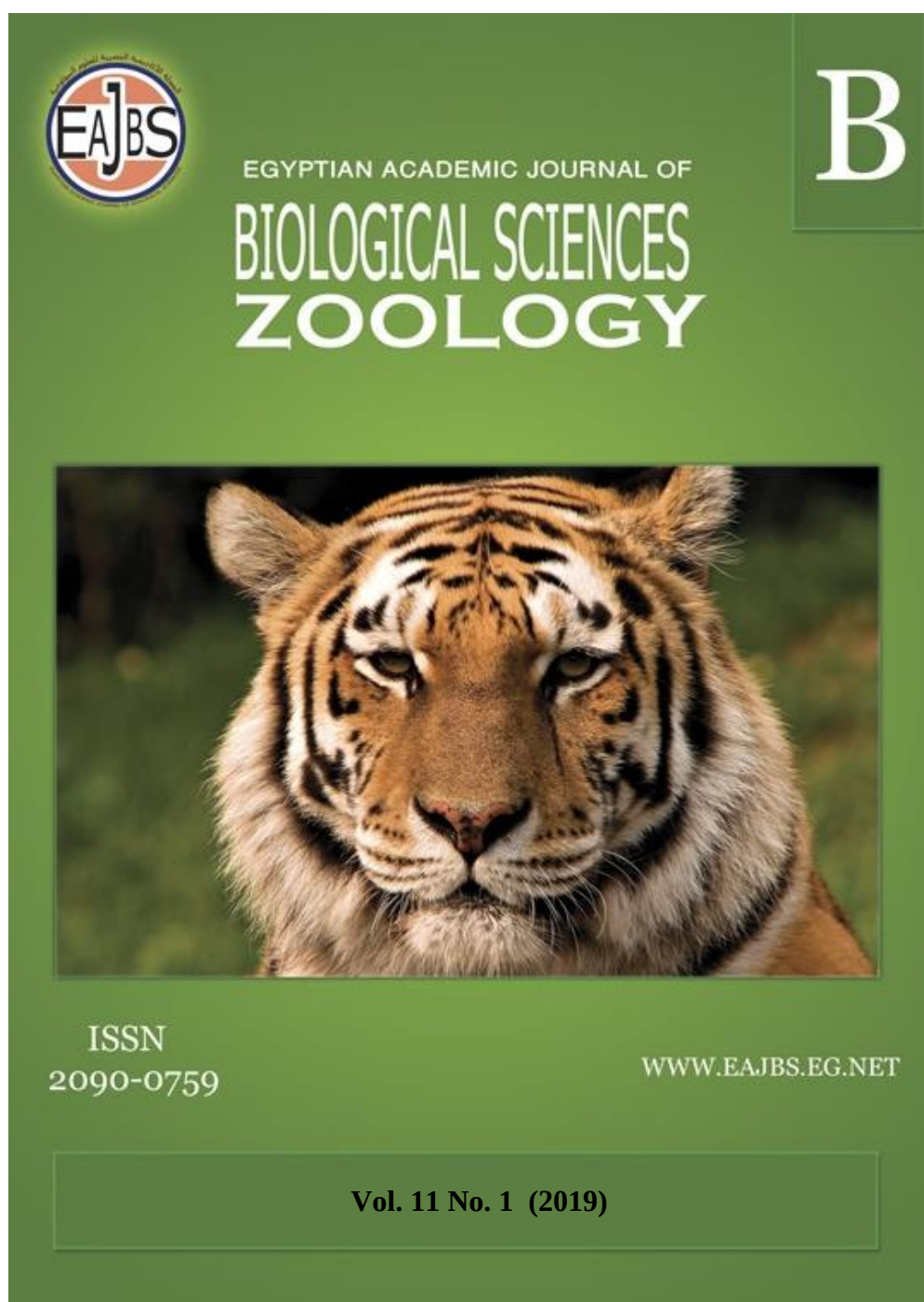


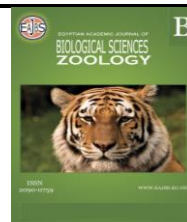
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Physiological Adaptations and Behavioral Responses of Janitor Fish (*Ancistrus sp. orange*) to High Temperature

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ABSTRACT

The study aims to determine the effect of high warming temperature on the behavioral responses of the *Ancistrus sp. orange* on the rates of ventilation, intake of food, and patterns of behavior at normal (21 0C to 26 0C) and high temperature (26⁺ 0C) in the present warming scenario. The non-experimental and experimental data were running in a One-Way ANOVA in order to determine the significant difference in the effect of temperatures, rates of ventilation, intake of food, and patterns of behavior. Statistical analysis was performed at 0.05 level of significance. Based on the findings of the study, it found out that *Ancistrus sp. orange* showed resistance in heat stress but is it projected to go through any physiological impairment when they exposed longer into an increasing temperature. Their food intake has no significant difference with a p-value of .594, but behavioral changes and ventilation rates were significantly affected with the increased high temperature with a p-value of .00).

INTRODUCTION

The janitor fish (*Ancistrus sp. orange*) is a decorative freshwater fish from Northern and Southern America that has been introduced to the aquatic habitats somewhere in America, Europe, and Asia (Fuller & Hill, 2002) like the Philippines. Their ever-increasing trade is one of the most significant passageways for aquatic invasion (Singh *et al.*, 2013; Raghavan *et al.*, 2013; Sandilyan, 2016; Muralidharan, 2017). Among of those ornamental fishes are the janitor fish of the family Loricariidae and genus *Pterygoplichthys* T.N. These fishes have established successful invasive populations throughout the world (Nico & Martin, 2001; Cha *et al.*, 2006; Armando *et al.*, 2007; Piazzini *et al.*, 2010; Gibbs *et al.*, 2013; Jones *et al.*, 2013).

The *Pterygoplichthys* spp. has many collective names comprising armored catfishes, janitor fish, sailfin catfishes, plecos, glass cleaner catfishes, or sucker-mouth catfishes (Daniels, 2006; Knight, 2010; Singh *et al.*, 2013). They have been documented from many water bodies and in many places where they become an invasive type of species for more than two decades (Meena *et al.*, 2016; Rao & Sunchu, 2017).

Pterygoplichthys spp. inhabits anthropogenically modified aquatic habitats (Ortega *et al.*, 2015 & Lopez *et al.*, 2009). These fishes were able to endure varied fluctuations in flow systems of water (Welcomme & Vidthayanom, 2014; Nico *et al.*, 2016), they resist hypoxia due to structural modifications in their gut that allow them to

take breaths air (da Cruz *et al.*, 2013). Their invasion as a non-native species was mainly determined with their capability to acclimate the physiochemical dynamics in their occupied territory (Kestrup & Ricciardi, 2014). Their janitorial skills, attracted by the number of aquarists due to their distinct appearance, strange behavior, routine characteristics, appealing among the aquarists (Rogers & Fletcher, 2012), and they grow fast that could be highly disruptive in small containers. As a result, they likely surpass the abilities of many hobbyists to contain them and are subject to release unintentionally that leads to environmentally unwise owners (Walsh *et al.* 2016).

As mentioned in the conceptual model of the environmental impacts of *Pterygoplichthys* spp., it identifies that there is a severe effect to different groups of organisms (receptors) if they become irresponsibly managed because they might be a model to use in assessing environmental warming, dealing resources, and planning future research (Scott, 2014). In a changing temperature nowadays, the tendency for rising temperatures may act like synergistic effects on water, which might be intensified when combined with effects (Portner *et al.*, 2004). Surface temperature is rising at an unprecedented rate since 1979 with the approximately mean global temperature increased to 0.13 °C per decade (IPCC, 2017). As experienced, warming temperature becomes more penetrating, more recurrent, and will last for a longer period in a tropical climate dynamics like the Philippines (IPCC, 2017).

Nowadays, many fishes are sensitive to warmer temperature than those they usually experience in nature for the past decades. The increase of even 1 °C temperature may be able to have a rapid effect on the geographical distribution and mortality rate of some aquatic fishes (Kennedy *et al.*, 2015). In fact, the rising of temperature is already affecting the abundance, distribution of species and compromising the entire ecosystem (Brierley & Kingsford, 2014).

Generally, fishes like *Pterygoplichthys* spp. fishes are ectothermic, meaning their body temperature relatively detects with the surrounding environmental temperature that result to their increased metabolic rates. Their characteristics in a warming temperature (Roessig, 2004) may be less severe in fishes; the peculiar characteristics of *Pterygoplichthys* spp. make them an interesting model for warming temperature researches (Perry *et al.*, 2015).

In addition, there is already a growing interest that stresses on the impact of warming temperature on water organisms, but there is no report for this concern, except in the findings in the aquaculture techniques of fishes. With this, the fundamental of this studies points out that changing into warming temperature might affect to their physiological growth, feeding, behavior, reproduction, survival, and fish color (Foster & Vincent, 2015; Lin *et al.*, 2009; Sheng *et al.*, 2016).

So, in the present study, the researcher attempts to observe the effects of the increased thermal temperature on the rates of ventilation, feeding rates, and behavioral responses of *Ancistrus sp. orange* both in normal and high temperature for better understanding of the warming environment effect to the future expected conditions, which may support the development of new model in studying *Ancistrus sp. orange* on warming temperature scenarios (Trajano, 2014; Bessa & Trajano, 2013; Reis *et al.*, 2016; Trajano & Bichuette, 2015) of fresh water organisms as well.

MATERIALS AND METHODS

Fish Collection and Storing Conditions:

Janitor fishes (*Ancistrus sp. orange*) were transported from an aquarium shop in October 2018. They were placed to the aquarium facilities in the laboratory and kept in

normal temperature with 825 mL of water (see Fig. 1). Water parameters were normal temperature of 21 °C to 26 °C, the high temperature of 26⁺ °C, and pH of 7.4. The *Ancistrus sp. orange* fishes were fed with fillet balls once a day, except for Sundays.

The aquaria were cleaned after two days with 10% (82.5 mL) of water changes were made. The janitor fishes (*Ancistrus sp. orange*) fishes were acclimatized to adapt to water conditions. Afterwards, janitor fishes (*Ancistrus sp. orange*) were acclimatized to two different temperature set-ups: i) the normal temperature (21 °C to 26 °C) and ii) the projected temperature that they may endure in the future. At the end of the experiments, the janitor fishes (*Ancistrus sp. orange*) were released in the river without injuries.



Fig. 1. The experimental set – ups of *Ancistrus sp. orange*. (a) Warm temperature; (b) Normal temperature

Food Intake, Ventilation Rates and Behavioral Pattern:

Six janitor fishes (*Ancistrus sp. orange*) were placed individually in 825 mL in the glass bowl aquaria inside the laboratory. The 3 janitor fishes (*Ancistrus sp. orange*) were observed in normal temperature (21 °C to 26 °C) in the lab and the other 3 janitor fishes (*Ancistrus sp. orange*) were placed on a warming temperature (26⁺ °C) outside the lab. The janitor fishes (*Ancistrus sp. orange*) were fed 5 fillet balls per day.

Their feeding rates, ventilation rates, and activity patterns were determined with 2 different temperature setups (21 °C to 26 °C and 26⁺ °C). Acclimation to warming temperature was done through a 2-hour gradual increase in the water temperature.

Ventilation of gill rates was counted before feeding based on their opercular beats per minute using a tally counter device. For their food intake, it was counted based on leftovers the following day since they are nocturnal. Their behavioral patterns were also observed on their ethogram presented in Table 1.

Table 1. Ethogram of *Ancistrus sp. orange* pattern activities.

Category	Behavioral Description
Sucker-mouthing	The <i>Ancistrus sp. orange</i> uses their suckermouth to hold into solid substrates/surface.
Swinging	The <i>Ancistrus sp. orange</i> sways its pelvic fin and bracing using their studded spines of their pectoral fins.
Feeding	The <i>Ancistrus sp. orange</i> scrapes / rasps food from different substrates/surface and swallows the food.
Inactivity	The <i>Ancistrus sp. orange</i> remains inactive most of the time in the glass floor / side.

Statistical Analysis:

The statistical analysis used in the study was One-Way Analysis of variance (ANOVA) in order to determine the effect of temperature on food intake and ventilation rates of *Ancistrus sp. orange*. Qualitative analysis was also used in their behavioral patterns. The statistical analysis was performed at a significance level of 0.05 using SPSS version 20 software.

RESULTS AND DISCUSSION

It is shown in Table 2 the mass and some physic-parameters of the distilled water used in the controlled and uncontrolled groups of *Ancistrus sp. orange*. Based on the result, there was a mass increased in the controlled group of at least .3g within the period of the study for 2 *Ancistrus sp. orange* and 1 has a decreased of .1g. As documented, fish #2 in the controlled group failed to consume enough food fillets in the first 2 days as an apparent factor to consider for its mass decreased.

For the uncontrolled group, fish #1 has only survived until the end of the study with a mass decreased of .8g. The rest, they did not survive (Table 1). This decreased in mass may be considered these factors such as unadjusted to the new environment, sudden changes in pH, abrupt changes in water temperature, insecurity if they are not comfortable in their surroundings, lack of sufficient oxygen, exposed to sunlight or physical stress (AquaNooga, 2018).

In this study, distilled water was used because tap water (Silencing, 2018) is more acidic (pH of 5.8) compared to distilled water with a pH of 7. This pH is desirable to consider when it comes to this kind of study. The pH meter was used to determine the quality of the water. As shown in Table 1, there was a decrease in the water quality after 2 days with a mean of at least -1.45. This shows that water quality was deteriorating if exposed into this kind of water condition. With this, fishes will not survive because their home is very limited and it could be contaminated easily with impurities in the water.

Dissolved oxygen (DO) was tested with the DO meter. Based on literature, each organism has its own DO tolerance range. Reading suggests that if the temperature raised to 21 °C, there would be 8.68 mg/L DO (Silencing, 2018) plus warm water holds less oxygen in water with the level below 1 mg/L are considered hypoxic and usually devoid of life. As presented in Table 1, there was a slight increase in water DO (-) of 4 fishes which is a desirable level for them to live or survive except for 2 fishes that had decreased in dissolved oxygen which they did not survive.

In addition, when water temperature increases, the dissolved oxygen (DO) level will decrease that can cause stress, less chance of getting oxygen, too low level of dissolved oxygen, or even death for many fishes in the water ecosystem (ScienceAnswers.com, 2018).

Table 2. Mass and physico-parameters of water used in the experiment.

<i>Ancistrus sp. orange</i>	Body weight	Water Quality (pH)	Dissolved Oxygen (DO)
F1 (Ntemp)	+ .3 g	- 1.47	- 2.18 mg/l
F2 (Ntemp)	- .1 g	- 1.48	- 0.61 mg/l
F3 (Ntemp)	+ .3 g	- 1.57	- 0.48 mg/l
F1 (Htemp)	- .8 g	- 1.41	- 0.02 mg/l
F2 (Htemp)	*	- 1.45	- 1.58 mg/l
F3 (Htemp)	*	- 1.44	- 0.74 mg/l

Legends: (Ntemp) Normal temperature, (Htemp) High temperature, (+) increased, (-) decreased, *dead

Effects of Normal and High Temperature in Their Food Intake:

As seen in Fig.2 , the lowest temperature recorded in the controlled set-up of *Ancistrus sp. orange* was 22 °C and the highest temperature was 33 °C. As shown below, there were 2 fishes did not eat their food fillets in the early part of the assays (both normal and high temperature) but most of them consistently consumed their foods daily within the period of the study. Their food intake has not affected much with the temperature until the end of the observations. *Ancistrus sp. orange* consistently consumed their food fillets. In my claim, the first 2 days from the start of the assay, fish #2 has disturbed the new environment.

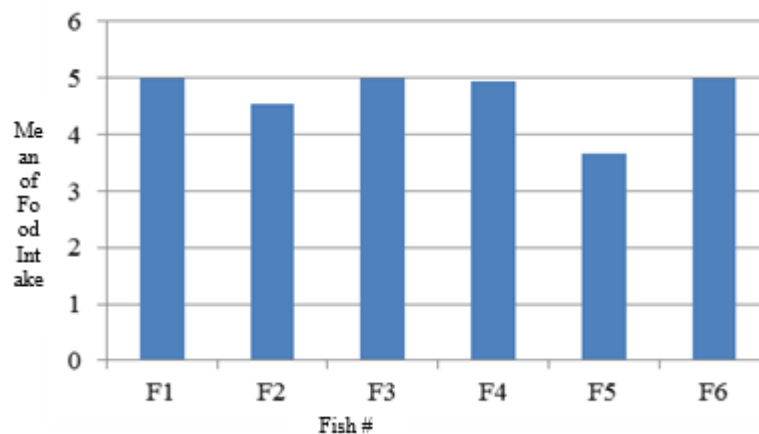


Fig. 2. Impact of both normal and high temperatures in the food intake in controlled and uncontrolled groups of *Ancistrus sp. orange*

In contrast, like most fishes, *Ancistrus sp. orange* is ectothermic and their body temperature fluctuates with external temperature. In the experiment, 2 *Ancistrus sp. orange* fishes were affected that high water temperature has harmful effects on several features of *Ancistrus sp. orange* life in an artificial ecosystem. The increase of water warming temperature has slight changes in their food intake and activity patterns.

In addition, the lack of differences in their food intake and behavior in 2 experimental set-ups with varied water temperature scenarios may be explained by the referred ability of *Ancistrus sp. orange* to adapt to different temperature fluctuations as they endure in their ecosystem. So, both factors if combined might act to decline and affect fish survival (Portner & Knust, 2007).

Effects of Normal and High Temperature in Their Ventilation Rates:

The effect of warming water temperature was expected to accompany by an increase in their ventilation rates. As shown in Figure. 3, there was a significant increase in their rates of ventilation of 800.67 mean difference beats compared to

normal water temperature. As indicated, the highest temperature recorded was 33 °C and has reached to 365 beats (Fish #1) based in their opercular beats using a tally counter per minute after it acclimatized for 2 hours in a warming water temperature when exposed into the sunlight.

As observed, the data suggest that such temperature (26+ °C) is already beyond the temperature tolerance or critical level. This indicates that fitness and survival level will be life threatening of the aquatic species due to oxygen deficiency (Portner et al., 2016) and higher temperature results in less dissolved oxygen in the water. It demonstrates that *Ancistrus sp. orange* significantly affected by warming water temperature. Their opercular beats were became faster when exposed to a rising temperature of 26+ °C.

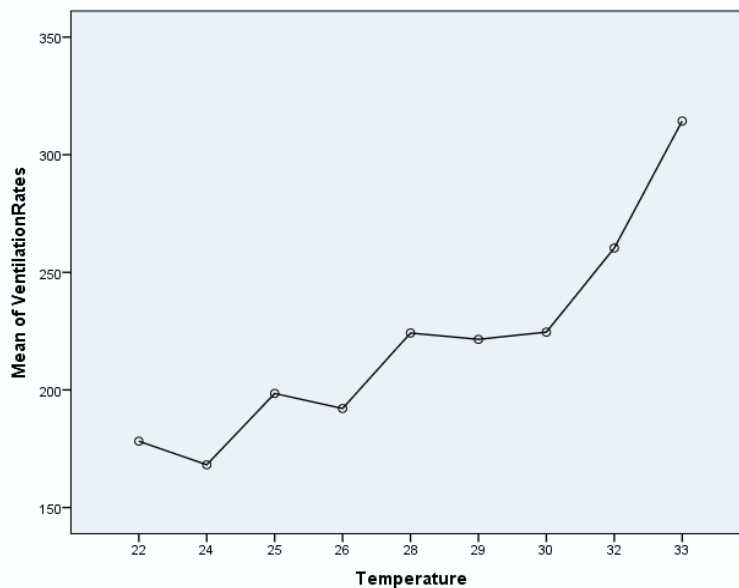


Fig. 3. Impact of high temperature in the ventilation rates of *Ancistrus sp. orange*.

Based on the literatures, when water will become heated, like exposing into the sunlight or near to thermal sources, water temperature increases, then the dissolved oxygen level will decrease that can cause strain or even death for many fishes, most importantly for the survival of aquatic animal like fishes (ScienceAnswers.com, 2018). This specifies that both factors might thus act as synergistic stressors (combined effects) and be overwhelming to the cardiorespiratory processes that might suffer a decline in the efficiency to sustain muscular activity, growth at a critical point, and the survival of the fishes (Portner & Knust, 2017) even they are highly adaptable to different environments (Hoover et al., 2014).

Data Analysis of the Food Intake and Ventilation Rates in Normal and High Temperature:

Table 3 shows the analysis on the food intake and ventilation rates when exposed to normal and high water temperatures. As presented above, the food intake of *Ancistrus sp. orange* has no significant correlation to warming temperature. The p-value of food intake is .594, which failed to reject the null hypothesis with the significant level of .05. Unexpectedly, their food intake remained almost unchanged with the increasing temperature except fish #2 which it presumed not totally adapted to the new environment.

Hence, their activity patterns were also expected to change in high temperature. The lack of differences in their food intake in various temperature scenarios may be

explained by the referred ability of the fishes to adapt temperature fluctuations as they endure in their newly adopted habitat.

As observed, *Ancistrus sp. orange* were very behaved most of the time and based on literature, they are very active in nighttime as nocturnal species.

In contrast, the *Ancistrus sp. orange* ventilation rates were significantly affected by warming temperature. As expected, their ventilation rates (Table 2) has a p-value of .00 less than the significant value of .05, which leads to rejecting the null hypothesis. As observed, their opercular beats relatively increased in the rising temperature of 26° C. Based on the record, the highest opercular beats recorded were 365 beats at 33 °C temperature (Fig. 3) as well as their activity patterns. When temperature exceeded beyond the normal degree, *Ancistrus sp. orange* started moving when directly exposed into the sunlight, upraised and expand their pelvic fins, and swam up to the surface water to get oxygen.

In general, *Ancistrus sp. orange* showed to be sensitive to warming water temperature. Based on study, the abrupt decline in their thermal sensitivity when they exposed to upper 26° C suggests that such temperature is already above their tolerance level, which leads to compromise their fitness and survival (Portner *et al.*, 2015) because warming temperature increases their oxygen demand that may result to less dissolved oxygen in water. In this situation, water quality becomes too low in oxygen leading the organisms to die (ScienceAnswers.com. 2018).

Moreover, it arose that warming temperature can be caused heat stress to water organisms like fishes and are projected to undergo physiological impairment and behavioral changes relative to their growth, survival, and environmental disturbance (Curtis & Vincent, 2016). Their limited environment will become harmful in a warming temperature scenario since their environment is so limited and they have no capacity for possible migrations in order to reproduce their species (Foster & Vincent, 2015).

Table.3 Results of One-Way ANOVA Analysis on the effect of high water temperature of *Ancistrus sp. orange* in relation to their food intake and ventilation rates.

		Sum of Squares	df	Mean Square	F	Sig.
Food Intake	Between Groups	8.706	8	1.088	.812	.594
	Within Groups	108.583	81	1.341		
	Total	117.289	89			
Ventilation Rates	Between Groups	98448.50	8	12306.06	8.284	.000
	Within Groups	120321.98	81	1485.457		
	Total	218770.48	89			

Conclusion

In conclusion, the researcher provides evidence that *Ancistrus sp. orange* was affected with heat stress and is likely to suffer physiological impairment and abrupt changes in their behaviors in the anticipated increase temperature. In addition, they might experience metabolic imbalance with likely significances for their endurance to warming temperature and may be enabled to ought certain level of adaptability and resilience to environmental disturbance but they may suffer and possibly may not survive. So, owners might be sensitive enough to consider their ecosystem because these fishes are charismatic to have the potential to become symbols of protection. Their unique aspect turns them into a flagship species for aquarists' conservation issues to understand their vulnerabilities and their responses to the future scenario for warming temperature.

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Declaration

The author declares that all works are authentic and this research paper has no competing interest exists.

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