

SUPPLEMENTARY MATERIAL

Methods

Animals and holding conditions

In early February 2012, juvenile barramundi (*Lates calcarifer* Bloch 1790) were obtained from Good Fortune Bay Hatchery, Bowen, Queensland, Australia. The pedigree of the fish was not known due to hatchery cross-breeding practices to maintain genetic diversity, yet it is most likely that the juveniles used in this study were the offspring of wild caught females fertilised by male broodstock that had been reared in captivity for 1-3 generations. The condition index ($k=100000M_b / TL^3$, where M_b is body mass in g and TL is total length in mm) of the fish used in this study ($k=1.3-1.6$) compared favourably with previously published values for wild caught individuals ([1] and references within), indicating negligible influence of hatchery practices on the gross morphology of the fish. Due to the protandrous hermaphroditic nature of barramundi [2], all juveniles used in the present study were assumed to be males.

Barramundi are considered ambush predators. In nature, the prey of barramundi measuring between 80 and 200 mm total length (as in the present study) consists mainly of crustaceans (especially penaeid shrimp) and fish [1,3]. Barramundi thus ingest meals in individual predatory events. The 2-min feeding period applied in this study nicely represents this type of feeding. Furthermore, barramundi typically consume prey which are less than 50% of barramundi length, with a few exceptions where prey as large as 61% of barramundi length has been observed [3]. We are not aware of any lower prey size limit, although small zooplankton prey such as copepods composed the majority of the diet of barramundi less than 80 mm total length but was absent from the diet of larger barramundi [3].

The fish were transported by road to the Australian Institute of Marine Science (AIMS), Townsville, Queensland, Australia, where they were held in 40 L rectangular tanks containing 30 L of seawater. Each tank was divided down the middle with an opaque plastic screen, creating two identical compartments holding one fish on each side (both sides had an individual water inlet and air stone). The tanks were supplied with recirculated, filtered seawater (particle filtered and UV sterilised) at $29\pm 1^\circ\text{C}$ (actual range) with a salinity of 35 ppt from a 250 L sump. Water in the sump was replaced with fresh seawater at a rate of 40 L h^{-1} to minimise any build-up of waste products. Photoperiod was set to 13:11 h light:dark. Fish were fed individually to satiation every second day with Ridley Aqua-Feed Marine Float pellets (Ridley Corporation, Melbourne, Victoria, Australia) by lifting the lid of each tank and introducing one pellet at a time until the fish stopped eating, except on experimental days where the fish were fed pellets corresponding to 0.6 to 3.4% of their body mass before being transferred to the respirometry setup.

Respirometry setup

Metabolic rate of the fish was estimated from rates of oxygen uptake (\dot{M}_{O_2}) obtained using automated intermittent-closed respirometry [4] and using best practices outlined in [5]. An illustration of the kind of data obtained from this exact setup can be seen in fig. 2 of [6].

The experimental setup comprised of eight polypropylene respirometers (1.43 L each) submerged in a darkened tank (experimental tank) containing 260 L of water. Temperature was controlled by a thermostatted water bath connected to a stainless steel coil submerged in the experimental tank. Water was continuously

circulated through each respirometry chamber using an inline Eheim pump in a recirculation loop, and the oxygen concentration of the respirometer water was measured continuously at 1 Hz in the recirculation loop using fibre optic oxygen sensors (FireStingO₂; Pyro Science GmbH, Aachen, Germany). Automated Eheim flush pumps refreshed the water in the respirometers for 5 min in every 10 min period and \dot{M}_{O_2} was calculated from the decline in oxygen concentration in the respirometers between flush cycles. Oxygen levels in the respirometers always remained above 80% air saturation.

Data analysis

All \dot{M}_{O_2} data (~250 measurements per fish) were analysed after importing the text file from the FireStingO₂ software into LabChart 7 (ADInstruments Pty Ltd, Bella Vista, NSW, Australia). Linear regressions between oxygen concentration and time were made for each measurement period and slopes derived from the regressions were used to calculate \dot{M}_{O_2} (mg min⁻¹ kg⁻¹). This was done by multiplying the slopes by the volume of the respirometry chamber after subtracting fish volume (assuming a density of 1 g mL⁻¹). Individual slopes were evaluated over 260 and 180 s for calculations of $\dot{M}_{O_{2,min}}$ and $\dot{M}_{O_{2,max}}$, respectively. Although background respiration was minor, it was always accounted for (subtracted from \dot{M}_{O_2}) when calculating \dot{M}_{O_2} by assuming a linear increase in background respiration between the mean of three \dot{M}_{O_2} measurements taken in the empty (i.e. without fish) respirometry chambers at the beginning and end of an experiment.

$\dot{M}_{O_{2,min}}$ was determined by first taking the mean of the lowest 10% of \dot{M}_{O_2} measurements after the SDA response was complete (see Fig. S2), after which outliers (± 2 s.d. from the mean) were excluded (no more than two data points were identified as outliers for any fish) and $\dot{M}_{O_{2,min}}$ was calculated as the mean of the remaining \dot{M}_{O_2} measurements. $\dot{M}_{O_{2,max}}$ was determined as the highest \dot{M}_{O_2} measurement recorded, which always occurred in the first minutes following the exhaustive exercise (chase) protocol. Aerobic scope (AS) was calculated as $\dot{M}_{O_{2,max}}$ minus $\dot{M}_{O_{2,min}}$.

Growth rates were calculated as specific growth rates (SGR, % day⁻¹) according to the equation

$$\text{SGR} = [(\ln M_f - \ln M_i) \times t^{-1}] \times 100,$$

where M_f and M_i is the body mass (g) of the fish at the end and start of the experiment, respectively, and t is the time (days) it took the fish to grow from M_i to M_f . The 20 fish for which growth rates were calculated were monitored for food intake by noting how many pellets they ate at each feeding over 7 weeks. Energy intake and growth efficiency were then calculated from the mean weight of a pellet (41 mg; measured for 100 pellets) and the digestible energy content of the feed (18.44 kJ g⁻¹; according to manufacturer specifications).

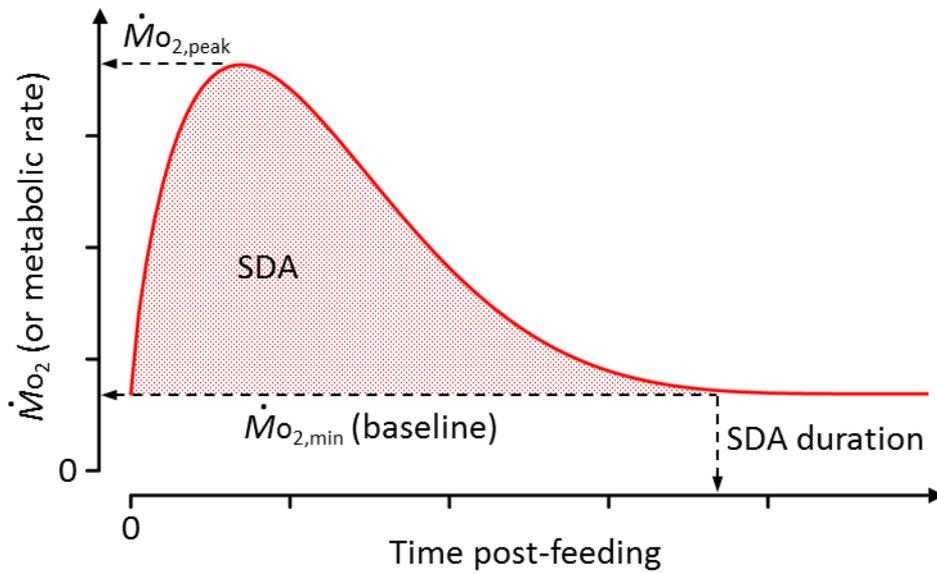


Fig. S1. Conceptual figure illustrating the various components of the specific dynamic action (SDA) response. \dot{M}_{O_2} is oxygen uptake rate, $\dot{M}_{O_2, \min}$ is minimum oxygen uptake rate (i.e. equivalent to standard aerobic metabolic rate). See [7] for further details.

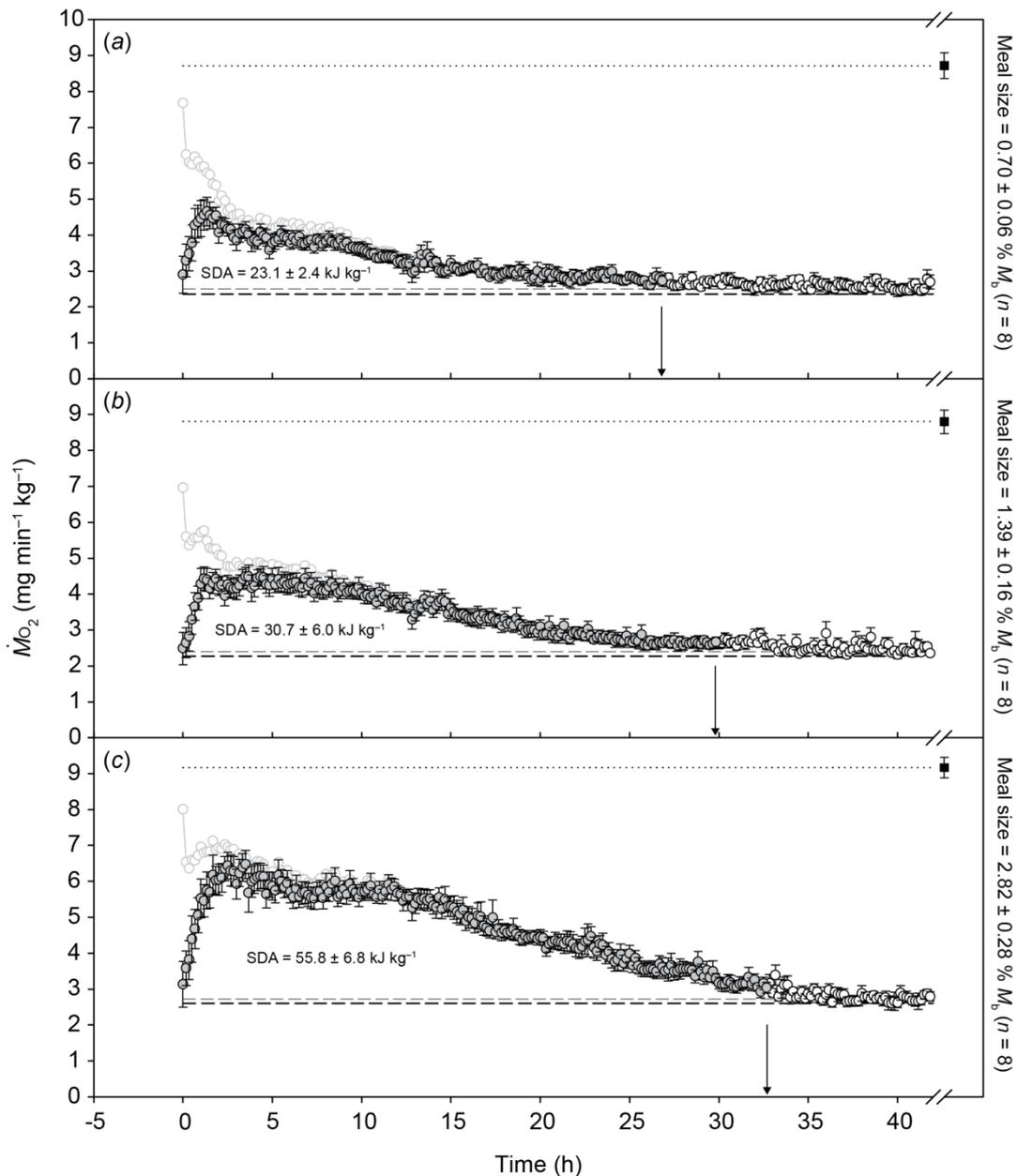


Fig. S2. Mean oxygen uptake rates (\dot{M}_{O_2}) of 24 juvenile barramundi (*Lates calcarifer*) over ~42 h following ingestion of food pellets (food was ingested at time 0 h). Error bars represent 1 s.e. in either direction. The data have been smoothed (five-point moving median) to account for brief periods of spontaneous activity. The amount of food eaten by individual barramundi ranged from 0.6 to 3.4% of fish body mass but is shown here in three average meal-size groups (means \pm 2 s.e.; panels *a*, *b*, *c*) for easy comparisons of the specific dynamic action (SDA) response which followed ingestion of the meals. Grey points with error bars represent the SDA response, and the amount of energy spent digesting the meals (i.e. SDA; kJ kg^{-1}) is noted within the panels (means \pm 2 s.e.). White points (dark outlines) with error bars represent \dot{M}_{O_2} after the SDA response was deemed complete, which was when three \dot{M}_{O_2} measurements had fallen below $\dot{M}_{O_{2,min}}$ (broken black lines) + 2 s.d. (broken grey lines) for individual fish. The white points (pale outlines) without error bars in the background represent the actual \dot{M}_{O_2} recorded, which had the \dot{M}_{O_2} of the unfed control group subtracted from it to differentiate between the increase in \dot{M}_{O_2} due to handling and due to SDA. Black squares and dotted lines represent $\dot{M}_{O_{2,max}}$. Vertical arrows note the SDA duration.

References

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