributes changes.

Key Words: microbiological quality, psychochemical quality, electroshock fishing, microbial contamination.

Introduction. Fish are highly perishable due to their biological composition, activity of endogenous enzymes, microbial contamination and conditions after harvest. Fish depending on the circumstances of capture, proper handling and preservation techniques to maintain their quality attributes. Quality is an aesthetic appearance and freshness or degree of spoilage has undergone (Huss 1995). Immediately after death, fish precede the autolytic changes that contribute to the quality loss (Huss 1995). Maeda et al (2014) documented that the deterioration of fish flesh after catch are affected by the adenosine triphosphate degradation and lactic acid accumulation. Thus, leads to a decrease in pH that effect on the physical properties of the muscle. Concurrently, the immune systems that prevent bacteria invade into the fish muscle were collapse (Huss 1995). Bacteria were allowed to proliferate freely and penetrate into the fish muscle. In addition, physical mishandling during capture relatively causes bruises and stress. These may accelerate spoilage and exposure to the microbial entry and subsequence deterioration. A good practice on capturing may produce high quality fish.

Electroshock is a technique that using electric charge and fields to control the movement and immobilize fish for allowing capture (Snyder 2003). The intensity of the electric charge and time of exposure influenced the physiological response of the fishes. Various researchers documented the effects of the electrofishing to the fish; severe spinal misalignment and fractured vertebrae (Maeda et al 2014; Snyder 2003), reduce growth rate (Gatz et al 1986; Dwyer & Erdahl 1995), reduce swimming stamina (Horak & Klein 1967), affect behavior (Mesa & Schreck 1989) and interfere with reproduction (Muth & Ruppert 1997). Electrofishing is an effective fish sampling method for researchers in helping to improve the quantitative studies of riverine fish (Penczak & Glowacki 2008).

The aim of this study was to determine the effects of electroshock on the fish quality with an emphasizing on the microbiology and physiochemical changes.

Material and Method. Sixty adult climbing perch, *Anabas testudineus* at 45.30±2.80 g and 14.50±1.00 cm were purchased from local aquaculture farm and brought to the hatchery. All samples were acclimatized for 24 h before randomly selection and placed in five separated tank filled with 250 L water accordingly. Samples were exposed to electric shock at 0, 50, 70, 90 and 110 V for 2 minutes using LR-24 Electrofisher (Smith-Root, Washington, USA), respectively. Pulsed direct current (PDC) waveform with a frequency of 30 Hz and a 12% duty cycle were setup as default setting. All analysis was done in triplicates.

Microbiological analysis. Total bacteria counts were determined by using the Maximum Recovery Diluent (Lab M, Lancashire, UK) as diluent and spread plate on Plate Count Agar (Lab M, Lancashire, UK) as described by Linton et al (2003) and Karim et al (2011). The plates were placed in incubator (BINDER BD 115, Germany) at 30^{o}C for 48 h. Plates of serial dilution at the countable range of 30-300 colonies were selected as the reliable counts. Microbiological counts were expressed as log colony forming units per gram of samples ($\log_{10}$ CFU g^{-1}).

pH measurement. Approximately 10±0.1 g of fish flesh were weighted and mixed with distilled water 1:5 w/v before homogenate using Waring 8010S laboratory blender (Torrington, USA). pH value of the homogenate were measured by using pH meter (HANNA Instruments HI 221, Europe). Three measurements were recorded and the mean value of pH was taken.

Water holding capacity determination. Water holding capacity (WHC) was measured according to the method of Lakshmanan et al (2007) with a minor modification. Fish (10±0.1 g) was accurately weighted and placed in a 50 mL centrifuge tube with 5 mm diameter of glass beads. The tubes were then weighted (W_1) before centrifugation for 20 min at 1,500 x g at 10°C (Centrifuge 5430R Eppendorf AG, Hamburg, Germany). After centrifugation, the supernatant was carefully removed from each centrifuge tube with 230 mm disposable glass Pasteur pipette and the tube were reweighed (W_2). The WHC was calculated as the amount of sample remaining after centrifugation. Three measurements were carried out on each sample and the mean value expressed as percentage WHC on a wet weight basis as follows:

WHC % = 100 % - (percentage of drip loss) %
Percentage of drip loss % = $[(W_1-W_2)/$ sample weight in g$]$ x 100

Where: W_1 - initial weight tube containing fish sample before centrifugation;
W_2 - initial weight of tube after the supernatant was removed after centrifugation.

Color measurement. Color measurements (*L*, a*, b** CIELAB values) were carried out using a Chroma Meter CR-400 (Konica Minolta, Japan). The instrument was standardized and calibrated before used. The *L*, a**, and *b** values correspond to lightness, black (0) or white (100), greenness (-a) or redness (+a), and blueness (-b) or yellowness (+b), respectively.

Statistical analysis. The entire experiment was replicated three times. Data were analyzed using ANOVA. Significant differences among were determined using LSD test at 0.05 level of probability. All statistical analysis was done using the IBM SPSS Statistic software (Version 20).

Results and Discussion. Total bacteria count in controls were higher (6.27±0.74 $\log_{10}$ CFU g^{-1}) compared to the bacteria counts found in electroshocked samples. Meanwhile, fish treated at 70 and 90 V gives amount of 4.48±0.37 and 4.65±0.48 $\log_{10}$ CFU g^{-1},

respectively showed a significantly ($p<0.05$) lower amount of microflora compared to other treatments (Table 1).

Table 1

Microbiological analysis of *Anabas testudineus* after exposed to electroshock treatment

Electric shock Voltage (V)	*Total bacteria count ($log_{10}CFU\ g^{-1}$)*
0	6.27 ± 0.74^{a}
50	$5.60\pm0.91^{a,b}$
70	4.48 ± 0.37^{b}
90	4.65 ± 0.48^{b}
110	$5.33\pm0.67^{a,b}$

All values are mean ± SD (n=3) indicate the significant differences ($p<0.05$).
Means with different superscripts (a, b, c) in the same column show significant difference ($p<0.05$).

pH value of fish that subjected to the electroshock (50-110 V) were not significantly ($p>0.05$) effected (Table 2). Similar trend were also found in regards to the water holding capacity (WHC) ($p>0.05$) (Table 2). Color analysis of untreated *A. testudineus* showed no significant different ($p>0.05$) of *L**, *a**, and *b** values compared to the samples treated at 50 and 70 V. Fish treated with higher electroshock (90-110 V) showed a significant ($p<0.05$) reduced in *L** value. The total color (*E*) of fishes treated with higher electroshock (90-110V) showed a significantly ($p<0.05$) lower amount compared to the mild electroshocked fishes.

Table 2

Psychochemical analysis of climbing perch, *Anabas testudineus* after exposed to electroshock treatment

Electric shock Voltage (V)	*pH value*	*Water holding capacity (WHC %)*	*Color attributes*			
			*L**	*a**	*b**	*E*
0	6.99 ± 0.22^{a}	89.35 ± 3.02^{a}	47.21 ± 4.16^{a}	$11.89\pm2.64^{a,b}$	13.32 ± 0.90^{a}	50.56 ± 3.63^{a}
50	6.68 ± 0.37^{a}	92.02 ± 1.95^{a}	46.97 ± 2.85^{a}	$10.08\pm0.59^{a,b}$	13.85 ± 1.92^{a}	50.00 ± 3.32^{a}
70	6.91 ± 0.05^{a}	94.02 ± 2.61^{a}	$41.53\pm2.02^{a,b}$	12.57 ± 3.61^{a}	13.38 ± 2.72^{a}	$45.49\pm3.52^{a,b}$
90	6.82 ± 0.29^{a}	92.04 ± 7.51^{a}	39.86 ± 4.41^{b}	8.39 ± 1.03^{b}	$9.85\pm1.69^{a,b}$	41.97 ± 3.89^{b}
110	7.02 ± 0.03^{a}	95.09 ± 4.04^{a}	39.47 ± 0.29^{b}	$12.11\pm1.01^{a,b}$	12.71 ± 0.51^{b}	43.21 ± 0.59^{b}

All values are mean ± SD (n =3) indicate the significant differences ($p< 0.05$).
Means with different superscripts (a, b, c) in the same column show significant difference ($p< 0.05$).

The electric field used in electroshock can be a stressor (Schreck et al 1976; Barton & Grosh 1996). Therefore the behavior response of the *A. testudineus* was also observed during the two minutes of electric charge exposure (Table 3). Fish at 70-110 V electroshock had the same intensity of aggression and jumping response (Table 3).

Meanwhile the stunned posture was stronger with increasing electric charge. Stunned posture occurs when the electric charge was transported across the nerve cells and leads to involuntary contraction. Muscle cramps at higher electric charge leads to an ineffective muscle movement during swimming. Therefore fish are immobilized and may floats at the surface. However, from the observation, there were no mortalities in all samples subjected to the electroshock.

Table 3

Behavioral observation of climbing perch, *Anabas testudineus* during two minute of electroshock exposure treatment

Clinical signs	*Electroshock voltage (V)*				
	0	*50*	*70*	*90*	*110*
Aggression	+++	0	+	+	+
Jumping	++	0	+	+	+
Stunned posture	0	+	++	+++	+++
Frequent surface to bottom movement	0	0	0	+	+
Erratic swimming	+++	+	+	++	+

Electric charge at 70 and 90 V showed a significantly reduced amount of bacteria accumulation might due to the destruction and no penetration of bacteria into the fish flesh. In addition, low electric charge causes mild lesion, harmlessly affects the tissue and cause injuries. However, the bacteria count found in fish flesh subjected at 110 V were increased might be influenced by the digestive bacteria and enzymes activities, and degradation of glucose and adenosine triphosphate (ATP) catabolites from the stressful autolysis post-mortem (Poli et al 2003). Fish were found to have a severe lesion which is characterized by the fracture and swollen of organs such as kidney, liver and intestine. The destruction of the intestine wall may permeate and contaminated the fish muscle by the bacteria. In addition, higher electric charge (110 V) might influence the oxidation of the hemoglobin and result an increasing respiratory action. Thus, denature the hemoglobin and displacement or release the heme group, which affect the activity of myoglobin-oxidising and metmyoglobin-reducing enzymes and these suggesting whitening effects (MacDoughall 2002). Likewise, Emery (1984) documented that the depression in muscular activity resume a build-up of lactic acid and depleting the oxygen level which subsequent to a pale looks and discoloration of the fish flesh.

Conclusions. Electrofishing is an efficient fish sampling technique. Malaysian Fisheries Regulation (1980) (Prohibited Method of Fishing) prohibits the use of the electric fishing as it may result the destruction on the ecosystem and associated biodiversity. However, electric fishing may help the researchers to improve and share information on the riverine fish studies. The practice of these fishing techniques needs permission and permits from the authorized department. The optimum and suitable electric charge for fish sampling that contribute to minimal attributes changes is suggested at 50-70 V.

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Authors:
Nurul Ulfah Karim, Universiti Malaysia Terengganu, School of Fisheries and Aquaculture Science, Malaysia, Terengganu, Kuala Terengganu 21030; Universiti Malaysia Terengganu, Institute of Tropical Aquaculture, Malaysia, Terengganu, Kuala Terengganu 21030, e-mail: ulfah@umt.edu.my
Rohisyamuddin Othman, Universiti Malaysia Terengganu, Institute of Tropical Aquaculture, Malaysia, Terengganu, 21030 Kuala Terengganu, e-mail: din@umt.edu.my
Abol Munafi Ambok Bolong, Universiti Malaysia Terengganu, School of Fisheries and Aquaculture Science, Malaysia, Terengganu, Kuala Terengganu 21030, e-mail: munafi@umt.edu.my
Marina Hassan, Universiti Malaysia Terengganu, Institute of Tropical Aquaculture, Malaysia, Terengganu, Kuala Terengganu 21030, e-mail: marina@umt.edu.my

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