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REPORT

Changes in aerobic metabolism associated with the settlement transition for the leopard coral grouper (*Plectropomus leopardus*)

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Abstract Metamorphosis is a critical aspect of coral reef fish ecology. This developmental milestone marks changes in form and function that permit successful transition of pelagic larvae to the demersal lifestyle on coral reefs. However, we know very little about the physiological changes that occur during this period, specifically potential changes in energetics associated with swimming. This is critical, as swimming is the mechanism by which pelagic larvae find a suitable reef on which to settle. Coral grouper larvae (Serranidae: *Plectropomas leopardus*) were collected at night as they came into the vicinity of a fringing reef to settle, and their physiological metamorphosis was characterized. Larvae and 24 h-settled juveniles were exposed to an endurance swimming test at ecologically relevant swimming speeds, and oxygen uptake rates were measured during activity. To describe how aerobic and anaerobic properties of tissues change during metamorphosis, we also measured whole body citrate synthase and lactate dehydrogenase activity, respectively, as well as mitochondrial density in the trunk and pectoral fins. Our approach accurately measures the oxygen uptake rates these life stages need during the recruitment process, with larvae having a 74% higher mass-specific oxygen uptake rate ($\dot{M}O_2$) than settled juveniles despite swimming at speeds that are only 1.5 body-lengths per second (BLs^{-1}) faster. Citrate synthase activity significantly decreased upon settlement; as larvae had 3.7 times higher activities than juveniles, suggesting that rapid changes in aerobic metabolism of tissues may be an important process during metamorphosis in this species. In contrast, lactate dehydrogenase did not significantly differ upon settlement. These findings highlight some physiological modifications that pelagic coral grouper larvae undertake within 24 h that contribute to successfully settling onto a coral reef.

Keywords Metamorphosis · Metabolic enzymes · Mitochondria · Ecophysiology · Endurance swimming respirometry · Mesopredator

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Introduction

Metamorphosis is a biological phenomenon that has captivated scientists for decades. Metamorphosis generally represents the developmental milestone when a less-developed, dispersive early life stage (e.g. larva) undergoes ontogenetic changes in morphology and physiology while selecting adult habitat conditions (Bishop et al. 2006). Metamorphosis has evolved independently across several animal lineages, being widespread across insect groups (e.g. butterflies and dragonflies), as well as amphibians and teleost fishes. Specifically for teleosts, flatfishes (Pleuronectiformes) have received

much research interest, given the unique shift from a benthopelagic symmetrical free-swimming larva, to a benthic, asymmetrical (i.e. eyes shift to one side of the body) juvenile/adult with a dorso-ventrally flattened body (Paris and Laudet 2008). However, a group of fishes that also undergo metamorphosis in highly variable and species-specific ways are the coral reef fishes (McCormick et al. 2002).

Despite the wide diversity of coral reef fishes in tropical reef ecosystems (approximately 6000–8000 species; Rabosky et al. 2018), their life-history is generally conserved across species. Adult reef fishes are demersal specialists and typically site-attached to a patch of coral (Leis 2006). Larvae are carried off the reef by currents and wave-action with the progression of larval development occurring in the pelagic environment (Leis and McCormick 2002). During this developmental period, larvae are pelagic-specialists, with body morphologies (i.e. body shape and muscle mass) that facilitate swimming at high speeds (20–100 cm s^{-1}; > 20 body lengths s^{-1}) not only to counteract ocean currents but also so that they can feed on plankton in the water column and locate and select for an appropriate reef habitat on which to settle (Fisher et al. 2005; Downie et al. 2021a). When coral reef fishes make the transition from pelagic to demersal reef habitats (i.e. the process known as settlement), they must change form (i.e. colours, body shape, and body spines) and function to compliment their new life style through a process called metamorphosis. Reef fish metamorphosis was previously viewed as an instantaneous event that occurred as soon as reef fish larvae settled onto the reef. While some reef fish species do complete metamorphosis coinciding with settlement (e.g. members of Pomacentridae), this developmental event is best described as a gradual transition that begins during the pelagic larval phase and ends when either the fish reaches the reef, or over several days or weeks post-settlement (McCormick and Makey 1997; McCormick et al. 2002). For example, goatfish (*Parupeneus multifasciatus*) and dartfish (*Pteroleotris evides*) make several changes in sensory systems and morphology, respectively, 2–3 weeks post-settlement to complete metamorphosis (McCormick and Makey 1997). Indeed, reef fish metamorphosis is an evolutionary response to selection pressures, particularly reef predators that have evolved to capitalize on reef-naïve, newly-settled larvae. Additionally, physical and hydrodynamic characteristics of reefs require different morphological and physiological adaptations than pelagic habitats (Fulton and Bellwood 2004; Fulton et al. 2005). Therefore, the developmental window of metamorphosis is critical for coral reef fish population dynamics and is widely considered a population bottleneck, due to the myriad challenges naïve larvae face upon reef settlement that result in high mortality (e.g. estimated 10% of newly settled larvae are eaten per day per cohort; Doherty et al. 1985). The changes that larvae make during metamorphosis ultimately have ramifications for their success in reefs later in their life cycle (i.e. contributing to adult stocks).

Recent advancements in endocrinology, molecular biology and physiological techniques (i.e. advancements in swimming respirometry design for larval and juvenile fishes) have significantly progressed our understanding of the changes reef fishes undergo during metamorphosis. Indeed, early studies on metamorphosis focused primarily on morphological changes (e.g. head shape, body coloration, and body spine abundance and size; McCormick et al. 2002) or changes to the sensory system (McCormick 1993; Shand 1994, 1997; Wright et al. 2005). Early physiological evidence suggested that newly settled larvae decrease their oxygen uptake rates to enhance survival during nocturnal hypoxia events on reefs (Nilsson et al. 2007). Recent evidence not only supports this notion but further suggests that larval reef fishes prepare for hypoxic reef conditions during the pelagic phase by switching haemoglobin subunits from low to high affinity for oxygen (Downie et al. 2023). Additionally, the role of thyroid hormones mediating metamorphosis and consequently changing metabolic processes during this period is becoming an area of research interest (Holzer et al 2017; Besson et al. 2020; Roux et al 2023; Huerlimann et al 2024; Zwahlen et al. 2024). While it seems likely that changes in aerobic metabolism are widespread for fishes whilst transitioning from active larvae to more sedentary juveniles, we still do not fully understand the underlying swimming physiology. Previous work has found that swimming performance can decrease by as much as 70–80% (i.e. median critical swimming speed) upon settlement, which may be because being able to manoeuvre through a complex reef habitat is more important than the constant fast (primarily) aerobically-driven swimming speeds required to survive in the pelagic environment (Leis et al. 2011). Indeed, many reef fishes use pectoral fin swimming to navigate reef habitats (Fulton et al. 2013); however, these fins are not as well-developed in young fishes (Thorsen and Hale 2005; Patterson et al. 2008; Green et al. 2011), which rely on body-caudal movements in the pelagic environment (Hale et al. 2006). Therefore, an important aspect of metamorphosis that supports successful settlement of reef fishes onto reef habitats may involve, not only the physiology underpinning aerobic metabolism, but also an immediate change in histo-morphological changes. Such changes in machinery are critical to promote survival of newly settled larva to reefs to contribute to future generations; which is especially critical for ecologically and economically valuable species, such as coral grouper.

The leopard coral grouper (Serranidae; *Plectropomus leopardus*) is an important Indo-Pacific predatory fish species responsible for structuring and moderating fish assemblages on coral reefs with studies showing changes in lower-trophic species composition correlating with loss of coral

grouper abundance (Frisch et al. 2016). The coral grouper are also one of the most economically important fishery species on the Great Barrier reef, with annual landings of $67 million AUD per annum, which accounts for 50% of all landings on the Great Barrier Reef (Frisch et al. 2016). Many critical physiological traits (e.g. active metabolic rates, metabolic enzyme activities) that support the impressive swimming capabilities of pelagic larvae and therefore support settlement remain unknown for the vast majority of reef fish species, including coral grouper. Currently, we have a basic understanding of laboratory (critical swimming speed of 42 cm s^{-1}; Leis and Fisher 2006) and in situ swimming speeds (18 cm s^{-1}; Leis and Carson-Ewart 1999) of pelagic coral grouper larvae and descriptions of colour and morphology changes at time of settlement (McCormick et al. 2002). Previous work has investigated reef habitat preference of pelagic larval coral grouper, but the physiological changes associated with settlement to these habitats are unknown (Doherty et al 1994; Light and Jones 1997).

While many changes occur during metamorphosis as larvae transition from pelagic to reef habitats (e.g. Roux et al 2023; Downie et al 2023; Huerlimann et al 2024), changes in aerobic and anaerobic metabolism related to swimming will be critical, given that hydrodynamic conditions between pelagic realms and reefs are distinct, and pelagic larvae of coral grouper occupy different ecological niches than demersal adults (active vs. ambush predator). Here, we present the first detailed measures of changes in metabolism across the pelagic to reef transition period for the common leopard grouper. We take an integrative physiological approach measuring cellular (i.e. citrate synthase, lactate dehydrogenase, and mitochondrial volume in pectoral and trunk musculature) and whole animal (i.e. oxygen uptake during an endurance test) metrics of swimming metabolism measured in larvae captured as they approach the reef and juveniles that have settled within 24 h of capture to better understand any immediate changes in swimming physiology initiated during the pelagic to reef transition period. Measurements of oxygen uptake during endurance tests at speeds relative to current speeds representative of pelagic and reef habitats provide estimates of the active energy demands larva and settled juveniles, respectively, required to swim in each habitat. We predicted that, within 24 h of settling onto a reef, juvenile coral grouper would exhibit: (1) a decrease in oxidative properties of swimming muscles in trunk (body-caudal) muscles, because predictably, active endurance swimming is not characteristic of juveniles (or adult lifestyle as an ambush predator); (2) increases in anaerobic properties of muscles to accommodate predator-escape responses as a newly settled larva and juvenile and for future ambush predation feeding strategies as an adult, which are behaviours critical for survival on reefs, and (3) increases in mitochondria in pectoral muscles for increased manoeuvrability through complex structures of reefs and coastal wave action. Measurable changes in physiological metrics would highlight the immediate changes in swimming required for larval success on coral reefs in order to contribute to future populations of coral grouper.

Materials and methods

Fish collection and husbandry

Fish were collected from and experiments were executed at Lizard Island Research Station (14°40′S, 145°28′E), which is located 240 km offshore of Cairns, Australia. Late-stage larvae of the common coral grouper were collected using light traps (Fig. 1b in Meekan et al. 2001) that were deployed in the late afternoon (~ 17:00) and subsequently collected early in the night (~ 22:00) (Fig. 1). We performed five night-time sampling sessions within one week (night of 14 November to the night of 18 November 2018; experiments ended on the afternoon of 19 November 2018) in which we captured sufficient larvae (generally 6–10 individuals) to run 2 larvae and subsequently 2 juveniles from the same set of light traps, with spares for metabolic enzymes and reserves in case we needed to replace individuals who failed to swim (Fig. 1). After a week (i.e. 20–21 November 2018), we observed a significant reduction in captured larvae (generally 1–2 larvae across all light traps per trip), which was an insufficient number of larvae per sampling session to run enough experimental replicates. No larvae were captured 8 days after the experiment started (i.e. after 22 November 2018). Larvae were identified using standard protocols and were within the published size range for larvae and juvenile coral grouper (Leis 1986). To further validate identification, a subset of 5 larvae were left to metamorphose in a separate aquarium for four days and examined under a microscope for meristic characteristics (Leis 1986). Half the larvae caught in light traps (3–5 fish) were immediately allocated to represent the 'larval' group. The larval tank was a darkened aquaria (lid on the top) with a few air stones on the bottom to keep the larvae off the bottom of the tank. This was done to prevent the larvae from settling before the second larva of that sampling cohort could be swum. The other half of collected larvae (3–5 individuals) were placed in separate aquaria and left for no more than 24 h prior to being allocated to experimental assays. Within 7–8 h of capture (07:00–08:00), newly captured grouper were exploring the bottom of the aquaria and were bright red in colouration and therefore represented the 'juvenile' group. Juveniles were swum at 12:00 and 17:00 the next day. We allocated more individuals than we could swim in a sampling period (i.e. 2 larvae and 2 juveniles) as precautionary measures in case the initially selected individuals did not choose to swim (i.e. 1 larva and

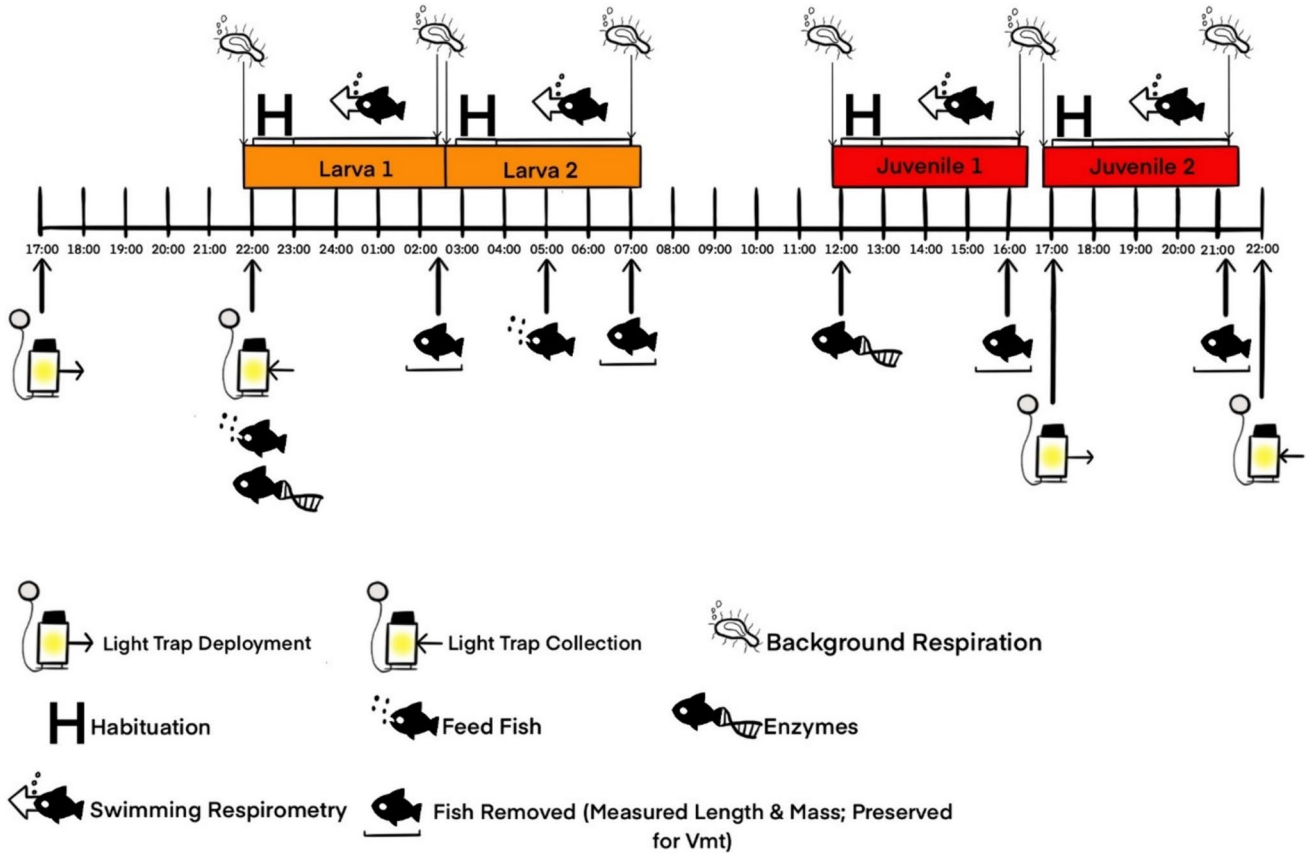

Fig. 1 General timeline for how experiments were executed on Lizard Island, including timestamps (24 h time) for when light traps were deployed and collected, start and end of experiments for larvae and juveniles (including background, habituation periods and 200 min swimming respirometry experiments), when physiological metrics were sampled (enzymes, morphometrics and tissues taken for mitochondrial volume), and when fish were fed. The workflow was repeated until we achieved the sufficient sample size for both larvae and juveniles (i.e. end sample size of 9 for each stage)

1 juvenile for the entire experiment) or mortalities during handling and experimentation (which did not occur), and to collect individuals for metabolic enzymes. All captured individuals were maintained in 40L flow-through tanks at the research station (28 °C, natural photoperiod; 4 individuals L^{-1}). Larvae were maintained in a darkened aquaria, swum in a swim tunnel (see below) within 1–4 h of capture and were not fed upon capture. Juveniles who were designated to be swum at 12:00 were only fed a small amount of *Artemia* spp. nauplii immediately upon capture, and juveniles designated to be swum at 17:00 were fed a small amount upon capture and at approximately 05:00 (7 h after capture; 12 h before experiments) (Fig. 1). All tanks were cleaned every three days or when required.

General workflow of the experiment

Here, we provide an overview and timeline of the experimental protocol (Fig. 1). Details for each physiological metric can be found below in subsequent subsections of the Methods. Upon capture (approximately 22:00) and sorting of larvae into experimental groups (larva or settled juveniles), the first larva is introduced into the swimming respirometer (approximately 22:00–22:10; see *'Swimming Respirometer'* below for details). The larva was habituated in the chamber for 1 h at a low flow velocity (approximately 1 body length per second; BL s^{-1}; approximately 1.7 cm s^{-1}) (Fig. 1). During this habituation period, the individuals designated for the juvenile group were fed a small aliquot of food, and 2–3 larva were euthanized in an ice bath and preserved in liquid nitrogen for metabolic enzyme assays (Fig. 1). Upon completion of the habituation period, the individual underwent an endurance test (see *'Endurance Test Experimental Protocol'* for details) for up to 200 min (Fig. 1). Upon completion of the test, the induvial was removed from the chamber, euthanized in an ice bath, morphometrics taken, and preserved for muscle dissection (see *'Morphometrics and Preparation for Histology'* for details). This workflow was repeated for the second larva swum per cohort (experiments start approximately at approximately 02:30), and

two juveniles per cohort (first swum at 12:00 and second at 17:00) (Fig. 1). In total, 9 larvae and 9 juveniles were successfully swum, and subsequently 9 individuals per developmental stage were collected for V_{mt}, however, not all muscle tissue samples were successfully measured for V_{mt} ($n = 5$ for both larva and juveniles) (Fig. 1). Individuals (2–3) sampled for metabolic enzymes were only taken during the habituation period for the first individual of either life stage per experimental cohort (approximately 10 larvae and juveniles each were sampled, but not all individuals were successful in measuring enzyme activity; CS activity: $n = 4$ larvae and 7 juveniles, LDH activity: $n = 4$ larvae and 5 juveniles) (Fig. 1).

Selection of swimming protocol

Due to limitations in the maximum speeds our swimming respirometer could achieve relative to the size of the coral grouper, the typical critical swimming speed test (U_{crit}) was not selected (i.e. coral grouper larvae could easily outswim our swimming respirometer's maximum speeds of 30 cm s^{-1}). Additionally, there has been a critical evaluation surrounding the ecological relevance of U_{crit}, as most fish rarely swim near maximum aerobic capacity (Downie et al. 2017, 2020), and we were not interested in measuring maximum metabolic rate or aerobic scope. We instead selected an endurance test as an alternative. Endurance tests swim fish at a constant, fixed speed until the fish fatigues or reaches 200 min of swim in which it is assumed the fish can swim indefinitely at that speed (Downie et al 2020). The benefit of endurance swimming within our experimental context is that we could select swimming speeds mimicking the current speeds larvae and juveniles would experience in the pelagic realm and on reefs, respectively, to understand the energy demands of swimming in both habitats. Larvae underwent a constant velocity test at 13.5 cm s^{-1} (approximately 8 BL s^{-1}), which mimicked the mean annual ambient current velocities around Lizard Island (Leis 2006). Juveniles were swum against velocities relevant for their depth at settlement (7.5–9.9 m; Leis and Carson-Ewart 1999), of approximately 10 cm s^{-1} (approximately 6 BL s^{-1}; Johansen 2014). It should be noted that we did not reciprocate flow velocities between life stages (e.g. swim juveniles at larval speeds and vice versa), as upon reaching a reef, settlement occurs and juveniles are unlikely to re-enter the water column. Additionally, the common coral grouper is rarely found in large numbers during early life history stages in nature. This posed a limitation on the number of specimens available for our research. Notably, after our initial experiments (i.e. after a week of initial sampling), our light traps yielded minimal fish captures, reflecting that our experimental duration coincided with the recruitment window at our designated location on Lizard Island. However, we do acknowledge that reciprocating the flows would be beneficial for future studies. We also acknowledge that developmental stage is correlated with swimming speed, and any comparisons are solely to either show the change in relationship between oxygen uptake and mass between the two stages (i.e. oxygen uptake not scaled for mass; mg O_2 h^{-1}) or quantify the different oxygen demands required for swimming in these distinct habitats using mass-specific oxygen uptake (mg O_2 g^{-1} h^{-1}). We therefore cannot prove that metamorphosis alone elicits any changes in oxygen uptake.

Swimming respirometer

Fish were swum using a glass, Blazka-style swimming respirometer (volume (V) = 125 ml; total length (L) = 14.5 cm; diameter (ø) = 2.7 cm), which allows for simultaneous measurements of oxygen uptake rates ($\dot{M}O_2$) while an individual fish swims at any given speed. To reduce the volume of the respirometer, thus providing a more accurate $\dot{M}O_2$ measurement for the size of animals swum, an insert (ø = 2.6 cm, L = 5.5 cm) was placed in the respirometer to create the working section. The working section of the respirometer (V = 50 ml, L = 6.5 cm, ø = 2.7 cm) fit a smaller chamber, which was where the fish swam (volume with swimming chamber = 40 ml). This smaller chamber (V = 5.1 ml, L = 4.5 cm, ø = 1.2 cm) was fitted with a flow straightener (made from capillary tubes of ø = 1.1 mm, L = 40 mm) to mitigate micro-turbulent flow and a downstream mesh barrier (mesh ø = 0.415 mm) to prevent the fish from being sucked into the propeller. The swimming respirometer was calibrated prior to experimentation using a high-speed camera (Casio Exilim High Speed Camera) and neutrally buoyant particles (hydrated *Artemia sp.* cysts, following Divi et al. 2018) to parameterize swimming speeds as well as visualize laminar flow (Downie et al. 2023). Visual inspection of the flat trajectory of the neutral particles moving through the smaller swimming chamber ensured that flow was not interrupted by either the insert or the presence of the smaller chamber within the working section of the respirometer, and the dimensions of the flow straightener were adequate. The swimming chamber was large enough for a fish to swim in any direction comfortably, thus reducing stress of enclosure. Blocking effects were reduced, as the fish cross-sectional area was less than 5% of chamber cross-sectional area, which would alter the flow within the chamber if it were more. Upon placing the fish in the chamber, the entire swimming respirometer was sealed underwater to prevent air bubbles from accumulating. An outer temperature jacket (V = 85 ml, L = 6.5 cm) maintained water temperature at experimental conditions (28 °C), which was effective even at high water velocities. Additionally, a black tarp (with a small window to view the swimming fish) was draped over the chamber to further reduce stress, and the

entire respirometry set-up was screened off from the rest of the experimental room using a curtain to prevent external stimuli.

An external flush pump was used to deliver clean seawater (temperature (T) = 28 °C; pressure (P) = ~ 1 bar; salinity (S) = 33; dissolved oxygen (DO) = ~ 6.1 mg ml^{-1}) to the respirometer in between measurement periods. Oxygen uptake rates (mg O_2 ml^{-1}) and temperature (°C) were simultaneously measured using probes (oxygen probe: OXROB3 Robust Oxygen Probe, PyroScience, Aachen Germany; Temperature sensor: TSUB36 Shielded submersible temperature sensor, Pyroscience, Aachen, Germany). Oxygen probes were calibrated to 100% air saturation using fully aerated seawater (T = 28 °C, S = 33, P = 1 bar, DO = 6.1 mg ml^{-1}), and to 0% oxygen (T = 28 °C, S = 33, P = 1 bar, DO = 0 mg ml^{-1}) using sodium sulphite (Na_2SO_3; UNIVAR Analytical Reagent, Ajax Finechem, New South Wales, Australia). Oxygen and temperature probes were connected to a Firesting oxygen meter (4-channel optical oxygen meter, Pyroscience, Aachen Germany), which constantly measured both of these variables throughout each experiment. Prior to any swimming experiment, all components of the swimming respirometer were washed in a 10% bleach solution to eliminate any microbial activity from the system and then rinsed with clean seawater.

Prior to each individual experiment, background respiration (i.e. microbial oxygen consumption) was measured for 10 min. To reduce microbial activity, the seawater used for all experiments was filtered using an ultra-violet filter (Blagdon Pro 24W ultra-violet clarifier, Dreative Pumps, South Australia, Australia). Upon completion of their respective endurance tests, focal fish were removed from the respirometer, euthanized in an ice bath, and a subsequent reading for any accumulated microbial activity in the chamber was performed for a final 10 min.

Endurance test experimental protocol

The swimming endurance tests followed a similar protocol as described in Downie et al. (2021b). Larval ($n = 9$) and juvenile ($n = 9$) fish were tested using the same experimental protocol (except for swimming speed; see *'Selection of swimming protocol'* Section for details) and swum, individually, in the same swimming respirometer. Larvae were swum upon capture during the night and finished early in the morning (22:00 to 07:00). For comparison, juveniles were swum within 24 h of capture (at 12:00 and 17:00). At this stage, juvenile fish had changed from light-transparent orange (i.e. larva) to red in colour, and their dorsal spine had reduced in length (McCormick et al. 2002). Individual fish were placed in the swimming chamber and allowed to habituate for 1 h at a velocity of 1 body length (BL) s^{-1} (approximately 1.7 cm s^{-1}). Upon completion of the 1 h habituation period, the individual swum at a constant, fixed speed of either 13.5 cm s^{-1} (approximately 8 BL s^{-1}; larva) or 10 cm s^{-1} (approximately 6 BL s^{-1}; juveniles). Oxygen uptake rates ($\dot{M}O_2$; mg O_2 s^{-1}) were measured throughout the swimming test using intermittent-flow respirometry, which included repeated cycles consisting of a 20 min measurement period followed by a 3 min flush period to replenish levels to 100% air saturation (DO = 6.1 mg L^{-1}; Rummer et al. 2016). Oxygen within the swimming chamber was never allowed to drop below 90% air saturation to prevent oxygen uptake rates of the fish being influenced by hypoxia (Rummer et al. 2016). The test ended if either: i) the fish rested on the downstream barrier (i.e. fatigue) or ii) the fish swam for 200 min, at which it is assumed the fish could swim at that speed indefinitely (Brett 1967). Upon test ending the fish was removed from the chamber and processed for morphology and muscle histology (see *'Morphometrics and Preparation for Histology'* Section below). Text files from the Firesting were imported and analysed in LabChart (ver 8, AD instruments, New South Wales, Australia) to calculate the slope at each 20 min interval. Oxygen uptake rates ($\dot{M}O_2$) at each 20 min interval were calculated as: $\dot{M}O_2\left(mg\,O_2 g^{-1} h^{-1}\right) = SV_{resp}M^{-1}$, where S is slope of the linear regression during the measurement period (mg O_2 s^{-1}), V_{resp} is the volume of the respirometer (minus the fish), and M is the mass of the fish (g) (Rummer et al. 2016). Details of respirometry set-up and protocol can be found in Supplementary Materials following the 'respirometry checklist' outlined by Killen et al (2021).

Morphometrics and preparation for histology

The acute swimming trials (200 min duration) were not expected to significantly change mitochondria volume densities in muscle tissues, and therefore for consistency, all swum individuals were used for measuring morphometrics and muscle histology of swimming tissues. After swimming, the fish was removed from the chamber and euthanized in an ice bath. Immediately after euthanasia, fish were measured for total length (i.e. tip of snout to tip of caudal fin), blotted dry and weighed (g) using a scale (Kern ABJ-NM/ABS-N, Kern, Balingen, Germany). Fish were then placed in 1 ml vials containing glutaraldehyde in a phosphate buffer (2% glutaraldehyde and 2% paraformaldehyde, phosphate buffer pH = 7.4, ProSciTech, Townsville, Australia) for tissue fixation (Hernández et al. 2016). After 24 h, a thin layer of red muscle from the left and right sides of the trunk musculature of the fish was isolated under a dissecting microscope. Pectoral fin adductor muscle groups were also removed from both pectoral fins of the fish (Hernández et al. 2016). Both muscle tissue types are key for providing thrust, and for further analysis, tissue samples were placed in a fresh vial of glutaraldehyde and phosphate buffer for tissue fixation.

Although light trap caught fish were randomly assigned to larval or juvenile groups, there were slight differences in mass between life stages. Fish were not measured before each trial, as this would have greatly stressed the individuals. A condition factor (Fulton's K) was calculated to examine whether the differences in mass were due to poor condition while fish were held in captivity. Fulton's K was calculated as: $K = \left(M/L^3\right) * 100$, where K is Fulton's K, M is mass (g) and L is length (cm) (Nash et al. 2006). However, it is important to note that shrinkage in body size has been found in other reef fish species during settlement and metamorphosis (McCormick et al. 2002).

Quantification of mitochondria volume density (V_{mt})

Transmission electron microscopy (TEM) was performed at the Advanced Analytical Centre at James Cook University (Cairns, Queensland, Australia). After fixation, muscle tissue samples were rinsed several times in fresh phosphate buffer with no fixative before being post-fixed for one hour in 1% aqueous osmium tetroxide solution. Specimens were rinsed with deionized (DI) water before being *en bloc* stained with 2% aqueous uranyl acetate for 1-2 h at room temperature in the dark. Specimens were then rinsed with DI water and dehydrated with a graded ethanol series (50, 70, 80, 90, and 100%). At the 100% stage, the ethanol solution was changed three times and left for 15 min each time. The specimens were then transferred to a 2:1 solution of 100% ethanol and Epon resin for 15 min, followed by a 1:1 solution of 100% ethanol and resin, and then by a 2:1 solution of Epon and 100% ethanol. The specimen was then submerged in three fresh changes of 100% resin for 15 min each before being left on a rotator in fresh 100% resin overnight. The following morning the samples were embedded into silicon moulds with fresh resin and polymerized in an oven at 60 °C for 24 h. Resin blocks were then cut on an ultramicrotome (Leica UC7 Ultramicrotome, Leica biosystems, Nussloch, Germany). Thick sections (approximately 1 µm) were first cut with glass knives, placed on slides, and stained with toluidine blue to assess orientation and presence/absence of areas of interest. Thin sections (50–70 nm) were then cut with a diamond knife and transferred to copper TEM grids. Sections on grids were then post stained with uranyl acetate and lead citrate to increase contrast before viewing (1500 × zoom) on a transmission electron microscope (Hitachi 7800 Transmission electron microscope, Hitachi High-Tech Global) and photographed (See Fig. 2 for an example of a micrograph). For each tissue sample, 10 micrographs were taken.

Micrographs of mitochondria were uploaded into ImageJ (ver 1.53). A grid was superimposed onto each micrograph. Grid calibration was performed to determine the proper grid line spacing size to count mitochondria, by placing grids with increasing sized spaces between lines. The largest grid size (ImageJ grid size setting = 105 pixels^2; total number of intersecting points on grid = 3888) that yielded the same number of mitochondria as grid spacing size decreased was selected. A point count method to quantify mitochondria for each micrograph was used (Weibel 1979). For each micrograph, any points that were counted outside of the cell membrane were subtracted from the total number of points (3888) to get the total number of points on the micrograph (P_{total}). The number of intersecting points that landed on mitochondria in the micrograph were counted (P_{mt}). Muscle mitochondria volume (V_{mt}) was calculated using the following formula (Weibel 1979):

$$V_{mt} = \left(P_{mt}/P_{total}\right) * 100$$

The V_{mt} from each of the micrographs were averaged together to calculate a representative mean V_{mt} for each tissue (Weibel 1979).

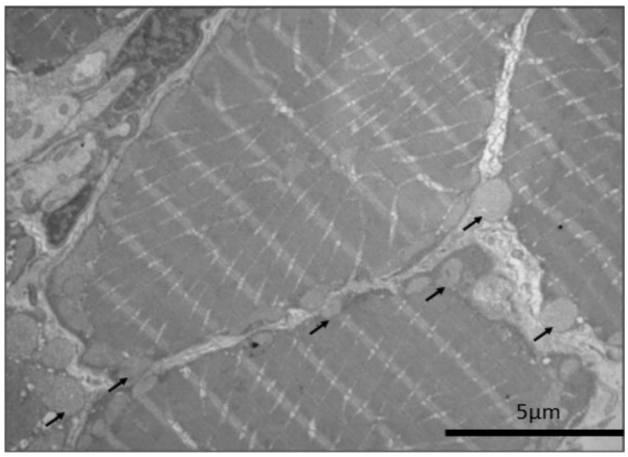

Fig. 2 Electron Microscope micrograph (1500 × zoom) of pectoral muscle of a larval leopard grouper (*Plectropomus leopardus*). Individual mitochondria are indicated by arrows

Metabolic enzymes

A sub-sample of larvae and juvenile coral grouper that were not swum were used for enzyme analyses. These individuals were not swum, as swimming trials could impact the activity of metabolic enzymes (e.g. lactate dehydrogenase if fish exhibited any burst swimming during the experiments). The maximum activity of two metabolic enzymes, citrate synthase (CS) from the aerobic citric acid cycle and lactate dehydrogenase (LDH) from the anaerobic glycolytic pathway, was determined in spectrophotometric assays, as described in Illing et al. (2020). Briefly, individually-frozen fish were freeze-dried (Christ Alpha 1–2 LDplus, 0.2 mbar, 24 h) and weighed (Mettler Toledo UMX2

Ultra-Microbalance, 0.1 µg readability), and whole-body homogenates created in a FastPrep ®-24 homogenizer (MP Biomedicals, Santa Ana, USA) using an ice-cold buffer containing 50 mM HEPES (pH 7.4 at room temperature), 5 mM EDTA, 0.1% Triton X-100, and 0.2 mM DTT (Dithiothreitol). The buffer volume was adjusted to the individuals' dry mass, and a 9 × dilution factor was applied for individuals > 15 mg dry weight. After centrifugation (400 g for 20 min at 4 °C; 5810 R centrifuge, Eppendorf, Hamburg, Germany), the supernatant was retrieved for both enzyme analyses, and individual protein content (mg ml^{-1}) was determined spectrophotometrically at 37 °C, using a Bicinchoninic Acid Protein Assay kit (BCA1, Sigma-Aldrich, St. Louis, USA). CS and LDH activities were assayed spectrophotometrically at 25 °C (SpectraMax® Plus384 Microplate Reader, Molecular Devices, San Jose, USA). Intra-assay variation (CV) was < 5%, and enzyme activities were expressed as µmol of substrate converted to product per minute per protein content (mg ml^{-1}). For further assay-specific CS and LDH steps, please see Illing et al. (2020). The valid measurements for larval CS and LDH were $n = 4$ for each, and juvenile CS and LDH were $n = 7$ and $n = 5$, respectively.

Statistical analyses

All analyses were performed in the R statistical and graphical environment (v. 4.2.0) (R Development Core Team 2021). Data were analysed with Bayesian statistics using package 'brms' (v.2.16.3) (Bürkner et al. 2021). All candidate models were run with three chains, 3000 iterations, a warm-up phase of 1000, and default, non-informative priors. Model selection was based on leave-one-out cross-validation techniques supplied by the 'loo' package (v.2.5.1) (Vehtari et al. 2022). Condition factor (Fulton's K), citrate synthase (CS) activity, and lactate dehydrogenase (LDH) activity were best explained by models using Gamma (link = 'identity') families and 'developmental stage' (either larva or juvenile) as categorical explanatory variable. Body mass was explained best by a Gaussian family model with 'length' as a continuous and 'developmental stage' as a categorical variable (no interaction). Mass-standardized oxygen uptake rate ($\dot{M}O_2$) was best explained using a 'lognormal' family distribution, with 'developmental stage' as categorical explanatory variable and Fish ID, nested in 'developmental stage' as random effect since multiple measurements were made on the same individual (i.e. $\dot{M}O_2$ measurements every 20 min for 200 min). The non-standardized oxygen uptake model used a' Gaussian family distribution, had 'mass' as another continuous variable included (interaction with 'developmental stage'), with Fish_ID nested in 'developmental stage' as a random effect. Mitochondrial volume density (V_{mt}) was best explained by a model that used a Gamma (link = 'identity') family, and 'developmental stage' and 'muscle tissue type' as categorical variables. All models were validated using MCMC diagnostics from package 'bayesplot' (v.1.9.0) (Gabry et al. 2022), and inspecting residuals using package 'DHARMa' (v.0.4.5) (Hartig and Lohse 2022). *Post hoc* comparisons were made using the 'emmeans' function in package 'emmeans' (Lenth et al. 2022). Predictions from the best fitting models were compiled with package 'tidybayes' (v.3.0.2) (Kay and Mastny 2022) and visualized with the packages 'ggplot2' (v.3.3.5) (Wickham et al. 2021), 'ggdist' (Kay and Wiernik 2023), and 'cowplot' (v.1.1.1) (Wilke 2020).

Results

Fish were in similar body condition (see Fig. 3A), with no difference found in Fulton's K between groups of larval (2.76 [2.51, 3.09]) and juvenile (2.56 [2.31, 2.84]) fish (all Bayesian posterior distributions provided as medians and 95% lower and upper highest posterior density intervals (HPDI) [$HPDI_{low}$, $HPDI_{high}$]). However, there was strong evidence (i.e. *post hoc* contrasts not intersecting with zero) for the tested larval fish to be heavier and taller than the juvenile fish (Fig. 3B) with a greater mass of 0.023 and 0.01 g per mm length, respectively (*post hoc* contrast 0.022 [0.038, 0.007]. The selected regression model explained 75 [52, 80] % of the observed variability in the mass-length relationship (Bayes_R2, median and lower and upper quantile intervals (QI) [QI_{low}, QI_{high}] reported).

The 200-min endurance swimming tests were successfully completed by all fish, regardless of life stage (~ 8 BL s^{-1} for larvae and ~ 6 BL s^{-1} for juveniles). We found strong evidence for differences in mass-standardized oxygen uptake rates ($\dot{M}O_2$) of larval (1.15 [1.03, 1.30] mg O_2 h^{-1} g^{-1}) and juvenile (0.87 [0.78, 0.98] mgO_2 h^{-1} g^{-1}) fish, with larval $\dot{M}O_2$ being 0.28 [0.14,0.43] units higher (all posterior distributions as medians and 95% [HPD_{low}, HPD_{high}]) (see Fig. 4A). When standardized for mass, $\dot{M}O_2$ was 75.5% higher in larvae than juveniles. Comparing the non-standardized $\dot{M}O_2$, we also found strong evidence for $\dot{M}O_2$ to be higher in larval than in juvenile coral grouper (0.049 [0.019, 0.080] (Fig. 4B). $\dot{M}O_2$ non-standardized for mass was 67% higher in larvae than juveniles. In addition, the observed trends were different, with larval and juvenile oxygen uptake changing with 1.56 [0.02, 3.01] and − 0.06 [− 1.25, 1.14] mg O_2 h^{-1} g^{-1} body mass, respectively. The selected regression models explained 42 [13, 59] % (mass-standardized $\dot{M}O_2$) and 78 [62, 84] % (non-standardized $\dot{M}O_2$) of the observed variability, respectively (Bayes_R2, median and QI_{low} and QI_{high} reported).

Mitochondrial volume density (V_{mt}) did not significantly differ between life stages or muscle types; although, weak evidence existed for larvae to have higher V_{mt} in trunk than

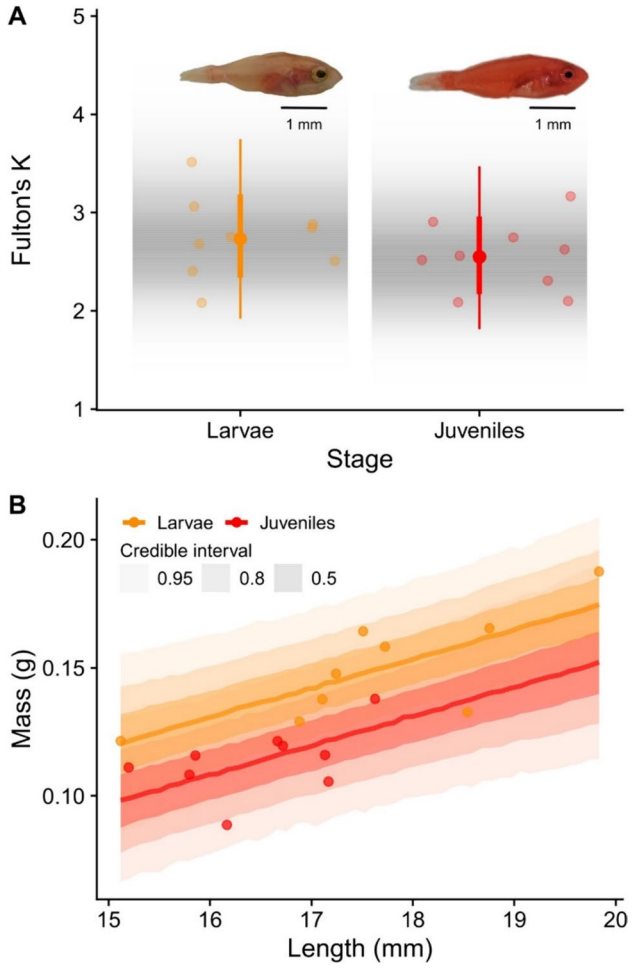

Fig. 3 **A** Fulton's body condition factor, and **B** mass to length relationship of larval ($n=9$) and 24 h-settled juvenile ($n=9$) common coral grouper (*Plectropomus leopardus*). 'Larvae' were captured from light traps moored off the Lizard Island fringing reef, and 'juveniles', while also light trap captured, were allowed to remain for 24 h post-capture in the laboratory under a natural light regime. Metamorphosis included a rapid change in coloration (i.e. from light transparent orange to red). Predicted Bayesian posterior distributions are shown with **A** symbols and error bars representing the median and 0.8 and 0.95 quantile-based credible intervals, and **B** the regression line representing the median and the coloured areas the 0.5, 0.8, and 0.95 quantile-based credible intervals, respectively. Semi-transparent circles show original data

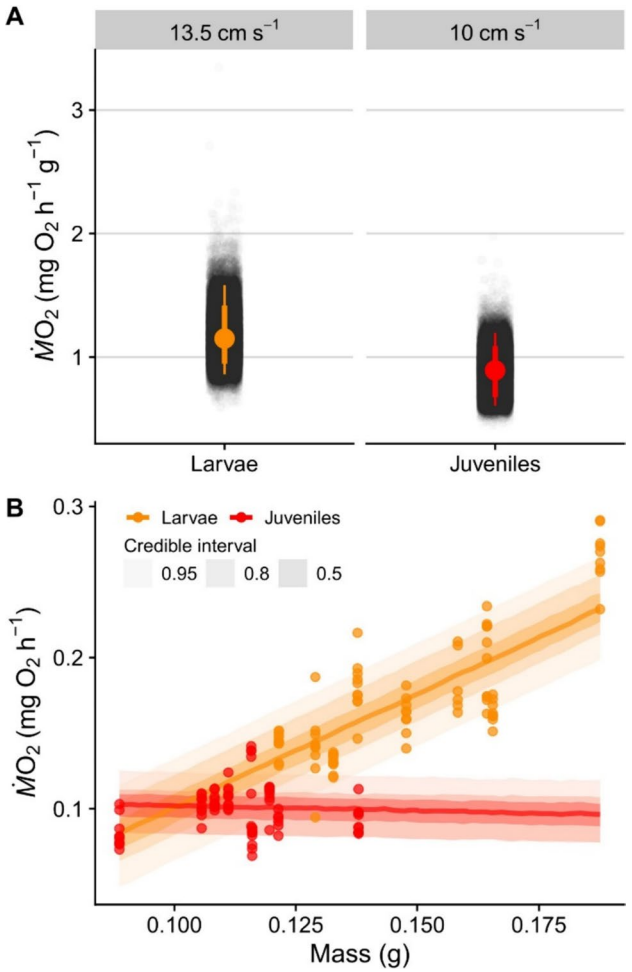

Fig. 4 **A** Mass-standardized, and **B** non-standardized oxygen uptake rates ($\dot{M}O_2$) during swimming for larval ($n=9$) and juvenile ($n=9$) common coral grouper (*Plectropomus leopardus*). $\dot{M}O_2$ was measured using endurance swimming tests (200 min duration) at velocities corresponding to natural flow regimes during each life stage. Larvae (swum at 13.5 cm^{-1}) and juveniles (swum at 10 cm^{-1}) were tested at flow velocities representing average current speeds around Lizard Island and at depth of settlement, respectively. Predicted Bayesian posterior distributions are shown with **A** symbols and error bars representing the median and 0.8 and 0.95 quantile-based credible intervals, and **B** regression lines representing the medians and the coloured areas the 0.5, 0.8, and 0.95 quantile-based credible intervals, respectively. Semi-transparent circles show original data collected for each tested fish every 20 min during the trials

juveniles (49.3% higher; Fig. 5A). Larvae had 8.92 [5.69, 12.97] and 5.82 [3.43, 9.14] % V_{mt} in their pectoral and trunk muscle tissues, respectively (Fig. 5A). Juvenile coral grouper exhibited 5.83 [2.68, 10.09] and 2.87 [1.68, 4.57] % V_{mt} in their pectoral and trunk muscle tissues, respectively (Fig. 5A). The only strong evidence for differences, found during *post hoc* comparisons, was between juvenile trunk and larval pectoral muscle tissues, with the latter showing about 6% more V_{mt} (5.98 [1.96, 10.43]) (all posterior distributions reported as medians and 95% [HPD_{low}, HPD_{high}]) (Fig. 5A). The selected V_{mt} regression model data explained 19 [4, 40] % of the observed variability (Bayes_R2, median and QI_{low} and QI_{high} reported).

Citrate synthase (CS) activity (in μ mol min^{-1} mg $protein^{-1}$) was 3.7 times higher in larval (2.74 [1.18, 6.14]) than in juvenile fish (0.74 [0.37, 1.38]), with strong evidence from a *post hoc* comparison (1.96 [0.07, 5.54]) supporting this trend (posterior distributions reported as medians and 95% [HPD_{low}, HPD_{high}]) (see Fig. 5B). The model with the best fit for the CS activity data explained 59 [6, 67] % of the observed variability (Bayes_R2, median and QI_{low} and QI_{high}

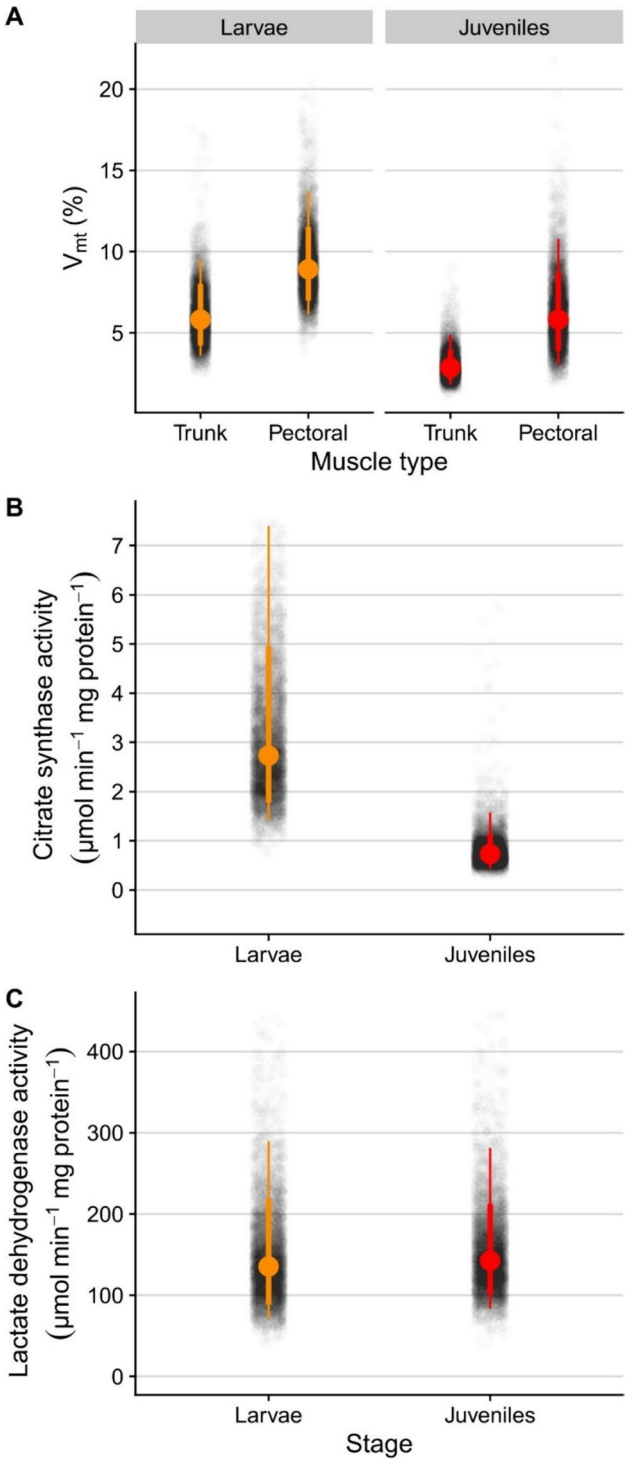

Fig. 5 **A** Mitochondrial volume density (V_{mt}), **B** citrate synthase activity (CS), and **C** lactate dehydrogenase activity (LDH) for larval and juvenile common coral grouper (*Plectropomus leopardus*). While V_{mt} was examined in trunk and pectoral muscle tissues from individuals ($n=8$) that underwent endurance swimming trials, citrate synthase and lactase dehydrogenase activities were tested with individuals ($n=11$ and 9, respectively) specifically collected for assessing metabolic enzyme baseline activity levels. In all three panels, predicted Bayesian posterior distributions are shown with symbols and error bars representing the median and 0.8 and 0.95 quantile-based credible intervals

reported). With regard to lactate dehydrogenase (LDH, in µmol min^{-1} mg protein^{-1}), measured activities were very similar for larval (135 [56.5, 257]) and juvenile (142 [70.1, 249]) coral grouper, with no evidence for differences in LDH activity across both stages (all posterior distributions as medians and 95% [HPD_{low}, HPD_{high}]) (see Fig. 5C). Due to high variability within the LDH data, the best model could still only explain 9 [0, 41] % of the observed variability (Bayes_R2, median and QI_{low} and QI_{high} provided).

Discussion

The present study addresses critical knowledge gaps relating to changes in energy demand during the pelagic to reef transition for coral grouper. Through the experimental replication of water flow conditions characteristic of the pelagic and reef environments experienced by larvae and juveniles, our findings yield a precise depiction of the oxygen requirements faced by early life stages of the common coral grouper during their transition from pelagic to reef habitats, crucial for successful recruitment (Downie et al. 2020, 2021a). These are among some of the highest $\dot{M}O_2$ estimates obtained by a fish prior to and during settlement. Additionally, common coral grouper juveniles exhibit a significant decrease in whole-body citrate synthase activity during metamorphosis, predictably reflecting a less-active lifestyle than pelagic larvae. Additionally, we found evidence for a decreasing trend (albeit not statistically significant) in muscle mitochondria within 24 h of settlement. These findings highlight some of the physiological changes that characterize metamorphosis within the first 24 h for *P. leopardus* larvae settling onto the reef. Moreover, these fine-scale changes in aerobic properties of muscles may help support successful pelagic to reef transition for coral grouper.

Our approach of swimming larvae and juveniles at constant speeds (i.e. endurance test) to simulate the hydrodynamic environment that each life stage experiences in its natural habitat provides an accurate representation of oxygen demands that support swimming during recruitment and metamorphosis (Downie et al. 2020). Stepped velocity tests (e.g. critical swimming speed tests; U_{crit}) are the most popular method for swimming fishes, but their ecological relevance is debatable, as fishes likely do not swim at maximum aerobic speeds in situ (Downie and Kieffer 2017; Downie et al. 2020; Illing et al. 2021). Swimming fish at speeds that simulate their natural habitat better represents their daily metabolic demands, but these types of experiments are seldom performed due to the long duration and because habitat relevant flow profiles may not be available. While we found that larvae had higher oxygen uptake rates (both mass-specific and non-mass-specific) than juveniles, we acknowledge that the changes we found between life stages are directly

related to the different speeds at which the two life stages were swum. Differences may also be due to body mass differences between stages, but the larvae and juveniles were in similar condition (as per Fulton's K) and were within the size ranges for these stages. It is possible that differences in mass between larvae and juveniles may be associated with metamorphosis. For example, six reef fish species (*Acanthurus triostegus*, *Acanthurus xanthopterus*, *Chrysiptera leucopoma*, *Pomacentrus nagasakiensis*, Apogonid sp., and *Sargocentron rubrum*) were found to 'shrink' in total length (by up to 5%) during metamorphosis despite being fed ad libitum, which was predicted to be due to re-allocation of energy during development, post-settlement (McCormick et al. 2002). While our results cannot state whether changes in oxygen uptake rates were due to metamorphosis, other studies have found that metamorphosis has been associated with decreases in oxygen uptake rates for other fish species. For example, decreases in oxygen uptake rates were documented in metamorphosing eel leptocephali (Bishop and Torres 1999), red sea bream (*Pagrus major*; Ishibashi et al. 2005), and flatfishes (Laurence 1975; Cunha et al. 2007). Such changes have been associated with increasing mass of anaerobically-driven white muscle relative to other more-metabolically active tissues (e.g. brain, viscera) as growth persists beyond metamorphosis as well as decreased locomotion associated with a benthic or demersal lifestyles. Given that juvenile coral grouper will develop large amounts of white muscle characterizing increased body mass and a less-active, demersal, ambush predator lifestyle, oxygen demands will predictably change well-beyond the 24 h metamorphosis window as well.

We found a decreasing trend in V_{mt} as fish transitioned from larvae to juveniles, but changes were not statistically significant. Some fish species have been noted to decrease or re-arrange muscle mitochondria densities after transitioning from pelagic to benthic/demersal habitats. For example, the Antarctic icefish, *Notothenia neglecta*, is very active in the water column during its pelagic larval stage, but upon transitioning to benthic habitats as a juvenile, this species exhibits a decrease in muscle mitochondria volume density, as they become less active and experience lower flow regimes (Johnston and Camm 1987). Atlantic cod (*Gadus morhua*) have also been found to change their red-muscle mitochondria density as they transition from pelagic to benthopelagic habitats (Personal communication in Finn et al. 2002). These studies were comparing the muscle structures of recently settled/settling juveniles with adults that have been established on the benthos but did not investigate the immediate changes associated with metamorphosis, as in the current study. In contrast, we did not detect strong changes (despite a decreasing trend) in muscle mitochondria between larvae and juveniles, or within muscle tissue groups (i.e. trunk or pectoral muscles) immediately upon settlement, but this may be due to the fine-scale approach of the histological techniques using a low sample size. The low sample size may have an impact here, as all larvae will not be at the exact same developmental competence upon settlement, and so the speed at which the transition from settlement and 24 h post-settlement is expected to be variable among individuals. Additionally, the time differences for when experiments were executed between individuals of the same stage captured within the same sampling session could elicit some differences in physiological performance; however, small sample sizes due to limited animals may mask any measurable differences. Regardless, as coral grouper continue to develop and grow, it would be expected for mitochondrial volumes to change in the specific muscles we measured (e.g. pectoral muscles) as they become more accustomed to demersal reef life.

Whole-body citrate synthase activity, which is an indicator of mitochondrial density in skeletal muscle (Vigelsø et al. 2014), decreased by 73% in juvenile coral grouper immediately upon completing metamorphosis. Therefore, mitochondrial densities may be decreasing in other tissues besides swimming muscle tissues, or the enzyme assay utilizing the whole animal detects changes in mitochondrial densities better than the fine-scale histological method. In contrast, for the false percula clownfish (*Amphiprion melanopus*), CS activity is lowest during embryonic stages and then increases during pelagic larvae and settled juvenile stages (Paul and Kunzmann 2019), suggesting differences in aerobic metabolism usage between clownfishes and coral grouper during early life history (described below). There are other examples of decreased CS activity during metamorphosis, which include lamprey (*Petromyzon marinus*; Kao et al. 1998) and whitefish (*Coregonus sp.*; Forstner et al. 1983). Additionally, lower CS may indicate preparations for hypoxic conditions (Zhou et al. 2000), which characterize the coral microhabitats newly settled coral grouper hide amongst, due to coral and plant respiration at night (Nilsson et al 2007), and may coincide with switching in Hb isoforms related to hypoxia tolerance found in clownfish (Downie et al. 2023).

We initially hypothesized that LDH activities would increase upon settlement to coincide with shifts from pelagic swimmer to reef ambush predator. Recent studies investigating changes in the transcriptome of metamorphosing reef fish larvae have found significant shifts in aerobic and anaerobic metabolic pathways associated with ecological niche and lifestyle. For example, genes regulating glycolysis and LDH to generate energy from glucose-based anaerobic metabolism are highly expressed in *A. ocellaris* larvae but decrease during metamorphosis, representing a switch to acquiring energy from aerobic metabolism through the citric acid cycle and β-oxidation of fatty acids (Roux et al. 2023). In contrast, for the Malabar grouper (*Epinephelus*

malabaricus), an ambush predator similar to *P. leopardus*, genes involved in the citric acid cycle are highly expressed as larvae, and then switch to anaerobic energy production via glycolysis and lactate formation beyond metamorphosis (Huerlimann et al. 2024). While we did not take a transcriptomics approach, a similar shift in metabolism may be occuring in *P. leopardus*, as made evident by a decrease in CS activity which may signify a shift to acquiring energy via anaerobic metabolism, supporting the development of ambush predation. While it is a good biomarker, changes in anaerobic metabolism may not solely be indicated by LDH activity, although ontogenetic changes in LDH activity appears to be highly species and life-stage specific. For example, in Japanese flounder (*P. olivaceus*), LDH activity increases during the larval phase, peaks during metamorphosis, and then decreases in juveniles (Overnell and Batty 2000; Ishibashi et al. 2007). A similar pattern has been observed in *A. ocellaris*, as LDH is low during embryonic and pelagic larval phases, increases before settlement, and then decreases as they metamorphose (Paul and Kunzman 2019). In contrast, LDH activity increases across ontogeny for the benthic plaice (*Pleuronectes platessa*) and pelagic herring (*Clupea harengus*) (Overnell and Batty 2000). Paul and Kunzman (2019) suggest that LDH activity decreases upon metamorphosis because burst swimming is not important for reef life as it would be for pelagic larvae swimming against currents. The elite swimming capabilities of larval coral reef fishes are highly aerobically driven (e.g. Fisher et al. 2005; Downie et al. 2023), but burst swimming may contribute to swimming against unexpected changes in currents. LDH may not have changed immediately following settlement, as within a few days of settlement coral grouper larvae seek out concealed microhabitats (e.g. rubble and holes to hide in) during the first few months of reef life and may not experience the same predation pressures as other coral reef fishes. Therefore, LDH activity may continue to change as coral grouper habituate to reef life, potentially increasing in tandem with the expansion of white muscle mass beyond the 24-h period linked with metamorphosis.

Our results highlight that the 'barrier to entry' to coral reefs does require some rapid physiological changes during metamorphosis for newly recruiting and settling reef fishes. Specifically, the decreases in CS activity indicate that coral grouper larvae must make quick changes to how energy is distributed to their tissues, in keeping with their ambush predator lifestyle versus high aerobically-driven swimming. Our oxygen uptake rate measurements provide an estimate as to the energy demands for swimming at each life stage and as fish transition from pelagic to reef life. Our swimming methodology offers potential avenues for advanced research, aiming to precisely account for the oxygen requirements of fish species in ecologically relevant contexts. As environmental changes bear down on global coastal ecosystems, notably coral reefs, incoming reef fishes must navigate these challenges. Factors like ocean warming and deoxygenation will have direct repercussions on metabolic processes, given their sensitivities to external environmental factors. It is possible that the metabolic changes experienced during settlement and metamorphosis may make settlement-stage reef fishes more susceptible or sensitive to sub-optimal environmental conditions. Taken together, the complexities of metamorphosis, which contribute to successful transitions from pelagic to reef environments, involve immediate physiological changes that support swimming and predict the ecological niche occupied by demersal reef fishes. Understanding these complexities and physiological changes offers a comprehensive perspective on the significance of metamorphosis in the life history of coral reef fish and how pelagic larvae are crucial contributors to future reef fish populations.

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Author contributions ATD and MIM designed the experiment and collected the fish. ATD and CMP executed swimming trials. ATD and BI undertook biochemical and statistical analyses. Histology was performed by JW. Funding was provided by MIM, ATD, and JLR. All authors contributed to writing the manuscript. All authors agreed on the final draft of the manuscript.

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Data availability Data (i.e. datasets, metadata and R code for statistical analyses and creation of figures) used for this research is openly available via the Open Science Framework (Downie et al. 2024/https://doi.org/10.17605/OSF.IO/23C5F).

Declarations

Competing interests We declare no competing interests.

Ethics statement Procedures used on these animals were assessed and approved by James Cook University Animal Ethics Committee (approval numbers A2425, A2408).

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