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Metabolic activity throughout early development of dusky kob *Argyrosomus japonicus* (Sciaenidae)

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The physiology of fishes in the early stages of development remains poorly assessed despite the importance of identifying energy bottlenecks in organisms faced with changing environmental conditions. This study describes the metabolic activity of dusky kob *Argyrosomus japonicus* throughout its early development, from hatchling to settlement stage. Standard, routine and active metabolic rates (SMR, RMR and AMR, respectively) were assessed to determine the species' metabolic scope and identify how metabolism changes with growth and development. Distinct metabolic changes occurred in association with developmental changes during the early life stages, with flexion-stage larvae showing significantly reduced metabolic scope (approx. 0.30 µmol O_2 ind.$^{-1}$ h^{-1}), representing an energy bottleneck. Based on these findings, it is likely that larvae of *A. japonicus* are most susceptible to environmental perturbations during flexion. The variability of metabolic rates during the diel cycle was also assessed and revealed that the early-stage larvae showed no preference for daylight, although settlement-stage juveniles were more active during daylight hours (RMR = 12.78 µmol O_2 ind.$^{-1}$ h^{-1}) than at night (RMR = 5.87 µmol O_2 ind.$^{-1}$ h^{-1}). These results suggest that metabolic measurements of the SMR of *A. japonicus* larvae can be taken at any time of the diel cycle until the settlement phase, when readings should take place at night.

Keywords: diurnal fluctuation, energetics, marine fish larvae, mass-specific metabolism, measurement protocols, oxygen consumption, physiology, respirometry

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Introduction

Metabolism quantifies the energy-consuming activities of an organism (Nelson 2016) and is used as an indication of how organisms partition energy resources to activities that allow them to survive, grow and reproduce (Post and Lee 1996). The metabolic profile, which is a composition of the various metabolic rates of an individual, therefore gives an indication of the efficiency of energy transformation and allocation (Fry 1971; Brown et al. 2004). McKenzie et al. (2016) suggested that an organism's physiology contributes towards its ability to survive under specific environmental conditions. As a result, physiological condition can be a reflection of the performance and fitness of an organism (Pörtner 2010). When combined with information on changing environmental conditions, physiological information can provide insight into species- and community-level responses (Pörtner and Farrell 2008). These kinds of data have served numerous ecological applications, including resource management, conservation (McKenzie et al. 2016) and climate-change assessments (Pörtner and Farrell 2008).

The measurement of oxygen consumption rate via respirometry experiments, a form of indirect calorimetry used to measure aerobic metabolism, is a commonly used method to describe the metabolic physiology of fishes (Cech and Brauner 2011). Various types of metabolic rates can be determined using respirometry, which for this study included the standard metabolic rate (SMR), routine metabolic rate (RMR) and active metabolic rate

(AMR) (Fry 1971). Each of these parameters is related to the metabolic activity of the organism under specific conditions during oxygen consumption measurements. For the purposes of this study, these are explained with reference to fish, as similarly described by Fry (1971). The SMR refers to the metabolic rate of a post-absorptive, acclimated, undisturbed, resting individual during the period of its circadian rhythm when it shows the lowest oxygen consumption rate. This measurement translates to the minimum amount of energy required for survival (Nelson and Chabot 2011) and includes energetic processes, such as biosynthesis of macromolecules, ion transport across membranes (e.g. osmoregulation) and other internal, life-sustaining processes, which are independent of growth, activity or reproduction. Normal physiological functioning is impaired below this metabolic rate (Chabot et al. 2016). The RMR is the mean metabolic rate of a fish in a resting state but exhibiting random swimming activity, and AMR is the metabolic rate of an active fish swimming at a sustained, constant speed.

Metabolic scope (hereafter referred to as MS) is calculated as the difference between AMR and SMR (Fry 1971) and represents the amount of energy available to the individual for activities over and above the energy required to maintain survival-related processes. This energy is apportioned to activities such as growth, feeding, reproduction and behavioural activities (Priede 1985), and ultimately

determines the survival and competitive ability of organisms and their ability to respond to changes in their environment (Killen et al. 2007; Clark et al. 2013). Due to this fundamental interrelatedness between physiology and fitness, understanding the physiology of an organism is essential in determining the link between physiology and ecological processes (Killen et al. 2010; Clark et al. 2013), such as life-history strategy, distribution, habitat use and behaviour.

Fishes show endogenous fluctuations in metabolic rate over the diel cycle and this generally reflects diurnal or nocturnal activity patterns (Marais 1978; Du Preez et al. 1986; Deacon and Hecht 1996). Therefore, the oxygen consumption of an individual may vary over a 24-hour diel cycle, and it is important to determine the optimal time for measuring the baseline metabolic rates of SMR and AMR to avoid overestimating these values.

The larval stages of fishes are usually subject to high mortality and this can be partially attributed to a narrow MS in the early stages of development (Killen et al. 2007). Metabolic scope is therefore a useful measure to identify potential survival bottlenecks during ontogenetic development, as the life stages with the lowest MS are likely to be the most vulnerable to unfavourable conditions because of reduced energetic capacity for adaptation (physiological or behavioural). In the case of larvae, a physiological approach is useful when determining constraints limiting recruitment into adult populations, particularly in environments that are predicted to change in the future (Killen et al. 2007).

Metabolic information on dusky kob *Argyrosomus japonicus*, particularly for the early life stages, remains limited (Fitzgibbon et al. 2007; Pirozzi and Booth 2009). *Argyrosomus japonicus* is an estuarine-dependent sciaenid that occurs in nearshore coastal waters and estuaries of the Indian and eastern Pacific oceans (Griffiths 1996; Fitzgibbon et al. 2007). It is a popular angling species in areas throughout its distributional range (Lenanton and Potter 1987; Gray and McDonall 1993) as well as an important aquaculture species (Silberschneider and Gray 2008). There has been no previous assessment of metabolic rates in the larval stages or of the fluctuations in metabolic rate during the diurnal cycle for this species. Such metabolic information will be useful for future studies by providing a reference to which time of day metabolic measurements should be taken (Kandjou and Kaiser 2014).

The aims of this study were to determine the baseline metabolic rates and metabolic activity during the early developmental stages of *A. japonicus*. An additional aim was to determine the 24-hour fluctuations in oxygen consumption of this species related to the diel cycle.

Materials and methods

Experimental animals
Experimental fish were obtained from a single, induced spawning event from broodstock (F1 generation) at the PureOcean aquaculture facility in East London, South Africa. Fertilised eggs were first hatched in darkness in three hatching cones, after which the hatched larvae were moved to a single 8 000-l cylindrical grow-out tank with a stocking density of 30 larvae l⁻¹. All individuals were reared

using a standard aquaculture protocol, where temperature (24 °C, range 23–25 °C), salinity (35), pH (8.15, range 7.65–8.65) and dissolved oxygen (8.3 mg l⁻¹, range 7.3–9.3 mg l⁻¹) were maintained at optimal levels for the rearing of *A. japonicus*. The light/dark cycle was kept at 16L:8D in order to replicate summer conditions in South Africa, where this summer-spawning species occurs. Larvae were fed with rotifers (*Brachionus plicatilis*, enriched with *Tetraselmis* spp.) and de-capsulated brine shrimp (*Artemia* spp.) in the early stages, and then weaned onto a pelleted diet (Skretting Gemma Wean 200–1 000 µm) at 18 days after hatching (DAH), as per aquaculture protocol to ensure optimal survival and growth. Rotifers and brine shrimp were provided in excess and maintained at an estimated concentration of 2 ind. ml⁻¹, which was checked regularly throughout the day to ensure consistent availability of food during the entire diel cycle. Rotifer and brine shrimp feeding was terminated at 13 DAH and 24 DAH, respectively. Water clarity was maintained at just below 1 m Secchi-disk reading with green algae (*Tetraselmis* spp.) to ensure optimum feeding conditions for the larvae until 24 DAH, as green-water rearing has been found to optimise larval feeding success (Chesney 2005; Shields and Lupatsch 2012). Once weaned onto the pelleted diet, the larvae were fed hourly throughout the photoperiod, using a belt feeder. Pellet size was increased according to growth of the larvae as per standard feeding protocols for this species.

Oxygen consumption measurements
Static respirometry was used to undertake 24-hour oxygen consumption measurements throughout development to include each life-stage of *A. japonicus*, from hatching through to the settlement stage (0–27 DAH) (Table 1). Care was taken to include developmental milestones, such as hatching, loss of the yolk sac, swimbladder formation, notochord flexion, first feeding and settlement (Table 1). The respirometry protocol was designed according to recommendations made by Clark et al. (2013), Chabot et al. (2016) and Peck and Moyano (2016), and included assessing suitable respirometer volumes, oxygen ingress and drift associated with bacterial respiration prior to experimentation. Small-volume static respirometry chambers (24-chamber glass microplates, Loligo Systems), with volumes of 200 µl, 750 µl and 4 ml, were used to determine oxygen consumption rate in pre-flexion larvae. Oxygen concentration was determined optically using a luminescent sensor spot located in each chamber, and which interfaced directly with a 24-channel microplate reader (SDR Sensordish® Reader; PreSens Precision Sensing GmbH). The microplate was housed in a water bath that maintained the temperature at 25 °C (with variation not exceeding ± 0.05 °C). Larvae were individually assigned to each of 20 chambers, with a further four chambers left empty to provide a control for bacterial respiration.

For post-flexion larvae, individual cylindrical glass respirometers with volumes of 5 ml and 15 ml were placed in a dark-sided water bath that maintained water temperature at 25 °C (± 0.05 °C) and minimised disturbance. The larger volume static respirometers were used to accommodate larger larvae. Oxygen measurement in these larger respirometers was accomplished by circulating water using

Table 1: Developmental stages of *Argyrosomus japonicus* larvae from 0 to 27 days after hatching (DAH)

DAH	Life stage	Description
0–3	Hatchling	From hatching to complete absorption of the yolk sac (at approx. 1.3 mm TL).
3–6	Early pre-flexion	From yolk-sac absorption to the start of notochord flexion; swimbladder forms during this stage; begins to actively feed on rotifers.
10–12	Late pre-flexion	Begins feeding on *Artemia* spp.
14–16	Flexion	Completion of notochord development and development of fin elements.
20–22	Post-flexion	Increased swimming activity; able to feed on pelleted diet.
26–27	Settlement	Completion of metamorphosis; appears and behaves like fully fledged young fish.

a peristaltic pump from the chambers through flow-through cells where dissolved oxygen concentration (mg l^{-1}) was recorded by luminescent sensor spots interfaced via a fibre optic cable to an optical oxygen meter (FireStingO$_2$ reader, PyroScience GmbH). The flow of water re-circulating through the flow-through cells ensured mixing of water in the chambers and maintained a uniform oxygen distribution within the chamber. The large-volume respirometry system consisted of four chambers per trial, of which three contained individual fish and one blank chamber served as a control. The seawater used in the respirometry trials was treated with 12.5% sodium hypochlorite (neutralised with thiosulphate) and ultraviolet light sterilisation to minimise the background respiration rates. The use of sodium hypochlorite is common practice in aquaculture to reduce bacterial growth in seawater systems (L Grant, Pure Ocean Aquaculture, pers. comm.).

A minimum of nine randomly selected individuals were used to assess oxygen consumption per life stage (Table 1). Selected individuals were placed in beakers filled with sterilised seawater approximately 6 h prior to each measurement, during which time they were not fed. These purging/acclimation chambers were housed in the water bath that was used for respirometry for between 1 and 4 h to ensure the individuals used for respirometry were in a resting, post-absorptive state and acclimated to the measurement temperature to ensure determination of SMR (the duration of purging, respirometer size, and acclimation time depended on the size and activity of the individuals). Individuals were placed in the respirometry chambers and oxygen consumption was measured for approximately 1 h at 6-h intervals (08:00, 15:00 and 22:00) over 18 h, resulting in one measurement period for each of morning, afternoon and night.

Measurement time and chamber volume were adjusted to avoid a reduction in oxygen saturation below 80% to ensure that hypoxia-induced stress and potential oxygen ingress were avoided. Oxygen concentration was measured in the chambers every 60 s during each 1-h measurement period. After all measurements were completed, fish were removed from the chamber, placed on filter paper (membrane filter, 0.2 μm) and excess water was removed using suction from a vacuum pump. Fish were then weighed to the nearest 0.0001 g. Due to their small size, it was not possible to weigh pre-flexion larvae, and hence individual weights could only be determined from 13 DAH onwards. For smaller individuals, a sample of 3–5 larvae were weighed at a time and an average weight was estimated. Due to the inaccuracy of weighing smaller larvae, oxygen consumption was

expressed as μmol O$_2$ fish^{-1} h^{-1}, and mass-specific oxygen consumption (mg O$_2$ g^{-1} h^{-1}) was calculated only for larger individuals (13 DAH onwards) and therefore was analysed separately. These measurement protocols were repeated over three consecutive days per life stage to avoid the influence of development on metabolic rate as development in *A. japonicus* occurs rapidly in the early stages.

Calculations of baseline metabolic rates
The first 5 to 10 minutes of the recorded measurements were excluded from the calculations to account for larval acclimation to the chambers. Any oxygen concentration readings that were below 80% saturation of seawater (5.3 mg l^{-1}) were also excluded from the calculations. A least-squares linear regression model for oxygen concentration over time was performed. Residual analysis was applied to determine whether the data were suitable for use in a linear regression model. The regression model was used to calculate ΔO_2, and the following equation was used to calculate oxygen consumption (MO$_2$: mg O$_2$ ind.$^{-1}$ h^{-1}) independent of mass for each life stage:

$$MO_2 = \frac{[\Delta O_2] \times V}{t}$$

where $\Delta[O_2]$ is the decrease of oxygen concentration in the water (Δ mg O$_2$), t is the total recording time (h), and V is the volume of the respirometer (litres). The average oxygen consumption values for the blank chambers were then subtracted from the final calculated oxygen consumption values for each individual to account for background respiration in seawater.

For flexion, post-flexion and settlement larvae (>13 DAH), mass-specific oxygen consumption (MO$_2$: mg O$_2$ g^{-1} h^{-1}) was calculated as follows:

$$MO_2 = \frac{[\Delta O_2] \times V}{t} \div M$$

where M is the wet mass of an individual larva, weighed to the nearest 0.0001 g when possible or an average weight calculated for larvae weighed in a group. A least-squares linear regression model (SMR = aM^b) was fitted. Mass-independent and mass-dependent oxygen consumption rates were analysed separately.

Standard (SMR) and active (AMR) metabolic rate were determined from the oxygen consumption rate by using the 5% and 95% percentile of all data obtained during the 18-h measurement period (Reid et al. 2012; Kandjou

and Kaiser 2014) for each life stage separately. The RMR was calculated by averaging all remaining metabolic rates obtained in the 18-h measurement period (Kandjou and Kaiser 2014). Metabolic scope (MS) was then calculated using the following equation: MS = AMR – SMR.

Prior to analyses, all oxygen consumption rates were converted from mg O_2 ind.$^{-1}$ h^{-1} to µmol O_2 ind.$^{-1}$ h^{-1} to achieve better resolution of low oxygen consumption rate values, with the exception of mass-specific metabolic rates which were expressed in mg O_2 g^{-1} h^{-1}. All oxygen consumption data were analysed descriptively without statistical testing due to the constraint of all larvae being contained in a single tank during the rearing process, hence not allowing for sufficient replication. Data were assessed for normality using visual distribution fitting and a Kolmogorov–Smirnov test at a significance level of $p < 0.05$. According to these tests, the oxygen consumption data for each life stage were normally distributed.

Results

Baseline metabolic rates
The RMR increased continuously throughout development barring a period of no apparent increase during the early pre-flexion stage (3–6 DAH). The RMR increased more rapidly from flexion during the later stages (20–27 DAH). There appeared to be more individual variation as the fish got older, with standard deviation (SD) peaking during the settlement phase (Table 2).

Both SMR and AMR remained relatively stable in early development. However, there was an increase in AMR during the settlement stage (22 DAH) when compared with the SMR (Figure 1). While the SMR increased gradually during the earlier life stages (0–22 DAH), the AMR peaked during the late pre-flexion stage (6–11 DAH), declined during the flexion stage (12–15 DAH), and increased rapidly after flexion, together with SMR (20–27 DAH; Figure 1a).

Metabolic scope was low during the early stages (0–6 DAH) and increased between 6 and 12 DAH. There was a reduction in MS (~0.30 µmol O_2 ind.$^{-1}$ h^{-1}) on day 14, which coincided with the beginning of flexion (Figure 1b). This was attributed to a decline in the AMR and a simultaneous increase in the SMR (Figure 1b). Metabolic scope was higher following flexion (Figure 1b).

The mean mass of larvae increased by 0.012 g per larva from flexion to post-flexion, and by 0.088 g per larva from post-flexion to settlement (Table 3). The mean mass-specific metabolic rate declined from the flexion to the post-flexion phase. However, it had increased by more than

five times by the settlement phase (Table 3). The relationship between mass-specific SMR and body mass (M) is shown in Figure 2.

Diurnal metabolic rates
Average oxygen consumption was similar in the morning, afternoon and night for fish in the early life stages (from hatchling to post-flexion) (Figure 3). However, average oxygen consumption during the settlement stage was highest in the morning (12.78 µmol O_2 ind.$^{-1}$ h^{-1}), followed by the afternoon (10.08 µmol O_2 ind.$^{-1}$ h^{-1}) and night (5.87 µmol O_2 ind.$^{-1}$ h^{-1}) (Figure 3). This resulted in an increase in the SMR during the daytime measurements in the settlement stage of *A. japonicus* larvae.
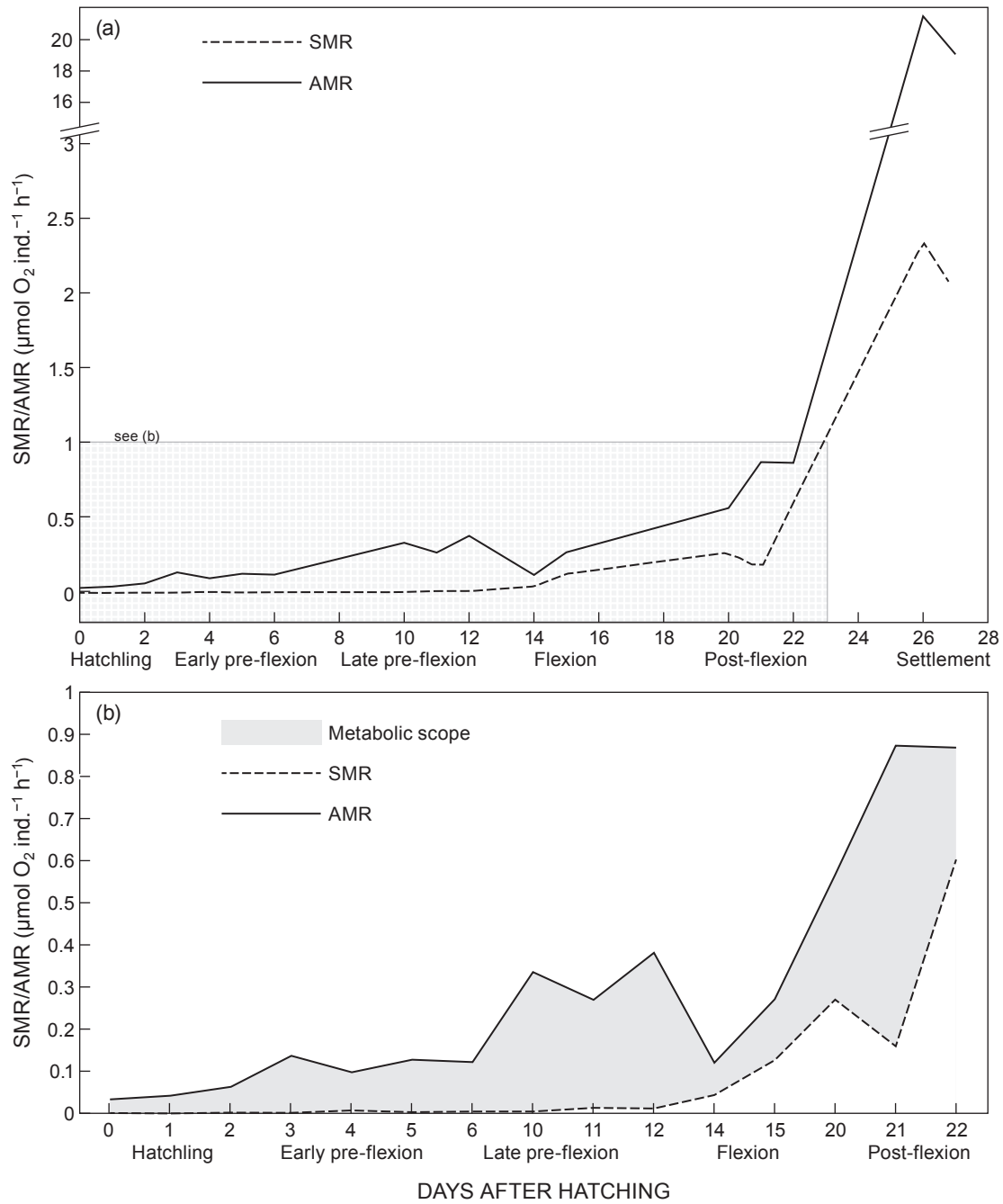
Discussion

Current research on the metabolism of *Argyrosomus japonicus* is limited to the larger juvenile stages (60–1 000 g per fish) of Australian populations (Fitzgibbon et al. 2007; Pirozzi and Booth 2009) and was assessed by these authors for the purposes of determining the energetic requirements of juveniles in aquaculture. There have been no assessments of the changes in metabolic rate with early ontogenetic development or of diurnal fluctuations in metabolism for this species. There was distinct metabolic structuring during the early stages of *A. japonicus* development, with the flexion stage (15 DAH) showing a reduced MS. There was no variation in metabolic rate with photoperiod in the early life stages; however, metabolic rate was highest in the morning during daylight in settlement-stage larvae.

The RMR increased with age and this is likely a result of the increased activity (represented by AMR) and energetic demands (represented by SMR) associated with an increase in body size and rapid morphological development in the early stages (Peck and Moyano 2016). A similar pattern was observed in the RMR of larval striped mullet *Mugil cephalus* during early development (Walsh et al. 1989). In *A. japonicus*, the initial increase in RMR appeared to be most pronounced after hatching, after which it increased at a more gradual rate in the later stages (early pre-flexion to flexion). In the early stages of development (prior to flexion), changes in metabolism were the least pronounced.

There appeared to be a slight decline in RMR during flexion, corresponding with a large decline in the active metabolic rate (AMR). Because AMR decreased rapidly during the flexion stage and SMR continued to increase, there was a reduced MS during this stage. This is similar to

Table 2: Descriptive statistics of routine metabolic rate (RMR; µmol O_2 ind.$^{-1}$ h^{-1}) for each early life stage of *Argyrosomus japonicus*. DAH = days after hatching
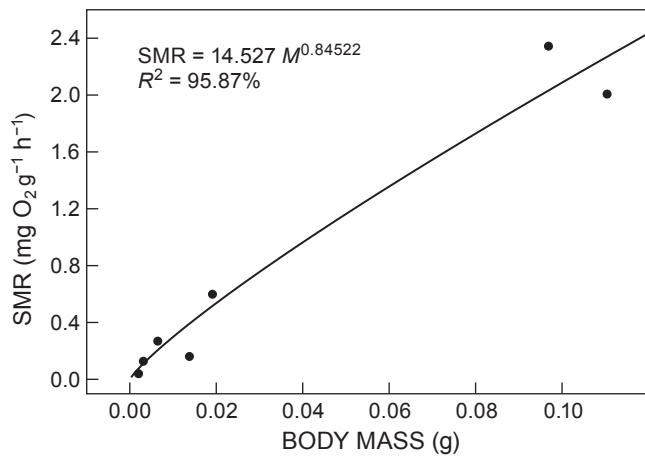
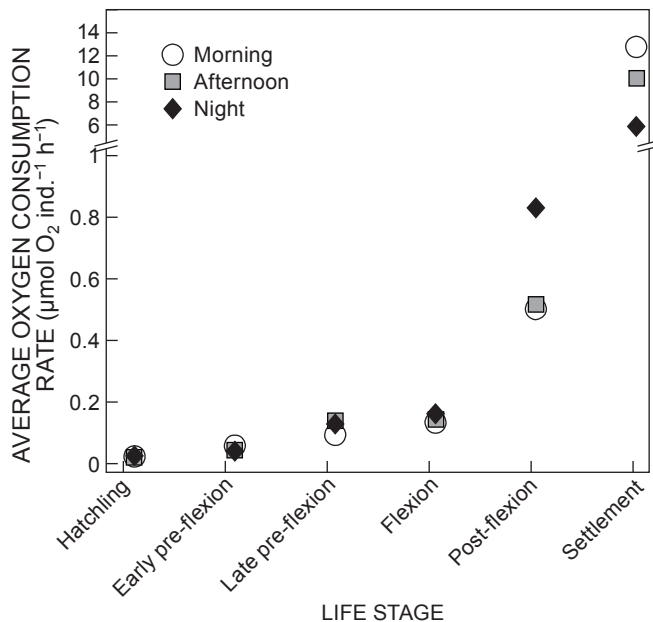| Life stage	DAH	*n*	Mean	Median	Min.	Max.	SD
Hatchling	0–3	98	0.022	0.018	0.00003	0.066	0.019
Early pre-flexion	3–6	105	0.049	0.041	0.00061	0.203	0.041
Late pre-flexion	10–12	99	0.139	0.121	0.00035	0.409	0.088
Flexion	14–16	13	0.132	0.127	0.00716	0.383	0.108
Post-flexion	20–22	21	0.590	0.647	0.15980	0.873	0.241
Settlement	26–27	13	10.471	8.338	2.00659	21.463	6.470

Figure 1: Standard (SMR) and active (AMR) metabolic rate throughout the development of *Argyrosomus japonicus* from (a) hatchling to settlement stage, and (b) hatchling to post-flexion stage, as determined by the 5th and 95th percentiles of metabolic rate ($\mu mol\ O_2\ ind.^{-1}\ h^{-1}$)

Table 3: Mass-specific (wet mass) routine metabolic rate ($mg\ O_2\ g^{-1}\ h^{-1}$) for flexion, post-flexion and settlement-stage *Argyrosomus japonicus* larvae. DAH = days after hatching

Life stage	DAH	n	Mean wet mass (g)	Mean	Median	Min.	Max.	SD	SE
Flexion	14–16	12	0.0026	1.446	1.398	1.1106	2.0381	0.293	0.084
Post-flexion	20–22	21	0.0149	1.390	1.060	0.3902	6.6537	1.295	0.282
Settlement	26–27	13	0.1031	5.516	4.813	2.6707	11.673	2.610	0.724

Figure 2: Model for the relationship between standard metabolic rate (SMR) and body mass of flexion, post-flexion and settlement-stage *Argyrosomus japonicus* larvae (mass = g wet weight)

$$SMR = 14.527\,M^{0.84522}$$
$$R^2 = 95.87\%$$

Figure 3: Average metabolic rate of *Argyrosomus japonicus* larvae at different life stages during different times of the 24-h diel cycle

the findings for the Senegal sole *Solea senegalensis* which also showed reduced metabolic rate during metamorphosis (Parra and Yúfera 2001). Similarly, Killen et al. (2007) found that shorthorn sculpin *Myoxocephalus scorpius* had the lowest MS just after metamorphosis. It has been suggested that there is an increase in energetic demand during periods of rapid morphological development (von Herbing and Boutilier 1996; Killen et al. 2007) and that larval fish potentially manage this demand by reducing activity during this time (Parra and Yúfera 2001).

The reduced MS observed during flexion suggests that energy bottlenecks (reductions in MS) may occur during periods where energy is being allocated specifically to development, thereby limiting the energy available for other energetically costly activities. Such activities could include maintaining homeostasis in changing environmental conditions (Killen et al. 2007). The flexion stage may be the period of highest mortality in the aquaculture of *A. japonicus* (L Grant, pers. comm.), which most likely occurs as a result of these bottlenecks. The recognition of energy bottlenecks is necessary as it allows for the successful identification of key life stages that should be considered when assessing vulnerability to changes in environmental conditions. This response may be species-specific. For example, the highest metabolic rate for the larvae of red seabream *Pagrus major* were observed during metamorphosis, suggesting that they make large amounts of energy available during flexion (Ishibashi et al. 2005). This suggests that species-specific information is required to determine the timing of energy bottlenecks during early development. The measurement of MS, which represents the energy available for adaptation, provides a useful metric as life stages with a limited MS often exhibit high mortality rates (Bailey and Houde 1989) and recruitment bottlenecks during unfavourable environmental conditions (Killen et al. 2007).

The MS of *A. japonicus* increased after flexion, with higher values observed at settlement. Killen et al. (2007) found that juveniles of three marine species (ocean pout *Macrozoarces americanus*, lumpfish *Cyclopterus lumpus*, and shorthorn sculpin *Myoxocephalus scorpius*) had a considerably higher MS than the larval stages. The authors attributed this to a rapid increase in AMR during the early juvenile phase when individuals begin swimming actively. In the case of the estuarine-dependent *A. japonicus*, this also coincides with the stage at which individuals begin recruiting into estuaries (Griffiths 1996; Cowley et al. 2008). The elevated MS associated with this developmental stage in *A. japonicus* could facilitate the physical demands required to navigate to estuaries and the tolerance that is required to withstand the highly fluctuating physico-chemical parameters in estuarine environments.

Changes in metabolic rate in early-stage *A. japonicus* may reflect changes in the activity of fish during early development. Although this study did not measure activity of the individual due to the difficulty of observing small larvae in the respirometry chambers, the link between metabolic rate and swimming was made by von Herbing and Boutilier (1996) in a study on large-sized larvae of Atlantic cod *Gadus morhua*. They found a positive relationship between metabolic rate and swimming activity, although this was only observed in the life stages following flexion (von Herbing and Boutilier 1996). This relationship was attributed to feeding activity and the onset of morphological development during flexion (von Herbing and Boutilier 1996).

In addition to determining the reference metabolic levels of early-stage *A. japonicus*, the relationship between metabolic rate and fish mass was explored. The dependence of metabolic rate on organism mass is well understood, and the scaling of metabolic rate with mass seems to be relatively universal among all animal species. This is termed 'allometric law' (Giguère et al. 1988). However, the scaling of metabolic rates with body mass in fish is poorly

understood (Killen et al. 2007). Studies that have attempted to assess the mass-specific metabolism in larval fishes have reported vast differences in mass dependence (Post and Lee 1996) and it has been suggested that the relationship between body mass and metabolic rate in fish larvae may vary among species (Burggren and Blank 2009). This study showed scaling of SMR with an increase in body mass, similar to the metabolic scaling usually observed in young fishes (Clarke and Johnston 1999). Clarke and Johnston (1999) suggested that an average universal scaling exponent of $b = 0.79$ is suitable for the relationship between metabolic rate and body mass in most fish species. Although this value is considered universal for most species, some studies have calculated scaling exponents that are higher than this average ($b > 0.79$) in fish larvae assessed throughout their complete life history (Killen et al. 2007). For example, Killen et al. (2007) found scaling exponents between 0.82 and 0.84 for short-spined sea scorpion *Myoxocephalus scorpius,* lumpfish *Cyclopterus lumpus* and eelpout *Macrozoarces americanus.* However, these authors stressed that the lack of standardised methods limits our current understanding of mass-dependent metabolic rates in larval fish as does the lack of data covering the complete life history. The metabolic scaling in this study represents a short period of development and should only be considered valid for the body-mass range that could be tested in this study.

This study also assessed the variation in metabolic rate within the 24-h diel cycle and provided the first assessment of rhythmic changes in metabolic rate throughout the early development of *A. japonicus* as related to diurnal changes. There were no clear changes in metabolic rate during the morning, afternoon and night in the early life stages (hatchling to post-flexion). This suggests that there are limited changes in activity patterns in the 24-h diel cycle during early development. There is a paucity of information on comparable diurnal metabolic research on larval marine fishes. Studies of adult fishes, including the black sea mullet *Liza saliens* (Shekk 1986), flounder *Paralichthys olivaceus* (Liu et al. 1997), Mediterranean yellowtail *Seriola dumerili* (De la Gándara et al. 2002), estuarine-dependent white steenbras *Lithognathus lithognathus* (Kandjou and Kaiser 2014) and spotted grunter *Pomadasys commersonnii* (Radull et al. 2002), found diurnal changes in activity patterns.

Metabolic rate was highest during daylight hours in settlement-stage larvae. Because measurements of metabolic rates reflect activity, this finding suggests that settlement-stage *A. japonicus* are more active during daylight than at night. The variation in metabolic rate in settlement-stage *A. japonicus* with time of day is potentially explained by the findings of a study by Ballagh (2011) who suggest that optimal feeding of early juveniles (10–50 mm TL) of *A. japonicus* (fed on a pelleted diet) occurred when all sensory functions were available in daylight conditions. It is also thought that settlement-stage larvae may be attracted to a turbidity plume, which is likely identified using vision, when recruiting into estuaries. The variation in metabolism in this study was conducted with the intention to determine at which time of day metabolic measurements should be taken. The results suggest that because activity does not change over the 24-h photoperiod in the early life stages of *A. japonicus* raised in a controlled laboratory environment,

metabolic measurements are not restricted to any specific time of day, other than during the settlement stage, when readings should be taken at night in order to avoid the over-estimation of SMR.

Conclusions

The results from this study provide a description of the metabolic activity of *A. japonicus* throughout early ontogenetic development. This contributes to our knowledge of metabolism in fish larvae and provides insight into the physiological bottlenecks in development that may render the species vulnerable to environmental changes. The study also provided the first insight into diurnal metabolic fluctuations during the early life stages of the species, which is required to optimise studies examining the metabolic structure. Future research should aim to examine the metabolic structure through the life stages of the species, including similar metabolic information for later-stage juveniles and adults. This baseline dataset is essential if further population-level questions, such as identifying metabolic bottlenecks at different life stages, are to be understood.

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