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Effects of feeding and hypoxia on cardiac performance and gastrointestinal blood flow during critical speed swimming in the sea bass *Dicentrarchus labrax*

A. Dupont-Prinet [a,b,*], G. Claireaux [c], D.J. McKenzie [a,b]

[a] Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France
[b] Institut des Sciences de l'Evolution de Montpellier (ISE-M), Unité Mixte de Recherche 5554, Centre National de la Recherche Scientifique, Université Montpellier 2,
Station Méditerranéenne de l'Environnement Littoral, 1 quai de la Daurade, 34200 Sète, France
[c] Optimisation des Régulation Physiologiques (ORPHY), Université Européenne de Bretagne–Campus de Brest, UFR Sciences et Technologies, 6 avenue Le Gorgeu, 29285 Brest Cedex 3, France

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ABSTRACT

Previous studies have shown that if European sea bass are exercised after feeding, they can achieve a significantly higher maximum metabolic rate (MMR) than when fasted. They can meet combined metabolic demands of digestion (specific dynamic action, SDA) and maximal aerobic exercise, with no decline in swimming performance. If, however, exposed to mild hypoxia (50% saturation), bass no longer achieve higher MMR after feeding but they swim as well fed as fasted, due to an apparent ability to defer the SDA response. This study explored patterns of cardiac output (Q_A) and blood flow to the gastrointestinal tract (Q_{GI}) associated with the higher MMR after feeding, and with the ability to prioritise swimming in hypoxia. Sea bass (mean mass ~325 g, forklength ~27 cm) were instrumented with flow probes to measure Q_A and Q_{GI} during an incremental critical swimming speed (U_{crit}) protocol in a tunnel respirometer, to compare each animal either fasted or 6 h after a meal of fish fillet equal to 3% body mass. Feeding raised oxygen uptake (M_{O2}) prior to exercise, an SDA response associated with increased Q_A (+30%) and Q_{GI} (+100%) compared to fasted values. As expected, when exercised the fed bass maintained the SDA load throughout the protocol and achieved 14% higher MMR than when fasted, and the same U_{crit} (~100 cm s^{-1}). Both fed and fasted bass showed pronounced increases in Q_A and decreases in Q_{GI} during exercise and the higher MMR of fed bass was not associated with higher maximum Q_A relative to when fasted, or to any differences in Q_{GI} at maximum Q_A. In hypoxia prior to exercise, metabolic and cardiac responses to feeding were similar compared to normoxia. Hypoxia caused an almost 60% reduction to MMR and 30% reduction to U_{crit}, but neither of these traits differed between fed or fasted bass. Despite hypoxic limitations to MMR and U_{crit}, maximum Q_A and patterns of Q_{GI} during exercise in fasted and fed bass were similar to normoxia. Estimating GI oxygen supply from Q_{GI} indicated that the ability of bass to prioritise aerobic exercise over SDA when metabolically limited by hypoxia was linked to an ability to defer elements of the SDA response occurring outside the GI tract.

1. Introduction

It has recently been shown that if European sea bass are exercised after feeding, in a standard incremental "critical swimming speed" (U_{crit}) protocol, they can achieve a significantly higher maximum metabolic rate (MMR) than when fasted (Jourdan et al., 2009). This ability allows the sea bass to respond to the metabolic demands of digestion (specific dynamic action, SDA) without any decline in their aerobic metabolic scope (AS) for exercise and, consequently, in their

U_{crit} performance. Thus, this teleost species has excess cardiorespiratory capacity beyond that utilised by aerobic exercise alone (Jourdan et al., 2009).

In salmonids, the evidence suggests that maximal aerobic exercise by fasted animals exploits their entire cardiorespiratory capacity (Farrell, 2007), as it is considered to do in birds and mammals (Taylor and Weibel, 1981; Bishop, 1999). Species such as the rainbow trout (*Oncorhynchus mykiss*) and chinook salmon (*O. tshawytscha*) show reduced U_{crit} performance after feeding. This is because the metabolic demands of the SDA response diminish the AS available for aerobic swimming, so the fish reach their MMR "ceiling" at a lower swimming speed than when fasted (Alsop and Wood, 1997; Thorarensen and Farrell, 2006). However, an ability to increase MMR and maintain aerobic exercise performance after feeding has been described before in some reptiles which infrequently consume very large meals (Secor et al., 2000; Bennett and Hicks, 2001; Hicks and Bennett, 2004).

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* Corresponding author. Institut des Sciences de l'Evolution de Montpellier (ISE-M), Unité Mixte de Recherche 5554, Centre National de la Recherche Scientifique, Université Montpellier 2, Station Méditerranéenne de l'Environnement Littoral, 1 quai de la Daurade, 34200 Sète, France.
E-mail address: aurelie.prinet@laposte.net (A. Dupont-Prinet).

The mechanistic bases of the excess cardiorespiratory capacity in reptiles have not been specifically explored (Hicks and Bennett, 2004). In all animals, however, the pumping capacity of the heart is believed to be a central factor in defining cardiorespiratory performance (Taylor and Weibel, 1981; Bishop, 1999; Hicks and Bennett, 2004; Claireaux et al., 2006; Farrell, 2007). Furthermore, the ability of the heart to allocate blood to competing organs systems, such as the gastrointestinal (GI) tract and the locomotor muscles, has also been explored as a factor limiting the ability of a species to meet the combined metabolic demands of the SDA and aerobic exercise (Thorarensen and Farrell, 2006; Altimiras et al., 2008).

A primary objective of the current study was, therefore, to measure and compare cardiac output (Q_A) in fasted versus fed sea bass during exercise to U_{crit}, to investigate the hypothesis that increased MMR after feeding would be directly linked to increased maximum Q_A. A second objective was to measure and compare patterns of blood flow to the gastrointestinal (GI) tract (Q_{GI}) under the same conditions, to see how GI blood flow allocation related to MMR and Q_A in fasted versus fed sea bass. Although the relationships of Q_A to Q_{GI} have been described in a teleost exercised to their MMR and U_{crit} when fasted (Thorarensen et al., 1996), they have never been investigated after feeding. Altimiras et al. (2008) found that feeding caused increased Q_A and Q_{GI} at low swimming speeds in sea bass but, at higher speeds, these cardiovascular responses to feeding were progressively lost and the Q_A response to exercise dominated. Altimiras et al. (2008) did not, however, exercise their animals to their maximal aerobic rates and so did not reveal the ability of sea bass to increase MMR after feeding (Jourdan et al., 2009), and how this might relate to Q_A and Q_{GI}. Nonetheless, Altimiras et al. (2008) calculated that the rates of blood flow and oxygen supply to the GI tract during exercise would not have been sufficient to sustain the measured increase in oxygen demand due to the SDA and, therefore, that some proportion of the response must represent metabolic processes in other tissues. This finding is significant for a further aspect of the current study, which investigated effects of hypoxia.

Jourdan et al. (2009) studied the potential effects of moderate hypoxia (50% of air-saturation) on responses to exercise and feeding in the sea bass. As expected (Fry, 1947, Fry, 1971; Beamish, 1978; Jones and Randall, 1978), hypoxia limited MMR, AS and U_{crit} in the sea bass. Furthermore, hypoxia abolished the ability of the sea bass to increase their MMR after feeding. A third objective of the current study was, therefore, to measure Q_A in sea bass that were exercised to U_{crit} in hypoxia, when either fasted or fed, to investigate whether hypoxic limitations to MMR, AS and U_{crit} were linked to limitations to maximum Q_A. Interestingly, Jourdan et al. (2009) found that, although hypoxia did not affect the development of the SDA response at low swimming speeds, the SDA load progressively disappeared as swimming speed increased towards MMR and U_{crit}. Therefore, although the hypoxic sea bass could not increase their MMR after feeding, they actually swam as well when fed as they did when fasted (Jourdan et al., 2009). That is, the sea bass somehow deferred the SDA load in order to prioritise exercise in hypoxia (Jourdan et al., 2009). Thus a final objective was to measure Q_{GI} during the hypoxic swim trials, to investigate the extent to which rates of blood flow to the GI tract might account for the magnitude of the SDA load (Altimiras et al., 2008) and, therefore, to gain insight into what elements of the SDA response were deferred in order to prioritise swimming performance.

Preliminary experiments were conducted to describe the SDA response in sea bass fed a large protein meal (a ration of fish fillet equivalent to 3% body mass) at the prevailing temperature of 18 °C, and identify the period of the peak response. Subsequently, fish were fitted with flow probes on their ventral aorta and on the major blood vessels supplying the GI tract (Axelsson et al., 2002; Altimiras et al., 2008), to compare responses to a U_{crit} exercise protocol when either fasted or during the peak SDA response following the meal. A second series of experiments then investigated how these responses to

exercise were affected by exposure to moderate hypoxia at 50% saturation during the U_{crit} protocol.

2. Material and methods

2.1. Experimental animals

Sea bass *Dicentrarchus labrax* of either sex (mass ~ 325 g, fork-length ~ 27 cm), were obtained from a commercial supplier and maintained under a natural photoperiod at the Station Méditerranéenne de l'Environnement Littoral, in a rearing tank (vol. 3000 L) supplied with natural sea water (salinity 34 ± 1‰) regulated at a temperature of 18 ± 1 °C. Animals were fed a commercial diet daily (Le Gouessant, Brest, France), but food was withheld for at least 24 h prior to use in any experiments. All experiments were performed in water maintained at 19 ± 0.1 °C.

2.2. Specific dynamic action (SDA)

Four fishes (320 ± 4 g) were studied for the dynamics of SDA. Each was lightly anaesthetised in tricaine methane sulphonate (MS-222; 0.1 g L^{-1}, Sigma-Aldrich) until righting reflexes were lost, then force-fed by introducing fish white muscle fillet, a ration of 3% of their body mass, into the stomach with a pair of forceps (Axelsson et al., 2002; Altimiras et al., 2008). The sea bass were then transferred to an automated swim-tunnel respirometer (chamber volume 49 l) which has previously been described in detail by McKenzie et al. (2001). Water current speed was set at 20 cm s^{-1} and this stimulated the sea bass to recover rapidly from the mild anaesthesia and start swimming gently. Instantaneous oxygen uptake (M_{O2}, in mg kg^{-1}h^{-1}) was measured once every 30 min by intermittent stopped-flow respiro-metry (Steffensen, 1989) using a fiber-optic oxygen meter (Fibox Oxygen Meter, PreSens, Germany) and associated software (Oxyview v. 5.32, PreSens) connected to a LoliDAQ automated control system and associated LoliResp software (Loligo Systems, Hobro, Denmark), as described by McKenzie et al. (2007). Measurements were made for 24 h, during which the fish recovered from the light anaesthesia and digested the fish fillet. At the end of each experiment, background M_{O2} was measured following removal of the fish from the respirometer, and values corrected accordingly.

2.3. Placement of flow probes

Individual sea bass were anaesthetised in tricaine methane sulphonate (MS-222; 0.1 g L^{-1}, Sigma) until breathing movements ceased. They were transferred to an operating table and their gills irrigated with aerated water containing 0.05 g L^{-1} MS-222. Ultrasound flow probes (2 S- and 1 S-type Transonic, resolution 0.1 mL min^{-1}; absolute accuracy ± 15%, EMKA, Paris, France) were placed around the ventral aorta and around the coeliaco-mesenteric arteries to measure total cardiac output (Q_A) and total gastrointestinal blood flow (Q_{GI}), respectively, as described by Axelsson et al. (2002) and Altimiras et al. (2008). After surgery, fish were transferred to opaque PVC chambers supplied with a flow of aerated sea water and allowed 24 h to recover.

2.4. Critical swimming speed protocol

Following this recovery period, fish were transferred to the swimming respirometer and allowed at least 12 h (overnight) recovery while swimming gently at a current speed of 20 cm s^{-1} (corresponding to 0.7 body length s^{-1}). At this low speed, the sea bass rested on the bottom and maintained position by gentle sculling of their pectoral fins and occasional tail flicks. The following day, fish were exposed to progressive increments in swimming speed, to 40, 60, 80 and then 100 cm s^{-1} every 30 min, until fatigue. Fish were considered to be fatigued when they were unable to remove