AMERICAN UNIVERSITY OF BEIRUT

METABOLISM OF MARBLED SPINEFOOT *SIGANUS RIVULATUS* AT VARIOUS OXYGEN AND SALINITY CONCENTRATIONS

by
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AN ABSTRACT OF THE THESIS OF

Jessica Vartan Babikian for Master of Science
Major: Biology

Title: Metabolism of Marbled Spinefoot *Siganus rivulatus* at Various Oxygen and Salinity Concentrations.

The present study was carried out to: (1) assess the oxygen requirements of juvenile *Siganus rivulatus* and its tolerance to short-term hypoxia, (2) establish standard metabolic rate of marbled spinefoot juveniles, (3) investigate the response of *S. rivulatus* to hypoxia, and (4) study the effect of salinity on metabolism of marbled spinefoot. In the first experiment, juvenile rabbitfish (15 fish per tank) were maintained for one hour in waters of various oxygen concentrations. They were then transferred to well-aerated tanks and observed for 72 hour. Survival was evaluated, fish behavior at low oxygen concentrations observed and recorded, and a broken-line analysis performed on the data. In the second experiment, a series of flow-through respirometry experiments were performed to estimate the standard metabolic rate of marbled spinefoot at 27 °C and 35 ppt. In the third experiment, a series of closed respirometry experiments were performed during which dissolved oxygen was allowed to drop to 0.5 mg/L. Oxygen consumption rates were calculated at 11 intervals along a declining continuum of oxygen concentrations and a broken line analysis was performed through the average respiration rates at every oxygen concentration to determine the critical oxygen tension P_{crit}. In the fourth experiment, fish were maintained at salinities of 25, 30, 35 and 40 ppt for 2 weeks. Flow-through respirometry was performed to measure respiration rates, with up to ten replicate runs per treatment. Results of the first experiment suggest that *Siganus rivulatus* can survive for one hour at oxygen concentration of 1.44 mg/L (100% survival) but not at oxygen concentrations below 0.65 mg/L (16% survival) where fish exhibited aquatic surface respiration. Results of the broken-line analysis performed on all data suggested a breakpoint of 0.69 mg/L. In the second experiment, we estimated a mean standard metabolic rate of 0.57 ± 0.02 mg O_2/g fish/h for marbled spinefoot. Results of the third experiment show that *S. rivulatus* is an oxyregulator until P_{crit} (1.7 mg/L O_2) is reached, becoming a partial conformer thereafter. In the fourth experiment, respiration rates were

similar among fish in salinities of 30, 35 and 40 ppt but increased significantly at 25 ppt. The findings of our study allow a better understanding of the respiratory behavior of marbled spinefoot and allow for a cost-effective approach towards ensuring a suitably aerated environment for the growth and well-being of rabbitfish under aquaculture conditions.

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CHAPTER I

INTRODUCTION

A. Fish Energetics and Metabolism

Fishes need energy to survive and to power activities such as swimming, growth and reproduction (Scholz and Waller, 1992). Energy is obtained by metabolism which can be defined as the sum of all physical and chemical processes within an organism that either use up or release energy (Brett and Groves, 1979). As such, metabolism is divided into two categories: Catabolism, the metabolic process by which complex molecules are broken down into simpler ones to produce energy, and anabolism, the process that requires energy to synthesize complex molecules from simpler ones. Fishes, like other animals, obtain energy from digestion of food whereby organic food molecules are broken down into simpler molecules that are then absorbed into the body and oxidized releasing energy. However, not all energy in the consumed food is utilized, and not all energy is metabolically useful. Accordingly, energy intake can be partitioned into three categories: Gross energy (GE), Digestible energy (DE), and Metabolisable energy (ME) (Smith, 1989). Gross energy is defined as the total amount of energy released by a series of complex chemical reactions that allow the complete oxidation of the ingested food. Part of GE is lost in faeces, and the remainder is digested and absorbed by the fish (DE). DE is partially utilized by tissues and the remaining is lost through the urine or across the gills. The energy that is used up by tissues is the metabolisable energy (Smith, 1989).

Energy budget equations have been used to evaluate fish energetics. Variations of these equations exist, however, they all can be modelled by the following equation:

$$C - F = A = R + U + P$$

where C stands for consumption and is the gross energy content of the consumed food, F is the energy lost in fecal waste, A is the energy absorbed, R is the energy expended/dissipated for respiration, U is energy lost in nitrogenous excretory products and P is energy assimilated for secondary production (Brafield, 1985; Winberg, 1956). In summary, fishes mainly utilize the energy ingested for growth, metabolism and excretion (Winberg, 1956).

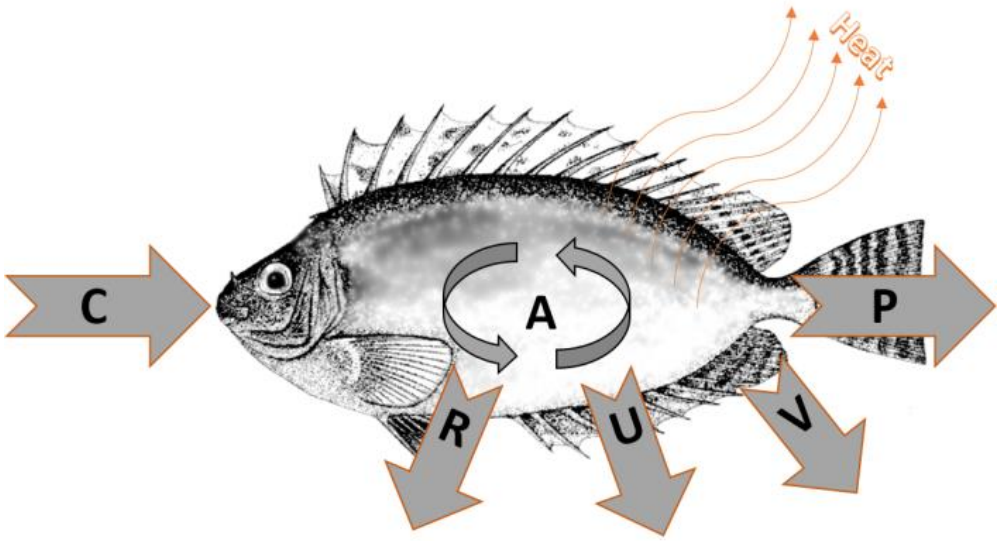

Figure 1. Fish Energy Budget (Adapted from FAO http://www.fishwisepro.com)

Researchers study energy metabolism and factors affecting it in various species of fish, in an attempt to better understand the physiology of metabolism and develop ways to better account for and cope with stress factors. Terms used to define metabolism in fish can

be narrowed down to three general definitions: Standard metabolic rate (SMR), Routine metabolic rate (RMR), and Active metabolic rate (AMR). Standard metabolic rate is similar to basal metabolic rate in homeotherms and is defined as the minimum amount of energy required by fish to survive (Fry, 1957; Cech, 1990). Standard metabolic rate is measured at a constant temperature, when the animal is at rest and in the post-absorptive or post-prandial state (Fry, 1957) in order to eliminate any energy expenses for digestion processes. Routine metabolic rate is the energy needed for swimming and other activities such as reproduction. It is measured while fish are starved and allowed to swim freely showing spontaneous swimming activity (Elliott, 1976). Active metabolic rate can be defined as "the maximum sustainable aerobic rate" (Winberg, 1956) whereby fish would be swimming at a maximum sustained swimming speed – a speed that can be maintained for at least 15 minutes (Brett, 1972). Other terms have also been associated with metabolism and these include the maximum metabolic rate (MMR), and the metabolic scope for activity (MS). MMR is measured during or after exhaustive exercise such as when fish are forced to swim at high speeds exceeding the maximum sustained swimming speed for a certain period of time (Schurmann and Steffensen, 1997). MS, as Fry described in 1971, is the difference between MMR and SMR. It allows for quantitative assessment of oxygen availability for aerobic activities such as swimming, digestion, growth and reproduction.

B. Measurement of metabolism in fish

Carrying out activities such as swimming, growth and reproduction entails energy expenditure (Scholz and Waller, 1992). The basic process by which energy is produced requires the presence of oxygen and is termed aerobic metabolism. Ingested food, mainly

protein and lipids, are digested and broken down into glucose, pyruvate and NADH, which are then oxidized in the mitochondria to produce adenosine triphosphate (ATP), an energy rich molecule. Oxygen is the final electron acceptor in the energy-producing terminal oxidation pathway in aerobic respiration. Accordingly, Fry (1971) categorized oxygen as a 'limiting factor' whereby its availability can directly limit the amount of energy metabolized (Kramer, 1987; Chapman and McKenzie, 2009). Oxygen consumption is directly related to metabolism and its measurements are thus good estimates of stress and the effects of various environmental conditions on aquatic animal physiology (Meade *et al.*, 2002).

Fish oxygen consumption rate is one of the most important parameters used as an indicator of metabolic rate (Ibarz *et al.*, 2003). A good method to estimate oxygen consumption and respiratory rate of fish is respirometry. The main types of respirometry that have been used by various researchers are closed respirometry, open or flow-through respirometry, and intermittent flow respirometry or the stop-flow, all of which follow the principle of "what goes in must come out" (Frappell *et al.*, 1989).

In closed respirometry, spontaneous activity is minimized by placing one or more fish in a closed respirometer – a size-limited tightly sealed chamber of known volume. With time, dissolved oxygen in the chamber will decrease in proportion to the metabolic demands of the fish. Mass-specific oxygen consumption rate can then be calculated by measuring the water volume inside the respirometer, the body weight of fish in chamber and the difference in oxygen content during a time interval. Oxygen consumption rate is thus calculated as:

$$VO_2 = (([O_2]_{t0} - [O_2]_{t1}) \bullet V/t) / BW$$

Where VO_2 is the oxygen consumption rate (mg O_2/g/hour), $[O_2]$ t_0 is the oxygen concentration at time t_0 (mg O_2/liter), $[O_2]$ t1 is the oxygen concentration at time t1 (mg O_2/liter), V is the water volume (liter), t is t1 – t0 (hour), and BW is the body weight of the experimental animal (g).

In open or flow-through respirometry, fish are placed in a chamber and water is allowed to flow through the chamber. Flow must be fast enough to make sure that oxygen in the chamber is never depleted and low enough so that oxygen changes can be detected. Oxygen content in water flowing into the chamber, final oxygen content of water in the chamber and flow of water exiting the chamber are measured. Mass-specific oxygen consumption rate is calculated as:

$$VO_2 = FR \bullet ([O_2]_i - [O_2]_f) / BW$$

Where VO_2 is the oxygen consumption rate (mg O_2/g/hour), FR is the water flow rate (L/hour), $[O_2]_i$ is the oxygen content in inflowing water (mg O_2/liter), $[O_2]_f$ is the final oxygen content of water in chamber (mg O_2/liter) and BW is the body weight of experimental animal (g).

Intermittent flow respirometry is a combination of both closed and flow-through respirometry whereby the experimental animal is placed in the chamber and water is allowed to flow through. After several hours when the animal has acclimated to the environment and is at rest, water flow is stopped and the system behaves as closed respirometry. For repeated measurements, the respirometer is flushed with air-saturated water by allowing water to flow through the chamber again as described by Steffensen (1989).

C. Factors affecting metabolism

Many studies in animal physiology evaluated the influences of various factors on metabolism. Several factors are known to affect fish oxygen consumption. A short communication by Kazakov and Khalyapina (1981) reported that Atlantic salmon spawners had lower oxygen consumption rates than juvenile fish, and showed that both females and males displayed reduction in oxygen consumption as they aged. The authors also concluded that mass-specific oxygen consumption rate decreased as fish body weight increased (from 152.7 to 90.1 mg O_2/Kg fish/hour in females and from 204.0 to 182.2 mg O_2/Kg fish/hour in males). The fact that large fish have relatively slower metabolic rates per unit weight than small fish is reported in literature for various fish species. Moss and Scott (1961) reported this inverse relationship between size and metabolism in bluegill, *Lepomis macrochirus* and largemouth bass, *Micropterus salmoides*. Similar observations were made by Sims (1996) working with juvenile dogfish, *Scyliorhinus canicula* and Glencross and Felsing (2006) working with barramundi, *Lates calcarifer*.

In addition to age and body weight, environmental factors such as temperature also affect oxygen consumption rates (Glencross and Felsing, 2006). Standard metabolic rate is often reported to increase exponentially as a function of temperature (Schurmann and Steffensen, 1997; Sylvestre *et al.*, 2007). This relationship is best described by a Q_{10} value which is defined as the relative increase in respiration caused by a temperature change of 10°C (Brett and Groves, 1979). Q_{10} values differ among species with values of 2.3 for goldfish (Brett and Groves, 1979), around 2.2 for some Asian carp species (Das *et al.*, 2004), circa 3.1 for barramundi (Glencross and Felsing, 2006) etc. Other environmental factors known to influence fish metabolic rates are salinity and dissolved oxygen.

D. Salinity and metabolism

Salinity is a key environmental factor and a potential source of stress that can have an impact on fish metabolism in both natural and aquaculture conditions (Varsamos *et al.*, 2004). Some fish species reside in habitats where environmental salinity never changes. Others are constantly exposed to changes in salinity to which they have to adapt in order to survive. The ability to cope with such a challenge requires energy consumption in a process known as osmoregulation (Evans, 1980; Saoud *et al.*, 2007), which allows fish to regulate the concentrations of water and ions intercellularly, intracellularly and sometimes in the external environment. Euryhaline teleost fishes tolerate a broad range of environmental salinities by maintaining the concentration of ions in lymph, plasma and interstitial fluid at relatively stable levels (Evans and Claiborne, 2009), and are thus able to live in various habitats (Varsamos *et al.*, 2005). When fish are salinity stressed, osmoregulatory mechanisms are employed whereby energy is utilized to maintain a relatively constant osmolality between cells and blood plasma (Varsamos *et al.*, 2005; Evans and Claiborne, 2009). This is mainly established by Na^+/ K^+ ATPase found on the basolateral side of chloride cells in gills, allowing active excretion of monovalent ions (Marshall and Bryson, 1998; Saoud *et al.*, 2007). This energy requiring process thus affects O_2 utilization and can be estimated using respirometry measurements.

Respirometry which measures oxygen consumption rates has been used by several authors in the attempt to study the effect of various salinities on fish metabolic rates (Febry and Lutz 1987; Woo and Kelly, 1995; Morgan *et al.* 1997; Gracia-Lopez *et al.* 2006). In one study dealing with tawny puffer *Takifugu flavidus* juvenile, the authors found a

significant effect of salinity on oxygen consumption whereby a parabolic relationship was established between oxygen consumption rates and salinity (5-35 g/L) (Shi *et al.*, 2011).

E. Hypoxia

1. *Causes*

Whether in nature or in aquaculture conditions, fishes are constantly exposed to various stressful situations to which they have to adapt in order to survive (Barrionuevo *et al.*, 2010). Often, even in their natural environments, they are exposed to changing environmental oxygen concentrations. Low dissolved oxygen concentration in water is termed hypoxia, a "constant environmental stressor for many fish species" (Barrionuevo *et al.*, 2010). This phenomenon occurs naturally in a wide range of aquatic habitats, but anthropogenic activities have increased the abundance and severity of hypoxic conditions (Diaz and Breitburg, 2009). Many factors attribute to an increase in hypoxia. Apart from the fact that oxygen is less soluble in water and diffuses more slowly in water than in air (Graham, 1990), stable water stratification prevents the bottom water from mixing with the oxygen-rich surface water, rendering water exchange less efficient and thus causing hypoxia (Scholz and Waller, 1992; Diaz and Breitburg, 2009). Fish might experience a gradual or rapid drop in oxygen concentrations resulting from organic pollutants or the decomposition of sewage or organic effluents (Jones, 1952; Moss and Scott, 1961). Eutrophication or excess nutrient enrichment leads to the formation of large plankton blooms which then rapidly decompose resulting in a decrease in dissolved oxygen concentrations (Scholz and Waller, 1992 Diaz and Breitburg, 2009).

2. $P_{critical}$ – *oxyregulators and oxyconformers*

Some fishes are able to modify their oxygen uptake rate as environmental oxygen decreases. When studying the relationship between oxygen consumption and environmental oxygen, it is necessary to assess the value of P_{crit} (critical oxygen tension), which is defined as the partial dissolved oxygen pressure PO_2 below which oxygen consumption rate becomes dependent on ambient oxygen (Verheyen *et al.*, 1994). Moreover, it is important to distinguish between oxygen regulators and conformers (Fry, 1971). Most fish species are oxy-regulators i.e. they maintain a relatively constant oxygen consumption rate regardless of dissolved environmental oxygen concentrations until Pcrit is reached (Cerezo Valverde *et al.*, 2006). Conformers on the other hand modify their oxygen uptake according to their environment, thus decreasing oxygen consumption rate with a decrease in ambient oxygen concentrations (Fry, 1957; Shelton, 1970; Hughes, 1973). Classical examples of oxy-regulators would be the plaice (*Pleuronectes platessa*) (Steffensen *et al.*, 1982), and the common common dentex (*Dentex dentex*) (Cerezo Valverde *et al.*, 2006). An example of an oxy-conformer would be the flounder (*Platichthys flesus*) (Steffensen *et al.*, 1982). Generally, organisms with high metabolic demand tend to be regulators whilst those with low metabolism conformers. Accordingly, pelagic predators are regulators whilst cold water bottom dwellers are conformers.

The extent of physiological damage resulting from hypoxic stress is largely dependent upon the species' capacity (Schurmann and Steffensen, 1997; Genz *et al.*, 2013) as well as the length of exposure. Even moderate levels of hypoxia can induce physiological halts in cases of prolonged exposure (Chabot and Dutil, 1999; Petersen and Gamperl, 2010). Various species of fishes can tolerate differing minimum concentrations of

oxygen in water. When performing studies on metabolism, it is important to assess Pcrit as this will help determine hypoxia tolerance in fish. Individuals with low Pcrit values are those that have good oxygen uptake and transport to tissues at low water oxygen and thus show high tolerance to hypoxia in contrast to those having high Pcrit values which tend to be more sensitive to hypoxia.

3. *Responses to hypoxia*

Various fish species have developed various mechanisms and strategies to tolerate hypoxia, mainly aiming at improving oxygen uptake and hence ensuring oxygen delivery to tissues (Wu, 2002; Braun *et al.*, 2006; Barrionuevo *et al.*, 2010) in order to maintain an acceptable amount of metabolic energy (Richards, 2009). There are thus various physiological, behavioral, biochemical and molecular responses to hypoxia. Oxygen is acquired by the process of breathing which involves ventilation. The latter can be defined as the movement of water containing dissolved oxygen over a respiratory surface i.e. the gills (Kramer, 1987). Primary responses to low ambient O_2 concentrations include changes in respiration patterns, behavior, gill structure and hemoglobin (Hb)-O_2 binding affinities (Sollid *et al.*, 2005; Gilmour and Perry, 2006; Sloman *et al.*, 2008; Wells, 2009). When these responses fail to enhance O_2 uptake, compensation is achieved *via* the alteration of metabolic pathways generating energy and the adjustment of metabolic energy utilization (Martinez *et al.*, 2006; Richards, 2009; Richards *et al.*, 2008).

Generally, fish exhibiting a decrease in dissolved oxygen level respond by increasing ventilatory frequency which will in turn reduce the energy present for other metabolic processes such as growth (Cerezo Valverde *et al.*, 2006). Increased ventilatory

frequency associated with hypoxia was observed in various species such as plaice *Pleuronectes platessa*, flounder *Platichthys flesus* (Steffensen *et al.*, 1982), sharpsnout sea bram *Diplodus puntazzo* (Cerezo and Garcia, 2004) and common dentex *Dentex dentex* (Cerezo Valverde *et al.*, 2006). However, there are some fishes that exhibit a decrease in ventilatory frequency in response to hypoxia. Burggren and Randall (1978), for instance, reported that the sturgeon *Acipenser transmontanus* decreased gill ventilation with decreasing oxygen concentrations. Many fishes also rely on aquatic surface respiration (ASR) to survive extended periods of hypoxia, ventilating at the oxygen-rich water surface (Verheyen *et al.*, 1994.; Kramer and McClure, 1982). Such behavioral responses were observed in many fishes under experimentally decreased dissolved oxygen concentrations. Silver catfish juveniles for instance showed increased opercular movements and searched for oxygen at the water surface when exposed to hypoxic conditions (Braun *et al.* 2006).

Another behavioral response adopted by a few fish species that are frequently exposed to hypoxia is aerial respiration or air breathing, whereby fishes 'gulp' air at the water surface. Many air-breathing freshwater teleosts have developed air breathing organs (ABOs) in addition to their well-developed gills. These ABOs include modified gastrointestinal tracts, swim bladders and labyrinth organs as means to extract oxygen from the atmosphere (Sloman *et al.*, 2009). The striped catfish *Pangasianodon hypophthalmus* for example is one of these species with a capacity for both aquatic and aerial respiration (Lefevre *et al.*, 2011).

Water-breathing fish need oxygen to generate ATP in aerobic respiration. When the previously mentioned responses fail to enhance oxygen uptake, and when oxygen drops below Pcrit, fishes suppress their metabolic rate by reducing total energy expenditure

and/or switching to anaerobic metabolism by making use of glycogen stores (Cooper *et al.*, 2002; Wu, 2002). However, energy production via anaerobic metabolism is limited by glycogen availability making the production of large amounts of ATP less feasible. Thus, relying on anaerobic metabolism is energetically unfavorable for fishes and is generally used as a means of last resort.

Fishes exposed to a decrease in ambient oxygen concentrations have been known to respond by increasing both water flow over the gills and blood flow inside the gills by increasing respiratory surface area (Hughes and Sunders, 1970). Recent studies have shown that a few fish species are capable of altering the size of the respiratory surface area in response to hypoxia and high temperatures by remodeling their gill morphology (Sollid and Nilsson, 2006). Gill remodeling involves the removal of an inter-lamellar cell mass (ILCM) to increase respiratory surface area which in turn promotes oxygen uptake. This is generally an acclimatization that happens over a few days and allows the fish to decrease metabolism. The hypoxia-tolerant crucian carp, *Carassius carassius*, was the first fish in which such morphological changes were seen (Sollid *et al.*, 2003). Under normoxic conditions or at low temperature (8°C), the lamellae of these fish were almost invisible being embedded in ILCM. However, under hypoxic conditions or at high temperature, apoptosis and suppressed mitosis led to the regression of ILCM, making the lamellae protrude exposing a larger respiratory surface area (Sollid *et al.*, 2003; Sollid *et al.*, 2005). Gill remodeling was also seen in other species such as the goldfish, *Carassius auratus*, and other cyprinids.

F. Marbled spinefoot *Siganus rivulatus*

The opening of the Suez Canal in 1869 allowed the invasion of a large number of Red Sea species known as Lessepsian migrants into the Mediterranean Sea (Por, 1978; Boudouresque, 1999, Galil, 2000; Bariche, 2004; Dulcic, 2004). One of the most successful of these colonizers is the marbled spinefoot or rabbitfish, *Siganus rivulatus* (Forsskal 1775), which was first reported in the Levantine area of the Mediterranean in 1927 from off the coast of Israel (Galil, 2007), and has subsequently established large populations along the Eastern Mediterranean coast (Bariche, 2005).

Siganus rivulatus, belonging to the family Siganidae, is a euryhaline herbivorous fish which naturally resides in shallow coastal waters over rocky substrates or sandy bottoms covered with vegetation (Ben-Tuvia, 1964; Galil, 2007). Marbled spinefoot is quite abundant along the Lebanese coast, existing in dense schools of 50 to several hundred individuals which can reach to a length of 31.9 cm and a body weight of 318.2 g (Popper and Gunderman, 1975; Bariche, 2005). This species is primarily herbivorous consuming mainly seaweed (Ben-Tuvia, 1972; Lundberg, 1995; Shakman, 2009). In captivity however, it exhibits omnivorous feeding habits readily consuming a wide variety of foods offered including chopped fish, molluscs, fishmeal, pellets, etc. (Ben Tuvia, 1971; Ben Tuvia, 1973; El-Dakar, 2011). Farmers can modify the feeding habits of the reared species by training them to accept the artificial feed offered (Parazo, 1990; Stephanou, 2007).

Marbled spinefoot is an economically valuable species and is considered a potentially suitable candidate for warmwater aquaculture (Stephanou, 2007; Saoud *et al.* 2008a, 2008b; El-Dakar *et al.*, 2011) because of its commercial importance and market value. Various studies were performed on the feasibility of farming *Siganus rivulatus* in the

Mediterranean region (Ben-Tuvia *et al.*, 1973; Popper *et al.*, 1973; Popper and Gundermann, 1975; Popper *et al.*, 1979; Stephanou and Georgiou, 2000), and interest in its culture is rapidly increasing. Aquaculturists in Cyprus and Saudi Arabia are currently farming marbled spinefoot in floating cages or self-cleaning tanks, but little information is available on the proper aquaculture protocols needed to ensure maximum production and profitability (Ghanawi *et al.* 2010).

In aquaculture, maximum production largely depends on the survival rates of juvenile stages (Shi *et al.*, 2011) and this requires that farmers understand their organisms well. Studies have shown that *Siganus rivulatus* is a euryhaline and eurythermal organism capable of tolerating wide ranges of salinity (10-50 ppt) (Saoud *et al.*, 2007) and temperature extremes (17-32°C) (Saoud *et al.*, 2008a), as well as rough handling, and over-crowding (Ben Tuvia, 1972). The nutritional requirements and the effects of various parameters on growth, survival and performance of *Siganus rivulatus* have also been assessed. However, no data is available on the respiratory behavior and oxygen consumption of marbled spinefoot, and no studies dealing with the effect of salinity and dissolved oxygen concentrations on respiration rates have been performed in this species. Such knowledge would have important implications in properly designing culture systems, optimizing growth performance and thus increasing yields.

The present study was designed to: (1) assess the effect of various oxygen concentrations on survival of juvenile *Siganus rivulatus*, (2) establish standard metabolic rate of marbled spinefoot juveniles, (3) investigate the response of this species to hypoxia and determine its hypoxia tolerance by assessing P_{crit}, and (4) study the effect of salinity on metabolism by measuring oxygen consumption rate at various salinities.

This study would potentially allow for a better understanding of metabolism and stress response of *Siganus rivulatus,* in an attempt to further optimize growth performance and thus increase yields. Moreover, knowledge of P_{crit} can possibly result in financial advantages for farmers, allowing for more accurate calculations of the power needed to sustain healthy aeration levels, and thus avoiding unnecessary expenses.

CHAPTER II

MATERIALS AND METHODS

A. Fish Acquisition and Holding

Rabbitfish *Siganus rivulatus* fingerlings were caught in traps off Ain El Mreisseh beach in Beirut and transported in aerated water to the marine research laboratory at the American University of Beirut (AUB). Fish were quarantined in an outdoors $1m^3$ circular tank (1.14m diameter; 1m depth) connected to a biological filter for four weeks, and were offered a 40% crude protein, 8% lipid commercial feed (Rangen Inc., Buhl, Idaho) three times daily to apparent satiation. Dissolved oxygen concentrations were maintained greater than 5mg/L, salinity at 35 ppt and water temperature at 27°C. The fish were fasted for 24-h prior to the start of all experiments in order to avoid post prandial metabolism.

B. Experiment 1: Effect of Various Oxygen Concentrations on Survival of *Siganus rivulatus*

Marbled spinefoot fingerlings (2.34 ± 0.09 g; 5.77 ± 0.08 cm, mean ± SE) were transferred to indoor environmentally controlled 180 L fiberglass tanks (60 × 60 × 50 cm; L × W × H) connected to a biological filter. They were offered a commercial feed twice daily at 0800 h and 1700 h.

The research setup consisted of plastic containers filled with 6 L of filtered seawater and aerated with various mixtures of oxygen to nitrogen in order to manipulate partial pressure of oxygen in the water. The chosen gas mixtures were: A: 80-20% (naturally occurring concentrations), B: 85-15% N-O, C: 90-10% N-O, D: 95-5% N-O, E: 97-3% N-O, F: 98.5-1.5% N-O and G: 100-0% N-O. Four replicate containers were assigned one of the gas mixtures. The various gas mixtures were bubbled in the assigned containers for one hour prior to the start of the experiment. Dissolved oxygen in each container was measured using the YSI-85 (YSI instruments, Yellow Stone, Ohio) oxygen probe and values recorded.

After having attained a stable oxygen concentration in each container, fifteen fish were introduced and maintained for one hour. Fish behavior at low oxygen concentrations was observed and recorded. Dissolved oxygen in each container was measured again. Subsequently, fish were transferred back to the indoor fiberglass tanks whereby normal aeration was restored. The fish were offered commercial feed at 4% average body weight daily, starting on the day right after experiment termination. Feed was divided into two equal morning and evening rations. Dead fish were removed, mortality recorded and feeding patterns observed for 72 hours.

C. Experiment 2: Standard Metabolic Rate of *Siganus rivulatus*

1. Respirometer design and operation

A Strathkelvin 929 6-Channel Dissolved Oxygen System was used for respiration measurements. The system comprised a calibrated oxygen meter (Interface 929, Strathkelvin Instruments) which can hold up to six oxygen electrodes (1302 microcathode oxygen electrodes, Strathkelvin Instruments) connected to a computer using Strathkelvin

929 software which runs on Microsoft Windows. Strathkelvin RC400 respiration chambers (730 ml, 102 mm in diameter) were used, and water in the chambers was mixed slowly using a magnetic stir bar under a perforated false bottom. Respiration measurements were assessed via closed cell or flow-through respirometry (refer to Strathkelvin 929 6-Channel Dissolved Oxygen System Instruction Manual).

Flow-through respirometry was used to determine respiration rates in the present experiment. The apparatus consisted of a glass water container (80×80×80 cm) and a 4000 ml Erlenmeyer flask, a submersible electric heater to maintain water temperature at 26.7 ± 0.08 C (mean temperature ± S.E.), a La Motte thermometer to monitor the temperature, and two submersible water pumps to maintain a standard water level in the Erlenmeyer flask (Figure 2). The container was filled with filtered seawater, and salinity was adjusted to 35 ppt. Water was continuously aerated using two air diffusing stones connected to an air pump. Before each experimental run, water was chlorinated and left for 24 hours before de-chlorination with sodium thiosulfate.

During each run, water was siphoned from the flask into the respiration chamber. Flow was adjusted using a plastic valve at the outflow. The respiration chambers and all water tubes used were washed with 10% HCl before each experimental run to reduce bacterial growth that might affect oxygen measurements.

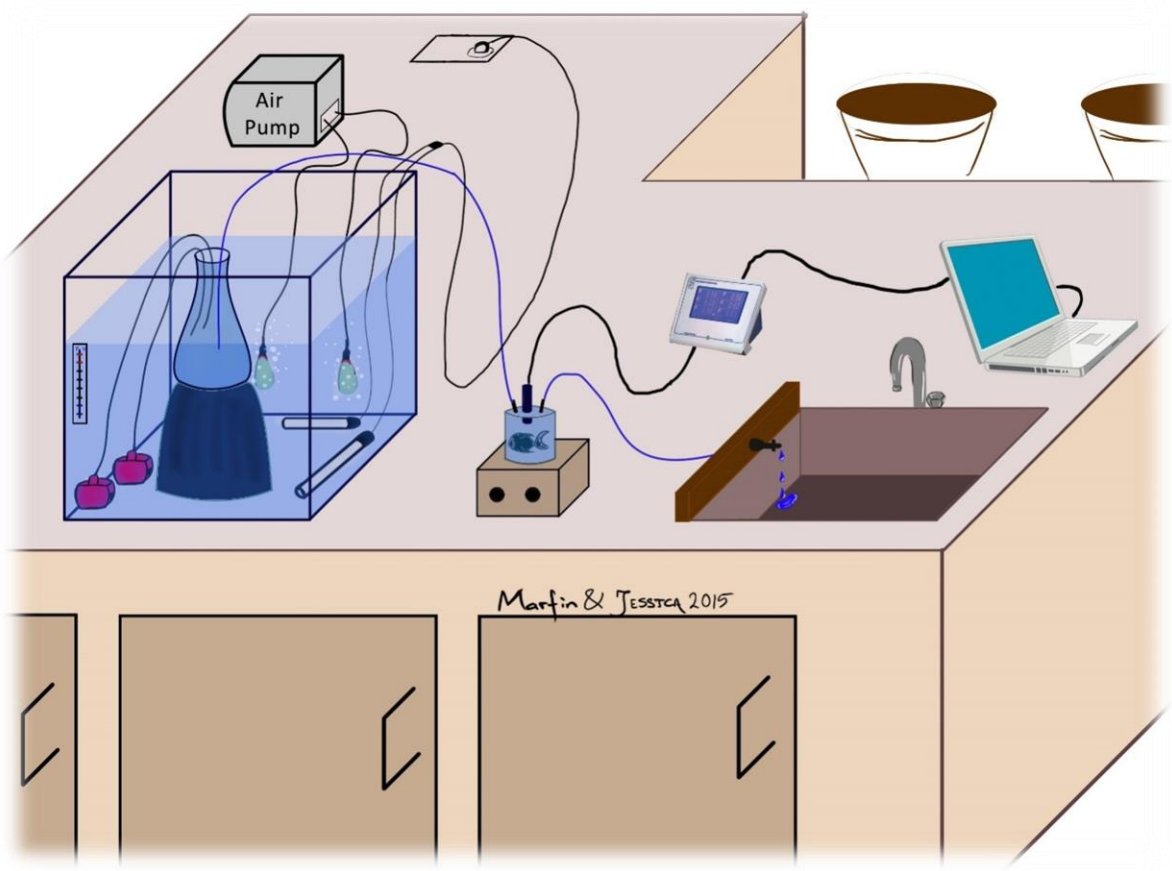

Figure 2. Schematic Diagram of the Flow-Through Respirometry.

2. Oxygen measurements

A two point calibration was performed for each oxygen electrode prior to each experimental run. Zero calibration was done by using an oxygen free solution prepared by adding sodium sulfite to marine water in a beaker. High point calibration was done in air-saturated water from the holding tank following recommendations in the Strathkelvin 929 6-Channel Dissolved Oxygen System Instruction Manual.

All fish were starved for 24 hours prior to the start of each experimental run. After setting the respirometer and calibrating the electrodes, each flow-through respiration chamber was

filled with water from the glass container and one to four fish were placed in each chamber. The chambers were tightly closed, electrodes were inserted and tubes assembled. Water flow was established through each chamber and flow rate was regulated by adjusting the valve at the outflow. Fish were allowed to rest for several hours in the chambers until respiration was stable before taking readings. Of the three chambers running simultaneously, one was always kept empty and used to calculate a correction factor for O_2 depletion. All chambers were covered with plastic sheets to avoid any disturbance that might stress the fish.

Oxygen consumption per unit weight of fish was calculated as follows:

$$VO_2 = FR \bullet ([O_2]i - [O_2]f) / BW$$

Where VO_2 is the oxygen consumption rate or respiration rate (mgO_2/g fish/hour), FR is the water flow rate (L/hour), $[O_2]i$ is the oxygen content in inflowing water (mgO_2 /L), $[O_2]f$ is the final oxygen content of water in chamber (mgO_2 /L), and BW is the body weight of experimental animal (g). Oxygen consumption was then corrected using the correction factor calculated from the equation:

Correction Factor = (O_2 at air saturation – O_2 in control chamber) / (O_2 at air saturation).

D. Experiment 3: Response of *Siganus rivulatus* to Hypoxia

Closed respirometry was used for the present experiment. The same respirometer design and procedure used in the second experiment for the flow-through respirometry was applied with some modifications. Three respirometry chambers were used simultaneously, with one fish placed in each chamber. After closing the chambers tightly and inserting the

electrodes into their corresponding chambers, water was allowed to flow through the chambers and flow rate was regulated such that dissolved oxygen inside each chamber was kept above 4 mg/L. Fish were allowed to rest for several hours (approximately 6 to 7 hours) under this condition until they acclimated to the environment and showed a more or less stable respiration. At this point, water flow was stopped by closing the valve at the outflow and the basic setup was switched from flow-through to closed respirometry. Dissolved oxygen was allowed to drop from 4 or 5 mg/L to 0.5 mg/L during which the decline of oxygen in each chamber was being recorded using the Strathkelvin microcathode oxygen electrodes. When oxygen reached 0.5mg/L, the valves were opened and a flush of water was allowed through the chambers for two minutes. At the end of each experiment, measurements of individual fish weight and fish volume were taken in grams and liters respectively. A 100ml graduated cylinder was used to measure fish volume whereby the cylinder was filled with 70 ml water, fish was placed in the cylinder and volume was obtained by calculating the difference between the initial and final water volumes in the cylinder. Fish from each experiment were placed in separate tanks and observed for three days. Mortality was recorded.

Measurements of mass-specific oxygen consumption rates were obtained using the 929 Strathkelvin software. The calculations were based on the following formula:

$$VO_2 = (([O_2]t_0 - [O_2]t_1) \bullet V/t) / BW$$

Where VO_2 is the oxygen consumption rate or respiration rate (mgO_2/g/hour), $[O_2]t0$ is the oxygen concentration at time t0 (mg O_2/liter), $[O_2]t1$ is the oxygen concentration at time t1

(mg O_2/liter), V is respirometer volume (L) minus fish volume, t is $t_1 - t_0$ (hour), and BW is the body weight of the experimental animal (g).

For each experimental run, oxygen consumption rates were calculated at 11 intervals of 0.5mg/L decline starting from a dissolved oxygen concentration of 6-5.5 mg/L to 1-0.5 mg/L. Average respiration rates were calculated for each interval for all the experimental runs performed and a plot of respiration rate versus oxygen concentration was obtained. In order to determine P_{crit}, a broken line analysis was performed through the average respiration rates at every oxygen concentration.

E. Experiment 4: Effect of Salinity on Oxygen Consumption of *Siganus rivulatus*

Research was performed in an indoor environmentally controlled recirculating system consisting of four batteries of four 180 L square fiberglass tanks (60×60×50 cm; L×W×H). Water was aerated by using a regenerative blower and submerged air diffusers. Each system was connected to a biological filter and settling tank equipped with a 300 W submersible heater that maintained water temperature at 27 °C. Measurements of salinity, temperature, and oxygen were carried out daily using the YSI-85. Four salinities (25 ppt, 30 ppt, 35 ppt, and 40 ppt) were chosen for this experiment. Salinities 30 ppt and 25 ppt were attained by adding dechlorinated tap water and salinity 40 ppt was achieved by removing the Styrofoam covers off the tanks allowing water to evaporate and salinity to increase. Following salinity adjustment and stabilization for at least 24 hours, marbled spinefoot fingerlings were randomly caught from the outdoor tank and equally divided among the

four treatments in the indoor recirculating system. These fish were allowed to acclimate for two weeks before the respirometry experiments.

In order to assess the effects of the various salinities on oxygen consumption of *Siganus rivulatus*, a series of flow-through respirometry experiments was performed. All fish were starved for 24 hours prior to the start of each experiment. The same respirometer design and procedure used in experiment 2 was used. Three respiration chambers were used during each run, one of which was always used as a control. One to two fish corresponding to any randomly chosen salinity treatment was placed in each of the two respiration chambers. When fish respiration became stable, flow rate was measured and dissolved oxygen values recorded for each chamber. Fish in each chamber were weighed, and mass specific oxygen consumption rates calculated accordingly. Oxygen consumption and respiration rates were calculated following the same calculation method used in the second experiment. Up to ten replicate runs were performed for each of the various salinities.

F. Statistical Analysis

All statistical analyses were performed using SAS 9.2 statistical software. Differences in percent survival and respiration rates of *Siganus rivulatus* among the various treatments were analyzed using a one-way ANOVA. Significant differences among treatment means were determined using Student Newman-Keuls means separation test. Differences were considered significant when $P< 0.05$.

CHAPTER III

RESULTS

A. Experiment 1: Effect of Various Oxygen Concentrations on Survival of *Siganus rivulatus*

1. Survival

Siganus rivulatus appears to survive well under relatively low ambient oxygen concentrations. Oxygen concentrations in the various treatments were: A= 6.90 mg/L, B= 5.60 mg/L, C= 3.80 mg/L, D= 2.50 mg/L, E= 1.80 mg/L, F= 1.40 mg/L. In treatment G, the oxygen concentrations recorded were 0.65 mg/L and 0.45 mg/L. All fish survived for 72 hours in the treatments containing oxygen concentrations from 1.44 to 7.04 mg/L (Table 1). Mortality was observed at 0.65 mg/L and increased at concentrations less than 0.65 mg/L, where more than half of the fish died during exposure or up to one hour later (Table 2). Among the survivors that were transferred to aerated tanks, only one fish from treatment G died 24 hours post transfer (Table 1). SNK mean separation test showed no significant differences in survival (100%) among treatments with oxygen concentrations from 1.44 to 7.04 mg/L (Table 2). Survival decreased significantly at 0.65 mg/L (83.5%) and at concentrations less than 0.65 mg/L (16%) (Table 2).

Results of the broken-line analysis performed on all data showed best fit with the models: $y = 446.67x - 207.33$ and $y = 0x + 100$, where y is the percent survival of fish (%)

and x is the ambient oxygen concentration (mg/L), and a breakpoint at 0.69 mg/L (Figure 3).

2. Behavior of Marbled Spinefoot during exposure to various oxygen concentrations

Fish exposed to oxygen concentrations from 1.44 to 7.04mg/L swam normally showing no signs of abnormal behavior. However, fish exposed to oxygen concentrations of 0.65 mg/L and less exhibited erratic swimming and random movements. Fish lost balance and orientation, and moved upwards ventilating and gasping at the water surface. Many fish were seen lying flat on the bottom of the containers with minimal or zero opercular movement. Only few of these fish recovered after normal aeration was restored but all survivors ate the feed offered showing no sign of stress within 12 hours post challenge.

B. Experiment 2: Standard Metabolic Rate of *Siganus rivulatus*

Fourteen experimental runs were performed using one to four fish per chamber. Average weight of fish in each chamber was 5.88g ± 0.38 (mean ± SE) (Table 3). Respiration rates ranged from 0.41 to 0.68 mg O_2/g fish/h with a mean standard metabolic rate of 0.57 ± 0.02 mg O_2/g fish/h (Table 3).

C. Experiment 3: Response of *Siganus rivulatus* to Hypoxia

Fifteen experimental runs were performed and respiration of each fish at decreasing oxygen concentrations were recorded (Table 4). A model of mean respiration rate versus ambient oxygen concentration was developed (Figure 4). The plot showed an increase in respiration rate from 0.45 to 0.63 mg O_2/g fish/hour as ambient oxygen concentrations decreased from 5.25 to 1.75 mg/L after which respiration rate decreased sharply to 0.40 mg O_2/g/h at 0.75 mg/L oxygen (Figure 4 and Table 4). Respiration rates at oxygen concentrations ranging from 5.75 till 3.25 were not significantly different from each other. At oxygen concentrations ranging from 4.75 to 3.25 mg/L, respiration rates were significantly different from each other. There was no significant difference among respiration rates when environmental oxygen concentrations ranged from 4.25 to 1.75 mg/L. Respiration rate decreased significantly at an ambient oxygen concentration of 0.75 mg/L (Table 4).

Results of the broken-line analysis calculated using mean values of respiration at various oxygen concentrations shows best fit with the models: $y = 0.23x + 0.24$ ($R^2 = 0.93$), and $y = -0.05x + 0.74$ ($R^2 = 0.98$), where y is the respiration rate (mg O_2/g fish/hour), and x is the ambient oxygen concentration (mg/L). The breakpoint was at x = 1.74 mg/L (Figure 5).

D. Experiment 4: Effect of Salinity on Oxygen Consumption of *Siganus rivulatus*

Respiration rate of *S. rivulatus* decreased as salinity increased from 25 ppt. At 25 ppt, respiration rate was 0.89 ± 0.10 mg O_2/g fish/h (mean ± SE), significantly greater than at 40 ppt. Respiration rate did not vary significantly among fish in salinities of 30, 35 and 40 ppt (Table 5; Figure 6).

Table 1. Rabbitfish (*Siganus rivulatus*) mortality after one hour exposure to various oxygen concentrations (0.39-7.04 mg/L).

Oxygen Concentration (mg/L)	Initial Number of Fish per Container	Number of Dead Fish after 1 hr	Number of Dead Fish after 24 hrs	Number of Dead Fish after 48 hrs	Number of Dead Fish after 72 hrs
7.04	15	0	0	0	0
6.80	15	0	0	0	0
5.60	15	0	0	0	0
3.80	15	0	0	0	0
2.64	15	0	0	0	0
2.30	15	0	0	0	0
1.84	15	0	0	0	0
1.44	15	0	0	0	0
0.66	15	2	1	0	0
0.65	15	2	0	0	0
0.56	15	13	0	0	0
0.56	15	11	0	0	0
0.45	15	15	0	0	0
0.41	15	9	0	0	0
0.39	15	15	0	0	0

Table 2. Fish survival after exposure to various oxygen concentrations (0.39-7.04 mg/L) for one hour. Values with different superscripts in the same column are significantly different based on SNK mean separation test ($\alpha = 0.05$).

Oxygen Treatment (mg/L)	Stocking number	N	Number of Dead Fish	% Survival
7.04	15	4	0	100[a]
6.80	15	4	0	100[a]
5.60	15	4	0	100[a]
3.80	15	4	0	100[a]
2.64	15	4	0	100[a]
2.30	15	4	0	100[a]
1.84	15	4	0	100[a]
1.44	15	4	0	100[a]
0.65	15	2	5	83.5[b]
<0.65	15	5	63	16[c]
PSE[1]				3.27

[1]PSE: Pooled Standard Error.

Table 3. The Standard Metabolic Rate of Juvenile *Siganus rivulatus* at 27 C and 35 ppt.

Measure	Fish Number	Fish Weight (g)	Respiration Rate (mg O2/g fish/h)
1	1	8.31	0.56
2	3	5.31	0.68
3	3	5.96	0.59
4	3	7.01	0.65
5	3	6.42	0.63
6	4	7.84	0.64
7	3	4.30	0.56
8	3	4.70	0.61
9	4	3.95	0.58
10	3	6.28	0.41
11	3	7.33	0.52
12	3	5.98	0.51
13	2	4.88	0.49
14	3	4.11	0.49
Mean			0.57
Standard Error			0.02

Table 4. Mean Respiration Rate (mg O_2/g fish/ h) and Standard Error of *Siganus rivulatus* at decreasing ambient oxygen concentrations from 5.75 to 0.75 mg/L. Values with different superscripts in the same column are significantly different based on SNK Mean Separation Test ($\alpha = 0.05$).

Oxygen Concentration (mg/L)	N	Mean Respiration Rate (mg O_2/g fish/h)	Standard Error
5.75	6	0.49^{bdc}	0.04
5.25	12	0.45^{dc}	0.03
4.75	15	0.49^{bdc}	0.03
4.25	15	0.52^{abc}	0.03
3.75	15	0.55^{abc}	0.03
3.25	15	0.57^{abc}	0.03
2.75	15	0.60^{ab}	0.03
2.25	15	0.61^{ab}	0.03
1.75	15	0.63^{a}	0.03
1.25	15	0.57^{abc}	0.03
0.75	15	0.40^{d}	0.02
Total	153	0.54	0.01

Table 5. Mean Respiration Rate (mgO_2/g fish/h) and Standard Error of *Siganus rivulatus* exposed to various ambient salinities of 25, 30, 35 and 40 ppt. Values with different superscripts in the same column are significantly different based on SNK Mean Separation Test ($\alpha = 0.05$).

Salinity (ppt)	Mean (mgO_2/g fish/h)	Standard Error
25	0.89[a]	0.10
30	0.41 [b]	0.04
35	0.43[b]	0.04
40	0.33 [b]	0.01
PSE[1]	0.06	

[1]PSE: Pooled Standard Error.

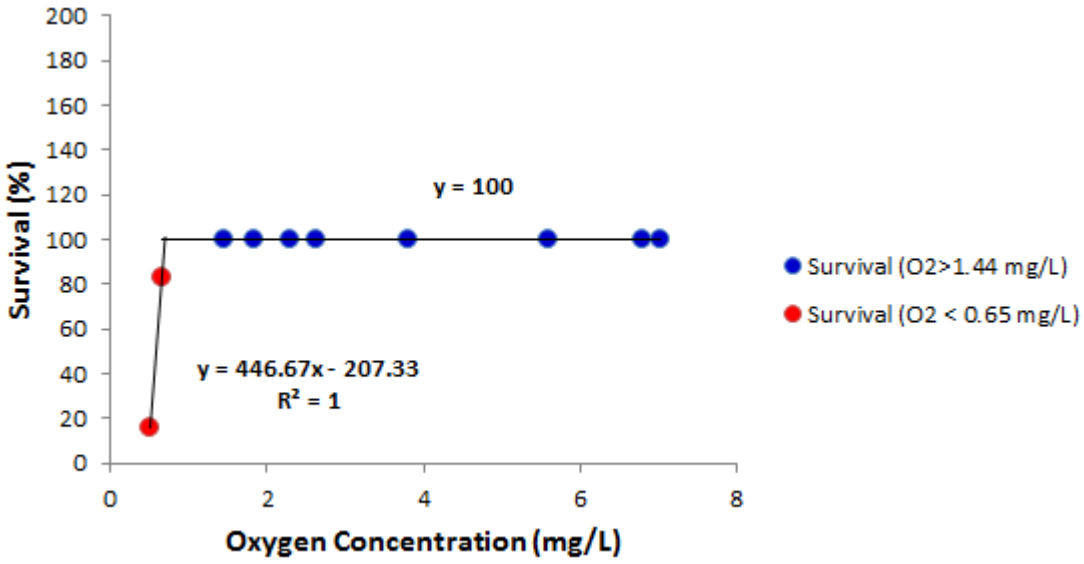

Figure 3. Breakpoint analysis of survival (%) as a function of various ambient oxygen concentrations (mg/L).

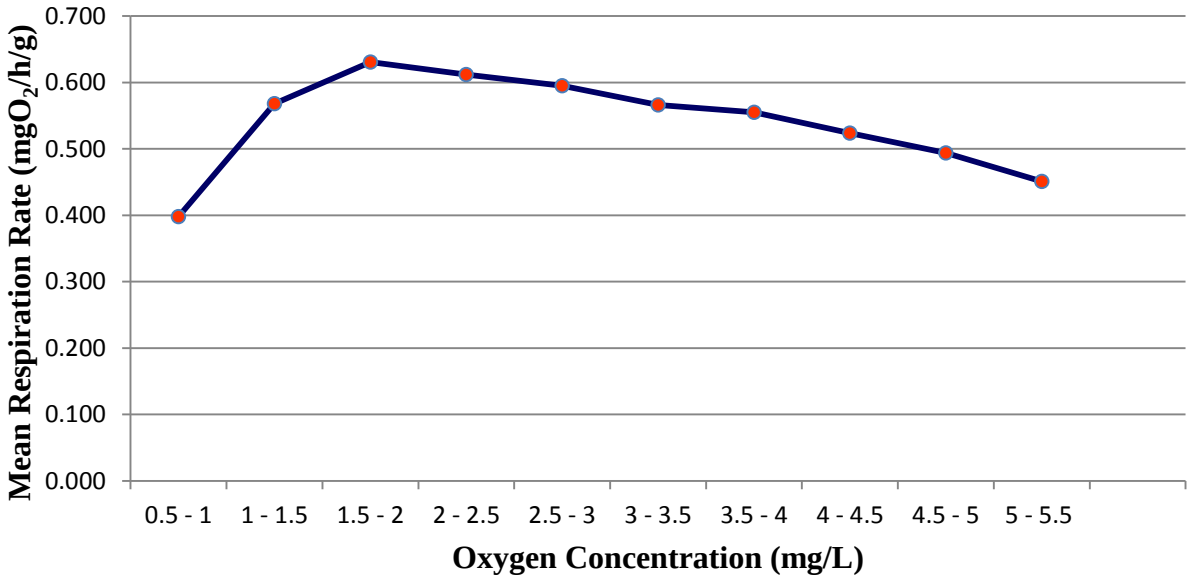

Figure 4. The mean respiration rate (mg O_2/g fish/h) of *Siganus rivulatus* as a function of ambient oxygen concentration (mg/L) during gradual hypoxic stress.

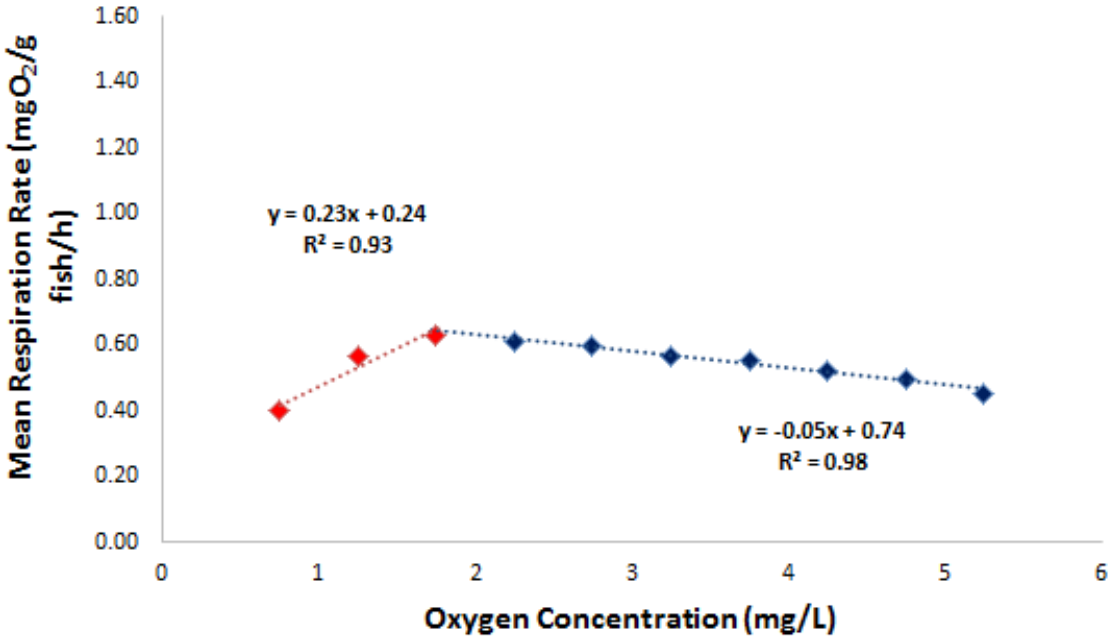

Figure 5. Broken-line analysis of Mean Respiration Rates (mg O_2/g fish/h) as a function of decreasing oxygen concentrations (mg/L).

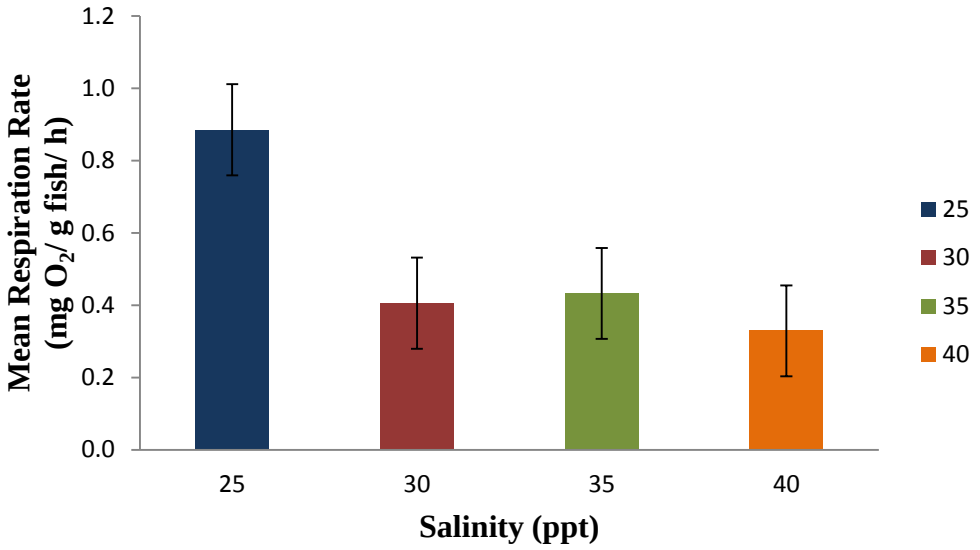

Figure 6. Mean Respiration Rates (mg O_2/g fish/h) of *Siganus rivulatus* at various salinities (25, 30, 35, and 40 ppt).

CHAPTER IV

DISCUSSION

A. Experiment 1: Effect of Various Oxygen Concentrations on Survival of *Siganus rivulatus*

1. Survival

Fishes are often exposed to changing environmental oxygen concentrations to which they have to adapt in order to survive (Barrionuevo *et al.*, 2010). Low dissolved oxygen has been shown to have deleterious effects on fishes in nearshore coastal waters causing stress, damaging ecosystems and resulting in loss of biodiversity. The occurrence of hypoxia is predicted to increase in the future as such factors as eutrophication and global warming become more frequent (Vaquer-Sunyer and Duarte, 2008). Scientists generally use three criteria to discuss fish tolerances to low oxygen concentrations. Fishes have a minimum DO requirement that allows them to sustain activities such as swimming, reproduction and growth. At DO levels less than the minimum, a second criterion, the sublethal oxygen threshold, corresponds to oxygen concentrations that induce stress causing reduced growth and reproduction, forced migration and increased vulnerability to predation. A third criterion called lethal oxygen threshold is the DO less than which mortality starts to appear in the population (Vaquer-Sunyer and Duarte, 2008). The present study provides useful information about these oxygen requirements of *S. rivulatus*. We

observed 100% survival at oxygen concentrations greater than 1.44 mg/L and increasing mortality as oxygen levels decreased. *S. rivulatus* can survive for one hour at DO of 1.44 mg/L and not at all at DO less than 0.65 mg/L. These results suggest that 0.69 mg/L is the minimum non-lethal oxygen concentration the fish can tolerate, and thus that marbled spinefoot has a lethal oxygen threshold of approximately 0.7 mg/L.

S. rivulatus is widely distributed in the Indo-West Pacific and has become well established along the eastern Mediterranean coast. This Lessepsian migrant mainly inhabits shallow waters of the Mediterranean, and the Red Sea coasts (Popper&Gundermann, 1975). In Lebanon, marbled spinefoot are found in various types of habitats, in open bays and enclosed tide pools, and in clear as well as polluted areas (Bariche *et al.*, 2004). Generally, fishes inhabiting hypoxic areas are well adapted to changes in DO and can tolerate relatively low DO levels (Mandic *et al.*, 2009). Inhabitants of tide pools and polluted areas must be able to cope with certain environmental conditions such as fluctuations in dissolved oxygen or even constant hypoxia. Accordingly, it comes as no surprise that *S. rivulatus* is able to survive at oxygen concentrations as low as 1.44 mg/l, less than the internationally accepted average requirement of fishes of 2-3 mg/L DO (Richards, 2011). Our results corroborate an earlier study showing that juvenile marbled spinefoot are capable of tolerating oxygen deficiency (Popper & Gundermann, 1975), but add to that by pinpointing the minimal tolerances acceptable.

2. Behavior of Marbled Spinefoot during exposure to various oxygen concentrations

The primary behavioral response of *S. rivulatus* to hypoxia was aquatic surface respiration (ASR). Fish exposed to low oxygen concentrations moved upwards ventilating at the oxygen-rich water surface. Similar results were reported by Carumbana and Luchavez (1979) working with *Siganus guttatus*. Aquatic surface respiration is widespread among non-air breathing fishes and is an adaptive strategy initiated at a specific oxygen threshold which varies from one species to the other (Kramer and McClure, 1982; Kramer, 1983). For instance, the oxygen threshold at which ASR was initiated in characoid fish was 1.75 mg/L, whereas in the angel fish it was initiated at a DO of 2.3 mg/L (Kramer and McClure, 1982). Our study showed that *Siganus rivulatus* exhibited ASR at an oxygen concentration of 0.65 mg/L.

In a study dealing with fishes of Panama, Kramer (1983) tested for ASR in fishes from various habitats. He reported that 72% of fishes from potentially hypoxic marine habitats such as tide pools exhibited ASR, and only 42 % of fishes from consistently well-oxygenated marine habitats ventilated at the oxygen surface. This suggests that marbled spinefoot must have evolved in environments prone to hypoxia. In aquaculture situations as in natural water body movement, rabbitfish attempting to respire at the surface would suggest hypoxic conditions with DO at or less than 1.44 mg/L. However, not all ASR is indicative of hypoxia. Excessive nitrite in water also causes ASR by reducing the oxygen carrying capacity of red blood cells, practically having the same effect as hypoxia (Saoud *et al.*, 2014).

B. Experiment 2: Standard Metabolic Rate of *Siganus rivulatus*

Prior to performing any studies on fish metabolism, it is necessary to estimate the standard metabolic rate of the species of interest at known optimal environmental conditions. For *Siganus rivulatus*, the chosen temperature and salinity at which the experimental runs were performed were 27°C and 35 ppt respectively, the optimal conditions for growth of cultured *Siganus rivulatus* (Saoud *et al.*, 2007; Saoud *et al.*, 2008a). Results suggest that at these parameters, the standard metabolic rate of *Siganus rivulatus* is 0.57 ± 0.02 mg O_2/g fish/h. However, it is worth mentioning that changes in temperature (Glencross and Felsing, 2006) and salinity affect standard metabolic rate (Varsamos *et al.*, 2004). Knowing that 27°C and 35 ppt are not necessarily the environmental temperature and salinity at which wild *S. rivulatus* live, the standard metabolic rate obtained in the present study might only be applicable to *S. rivulatus* aquacultured in recirculating systems. Values would be different if the fish were reared in sea cages or ponds exposed to the vagaries of nature. Accordingly, if our results are to be used for comparison in future experiments, those experiments have to be performed at the same temperature and salinity used in the present work.

Part of fish's daily energy expenditure goes to basic needs to sustain life (Auer *et al.*, 2015), and excess energy is expended for functions such as growth and reproduction. Standard metabolic rates vary among fishes, some having SMRs two to three times greater than those of other fishes (Auer *et al.*, 2015). The standard metabolic rate (0.57 ± 0.02 mg O_2/g fish/h) of juvenile marbled spinefoot determined in the present study is different from the SMR calculated for other species of the same age group maintained at their own

optimal temperatures. The SMR of juvenile Atlantic cod, for instance, at a temperature of 10°C was reported to be 0.06 mg O_2/g fish/h (Schurmann and Steffensen, 1997), and that of carp at 20°C was reported to be 0.22 mgO_2/g fish/h (Brett and Groves, 1979). Having low SMR would enable fish to allocate more energy for growth and reproduction (Wieser, 1994) making them better suited for aquaculture. Compared to other species marbled spinefoot appear to have a high standard metabolic rate which might translate into a high FCR. However, empirical data suggest otherwise. *S. rivulatus* juveniles tend to have an FCR between 1.1 and 1.5 (see Barakat *et al.* 2011).

C. Experiment 3: Response of *Siganus rivulatus* to Hypoxia

Results tend to suggest that *Siganus rivulatus* behaves as an oxyregulator at high oxygen concentrations, maintaining an increased respiration rate until a mean critical oxygen tension is reached. However, at oxygen concentrations below P_{crit}, marbled spinefoot becomes an oxyconformer decreasing its oxygen uptake with decrease in ambient oxygen concentration. Similar behavior was observed in other fishes, such as sharpsnout seabream (*Diplodus puntazzo*) (Cerezo and Garcia, 2004) and common dentex (*Dentex dentex*) (Valverde *et al.*, 2006). At DO greater than P_{crit}, fishes try to enhance the uptake and transport of oxygen by means of respiratory and circulatory mechanisms (Valverde *et al.*, 2006).When oxygen concentrations fall below P_{crit}, SMR can no longer be maintained (Ott *et al.*, 1980), and fishes suppress their metabolism by reducing total energy

expenditure to delay the activation of anaerobic pathways of ATP production (Richards, 2011).

In the present study, marbled spinefoot decreased its respiration rate at DO below P_{crit}, suggesting a decrease in metabolic rate. This decrease could be explained by an alteration in hemoglobin-binding affinity to oxygen as a response to lowered ambient oxygen concentrations. Studies have shown that fish generally respond to hypoxia by increasing their hemoglobin-O_2 binding affinity in order to improve oxygen uptake (Richards, 2010). This makes the unloading of oxygen at the level of tissues more difficult, obliging the fish to reduce their oxygen demand by reducing energy expenditure and thus compensate for the lower dissociation rate. Specific hematological studies on oxygen binding affinity of hemoglobin at various O_2 partial pressures are required to understand the mechanisms by which *S. rivulatus* specifically reacts to hypoxia.

Fishes inhabiting hypoxic or O_2-variable areas are well adapted to changes in environmental DO and can tolerate relatively low DO levels (Mandic *et al.*, 2009). Moreover, hypoxia tolerant species have an improved O_2 uptake and lower P_{crit} values when compared to hypoxia sensitive species (Richards, 2011). Mandic *et al.* (2009) attributed low P_{crit} values of sculpins to physiological selection-driven modifications induced by hypoxia such as large gill surface area and high hemoglobin-oxygen binding affinity which are not present in sculpins that are unable to tolerate hypoxia. Results of our study suggest a P_{crit} of 1.7 mg O_2/L for *Siganus rivulatus*. Being able to extract oxygen and maintain SMR down to the relatively low value of 1.7 mg/L O_2 suggests that *S. rivulatus* must have evolved in environments prone to hypoxia. Selective environmental adaptations to hypoxia

are yet to be discovered, but our findings that marbled spinefoot is able to suppress metabolism below 1.7 mg/L can be considered as an adaptive strategy for survival in hypoxic conditions. The evident capacity of *S. rivulatus* to tolerate hypoxia further suggests that this species is a good candidate for intensive aquaculture where fish are often inadvertently exposed to hypoxia.

Dissolved oxygen is one of the important water quality parameters in aquaculture that farmers should take into account when raising fish (Braun *et al.*, 2006). A decrease in ambient oxygen concentration might especially occur in intensive aquaculture when fishes are stocked at high densities. Results of the third experiment assessing responses to hypoxia showed that as DO levels decreased, marbled spinefoot increased its metabolic rate up until P_{crit} was reached, entailing energy expenditure. Aeration to maintain high oxygen concentrations is expensive. Furthermore, it seems that the larger the gap between P_{crit} and the ambient oxygen concentrations, the more energy is lost by *S. rivulatus* in an attempt to maintain homeostasis. All this adds up to both losses at the level of energy spent to power up aerators as well as energy lost by fish that could otherwise be translated into growth. It is therefore important that farmers know the minimum oxygen concentration required by aquacultured species which would allow for a cost effective approach towards ensuring a suitably aerated environment for the growth and well-being of the fishes.

P_{crit} variability among species are related to body weight and environmental factors such as temperature. Most studies have shown that P_{crit} decreases with an increase in body weight (Fernandes and Rantin, 1989), and increases with an increase in temperature (Schurmann and Steffensen, 1997). Some studies however disagree with the general

consensus. Cerezo and Garcia (2004) for instance reported the P_{crit} in sharpsnout seabream to be independent of both body size and temperature. In our study, it is worth mentioning that P_{crit} obtained is only valid at the specific conditions tested i.e. temperature 27°C, and for rabbitfish of sizes ranging from 1.74 to 4.40 g. Because fishes are poikilothermic, we expect P_{crit} to increase with an increase in temperature but to what level it will increase remains to be established.

D. Experiment 4: Effect of Salinity on Oxygen Consumption of *Siganus rivulatus*

In a study assessing the effect of various salinities on fish energetics, Saoud *et al.* (2007) showed that energy expenditure of *S. rivulatus* at 35 ppt was least as seen by gill Na^+ -K^+ -ATPase (NKA) activity. Salinities greater or less than 35 ppt entailed an increased NKA activity and thus an increase in energy expenditure.

Results of the present study showed no significant differences in oxygen uptake of fish reared at salinities 30, 35, and 40 ppt, as seen by respiration rate measurements. However, oxygen uptake increased at 25 ppt. Our findings complement and corroborate the results by Saoud *et al.* (2007). As salinity decreases, NKA activity increases, resulting in an increased demand for energy, thus oxygen. The fact that Saoud *et al.* (2007) found differences in gill NKA activity between fish at 30, 35 and 40 ppt but these differences were not observed in respirometry could be attributed to one of two possibilities. Either fish reduce body metabolism during salinity stress, thus counteracting the increase in gill

metabolism, or variation in the data within treatments masked out any differences between treatments.

CHAPTER IV

CONCLUSION

The present work provides useful information about the oxygen requirements of *S. rivulatus* and its tolerances to short-term hypoxia. According to our findings, marbled spinefoot has a lethal oxygen threshold of 0.7 mg/L and a sublethal oxygen threshold within the range of 1.44 to 1.7 mg/L. The primary behavioral response of *S. rivulatus* to hypoxia is aquatic surface respiration allowing the species to improve oxygen uptake when challenged with decreased oxygen concentrations. Moreover, marbled spinefoot is an oxyregulator until a DO of 1.7 mg/L below which it behaves as an oxyconformer suppressing its metabolism. These results are especially useful in aquaculture; farmers culturing *S. rivulatus* should make sure oxygen does not go below 1.44 ppm for more than one hour during fish transportation and handling. In aquaculture situations, rabbitfish attempting to respire at the surface would suggest hypoxic conditions with DO at or less than 1.44 mg/L. Knowledge of P_{crit} would result in financial advantages for farmers, allowing for a cost-effective approach towards ensuring a suitably aerated environment for the growth and well-being of fishes, and thus avoiding unnecessary expenses.

The present study also allows for a better understanding of metabolism and stress response of *Siganus rivulatus*. Our results suggest that standard metabolic rate of juvenile marbled spinefoot at 35 ppt is 0.57 ± 0.02 mg O_2/g fish/h with lower salinities causing an increase in metabolism. Despite the euryhalinity of marbled spinefoot, farmers should

maintain salinity within the optimal range of 30 to 40 ppt in order to avoid stress and reduce mortality to ensure maximum productivity.

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