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بعض جوانب علم وظائف الجهاز التنفسي في سمك السبيطي المستزرع سباريدينتيكس هاستا (Valenciennes 1830) بمملكة البحرين

Khadija Zainal

Department of Biology, College of Science, University of Bahrain, PO Box 32038,
Kingdom of Bahrain

الملخص:

تم جمع عينات من سمك السبيطي المستزرع (سباريدينتيكس هاستا) من مركز استزراع الأحياء البحرية الوطنية (ناماك) في راس حيان بمملكة البحرين. تم تعريض الأسماك تدريجياً إلى مستويات منخفضة جداً من تراكيز الأوكسجين المذاب وعلى مدى 24 ساعة واستمرت القياسات لمدة ثلاثة أيام متتالية لكل سمكة، وذلك باستخدام جهاز قياس معدل الأيض والمتمثل بمدى استهلاك الأوكسجين من الماء (نظام قياس التنفس المتقطع) (ملغم لكل ليتر و لكل جرام من وزن السمكة في الساعة الواحدة). بشكل عام استجابت الأسماك للظروف المحيطة استجابة غير منتظمة و كذلك أكدت القياسات بان هذه الأسماك تتميز بمقدار منخفض من استهلاك الأوكسجين. ولوحظ بانه كلما قلت نسبة الأوكسجين بالماء كلما ارتفعت نسبة التنفس لديها (أي ارتفعت نسبة تهوية الخياشيم) محاولة منها استخلاص أكبر قدر ممكن من الأوكسجين من المياه المحيطة بها في الحوض. وبشكل عام أيضاً ترتفع نسبة الاستهلاك عندما تضطرب هذه الأسماك في بداية كل تجربة. تمخضت الدراسة عن نتائج مماثلة في حالة كل سمكة حيث تفقد السمكة توازنها عند ما تصل نسبة الأوكسجين إلى الحد دون المميت وفي حالة الاستمرار في نقص الأوكسجين تموت السمكة. أكدت الدراسة بان سمكة السبيطي المستزرع بالتحديد تتميز بعدم القدرة على تحمل نسب منخفضة جداً من الأوكسجين المذاب. وإحصائياً، توجد علاقة طردية بين معدلات استهلاك الأوكسجين وتراكيز الأوكسجين المذاب مما يدل على عدم القدرة على تنظيم عملية الأيض في حالات عدم توفر الأوكسجين لهذا تعتبر هذه الأسماك من الأكسيكونفورمر أي عدم تحملها الاستمرار في الظروف اللاهوائية. كما ودلت النتائج على وجود علاقة غير خطية بين الحد الأدنى الأساسي لاستهلاك الأوكسجين وحجم السمكة (وزن الجسم). وبهذا قدمت الدراسة الحالية بيانات أساسية بالإمكان استخدامها في دراسات مستقبلية ذات الصلة بسمك السبيطي الصغار منها أو أنواع أخرى من الأسماك.



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ORIGINAL ARTICLE

Aspects on respiratory physiology of cultured Sea bream, *Sparidentex hasta* (Valenciennes 1830), Kingdom of Bahrain



Khadija Zainal

Department of Biology, College of Science, University of Bahrain, P.O. Box 32038, Kingdom of Bahrain

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Sublethal oxygen

Abstract Cultured fish Sea bream, *Sparidentex hasta* collected from the National Mariculture Center (NaMaC) at Ras Hayan in the Kingdom of Bahrain, were subjected to extreme low dissolved oxygen concentrations under controlled experimental conditions for 24 h and continued up to three consecutive days. The metabolic rate as represented by oxygen consumption rate (mg/l/g/h), was measured using intermittent computerised respirometry system. The fish responded in general, by irregular and low metabolism. Elevated ventilation in gills was observed in order to intake more oxygen from surrounding water. An increase in oxygen consumption rate was recorded at different stress levels including initial handling and movements as well as at the onset of hypoxia. Similar findings were obtained for the level at which fish mortality attained 100% representing lethal points.

The fish regulatory ability to withstand declining oxygen concentration in the water was limited. A typical steep straight line relationship was found between oxygen consumption rate and oxygen concentration indicating a non regulatory ability and extreme in-tolerance to hypoxia. Therefore, the fish is considered as oxy-conformer, i.e., unable to continue metabolism at anaerobic condition. Correlation between minimum (basic) oxygen consumption rate and body weight was of non-linear form. The present study provides comparative data to base on for further prospective related studies on juvenile Sea bream and other fish species.

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1. Introduction

Sparid fishes are widely distributed and commercially significant for both fisheries and aquaculture industry around the world (Pavlidis and Constantinou, 2011). This group inhabit tropical and temperate coastal waters (Bauchot and Smith,

1984). Sea bream, *Sparidentex hasta* (locally known as Sobaity) has been reported as the only species in the genus *Sparidentex* by FAO-FishStatPlus (2008), however, a new sparid species *Sparidentex jamalensis* has been recently recorded from mangrove swamps in Pakistan (Siddiqui and Rafaqat, 2014). *S. hasta* is a native species to the Arabian Gulf waters within Bahraini, Kuwaiti, Saudi Arabia, Omani and Qatari waters. It is known as Sobaity Sea bream or Silvery Sea bream. It is also widely distributed along the western Indian Ocean and

E-mail address: Kzainal@uob.edu.bh

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coasts of India (Bauchot and Smith, 1984). In addition, according to the FAO-FishStat Plus (2008), the worldwide production of the sparidae fish was 693,732 metric tons in 2006. Approximately, 35% of that contribution was produced by aquaculture. *S. hasta* is a pelagic fish. It was selected in the National Mariculture Centre to enhance local fish stock and fisheries in the Arabian Gulf as well.

Sea bream has been successfully cultured at NaMaC in the Kingdom of Bahrain. The fish is maintained in well aerated tanks and fed three times to satiation or at 3% of their body weight (NaMaC information leaflet). Eggs are provided with adequate aerations and sterile re-circulating sea water connected to sand filtering system. Larvae are fed on live food organisms by gradually weaning from the artificial diet. Following 40 days, larvae are transported to incubation tanks where they can be harvested at regular intervals. During this time, the fingerlings (2–4 cm total length) are separated according to their sizes and are either exported, released into the sea or left to grow. The adults from these batches were used in the present experimental studies.

Tharwat and Al-Gaber (2006), reported an average length of 30 + 2.3 cm; average weight of 190 + 4 g, and a minimum size at sexual maturity of about 24 cm for *S. hasta* in the natural waters of the Arabian Gulf. A maximum total length of 50 cm has also been recorded by Randall (1995), however, *S. hasta* and other sparid fishes may grow up to 75 cm (Karam, 2011).

The metabolic requirements of cultured species are important at all stages of fish life cycle in order to maximise the optimum conditions for growth (Nerici et al., 2012). The metabolic rate represented as oxygen consumption rate for investigated species will provide information concerning the development of an oxygen management strategy under farming conditions.

The present study is the first initiation experimental study on *S. hasta* metabolic rates as represented by oxygen consumption rates at normal levels of oxygen availability (active, routine and resting), as well as under stress conditions (hypoxia and anoxia). The present study aimed to determine the oxygen requirements of the adult individuals of *S. hasta* and to verify tolerance under limited oxygen conditions.

2. Materials and methods

Twenty four Sea bream fishes were obtained from the Mariculture centre at Ras Hayan-Bahrain during 2009 and 2013. At the centre, the adult fishes were kept in tanks under controlled conditions with temperature ranging between 15 and 32 °C and oxygen concentration of about 5–6 mg l⁻¹. These fishes were originated from brood-stock eggs, spawned in captivity as mentioned above.

At the Department of Biology laboratory in the University of Bahrain, fish individuals were maintained in well aerated re-circulated sterile artificial seawater at fixed room temperature of 20 °C and salinity of around 40–45‰. The photoperiod system was adjusted using an automatic timer set at 12:12 light and dark times respectively. Fishes were fed on commercial feed obtained from the Mariculture centre except one day prior to the experimentation. The water level in the aquarium was regulated manually.

The volume of the respiratory chambers and tubing was reduced to the minimum in order to achieve a gradual reduction in oxygen concentration during the measurement phase.

Fish individuals collected during 2009 were weighing between 7.57 and 70.9 g., with total length between 74 and 165 mm. The fish individuals collected during 2013 were slightly larger with weight range (36.5–97.6 g) and total length of 13.5–180 mm. Fish gender was determined by dissection at the end of each experiment.

2.1. Respirometry arrangement and data analysis

Initially, the experimental fish were allowed at least one day to acclimatise in the respirometer and were unfed at least for 24 h prior to the experiment to avoid effects of the specific dynamic action (Atsunobu and Toshiomi, 1973; Jobling, 1981).

The experimental set up was based on a computerised intermittent flow through system (Forstner, 1983; Steffensen et al., 1984; Steffensen, 1989; Kaufman et al., 1989). Fig. 1a shows a photograph and Fig. 1b a diagram of the respirometry setup used in the present study. Different sizes chambers were used to accommodate different sizes of fish (male and females). The ratio of fish volume to the chamber volume was adjusted so that the reduction in the dissolved oxygen is not too fast (Steffensen, 1989). The chamber was placed in a larger reservoir water tank at the ambient oxygen level, salinity and temperature. The oxygen electrode incubated in a holder was also submerged in the tank. The tank and all the tubing were cleaned on a weekly basis or as soon as the experiment was ended. The chamber was moved from side to side to get rid of any air bubbles before recording.

A total of 77 experiments were conducted and were continued up to a minimum of 24 h and a maximum of three days. Out of the total, 34 experiments were run under controlled normoxic conditions (normal ambient oxygen concentration) whereas, 43 experiments were run under hypoxic condition (progressively reducing ambient oxygen concentrations). If a fish survived at first exposure, it was exposed again to the hypoxic condition (extremely low oxygen concentration but not to a total lack of oxygen). Anoxic exposure (i.e., total lack of oxygen) experiments were also conducted in order to investigate survival rate at the extreme condition. Fully recovered individuals were returned to the maintenance tank. If they showed a sign of recovery after 2–5 weeks, they were examined once more in normoxia and hypoxia series of experiments as required otherwise, they were discarded.

The oxygen consumption per unit time was determined for each fish individual at normoxia (active and resting), stressed (hypoxic) and back to normal (recovered). Each experiment lasted for a maximum of 4–5 days provided that the fish behaviour was considered relaxed. At the end of each run, the experimental fish was weighed; total length was measured and moved back into well aerated maintenance aquarium tanks, fed and observed for survival.

Normoxia measurement of the oxygen consumption rate during the closed phase was continued until the oxygen concentration was dropped from 100% to around 80–90%. The treating period of 15 min was followed by a flushing period of another 15 min. These recordings were continued over night and up to three days during which, the oxygen level was not allowed to drop below 80% level. The experiments were stopped once the oxygen consumption declined to a low rate that was stable for at least 12 h indicating the best possible recordings of the basic level (resting).

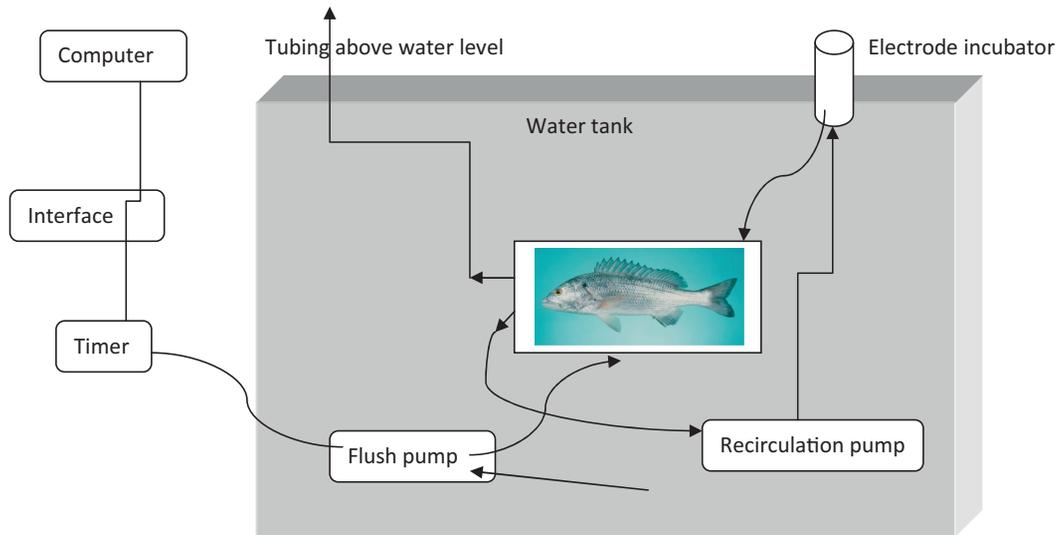


Figure 1a A diagram of the experimental set up, respirometer and tubing are submerged in water tank. Arrows indicate the direction of water flow.



Figure 1b A photograph of the experimental setup.

On the other hand, hypoxia experiments were run during the closed phase whereby, the oxygen level allowed dropping progressively towards zero. The average values of the lowest level of oxygen concentrations were called a sub-lethal oxygen concentration. At this point fish can no longer tolerate the hypoxic condition, but be able to recover once flushing restarted. Fishes were also monitored carefully for any sign of stress indicator such as changes in ventilatory rate.

At the beginning of each run, the oxygen electrode was calibrated using two calibration points; zero and saturation levels. Data were acquired using Logger-Pro software (Qubit) installed as interface.

Oxygen consumption rate was calculated during the closed circuit using the following equation (Steffensen, 1989):

$$MO_2 = [O_2(t_0) - O_2(t_1)]V \frac{1}{t} \frac{1}{BW} \quad (1)$$

where MO_2 is the oxygen consumption rate ($\text{mg O}_2/\text{L/g/h}$), $Q_2(t_0)$ is the oxygen concentration at time t_0 ($\text{mg O}_2/\text{L}$), $O_2(t_1)$ is the oxygen concentration at time t_1 ($\text{mg O}_2/\text{L}$), V is the respirometer chamber volume minus the fish volume in litres (L) and $t = t_1 - t_0$ (h) and BW is the body weight in grammes.

The frequency of gill ventilation rate during normoxia (active and at rest) and hypoxia was recorded by visual observation for mouth and opercula movement using a manual counter.

Although, recordings were continuous, the data were extracted for the closed phase only, whereas, data recorded for the flushing phase were discarded. The data were transferred to Excel sheet files for further calculations. Experimental fish individuals were compared using average values and standard deviation of minimum and maximum levels of responses to oxygen concentrations sub-lethal points. Data were analysed using SPSS version 8 and Microsoft Excel packages.

3. Results

The majority of fish individual were females (71%). Length–weight relationship for the pooled data of both 2009 and 2013 ($n = 24$) is presented in Fig. 2. The linear regression line by which body weight predicted from length and vis-versa showed that the correlation as fitness was $R^2 = 0.77$.

The fish was able to withstand starvation for up to two weeks under the experimental conditions in the respirometer and survived for approximately three months in full aeration condition. However, the survival was extremely dropped at treatment exposed to lack of oxygen.

A typical oxygen concentration (mg l^{-1}) recorded over a time period during both flushing and closed phases for a female *S. hasta* weighing 70.5 g is presented in Fig. 3. Oxygen consumption rate MO_2 ($\text{mg O}_2/\text{g/h/l}$) vs., time is presented in Fig. 4. Data (457 points) during the closed period were extracted from the total recording time obtained for fish weighing 70.5 g during normoxia. The lower values for the MO_2 recorded during the quiescent phases, whereas, higher levels are seen at spontaneous activity.

In addition, a reduction in oxygen concentration during hypoxic condition and the corresponding calculated MO_2 (Fig. 5). The results revealed that once the oxygen concentration declined, a progressive reduction in the MO_2 was

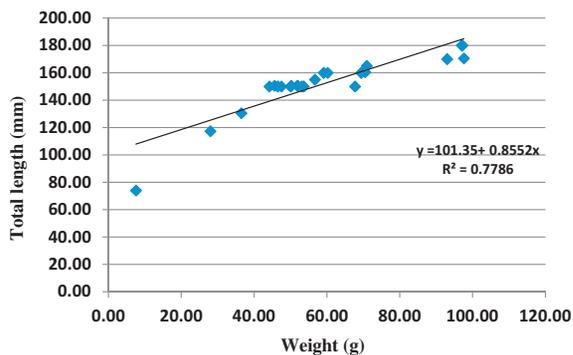


Figure 2 Correlation between body weight (g) and total body length (mm) of total number of 24 *S. hasta* collected from NaMaC (2009–2013).

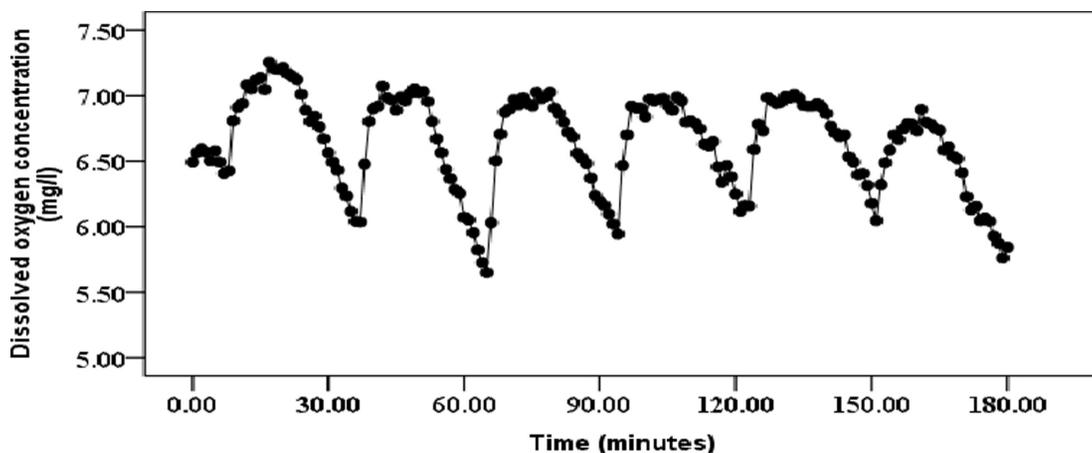


Figure 3 Monitoring of oxygen concentration (mg/l) for *S. hasta* during flushing (higher values) and closed periods. Data points are presented for consecutive 3 h.

obtained. Following to these results, *S. hasta* is considered as regulatory conformer.

Although some temporal variation can be observed between fish individuals considering the average basic (routine) metabolic rate (t -test, $p < 0.05$), no sexual difference between male and female was significantly defined ($p > 0.05$). Therefore, data of combined sexes were analysed.

The level of metabolic rate remained high for few hours following handling before stabilisation. The average value of MO_2 of 17 fish individuals for both males and females (body weight of 37–98 g) is presented in Table 1. The differences between maximum and minimum values of MO_2 are also included.

At extreme hypoxic levels, the mortality attained was 100%. Table 2 presents the average values of the sub-lethal points of oxygen concentration levels. Similar values were obtained for the sub-lethal points of first exposure, second time and after fully recovered for each individual. Statistically, there was no significant difference ($p > 0.05$) between repeated measurements of the sub-lethal points. Almost all individuals behaved similarly and at an oxygen concentration range of 0.9–1.5 $\text{mg O}_2 \text{ L}^{-1}$, the fish undergoes a state of extreme stressful condition and enters a state of comma following a series of coughs. Survival at this stage was found to be noticeably limited. The fish has been observed turning upside down and sinking to the bottom of the chamber or moving vigorously. Further abnormal behavioural sign observed was represented by spreading out dorsal fin.

An indication of stress response behaviour was observed on elevated gill ventilation rate at the onset of an extreme hypoxic stress (Fig. 6), which was followed by a sharp decline. Recovery from the stress was observed as an overshooting in the frequency of ventilation before leveling off.

4. Discussion

S. hasta from Mariculture centre is protandrous hermaphrodite. It is well known that as sparid species have the ability to change sex at the onset of maturity and mature as males (Lone et al., 2001) followed by sex change to females. The spawning season takes place from January to March (Yousif et al., 2003). The combining of the data of all individuals was therefore justified.

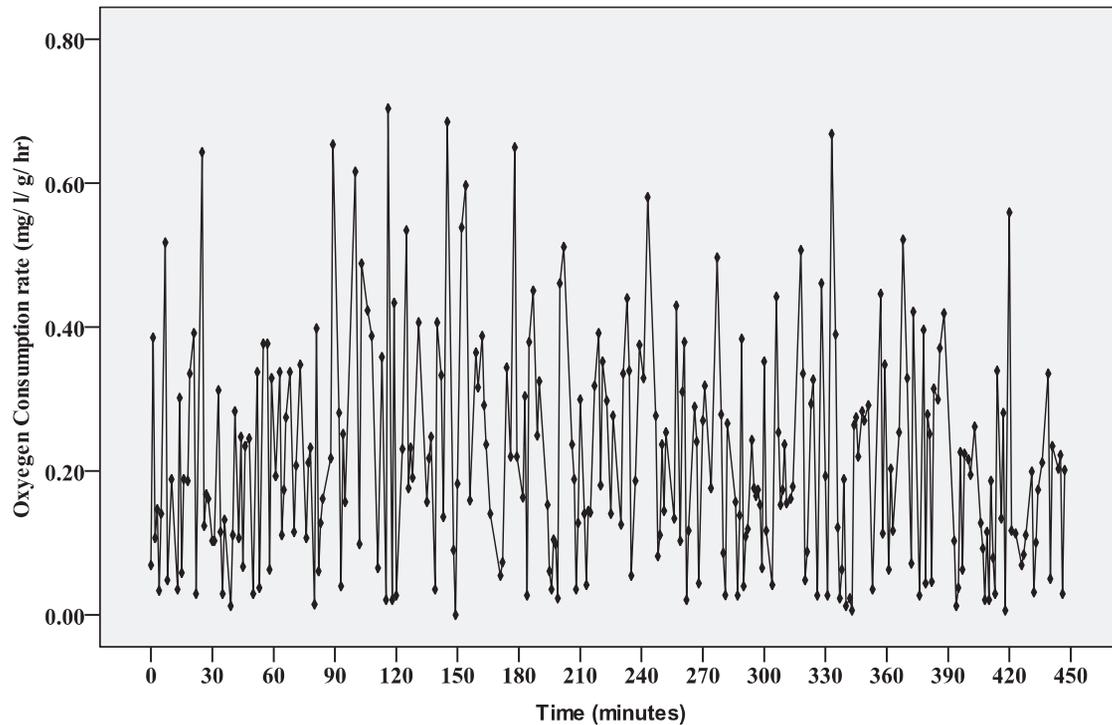


Figure 4 Monitoring of MO_2 (oxygen consumption rate mg/l/h/g, body weight) of *S. hasta* for 8 h.

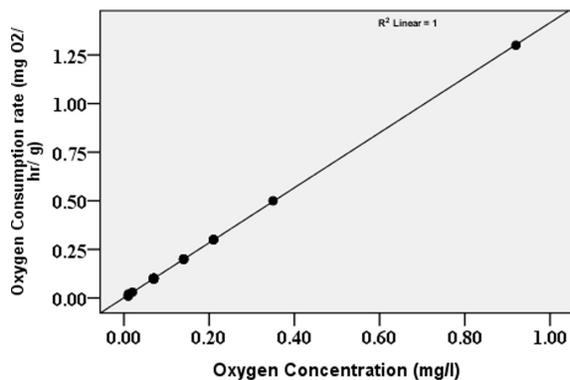


Figure 5 Typical performance of *S. hasta* weighing 53 g, during a hypoxia experiment.

As first reaction of fish against handling and experimental conditions, the fish shows different signs of stress behaviour such as escaping but eventually quiescent behaviour follows after 1–2 h. This is indicated by both elevations in the oxygen consumption and ventilation rates.

The results and daily observations revealed that active level of metabolic rate can be recorded for an active fish (during moving or following initial handlings). However, routine metabolic rate can be identified as metabolic rate for unfed, not starved fish but that accompanied by some spontaneous activity. The standard metabolic rate (basic or resting) is represented by the rate measured for unfed, resting, not starved but not influenced by any spontaneous activity (Hermann and Enders, 2000). The distinction between these levels has been pointed out in other fish species for example, the horse mackerel, *Trachurus trachurus* (Hermann and Enders, 2000). During the present study, although it was possible to record

MO_2 on a very quiescent fish, the results indicated a non regulatory ability characterised the investigated fishes. The minimum metabolic rate (oxygen consumption rate) has been considered as routine interspersed with resting or basic levels due to some spontaneous movement, which cannot be avoided. The difference between maximum and minimum rates of oxygen consumption is considered as an aerobic scope for hypoxia tolerance, which seems to be very narrow range. Feeding also results in a higher metabolic rate due to the Specific Dynamic Action (SDA) effect for example, (Requena et al., 1997).

As reported for other fish species, *S. hasta* showed correlation between respiratory responses to activity as found by Granter and Tabrosky (1998) and to hypoxia as concluded by McBryan et al. (2013). *S. hasta* exhibited a sharp decline in oxygen consumption indicating that the capacity to withstand further oxygen reduction of 1 mg/l +0.2 is limited, reflected by abnormal behaviour observed on movement. Typically, acute hypoxia causes hyperventilation and bradycardia and an elevation in gill vascular resistance in teleosts (Holeton and Randall, 1967). Hyperventilation was frequently observed during the present investigation, as a compensation strategy as a response to reduce dissolved oxygen level.

Almost all fish individuals showed similar responses at low oxygen concentration levels. The stress responses represented by speeding up ventilation, coughing and vigorous movement were explained in terms of avoidance of oxygen depleted water and or obtaining more oxygen from the surrounding water. Therefore, *S. hasta* can be considered as oxy-conformer which cannot survive extreme hypoxia. An oxy-conformer fish's metabolic regulatory ability to withstand hypoxia can be demonstrated by a steep curve of oxygen consumption against oxygen concentration levels as indicated in the present study. Furthermore, a 100% mortality rate at zero oxygen concentration showed that the species is unable to survive the anaerobic condition and a total dependency on oxidative metabolism.

Table 1 Average oxygen consumption rate (MO₂) calculated for 17 individuals of *S. hasta*.

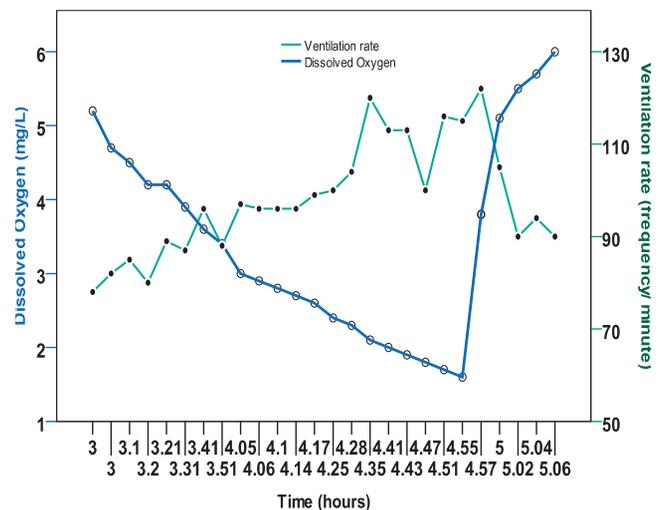
Total number of recording	Fish sex	Body weight (g)	MO ₂ min.	MO ₂ max.	(Max–Min)	MO ₂ average	S.d.
9	f	68	0.02	0.2	0.18	0.07	0.06
23	m	52	0.001	0.063	0.062	0.018	0.015
11	f	71	0	0.7	0.7	0.009	0.013
12	f	52	0.001	0.018	0.017	0.006	0.005
5	m	46	0.02	1.3	1.28	0.38	0.53
9	f	54	0.02	0.24	0.22	0.134	0.079
10	f	53	0.03	0.96	0.93	0.403	0.303
9	f	50	0.03	0.55	0.52	0.287	0.2
9	f	60	0.02	0.15	0.13	0.043	0.044
24	f	37	0.02	0.12	0.1	0.027	0.025
12	f	97	0.01	0.05	0.04	0.043	0.01
33	m	97	0.003	0.11	0.107	0.027	0.019
16	f	93	0.003	0.03	0.027	0.029	0.007
5	f	48	0.004	0.16	0.156	0.035	0.062
24	m	50	0.02	0.071	0.051	0.012	0.014
17	m	98	0.003	0.216	0.213	0.084	0.05
24	f	47	0.001	0.045	0.044	0.011	0.012

Table 2 Survival of fish individuals of *S. hasta* following exposures to hypoxia during different conditions.

Fish No.	Gender	Body weight (g)	Lethal point (first run)	Lethal point (second run)	After 15–35 days
1	f	68	na	na	na
2	m	52	0.9	na	na
3	f	71	na	na	na
4	f	52	0.9	na	na
5	m	46	0.8	na	na
6	f	54	1.0	na	na
7	f	53	1.0	na	na
8	f	50	na	na	na
9	f	60	0.8	0.7	na
10	f	37	0.6	na	na
11	f	97	0.7	0.9	0.8
12	m	97	0.8	0.9	0.9
13	f	93	1.1	1.1	0.9
14	f	48	1.1	0.7	na
15	m	50	0.9	0.8	1.1
16	m	98	0.9	0.8	0.9
17	f	47	0.8	0.8	0.9
18	na	28	1.0	na	na
19	na	8	1.3	1.1	na
20	na	71	1.0	na	na
21	na	57	1.1	1.0	na
22	na	59	1.0	1.1	na
23	na	69	0.9	0.9	na
24	na	44	0.9	0.8	na
Avg.		59	0.9	0.9	0.9
s.d.		22	0.2	0.1	0.1
Total		21	13	6	6

On the contrary to the expectation of possible adaptive tolerance to hypoxia reflected in a change in the lethal points by repeated treatments, the points at which the fish mortality attained 100% were consistent throughout the investigation. Such finding indicated a lack of any sign of short term adaptation response.

In comparison, a wide range of marine species are characterised by the ability to regulate metabolic rate at an onset of

**Figure 6** Typical ventilatory responses of *S. hasta* to the reduction in dissolved oxygen concentration.

hypoxia and even at anoxia by several minutes to several hours before losing regulatory ability at a critical point of oxygen concentration (McBryan et al., 2013). On the other hand, these species are able to survive using anaerobic metabolism conditions. The sharp snout Sea bream (*Diplodus puntazzo*) has been found to maintain a constant oxygen consumption rate regardless of dissolved oxygen level until a critical saturation point (Gerezo and Garcia, 2004). Consequently, the optimum oxygen saturation for *D. puntazzo* was estimated to be above 70%.

Although, *S. hasta*, survive hypoxia exposure for a limited time, this is not unusual, since some species or individuals of fishes can be completely oxy-conformers (Richard, 2011). The difference between minimum and maximum oxygen consumption rate is of great importance to be used in ecological studies notably related to climate change (Clark et al., 2013).

The survival rate of the cultured individuals of *S. hasta* was found to be limited in case of releasing in seawater less than 90% dissolved oxygen saturation level. The extreme summer seawater temperature (~39 °C) is significantly exceeding the

current experimental temperature of 20 °C under which the oxygen consumption was determined. High temperatures substantially affect the levels of oxygen availability in the coastal waters reducing the survival rate to a critical point. Such phenomenon has been observed in the Bahraini waters in August 2010 where the temperature exceptionally increased above 36 °C. Hypoxia resulting due to oxygen limitation reduced embryo development and survival rate as reported by Hassell et al. (2008) on black Sea bream (*Acanthopagrus butcheri*).

The results of the present study could be based on for prospective related studies on other local species particularly those released by NaMaC such as groupers (*Epinephelus coioides*) and rabbit fishes (*Siganus canaliculatus*). Such studies should include tolerance capabilities under extreme environmental conditions for the cultured individuals as well as those obtained from wild population.

5. Conclusion

The metabolic rate of *S. hasta* obtained from Mariculture was low and irregular. Elevated ventilatory responses were recorded as a compensatory behaviour during activity and exposed stress. A non-regulatory metabolic ability was observed at different levels of stress exposures. The correlation between minimum (basic) oxygen consumption rate and body weight was found to be of non-linear form. On temporal variation, no significant differences ($p > 0.05$) have been noticed between the results obtained for the fish set of the year 2009 and of the year 2013. A typical steep straight line relationship between oxygen consumption rate and oxygen concentration indicated a non regulatory ability and extreme intolerance to hypoxia exposure. Therefore, *S. hasta* is considered as oxy-conformers and the sub-lethal point was at 1 mg/l dissolved oxygen.

Acknowledgments

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