Determination of the average intraocular pressure values, optimum anesthesia dose and phenotypic characteristics in Oscar fish (Astronotus ocellatus)

Research Article

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ABSTRACT

This research was carried out to determine the intraocular pressure values, optimum anesthesia dose and phenotypic characteristics in Oscar fish (Astronotus ocellatus). A total of 11 adult, male Oscar fish were used in the study. Optimum anesthesia dose, pre-and post-anesthesia intraocular pressure values and phenotypic measurements (total length, fork length, standard length, dorsal fin base length, head length, body length, eye diameter, body depth, pectoral length, pelvic fin bottom length, anal fin bottom length) were determined and reference values specific to this fish species were obtained by performing a statistical analysis. When exposed to 3 cc/L of the concentrations, fish achieved a deep state of anesthesia (induction time 1.31 min; recovery time 6.42 min). The most balanced anesthesia without risking vital functions was achieved using Clove oil with a dose of 2.5 cc/L. Compared to the other dimensions, total length, fork length and standard length were the most consistent body measurements with high positive correlations. No statistically significant difference ($p > 0.05$) was seen in both eyes between the intraocular pressure values before (5.36-5.86) and after (5.59-5.36) anesthesia. It is recommended that the ideal concentration of clove oil was 2.5 cc/L to reduce stress and injury damage during handling procedures. Additionally, intraocular pressure values, body measurements and Clove oil optimum anesthesia dose obtained in this study can be used as reference values for Oscar fish raised in aquarium conditions.

Keywords: Anesthesia, clove oil, intraocular pressure, Oscar fish, phenotype

INTRODUCTION

In terms of the aquarium fish farming sector, precise anesthesia dosages that can be taken as reference are needed for transportation, operative interventions and experimental studies. Regarding the Oscar fish as a species with economic importance, there is no reference source with data on adult body phenotypic measurements, normal intraocular pressure values and optimal anesthesia dosage. On the other hand, since there is a limited number of studies aiming to establish physiological reference values for various parameters of Oscar fish, especially intraocular pressure, the need for such studies in the field has not yet been met (Keeney et al., 2019; Link et al., 2004) because it is not commonly preferred in aquaculture due to its slow growth rate and troublesome maintenance (Pinheiro et al., 2018). Nevertheless, studies that will provide reference data in the field of aquarium fish farming, which is a dynamic sector, are welcomed by both breeders and researchers.

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In recent years, different types of anesthetics have been used in aquaculture and scientific research to eliminate the negative effects on the health and growth of fish and to reduce the stress responses, minimize physical injuries, taking photographs, processing artificial reproduction and making several measurements (Roubach et al., 2005; Küçük et al., 2016; Chatigny, 2017; Fernandes et al., 2017). On the other hand, intraocular pressure (IOP) is required to maintain the eye into a shape allowing it to function and it is sustained by the balance between the production of aqueous humor by the ciliary body and the resistance to its outflow from the eye in vertebrates (Zouache et al., 2016).

Oscar fish are mostly known as omnivorous but they seem to prefer a carnivorous diet. They mainly feed on aquatic and terrestrial insects, small fish and invertebrates, fruits, benthic algae and plants (Yılmaz & Arslan, 2013). The Astronotus ocellatus species was chosen in this study due to lack of work on it. It is difficult to find breeders who have large numbers of these fish in their aquariums.

It was aimed to determine the optimum anesthesia dose, analyze the pre and post-intraocular pressure changes and determine some phenotypic features to provide scientific information about Oscar fish. In this respect, it is hoped that the data obtained from this research will fill an important gap to some extent and will shed light on future studies for both industry and experimental studies.

MATERIAL and METHOD

Fish material and experimental conditions

A total of 11 adult, male Oscar fish reared in the 100 cm x 35 cm x 45 cm size aquarium were used as the study material and the experiments were performed in duplicate under the similar experimental conditions in a two-week interval after the end of the first applications.

Feeding was made on the base of 5% of live weight with commercial feed. Bottom cleaning of the aquariums was done twice at 3-day intervals and water change up was performed up to 30% each time. All experiments were conducted at 27.2°C water temperature with a standard 16-h light: 8-h dark photoperiod. As routine measurements pH, salinity, Total Dissolved Solids (TDS), conductivity, aquarium temperature measurements were recorded with a waterproof ExStik® II pH/conductivity meter, EC500, "Extech" brand device, ambient temperature and humidity with a "Thermo HYGRO" device (Table 1).

Table 1. Ambient conditions during the trials

Ambient temperature (°C)	29.1
Humidity (%)	40.0
Salinity (mg/L)	333.0
Aquarium water temperature (°C)	27.2
Total Dissolved Solids (TDS)	518.0
pH	8.2

Anesthesia

Clove oil used in the research was supplied from "Kimbiotek Chemical Substances Joint Stock Company, Turkey". For anesthesia 1.5, 2, 2.5, and 3 cc/L clove oil dosages were tried to determine the optimal dosage in Oscar fish (Figure 1). Each dose was tested at 3-day intervals on the same animals. The solutions were prepared by dissolving clove oil in 95% ethanol (1:10 ratio) as described by Anderson et al [1997] to facilitate mixing.

Figure 1. Experimental setup: A simple recirculating system maintains anesthesia by delivering anesthetic water from a reservoir to the gills and recycles the effluent back to the fish through the use of a submersible pump.

The fish was anesthetized and positioned with the lateral side up during measurements (Figure 2). The induction and recovery times of the fish were monitored with a digital stopwatch, and the stimulation reaction was determined by contact with a plastic pipette.

Figure 2. Fish position on a small fish anesthetic unit at the time of anesthesia

Intraocular pressure (Ocular tonometry) measurements

After determining the optimum anesthesia dosage, the fish were individually placed in containers for preparation of the measurements. Before and after the anesthesia, bilateral intraocular pressures of the fish were measured with a digital tonometer, TonoVet (Icare, Finland) using the needle of the device brought into contact with the outer surface of the fish eye (Figure 3).

Figure 3. Intraocular pressure measurements using a digital tonometer

Bodyweight and other phenotypic measurements

Fish were weighed with a digital weighing scale precise to 0.1 g. Other phenotypic measurements (total length, fork length, standard length, dorsal fin base length, head length, body depth, eye diameter, pectoral fin length, pelvic fin bottom length, anal fin bottom length) were taken on a flat platform while the fish were under anesthesia. Metric measurements were taken using a ruler on a straight line over the curves of the body (Figure 4).

Figure 4. Phenotypic measurements of fish on the anesthetic unit

Morphological measurements were taken using the procedure below (Figure 5)

Total length: The distance measured from the anterior part of the head to the end of the most posterior part of the caudal fin.

Fork length: The distance measured from the anterior tip of the jaw to the endpoint of the caudal fin.

Standard length: The distance measured from the anterior part of the head to the end of the vertebral column/caudal peduncle.

Dorsal tail bottom length: The distance measured between the anterior and posterior base of the fin at the base of the dorsal tail.

Head length: The distance measured from the tip of the mouth to the most distant point on the opercular membrane.

Body depth: The vertical line at the widest part of the body.

Eye diameter: Maximum diameter of the eye.

Pectoral fin length: The distance measured between the anterior and posterior lines of the pectoral fin.

Pelvic fin bottom length: The distance measured between the anterior and posterior base of the pelvic fin.

Anal fin bottom length: The distance measured between the anterior and posterior base of the anal fin.

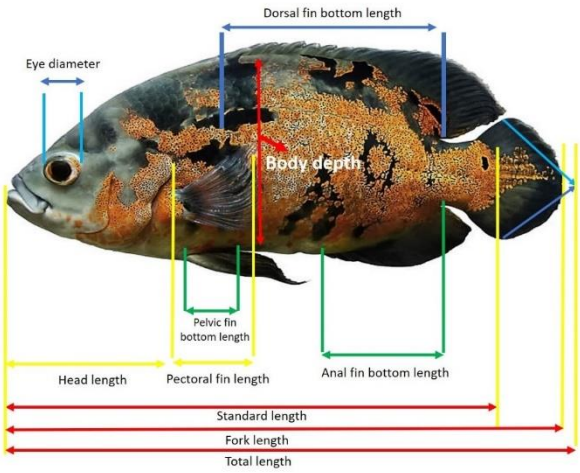

Figure 5. Morphological measurements of Oscar fish

At the end of all measurements, each fish was returned to the aquariums for recovery with clean water allocated for it.

Statistical analysis

IBM SPSS Statistics 25.0 package program was used for statistical analysis. ANOVA with the Tukey test was applied to compare the induction and recovery times ($p < 0,05$). Intraocular pressures were compared by Paired Samples t-Test ($p < 0.05$) while descriptive statistics were used to demonstrate the phenotypic measurements.

RESULTS

Behavioral characteristics observed in fish during the anesthesia induction were loss of balance, decreased respiratory rate, decreased reactions to external stimuli, immobility at the bottom of the water, stopping of gill and mouth movement, respectively. Observations during recovery from anesthesia were light movements in swimming, gills and mouth, increased response to external stimuli, improvement of balance, normal movement and regaining the swimming balance, respectively.

When applying different doses to determine the optimal dosage, 3 cc/L clove oil was noticed to cause deep anesthesia in the fish which gave the impression to be detrimental. Those fish were immediately taken to clean water and sober up before they died. It was decided that a dose of 3 cc/L would not be appropriate, since the anesthetic agent to be used was aimed to create anesthesia at the level of surgical anesthesia. On the other hand, the most balanced anesthesia without risking vital functions was achieved with a dose of 2.5 cc/L whereas 1.5 and 2 cc/L were found inadequate in terms of timing and efficacy (Table 2).

Table 2. Intergroup analysis of the effect of different doses of Clove oil on induction and recovery from anesthesia in the Oscar fish

Process	n	Dosage (cc/L)	$\bar{x} \pm S_{\bar{x}}$	p
Anesthesia induction (min)	11	1.5	14.63 ± 0.4^{a}	0.000
	11	2.0	6.95 ± 0.2^{b}	
	11	2.5	3.35 ± 0.1^{c}	
	11	3	1.31 ± 0.1^{d}	
Recovery from anesthesia (min)	11	1.5	0.40 ± 0.1^{a}	0.000
	11	2.0	3.21 ± 0.1^{b}	
	11	2.5	3.55 ± 0.2^{b}	
	11	3	6.42 ± 0.2^{c}	

$p < 0.05$, p: ANOVA. Means within a column with the different letter are significantly different

Those fish that had long induction time emerged from anesthesia early while the ones with a short induction time were delayed from anesthesia.

As seen from the measurement results, the body size and live weight of Oscar fish were much higher compared to many other aquarium fish (Table 3).

Table 3. Descriptive values for the phenotypic characteristics of Oscar fish

	n	x̄ ± SD	Min	Max
Live weight (g)	11	471.21 ± 67.80	368.1	593.0
Total length (cm)	11	27.47 ± 0.88	26.0	29.5
Fork length (cm)	11	27.35 ± 0.94	25.5	29.0
Standard length (cm)	11	22.88 ± 1.21	21.3	25.4
Dorsal tail bottom length (cm)	11	11.94 ± 2.14	7.4	14.7
Head length (cm)	11	8.12 ± 0.70	7.0	9.2
Body depth (cm)	11	11.05 ± 0.67	9.7	12.2
Eye diameter (cm)	11	1.43 ± 0.11	1.2	1.6
Pectoral fin length (cm)	11	6.68 ± 0.83	6.0	9.9
Pelvic fin bottom length (cm)	11	1.80 ± 0.48	1.2	3.0
Anal fin bottom length (cm)	11	5.89 ± 0.79	4.5	7.5

No statistically significant difference was found between intraocular pressure values in both eyes during the pre-and post-anesthesia periods ($p > 0.05$) (Table 4).

Table 4. Intraocular pressure measurements before and after anesthesia

	n	Pre-anesthesia (x̄ ± Sx̄)	Post-anesthesia (x̄ ± Sx̄)	p
Right eye IOP	11	5.36 ± 0.24	5.59 ± 0.26	0.107
Left eye IOP	11	5.86 ± 0.29	5.36 ± 0.19	0.625

$p < 0.05$; p: Paired samples t-test

Regarding the relationships between the body measurements, high positive correlations were determined between the main three dimensions. They were total length, fork length and standard length. They were all positively correlated with each other and with head length, body depth, and anal fin bottom length while negative correlations were found between body weight and the total length, fork length, standard length, head length, body length, and anal fin bottom length (Table 5).

DISCUSSION

The results of the current study showed that clove oil could be used effectively at a dosage of 2.5 cc/L in Oscar fish without any harmful effects. Depending on this dose with balanced anesthesia without risking vital functions, the mean induction time was 3.4 min with a range from 3.1 to 3.7 min and mean recovery time was 3.7 min with a range from 2.3 to 5.1 min. At other doses, induction occurred too late without obvious symptoms or too early in a deep anesthesia situation. In accordance with our results, researchers refer to clove oil as an attractive anesthetic for fish due to its efficacy, low price and safety (Fujimoto et al 2018, Silva-Souza et al 2015). It also refers to a natural substance that has no side-effects on fish and does not represent any ecological or hygienic risks (Hamackova et al 2006). Another advantage of Clove oil is that it can easily be found in pharmacies, spice shops or markets.

Regarding the phenotypic properties, Oscar fish is not among the ubiquitous species and attracts attention with their large body structures. It is difficult to keep and feed them compared to most of the other aquarium fish species. Since it is not a widely grown fish

species, it is not easy to come across the literature regarding most of the phenotypic measurements of this species. Considering the available reports of studies on body measurements of Oscar fish, several researchers reported the mean length for adults varying between 19-33 cm and weight between 0.4 kg and 1.5 kg generally referring to the wild types (Castro-Castellón et al 2020, De Boeck et al 213, Paes et al 2011, Rodrigues et al 2017, Trindade and Queiroz 2012, Yılmaz and Arslan 2013). The average total length of adult Oscar fish reared under laboratory conditions was found as 27.5 cm with a range from 26.0 to 29.5 and average live weight as 471.2 g with a range from 368.1 g to 593.0 g in the current study. The standard length which was determined as 23 cm (21-25 cm) in this study was similar to those reported by some researchers in the range of 16-28 cm for Oscar fish (Trindade and Queiroz 2012, Yılmaz and Arslan 2013). Any discussion could not be made on other phenotypic measurements due to the lack of accessible literature.

Table 5. Correlations between morphometric features of Oscar fish

		Total length	Fork length	Standard length	Dorsal fin bottom length	Head length	Body depth	Eye diameter	Pectoral length	Pelvic fin bottom length	Anal fin bottom length
Live weight	r	-.732*	-.767**	-.661**	.270	-.693**	-.671**	-.517*	.172	-.505*	-.628**
	p	.024	.003	.010	.466	.017	.036	.131	,214	.099	.047
Total length	r	1	.907**	.829**	.035	.736**	.529*	.415	-,272	.490*	.604**
	p		.000	.000	.830	.005	.031	.094	,336	.124	.017
Fork length	r		1	.923**	-.072	.780**	.717**	.585**	-,250	.511	.676**
	p			.000	.794	.005	.008	.039	,417	.107	.021
Standard length	r			1	-.104	.731**	.678**	.518*	-,202	.492*	.645**
	p				.615	.003	.016	.099	.821	.165	.011
Dorsal fin bottom length	r				1	.069	-.196	.164	-.031	.413	-.228
	p					.799	.473	.573	.934	.189	.507
Head length	r					1	.439*	.377	-.264	.235	.646**
	p						.212	.267	.422	.415	.022
Body depth	r						1	.791*	.006	.480	.668**
	p							.015	.996**	.148	.039
Eye diameter	r							1	.214	.530*	.311
	p								.533	.094	.381
Pectoral length	r								1	-.112	-.150
	p									.567	.404
Pelvic fin bottom length	r									1	.205
	p										.612

*p < 0.05; **p < 0.01

Intraocular pressure values provide important clues for the health of fish in routine clinical examinations. Therefore, a complete ocular evaluation should be included in every routine physical examination (Williams and Whitaker 1997). For example, elevated intraocular pressure (IOP) is recognized as the main risk factor in Glaucoma (Adornetto et al

2020). The changes observed in intraocular pressure values due to the effect of anesthesia are among the indicators of the sensitivity of animals towards anesthetic substances. The fact that there was no statistically significant difference between the intraocular pressure values before (5.36-5.86) and after (5.59-5.36) anesthesia in both eyes in Oscar fish in the present study could be considered as an indicator of how resistant these fish were against side effects of anesthesia. Parallel to the findings of this study, the normal range for intraocular pressure in fish is reported between 2.24 and 13.63 (Lynch et al 2007, Waser and Heisler 2005, Zouache et al 2016) depending on the corneal thickness and other biomechanical properties of the eye (Bennett et al 2018).

The high positive correlations between body measurements indicate that these fish have a consistent body structure. On the other hand, negative correlations between body weight and body measurements indicate variability in body weight values. This situation can be explained by nutritional skills depending on the social hierarchical order and individual physiological differences. Among the body measurements, total length, fork length and standard length were the most consistent ones. They were all positively correlated with each other and with head length, body depth, and anal fin bottom length. Therefore, this study suggests that considering a standard body shape total length, fork length and standard length should be used rather than the other measurements.

It is seen that not only pet animals but also aquatic creatures are becoming popular by people day by day. In this context, the diagnosis and treatment of diseases of such organisms has an important place in veterinary medicine. The fact that the clove oil used in this study did not cause a serious change in intraocular pressure is extremely valuable data, especially for ophthalmological operations and intraocular procedures.

The fact that there was no significant change in the intraocular pressure value showed that these fish were not metabolically affected at the optimum anesthetic dose.

CONCLUSION

Oscar fish was resistant to side-effects of anesthesia having a wide tolerance since the metabolic activity was not affected under the 2.5 cc/L dose. Therefore, Clove oil can safely be used for surgical anesthesia purposes. Additionally, intraocular pressure values, body measurements and Clove oil optimum anesthesia dose obtained in this study can be used as reference values for Oscar fish raised in aquarium conditions.

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