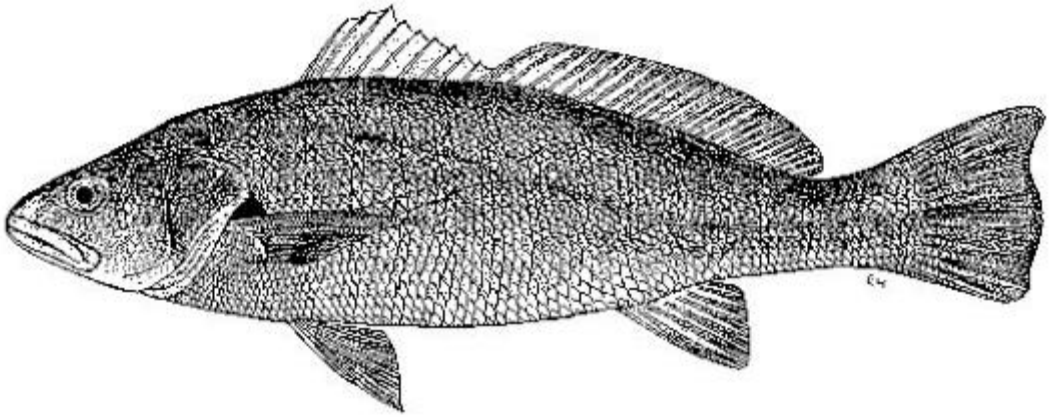
# An assessment of metrics to determine a rapid, benign method of classifying the metabolic physiology phenotype of individual fish, using dusky kob (*Argyrosomus japonicus*) as a case study



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Of

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By

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## Declaration

I, Christian Dieter Hempel, hereby declare that the work described in this thesis was carried out in the Department of Ichthyology and Fisheries science at Rhodes University, under the supervision of Professor Amber Childs, and Professor Warren Potts. The components of this thesis comprise original work by the author and have not been submitted to any other institution.

Signed: 

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## Abstract

Climate change is a major driving factor in the redistribution of species across the globe. The overall global trend of ocean warming has already driven fish distributions to higher latitudes and deeper depths. This shift in fish distribution can have several negative implications on coastal communities who rely on these fish as a source of food and income. Similarly short-term thermal variability in the coastal ocean, largely driven by increases in the frequency and intensity of upwelling events and marine heatwaves, may push fishes beyond their thermal limits. As ectotherms, fish are required to respond rapidly to such changes in coastal temperatures. For many species, behavioural adjustments, such as movements to more suitable habitats represent the first response to changing thermal conditions. Advanced techniques, such as biotelemetry have allowed scientists to track fish in their natural environments providing important information on movement ecology, and the response to changes in environmental conditions. While this technique provides valuable information on movement patterns it has limited utility in understanding the mechanisms driving these patterns. To understand the mechanisms driving fish behaviour, it is necessary to link behaviour in the wild with the physiological attributes of individual animals. Ideally, the physiological phenotype of the species should be categorised prior to tagging and tracking their movements in the wild. However, clear methodologies to do this have been elusive.

This study aimed to fill this knowledge gap by investigating an appropriate laboratory technique for rapidly assessing the physiological attributes of individual fish without compromising their health prior to an acoustic telemetry study. Importantly, the physiological technique should provide appropriate information with which to categorize the physiological phenotype of an individual before it is tagged and released into the wild. The dusky kob, *Argyrosomus japonicus* (Sciaenidae), was selected as a candidate species, as they have shown extensive diversity in their behaviour in the wild which has been attributed to individual

thermal tolerance. They are also currently listed as endangered on the IUCN Red List of threatened species with research gaps on the impacts of climate change and overexploitation on the sustainability of future populations; and hatchery-reared fish were readily available from Kingfish Enterprise in East London, South Africa, which allowed for the physiological phenotypic assessments without compromising wild-caught populations.

A total of 40 juvenile *A. japonicus* were obtained from Kingfish Enterprises and transported back to a laboratory in the NRF SAIAB's Aquatic Ecophysiology Research Platform (AERP) at the Department of Ichthyology and Fisheries Science (DIFS), Rhodes University. Thirty fish were randomly selected from the original 40 and randomly split into two groups (Batch A and Batch B) and housed separately in two 5900 L tanks. For the purpose of repeated measures, each fish was tagged with a passive integrated transponder (PIT) for individual identification throughout the experiments, and was given a colour coded (either red, blue, or white) spaghetti tag for visual identification. The dynamic method, characterized by the critical thermal minimum ($CT_{min}$), and critical thermal maximum ($CT_{max}$) was conducted using a repeated measures approach to assess the thermal tolerance of individual fish. Temperature was decreased or increased at a rate of 1 °C per hour to simulate marine upwellings or marine heatwaves until fish reached their critical thermal end points. Thermal breadth was calculated as the difference between $CT_{min}$ and $CT_{max}$. To assess thermal stress prior to reaching critical thermal limits, opercula beats (OB), as a proxy for ventilation rate, were counted for each fish at every degree change using GoPro cameras. A subsample of eight individuals were selected and intermittent flow respirometry was used to determine their standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS) at three test temperatures (12, 18, and 24 °C) using a repeated measures approach.

The results of the thermal tolerance trials indicated that on average hatchery-reared *A. japonicus* had a broad thermal tolerance, with an average $CT_{max}$ of 32.7 °C (SD = 0.59), $CT_{min}$

of 8.2 °C (SD = 0.28) and an average thermal breadth (TB) ($CT_{max}$ - $CT_{min}$) of 24.5 °C (SD = 0.69). Substantial individual variability was also observed in the critical thermal endpoints and TB of *A. japonicus* where $CT_{min}$ ranged from 7.5 to 8.6 °C, $CT_{max}$ ranged from 31.7 to 33.7 °C, and TB ranged from 23.3 to 26.0 °C. Categorisation of fish into physiological phenotypes based on their $CT_{max}$, $CT_{min}$ and TB estimates revealed that the sampled population exhibited a high degree of phenotypic variability. Using the percentile ranking method, individuals were categorised as high performers (broad TB, low $CT_{min}$, high $CT_{max}$), low performers (narrow TB, high $CT_{min}$, low $CT_{max}$) and intermediate performers (those individuals that ranked as neither high nor low performers). Individuals were also classified as either "broadly-adapted" (low $CT_{min}$, high $CT_{max}$), "narrowly-adapted" (low $CT_{max}$, high $CT_{min}$), "cold-adapted" (low $CT_{min}$, low $CT_{max}$) and "warm-adapted" (high $CT_{min}$, high $CT_{max}$).The piecewise linear breakpoint analyses conducted on the ventilation rate (used as a proxy for thermal stress) data indicated the onset of thermal stress at temperatures below 12 °C and above 30 °C. Given that these temperatures are occasionally observed in estuarine and in the nearshore marine environment, these breakpoints may be useful to test hypotheses on thermal drivers using telemetry data.

Respirometry data showed a positive relationship between metabolic rates (SMR, MMR) and temperature. The high performance (AS) at all the test temperatures confirmed the eurythermic nature of the species. As with the thermal tolerance data, there was considerable intraspecific variability in SMR, MMR and AS and the percentile rankings were used to identify high, low and intermediate performers across the three temperatures.

Overall, the diversity in thermal tolerance and performance of these hatchery *A. japonicus* was surprising because they were from a limited adult gene pool and were reared in a relatively warm and stable thermal environment. However, the diversity among individuals does suggest

that the natural population of this species may have considerable adaptive potential, but a similar study on wild *A. japonicus* will be required to confirm this.

The rankings for the different physiological metrics were used to obtain an overall sum rank which was assumed to provide a numerical representation of the physiological phenotype. Of the various metrics measured, the AS across all test temperatures followed by the $CT_{min}$ and $CT_{max}$ showed the greatest alignment with the sum rank. When the handling stress, confinement stress, thermal stress, and time taken to complete the experiment were considered, the most appropriate metrics for assessing the physiological phenotype before a telemetry experiment were $CT_{min}$ and $CT_{max}$.

In conclusion this study contributed to the development of a rapid, benign method to assess the physiology of fishes prior to a telemetry experiment. Such experiments will allow researchers to explore correlations between physiological performance and fish behaviour in the wild, furthermore improving our understanding of their adaptive capacity to impacts of climate change.

**Key words:** fisheries management, climate change, *Argyrosomus japonicus,* thermal tolerance, respirometry, thermal stress

# Chapter 1: Introduction

The condition of coastal marine ecosystems is declining rapidly due to multiple anthropogenic effects, such as overexploitation, mining, carbon emissions, oil, and gas extraction, and increasing human populations in coastal areas (Potts et al. 2015). These impacts are aggravated by the ever-increasing pressures of climate change, such as ocean acidification, increasing global sea surface temperatures, and intensifying of upwelling events (Harley et al. 2006). Anthropogenic pressures combined with climate change have serious negative implications for coastal fish populations around the globe (Hoegh-Guldberg and Bruno 2010, Cooke et al. 2019).

As ectotherms, fish are particularly vulnerable to temperature fluctuations, as even minor changes can directly affect their physiological processes (Pankhurst and Munday 2011, Potts et al. 2015). Increasing temperatures can reduce the ability of fish to meet their oxygen demands, negatively impacting their reproduction, growth and development (Pörtner and Peck 2010, Pankhurst and Munday 2011). Exposure to temperatures beyond an individual's thermal limit can result in physiological stress, tissue damage, hypoxia, or even mortality (Pörtner and Peck 2010, Ern et al. 2016, Slesinger et al. 2019). In response to temperature change, fish adjust their behaviour to avoid these unfavourable thermal conditions, shifting their distributions to higher latitudes and deeper depths (Pörtner and Knust 2007, Hoegh-Guldberg and Bruno 2010, Howes et al. 2015, Johnson and Layman 2020). This shift in fish distribution can have several negative implications for fishing communities that rely on fish resources for food and income.

To predict the response of fish to climate change, researchers have used various modelling and analytical approaches including species distribution models (SDMs), ecophysiological models, coupled biophysical models, and analyses of historical data (Hinrichsen et al. 2011, Tanner et al. 2016, Ward et al. 2016, Zhang et al. 2019, Jenkins and Stevens 2022). Species Distribution

Models are numerical tools that combine observations of species occurrence or abundance with environmental variables such as temperature and oxygen levels, allowing for future projections, through the use of ecological niche models, on habitat suitability under changing climate conditions (Elith and Leathwick 2009, Jenkins and Stevens 2022). Ecophysiological models focus on the physiological thresholds of fish, such as thermal tolerance and hypoxia sensitivity to assess their vulnerability to environmental stressors (Ward et al. 2016, Earhart et al. 2022). Coupled biophysical models integrate oceanographic processes with biological factors to predict changes in fish spawning grounds and larval transport under changing climate conditions (Hinrichsen et al. 2011). Historical data sources, such as otolith analyses and genetic markers, offer medium- long term perspectives on fish responses to climate variability by tracking past changes in growth patterns and population structure (Tanner et al. 2016). While these models and techniques are advancing rapidly, they often fail to incorporate the role of individual physiological variability, which could improve the accuracy of predictions and provide a better understanding of species adaptive potential in a changing climate (Ward et al. 2016).

Traditionally, SDMs and ecophysiological models assume that all individuals within a population exhibit the same physiological characteristics and responses to environmental changes. However, emerging research highlights the importance of individual variability in shaping population level responses (Poloczanska et al. 2013, Ward et al. 2016). Individuals within a population exhibit varying thermal tolerances (Ward et al. 2016). Those with a narrow thermal tolerance tend to migrate polewards in response to increasing temperatures, whereas those with a broader thermal tolerance may remain within their existing distribution. This may be why many of the range shifts observed in ectotherms may be occurring faster at the leading edge when compared to their trailing edge (Polocsanzka et al. 2013). Despite its importance,

individual variability has received little to no attention in the context of climate change predictions, creating a gap in our understanding of the adaptive potential of species.

Besides the lack of knowledge on individual variability, there is a notable gap in understanding the link between individual physiological phenotypes and behaviour (Ward et al. 2016, Bailey et al. 2022). Although Bailey et al. (2022) highlighted the link between individual physiological phenotypes and behaviour, there is limited empirical evidence supporting this in the wild. One of the primary challenges in studying these links is the difficulty of monitoring wild behaviour of a fish whose physiological traits have been quantified. One approach may be to first quantify the physiological profile of a fish in the laboratory and then observe its natural behaviour using methods such as Remote Underwater Video (RUV) (Schramm et al. 2020), Baited Remote Underwater Video (BRUV) (Whitmarsh et al. 2017), or acoustic telemetry (Villegas-Rios et al. 2017). However, to ensure that this approach is successful, a benign physiological assessment would be required to ensure that the behaviour of the individual in the wild is not altered as a result of the physiological assessment.

Several physiological performance methods have been used to assess individual phenotypes across thermal gradients. They include laboratory-based respirometry trials which estimate the aerobic scope (AS) of fish at a range of temperatures (this requires the measurement of standard metabolic rate (SMR) and maximum metabolic rate (MMR) (Rupia et al 2016, Bailey et al. 2022, Bailey 2023, Nabani 2023); heart rate monitoring which measures the cardiovascular response to thermal stress (Muller et al. 2020, Skeeles et al. 2020); or swimming performance tests where the critical swimming speed (Ucrit) is measured across a range of temperatures (Plaut 2001, Farrel 2008, Skeeles 2020). Unfortunately, all these methods require prolonged acclimation and measurement periods, are highly stressful, particularly when individuals are subjected to repeated assessments at different temperatures, and the fish are handled and confined. These experiments not only require fish to be out of their natural environment for

protracted periods, but can also compromise fish health through physiological stress, such as increased blood cortisol levels (Foo and Lam 1993), cold shock when exposed to extreme cold temperatures (Szekeres et al. 2016, Reid et al. 2022), and the induction of heat shock proteins when exposed to warm temperatures (Iwama et al. 1998, Iwama et al. 1999); and through physical damage, including damage to fins and body tissue, which can have profound negative effects on their behaviour in the wild (Brownscombe et al. 2017). There is an urgent need to develop alternative, less stressful and rapid techniques for categorising the physiological phenotype of fishes to link physiological characteristics with behaviour in the wild.

Several other minimally invasive, less stressful techniques have been used to assess the physiological performance of fishes. These include, measurements of SMR and routine metabolic rate (RMR), which do not require the fish to be exercised to exhaustion, as in the case of MMR (Norin and Clark 2015, Chabot et al. 2016); minimally invasive blood sampling for estimating the cortisol, glucose or lactate levels (Iwama et al. 1995); ventilation rate measurements to estimate rates of respiration (Millidine et al. 2008) and assessment of the thermal endpoints (critical thermal maximum ($CT_{max}$) and minimum ($CT_{min}$)) to determine the upper and lower thermal limits of individual fish (van der Walt et al 2021). Of these techniques, the assessment of thermal endpoints holds promise because they can be performed rapidly, with minimal acclimation time and can be employed in simple laboratories or even in field settings.

### 1.1 Aims and objectives

The aim of this study was to assess the potential of less invasive methods to categorise the physiological phenotype of individual fish using the dusky kob (*Argyrosomus japonicus,* Temminck & Schlegel 1843) as a model species. By doing this it is hoped that this thesis will contribute to the development of a framework in which one can assess the link between the

physiological phenotype and their behavioural phenotypes of fishes in the wild to improve our understanding of their adaptive capacity to the impacts of climate change.

This was be achieved by using two contrasting laboratory-based techniques to assess a range of metrics categorising physiological phenotypes and to assess the amount of stress associated with the use of each technique and metric.

The specific objectives were to:

1. Determine the thermal tolerance of individual fish using critical thermal maximum ($CT_{max}$) and minimum ($CT_{min}$) limits and use these metrics to categorise each fish into high, intermediate or low performance phenotypes.
2. Determine the metabolic rates (SMR, MMR) and aerobic scope (AS) of selected individual fish representative of each high, low and intermediate category (identified in objective 1) using intermittent flow respirometry, and to categorise each fish into high, intermediate or low performance phenotypes based on the metabolic metrics.
3. Assess the link between thermal tolerance and respirometry by ranking the relative physiological performance of each fish for each metric and the accuracy of the alignment of these rankings.
4. Determining the optimal method to predict individual physiological performance based on accuracy, stress, and duration of experiment.

This thesis is divided into four chapters. The first chapter has provided a general introduction to the study topic with aims and objectives, the second chapter includes information on the study species and materials and methods, the third chapter presents the findings of this study, and the final chapter includes the discussion.

# Chapter 2: Species Profile and Materials and Methods

## 2.1 Species Profile

The populations of many coastal fishery species in South Africa are severely overexploited, with the dusky kob, *Argyrosomus japonicus* (Figure 2.1), being no exception. This species is a large globally distributed estuarine-dependant Sciaenid (maximum size 1800 mm and 75 kg) found in South African waters between the Cape Agulhas and Mozambique border (Griffiths and Heemstra 1995), along the southern Australian coastline (Kailola et al. 1993), and from Hong Kong North-wards along the Chinese coast to southern Korea and Japan (Trewavas 1977).

**Figure 2.1** A recreational fisherman (Christian Hempel) holding a dusky kob (*Argyrosomus japonicus*, Pisces: Sciaenidae).

The dusky kob is an apex predator, with adults primarily exhibiting piscivorous feeding behaviour but also consuming cephalopods and crustaceans (Griffiths 1996). Juveniles mainly

feed on mugilids, the pelagic estuarine round herring *Gilchristella aestuaria*, mysids, as well as other crustaceans (Marais 1984). *Argyrosomus japonicus* is an important commercial and recreational fisheries species, due to its large size, palatability, and predatory nature (Griffths and Heemstra 1995, Griffths 1996).

Adult dusky kob spawn at sea, with early juveniles (30-150 mm total length (TL)) recruiting into estuaries (Griffiths 1996), which serve as crucial nursery areas, as they provide thermal refuge, safety from marine predators, and a range of food types which facilitate early growth and survival (James et al. 2013, Childs et al. 2015). Once juveniles reach a size of 150 mm TL they begin to alternate between surf and estuaries (Griffiths 1996). Upon reaching adulthood, *A. japonicus* (>1070 mm TL) predominantly inhabit shallow coastal waters (<100 m depth) (Griffiths and Heemstra 1995). Given the species' reliance on multiple habitats throughout its life cycle, it is vulnerable to changes in the thermal conditions of both estuarine and coastal environments (James et al. 2013, Potts et al. 2015).

Previous telemetry studies on *A. japonicus* have highlighted estuarine-marine connectivity (Cowley et al. 2008, Childs et al. 2015), and partial migration behaviour (Childs et al. 2015). Partial migration is the phenomenon where only a subset of a population exhibits movements (these could be large scale spawning migrations or exploratory/ranging movements) while others display sedentary behaviour (Johnson and Johnson 1993, Kerr et al. 2009, Chapman et al. 2012b, 2012a, Maggs et al. 2019). According to Kerr et al. (2009), partial migrations is common across many fish species as a behavioural strategy and allows for the maintenance of fish stocks. In the case of *A. japonicus*, research by Childs et al. (2015), found that juveniles residing in estuaries were recaptured at nearly twice the rate of their marine counterparts, suggesting that their partial migration increases their resilience to exploitation and facilitates the persistence of this species.

Childs et al. (2015) suggested that the partial migration observed in *A. japonicus* may be due to individual differences and phenotypic plasticity within the population. More specifically, Childs (2013) suggested that a conditional strategy, where an individual's genetic make-up allows for the adoption of resident or migratory behaviour based on the interaction between an individual's physiology and its environment (Kerr et al. 2009), may be the mechanism driving partial migration in the species. Such intra-population variability makes dusky kob an ideal candidate for identifying individual physiological performance phenotypes and determining the most benign and least invasive method for such an assessment.

### 2.2 Experimental Approach

To determine the optimal method to assess individual physiological thermal performance phenotypes, two well established laboratory-based methods were used, the dynamic thermal tolerance method and respirometry (see section 2.6 and 2.7, respectively). To allow for comparisons within each method and between methods, a repeated measures approach using the same individual was adopted for thermal tolerance (Allison et al. 2021) and respirometry (Bailey 2023, Nabani 2023).

For this study, aquaculture-reared *A. japonicus* were obtained from Kingfish Enterprise in East London. These aquaculture fish originate from wild-caught brood stock, ensuring that their phenotypes remain comparable to wild-caught individuals. Using aquaculture-reared fish ensures standardized rearing conditions, including known environmental histories and controlled diet, which help minimize confounding factors, therefore facilitating a repeated measures approach. Aquaculture-reared fish are more readily available, and can also be sampled with minimal ethical and logistical concerns associated with wild sampling.

### 2.3 Fish Collection

Forty juvenile fish were collected from Kingfish Enterprise in East London in September 2023 and transported in a 1000L tank filled with seawater collected from the aquaculture tanks via a Toyota Landcruiser. The tank was connected to two oxygen cylinders and one battery operated aerator. All specimens were transported to the NRF-SAIAB Aquatic Ecophysiology Research Platform (AERP) laboratory at the Department of Ichthyology and Fisheries Science (DIFS), Rhodes University, Makanda, South Africa.

### 2.4 Acclimation system

All specimens were housed in an acclimation system comprising two separate cylindrical holding tanks (5900 L each). Water from the holding tanks were filtered using a bubble bead filter (BBF-200-COMP, Wilper Koi Products) and a fluidised biological filter (750 L slime tank fitted with Super ActiFlo Media), before flowing into a sediment sump (750 L slimline). and into the holding tanks after excess protein was removed using a protein skimmer (UltraZap, Red Devil DC 5000s pump), and the water was sterilized, using an Ultraviolet sterilizer (UVS-30, Wilpet Koi products). The holding tanks were aerated using a air blower (2.2Kw) which was mounted to the wall outside of the laboratory. A Speck Porpoise 0.75l W pump was used to recirculate water through the system. Water temperature was maintained by a heat pump set at 18 °C and an air conditioner was used to maintain the ambient air temperature at 18 °C. No environmental enrichment (in the form of structural habitat) was added to the tanks as these fish are known to be a shoaling species (Griffiths 1996, Whitfield and Blaber 1978, Childs 2013).

Fish were acclimated in the holding tanks for one month at a constant temperature of 18 °C. Water quality parameters including dissolved oxygen, ammonia, nitrite and nitrate were monitored daily. The two holding tanks were maintained under similar conditions throughout

the experimental period in order to minimise bias and ensure consistency in environmental factors such as water quality, temperature, lighting and feeding regimes. Fish were fed a formulated pellet diet produced by Kinfish Enterprise every second day. The pellets were thrown into the tank in small handfuls to prevent uneaten pellets from sinking to the bottom of the tank.

**2.5 Tagging**

Thirty fish (mean total length = 442.28 mm, range: 413-487 mm TL) were selected to undergo the experimental procedures. In order to identify individuals, each fish was tagged using a Virbac Backhome super mini Passive Integrated Transponders (PIT tag) and with PDX extra small colour coded plastic tipped dart tag (Hallprint, Australia) for visual identification during experiments. Fish were captured using sterilized silicone nets and placed into a 50 L plastic container containing seawater and 0.04 ml/L of clove oil (Bailey 2023). Once fully anaesthetised, the fish was placed dorsal side up in a high-density foam-lined, V-shaped cradle. Gills were continuously submerged in fresh seawater during the procedure (Bailey 2023). Each individual was measured (mm total length (TL)). A certified South African Veterinary Council (SAVC) researcher then PIT tagged the fish below the dorsal fin (Figure 2.2). Following the tagging procedure, fish were placed into an aerated recovery bath. Once recovered, the fish were placed back into the holding tanks until they resumed normal feeding behaviour (Allison et al. 2021). Once fish resumed normal feeding behaviour, the same procedure was followed where fish were tagged with either red, blue or white colour coded Hallprint plastic tipped PDX dart tags (n=10 per colour) below the dorsal fin, randomly split into two groups (Batch A and Batch B), and housed separately.

Fish were left to recover from the tagging for one month before the commencement of the thermal tolerance trials. During the recovery period, one tagged individual jumped out of the holding system and did not survive, reducing the total number of experimental fish to 29.

**Figure 2.2** *Argyrosomus japonicus* being tagged with passive integrated transponders (PIT) for the purpose of individual identification.

### 2.6 Thermal tolerance

The dynamic method, characterised by the critical thermal maximum ($CT_{max}$) and critical thermal minimum ($CT_{min}$), is a well-established and reliable method used to determine the thermal tolerance of aquatic animals by increasing or decreasing temperature until critical limits are reached (van der Walt et al., 2021; Mora and Maya, 2006). To assess the thermal

tolerance of individual fish, a repeated measures approach following Allison et al. (2021) was employed.

*2.6.1 Experimental system*

The experimental set-up included four 500 L circular plastic tanks connected to a fifth 500 L sump connected to a recirculating system. Temperatures were controlled by an external in-line heat pump (Aquaheat, SF040P G/EVAP). The experimental tanks were linked to a system that consisted of a protein skimmer (UltraZap, Red Devil DC 5000s pump), a bubble bead filter (BBF-200-COMP, Wilper Koi Products), an Ultraviolet sterilizer (UVS-30, Wilpet Koi products), a fluidised biological filter (500 L slime tank fitted with Super ActiFlo Media), and a sediment sump (750 L slimline). A pool pump (Speck Porpoise 0.751 W) was used to recirculate water through the system. The experimental tanks were aerated by an air blower (2.2 kW) which was mounted to the wall outside of the laboratory.

*2.6.2 Thermal tolerance ($CT_{min}$ and $CT_{max}$) experimental procedure*

The fish were fasted for 24 hours prior to the experiment. Three individuals, each with a different colour tag (red, blue and white) were translocated into each experimental tank. This was done to reduce stress as *A. japonicus* is a shoaling species. Prior to the start of the trial, the dissolved oxygen (DO) (ml $L^{-1}$), temperature (°C), salinity (ppt) and pH were measured using a digital multiparameter probe (Hach HQ40d). Fish were allowed to acclimate for one hour, before the temperature was either increased (in the $CT_{max}$ experiment) or decreased (in the $CT_{min}$ experiment) at a rate of 1 °C per hour. This rate of change was set to simulate a marine upwelling and marine heatwave experienced in the wild.

To assess thermal stress prior to reaching critical thermal limits, opercula beats (OB), as a proxy for ventilation rate, were counted for each fish at every degree change (Allison et al. 2021). To reduce the influence of human interaction, a GoPro Hero 11 camera mounted to PVC sheeting

and a 25 mm PVC quick release spring clip was used to record OB. The cameras were attached to ensure that the fish were always in the field of view. The GoPro Quik mobile app was used to remotely turn the cameras on for the first five minutes of each degree change and this footage was used to estimate the OB, which was measured as beats per minute for each individual.

Once the temperature extremes approached the thermal endpoints (below 9 °C and above 30 °C), a team of volunteers monitored the fish's behaviour closely from a distance of one metre, noting any signs of distress or instability, such as erratic swimming, or loss of equilibrium (Lutterschmidt and Hutchison 1997) (Figure 2.3). Once these signs were observed, the fish was removed from the tank using a silicone landing net and the time and temperature were recorded. The fish were then placed into an aerated recovery bath (13°C for $CT_{min}$ and 25°C for $CT_{max}$) for 10-15 minutes until equilibrium was regained. Once recovered, each fish was measured (to the nearest mm total length (TL)) on a measuring sling and released back into their housing tanks. Fish were then allowed to recover for one week, before it was exposed to a second trial (either $CT_{min}$ or $CT_{max}$). Batch A (n=15) was exposed to $CT_{max}$ first followed by $CT_{min}$ whereas batch B (n=14) was exposed to $CT_{min}$ first and then $CT_{max}$.

**Figure 2.3** A volunteer (Joshua Frachet) observing a fish for signs of loss of equilibrium during a $CT_{max}$ experiment.

*2.6.3 Thermal tolerance data analyses*

Thermal breadth, defined as the difference between $CT_{max}$ and $CT_{min}$, was estimated for each individual to assess variability among individuals. Pearson's correlation tests were performed to examine the correlation between thermal breadth and fish length, as well as between $CT_{min}$ and $CT_{max}$. Prior to analyses, normality was assessed to ensure the assumptions of Pearson's correlation were met to evaluate the relationship between ventilation rate (opercular beat rate) and temperature. A linear regression model was applied, with opercular beat rate log-

transformed to meet statistical assumptions. A piecewise linear breakpoint analysis was conducted using the 'segmented' package in RStudio to identify the temperatures at which respiratory performance deviated from linearity, indicating the onset of thermal stress before reaching critical thermal limits. All statistical assumptions were tested prior to analysis, and all analyses were conducted using the latest version of RStudio (R 4.3.3).

Using Excels Rank and Percentile function; each fish's percentile rank was calculated for $CT_{min,}$ $CT_{max}$ and thermal breadth. This approach was adapted from the methodology described by Bailey (2023) and, Nabani (2023). The percentile ranks were converted into scores ranging from 1 to 4 based on the quartile divisions. For $CT_{min}$, fish in the 0-25$^{th}$ percentile received a score of 4, fish in the 26$^{th}$-50$^{th}$ percentile received a score of 3, fish in the 51$^{st}$-75$^{th}$ percentile received a score of 2, and fish in the 76$^{th}$-100$^{th}$ percentile received a score of 1. For $CT_{max}$ and thermal breadth, fish in the 0-25$^{th}$ percentile received a score of 1, fish in the 26$^{th}$-50$^{th}$ percentile received a score of 2, fish in the 51$^{st}$-75$^{th}$ percentile received a score of 3, and fish in the 76$^{th}$-100$^{th}$ percentile received a score of 4. $CT_{min}$ and $CT_{max}$ were then combined and ranked to receive an overall phenotypic ranking.

Fish were selected for respirometry based on their $CT_{min}$ and $CT_{max}$ values. From each batch, four individuals were chosen: one with a low $CT_{min}$, one with a high $CT_{max}$, one with an average $CT_{min}$, and one with an average $CT_{max}$. To ensure compatibility with the respirometer, only fish measuring less than 450 mm in total length were selected.

**2.7 Respirometry**

Intermittent flow respirometry, a well-known and reliable method to assess the effect of temperature on the metabolic rate of fish (Clark et al. 2013, Duncan et al. 2019), was used to determine the standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS) of *A. japonicus*. To assess individual variability within metabolic rates, a repeated

measures approach was employed (using the methods outlined by Bailey (2023) and, Nabani (2023)).

*2.7.1 Experimental system*

Physiology experiments were conducted in an experimental room which housed an 800 L cylindrical plastic lined tank connected to a filtration system comprised of a protein skimmer, bubble bead filter (BBF-100 COMP), fluidised bed filter and UV sterilizer (UV 55 W, Ultrazap Pro UVS-55). Oxygen was maintained at 100% saturation in the cylindrical tank using a 2.2 kW blower. Four cylindrical intermittent flow perspex respirometers (45 cm length and 29 cm diameter) (Figure 2.4 a) were placed into adjacent 250 L glass water-baths, which were lined with black plastic sheeting to avoid the fish from detecting one another. Each respirometer consisted of a flush pump (saltwater submersible) inlet which pumped water into the respirometer chamber after each measurement period, a recirculation loop made from clear oxygen-impermeable material, a peristaltic pump (masterflex HV-07522 with 4 multi-channel F/S pump) connected to a FireSting02 oxygen sensor (FSO2-4, Pro Science GmbH), which was used to take measurements of oxygen concentration from the recirculation loop connected to the respirometer chamber (Figure 2.4 b). Pro Science GmbG was used to read oxygen measurements taken by the FireSting oxygen sensor every five seconds. An external heat pump was used to adjust the temperature according to the experimental needs.

(a) (b)

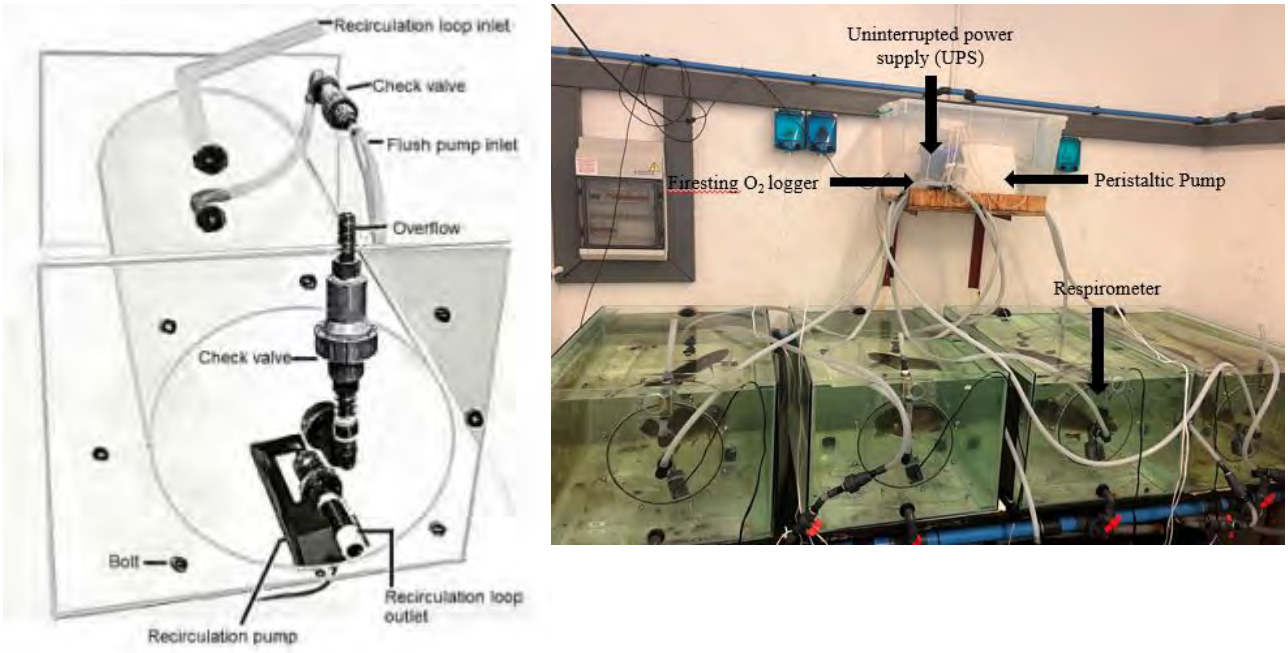



**Figure 2.4** Experimental set-up with a) an intermittent flow respirometer (from Nabani 2023), b) four respirometers, peristaltic pump, and oxygen sensor.

*2.7.2 Respirometry Experimental Procedure*

Of the twenty-nine experimental fish, eight were selected for respirometry experiments. Fish were fasted 36 hours prior to the experiments to reduce postprandial effects. After this fasting period, four fish were placed into the respirometry chambers (one fish per respirometry chamber). Respirometer lids were sealed shut using bolts and fish were allowed to acclimate to their new environment for 12 hours at 18 °C. After the acclimation period, the temperature was either increased or decreased at 1°C per hour until the test temperature (12 °C and 24 °C) was reached. This rate of change is used to simulate a marine upwelling or marine heatwave commonly experienced by these fish in the wild. The fish were given six hours (18 °C treatment) or 24 hours (12 °C and 24 °C) to acclimate to their new thermal environment.

During experiments, the rate of oxygen consumption ($MO_2$) was recorded every five seconds over a 15-minute measurement period followed by a 15-minute flush period at 18 °C. At 12 °C the measurement period was adjusted to 20 minutes followed by a 10-minute flush period. At

24 °C the measurement period was adjusted to 5 minutes followed by a 15-minute flush period. The oxygen measurements for SMR were taken at night between 02:00 and 07:00 when fish were less active and undisturbed. Following the SMR measurement phase, individual fish were removed and placed into the well-aerated 1000 L cylindrical plastic-lined tank (Figure 2.5), where it was then chased with a net for 10 minutes or until exhaustion (loss of equilibrium) and exposed to air for 30 seconds before being returned to the respirometer. After the chamber was sealed (within 3-4 minutes) $MO_2$ were measured for 8 minutes. Following this 8-minute measurement period, the respirometer was placed back onto the intermittent flush-measure cycle and fish were left in the respirometers for a further 4 hours to recover from the MMR procedure.

After the recovery period, critical $O_2$ tension (P crit) was measured for fish at the extreme temperatures (12 °C and 24 °C). To do this, the flush was switched off eliminating the flow of oxygenated seawater into each of the respirometers. The fish were then observed for any behavioural changes in response to the declining oxygen levels. Once the fish started showing signs of loss of equilibrium or erratic swimming behaviour, the time and $O_2$ concentrations were recorded, and the flush was switched back on. Following this procedure, fish were returned to their holding tanks to recover. Due to the extreme stress associated with this method, it was decided that a repeated measures approach would not be implemented, to prevent unnecessary stress or mortality. Batch A was tested at 12 °C and Batch B was tested at 24 °C. P crit was conducted as the final respirometry experiment for each batch. Once individuals were removed from the respirometers, the empty respirometers were sealed, and oxygen was measured for 3 hours after each MMR measurement to account for any background respiration.

Once removed from the respirometers, fish were placed back into their housing tanks for a recovery period of seven days before the process was repeated at a different test

temperature. All fish were exposed to the three test temperatures following a repeated measures approach.

**Figure 2.5** A volunteer (Samkele Ngcefa) chasing a dusky kob (*Argyrosomus japonicus*) to exhaustion as part of the protocol for estimating the maximum metabolic rate (MMR).

### *2.7.3 Data Handling*

Data generated throughout the respirometry experiments consisted of a series of measurements and flushing periods (Figure 2.6). The RespR package was used to estimate SMR and MMR in RStudio software (Harianto et al. 2019) and a quality threshold of $R^2 > 0.9$ was used to filter a linear decline in oxygen.

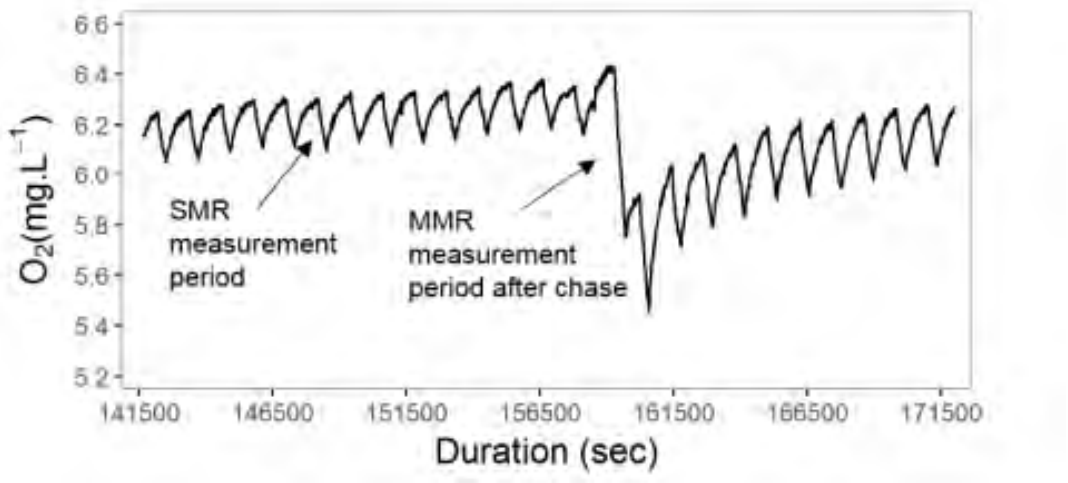



**Figure 2.6** Example of an intermittent flow respirometry dataset (from Duncan et al. 2019). Each decline represents the measurement period and increases in oxygen concentration represent the flush period. The steep decline in oxygen concentration represents the measurement period during the maximum metabolic rate procedure.

The rate of oxygen consumption ($mg.kg^{-1}.h^{-1}$) for each measurement period ($RO_2$) was calculated using Equation 2.1 which was adapted from (Svendsen et al. 2019). To determine the effect of mass on metabolic rates, temperature was corrected following equation 2.2 (Clarke and Johnston 2002). $RO_2$ data was then mass corrected ($MO_2$) using the mass scaling relationship using Equation 2.3. Aerobic scope was determined by subtracting the mass normalised metabolic rates using Equation 2.4.

$$RO_2 = \left(\left(\frac{V_{re} - M}{W}\right)\left(\frac{\Delta[O_{2a}]}{\Delta t}\right)\right) - \left(\left(\frac{V_{re} - M}{W}\right)\left(\frac{\Delta[O_{2b}]}{\Delta t}\right)\left(\frac{V_{re}}{(V_{re} - M)}\right)\right)$$

(Equation 2.1) where $V_{re}$ is the total volume of the respirometer in litres; M is the mass of the specimen in kg expressed as I; W is the mass of the specimen in kg, $\frac{\Delta[O2a]}{\Delta t}$ is the slope of the linear decrease in oxygen concentration during the measurement period and $\frac{\Delta[O2b]}{\Delta t}$ is the slope of the linear decline in oxygen concentration when the specimens were removed from the respirometers (background respiration).

$$RO_{2(\text{temp corrected})} = RO_2 \times \frac{-E}{e^{kt}}$$

(Equation 2.2) where E is the average activation energy of ectotherms, k is the Boltzmann constant, and T is the absolute temperature in kelvin.

$$MO_2 = \frac{RO_2}{M^a}$$

(Equation 2.3) Where $MO_2$ is mass normalised SMR or MMR, $RO_2$ is either standard or maximum metabolic rate, M is the mass of the fish and α is the allometric mass scaling.

$$AS = MMR - SMR$$

(Equation 2.4) Where MMR is the mass normalized maximum metabolic rate, and SMR is the mass normalized standard metabolic rate.

Three models were tested to determine the most appropriate representation of the relationship between temperature and metabolic rate across fish. The first model was a linear mixed-effects model using the *lme4* package, with temperature as a fixed effect and fish id as a random intercept. This model allowed for individual variability in baseline metabolic rate among fish while estimating a common slope for the temperature effect. To capture potential variation in the temperature response across fish, a model with temperature as both a fixed effect and a random slope was fit. However, results showed zero variance for the slope term, indicating that the temperature effect did not vary significantly between fish. Therefore, this model was not pursued further. The final model included a temperature as a fixed effect and a random intercept and slope for temperature across individual fish and had a superior fit.

To identify physiological phenotypes, the percentile-based ranking method was employed for SMR, MMR, and AS at 12, 18, and 24 °C (Bailey 2023, Nabani 2023). The percentile ranks were converted into scores ranging from 1 to 4 based on the quartile divisions. For SMR, fish

in the 0-25$^{th}$ percentile received a score of 4, fish in the 26$^{th}$-50$^{th}$ percentile received a score of 3, fish in the 51$^{st}$-75$^{th}$ percentile received a score of 2, and fish in the 76$^{th}$-100$^{th}$ percentile received a score of 1. For MMR, and AS, fish in the 0-25$^{th}$ percentile received a score of 1, fish in the 26$^{th}$-50$^{th}$ percentile received a score of 2, fish in the 51$^{st}$-75$^{th}$ percentile received a score of 3, and fish in the 76$^{th}$-100$^{th}$ percentile received a score of 4. Rankings for SMR, MMR, and AS across all temperatures were then combined and ranked to determine a total SMR, MMR, and AS rank for each fish.

*2.7.4 Linking thermal tolerance to respirometry*

To compare individual fish across the two physiological methods (thermal tolerance and respirometry), a percentile-based ranking approach was employed (Bailey, 2023; Nabani, 2023). Using Excels Rank and Percentile function; each fish's percentile rank was calculated within its respective metric. The percentile ranks were converted into scores ranging from 1 to 4 based on the quartile divisions. The individual ranks for all metrics were then summed for each fish to create a total rank sum. This total provided an overall ranking for each fish across the different metrics. The sum rank provides a robust determination of an individual's overall ranking (it's physiological phenotype) based on all of the metrics.

To evaluate the accuracy of each metric in determining individual physiological phenotypes, the rank of each fish at each metric was compared with the sum rank. If the metric rank for each fish aligned with the sum rank, it was scored a 1, and if it did not align, it was scored a 0. The scores were then summed for each fish. Thus, if the ranks for a metric matched the sum rank for each of the eight fish that metric would score an 8. Conversely, if the ranks of the metric did not match the sum rank for any of the fish, the overall score for the metric would be 0.

*2.7.5 Determining the optimal method to predict individual physiological performance based on accuracy, stress, and duration of experiment*

To evaluate the level of stress associated with each metric, a scaling method was used that considered overall handling, confinement, and thermal stress. To determine the level of stress involved with overall handling, four stress-inducing factors were considered, netting (number of times the individual was netted during the experiment), handling (number of times the individual was physically handled), air exposure (instances of air exposure ≥30 seconds per trial), and chasing (number of times the individual was chased per trial). These were then summed to generate a total score, which was then ranked based on the percentile-based method. The percentile ranks were converted into scores ranging from 0 to 3, with 0 representing extreme levels of handling, 1 indicating high levels, 2 reflecting intermediate levels, and 3 low levels of handling. Metrics in the 0-25th percentile received a score of 3, metrics in the 26th-50th percentile received a score of 2, metrics in the 51st-75th percentile received a score of 1, and metrics in the 76th-100th percentile received a score of 0.

Confinement was scaled from 0 to 1, where 0 indicated no confinement, and 1 represented confinement (in this case, in a respirometer) (Portz et al. 2006). Thermal stress was scaled from 0 to 3, with 0 representing extreme thermal stress (where the fish loses equilibrium, Ern et al. 2023), 1 representing high thermal stress (close to the fish's thermal breakpoints (Allison et al. 2021, Figure 3.1), 2 reflecting intermediate thermal stress (below the fish's breakpoints, Allison et al. 2021, Figure 3.1), and 3 indicating no thermal stress (close to the fish's optimal temperature, which was determined to be between 17.5 °C and 18 °C, Acoustic Tracking Array Platform, unpublished data).

To evaluate the time taken to complete each trial, a scaling method was applied with a scale of 0 to 3. With 0 representing trials that took 72 hours or more, 1 representing trials that took 48

to 71 hours, 2 representing trials that took 25 to 47 hours, and 3 representing trials completed in less than 24 hours.

The accuracy, overall handling, confinement, thermal stress and time scores for each fish were then summed to generate a total score, allowing for the prediction of both the most accurate, benign, and time-effective metric. This total score helped to identify which metrics minimised stress, were limited in duration and maintained the highest levels of accuracy in predicting the physiological performance of individual fish.

# Chapter 3: Results

## 3.1 Thermal Tolerance

The $CT_{max}$ of individuals exhibited variability within the sampled population, ranging from 31.7 °C to 33.7 °C with a mean of 32.7 °C (SD = 0.59) (Table 3.1). Similarly, the $CT_{min}$ of individuals also varied among individuals and ranged between 7.5 °C and 8.6 °C with a mean of 8.2 °C (SD = 0.28) (Table 3.1). The thermal breadth among the sampled fish ranged from 23.3 °C to 26.0 °C, with a mean thermal breadth of 24.5°C (SD = 0.69) (Table 3.1). There was a weak negative correlation between fish length and CTmin (r = - 0.177), there was a weak positive correlation between CTmax and fish length (r = 0.126), and there was also a weak positive correlation between thermal breadth and fish length (r = 0.181).

**Table 3.1** Details of individual *Argyrosomus japonicus* exposed to thermal tolerance trials, including fish ID, fish length (mm total length, TL), critical thermal minima ($CT_{min}$) critical thermal maxima ($CT_{max}$), thermal breadth (difference between $CT_{min}$ and $CT_{max}$) and phenotypic ranking based on thermal breadth (1 being a low ranked individual, narrow thermal breadth and 4 being a high ranked individual, broad thermal breadth). Fish selected for respirometry are indicated by an asterisk.

| Fish ID | Fish length (mm TL) | $CT_{max}$ | $CT_{min}$ | Thermal Breadth | Phenotypic Ranking |
|---|---|---|---|---|---|
| 1* | 426 | 32.7 | 8.2 | 24.5 | 3 |
| 2* | 425 | 31.9 | 8.6 | 23.3 | 1 |
| 3* | 445 | 33.6 | 8.3 | 25.3 | 3 |
| 4* | 430 | 32.5 | 8.2 | 24.3 | 2 |
| 5* | 434 | 32.3 | 7.8 | 24.5 | 3 |
| 6* | 430 | 31.7 | 8.1 | 23.6 | 2 |
| 7* | 435 | 33.7 | 7.9 | 25.8 | 4 |
| 8* | 445 | 32.6 | 8.1 | 24.5 | 3 |
| 9 | 417 | 32.9 | 7.7 | 25.2 | 4 |
| 10 | 430 | 33.3 | 8.3 | 25 | 3 |
| 11 | 420 | 32.1 | 8.1 | 24 | 2 |
| 12 | 425 | 32.6 | 8.6 | 24 | 1 |
| 13 | 435 | 32.5 | 8.4 | 24.1 | 1 |
| 14 | 465 | 33 | 8.1 | 24.9 | 4 |
| 15 | 450 | 32 | 8.5 | 23.5 | 1 |
| 16 | 449 | 33.2 | 8.1 | 25.1 | 4 |
| 17 | 442 | 33.3 | 8.4 | 24.9 | 2 |
| 18 | 460 | 32.4 | 8.3 | 24.1 | 2 |
| 19 | 413 | 32.5 | 8.6 | 23.9 | 1 |
| 20 | 435 | 33.3 | 8.1 | 25.2 | 4 |
| 21 | 480 | 33.7 | 7.7 | 26 | 4 |
| 22 | 479 | 32.6 | 8.3 | 24.3 | 2 |
| 23 | 487 | 32.6 | 8.4 | 24.2 | 1 |
| 24 | 440 | 32.6 | 8.6 | 24 | 1 |
| 25 | 454 | 33.3 | 8 | 25.3 | 4 |
| 26 | 444 | 32 | 8.1 | 23.9 | 2 |
| 27 | 445 | 32 | 8.3 | 23.7 | 1 |
| 28 | 460 | 31.7 | 7.5 | 24.2 | 2 |
| 29 | 426 | 33.2 | 8.2 | 25 | 3 |

### *3.1.1 Thermal tolerance phenotypic ranking*

Using the percentile ranking method of individual $CT_{min}$ and $CT_{max}$ endpoints, individual variability of thermal tolerance was observed within the sampled population (Table 3.3). Using $CT_{min,}$ a large proportion 44.2% (n = 13) were ranked as high performers, 27.6% (n = 8) ranked

as low performers, 17.2% (n = 5) ranked as low-intermediate performers, followed by 10.3% (n = 3) ranked as high-intermediate performers (Table 3.3). Using $CT_{max}$ estimates, the majority, 34.5% (n = 10) of individuals were ranked as low-intermediate performers, 24.1% (n = 7) ranked as low performers, 24.1% (n = 7) ranked as high performers, followed by 17.2% (n = 5) ranked as high-intermediate performers (Table 3.3). The $CT_{min}$ and $CT_{max}$ rankings were combined to get an overall ranking, which showed that 27.6% (n = 8) were ranked as low performers, 27.6 (n = 8) were ranked as low-intermediate performers, 24.1% (n = 7) were ranked as high performers, followed by 20.7% (n = 6) ranked as high-intermediate performers (Table 3.3). Some individuals exhibited low rankings in either $CT_{min}$ or $CT_{max}$ but performed well in the other, and vice versa, while others ranked highly in both $CT_{min}$ and $CT_{max}$ Table 3.3). A total of 13.8% (n = 4) of individuals ranked highly for $CT_{min}$ but poorly for $CT_{max}$, indicative of "cold-adapted" individuals (Table 3.3). A total of 3.4% (n = 1) individuals ranked highly for $CT_{max}$ but poorly for $CT_{min}$, representing "warm-adapted" individuals (Table 3.3). Additionally, 13.8% (n = 4) individuals ranked highly in both $CT_{min}$ and $CT_{max}$, classifying them as high thermal performers (Table 3.3).

*3.1.2 Ventilation rate*

Opercular beat (OB) rates ranged from six beats $min^{-1}$ at 11 °C to 107 beats $min^{-1}$ at 33° C (Figure 3.1). A significant positive relationship was found between opercular beat and temperature ($R^2 = 0.619$, p-value < 0.01, Table 3.2). A piecewise linear breakpoint analysis indicated a departure from linearity at 12 °C and 30 °C (Figure 3.1). A slight increase in OB rates was recorded below 12 °C, where signs of thermal stress, such as loss of coordination, reduced movement, and occasional surface bobbing, were observed. These activities continued and got progressively worse until $CT_{min}$ was reached. Opercular beat rates above 28 °C increased rapidly in conjunction with increased swimming activity and decreased

orientation until $CT_{max}$ was reached. Ventilation rate was not included in the calculation of individual rankings as this analysis was done at a population level only.

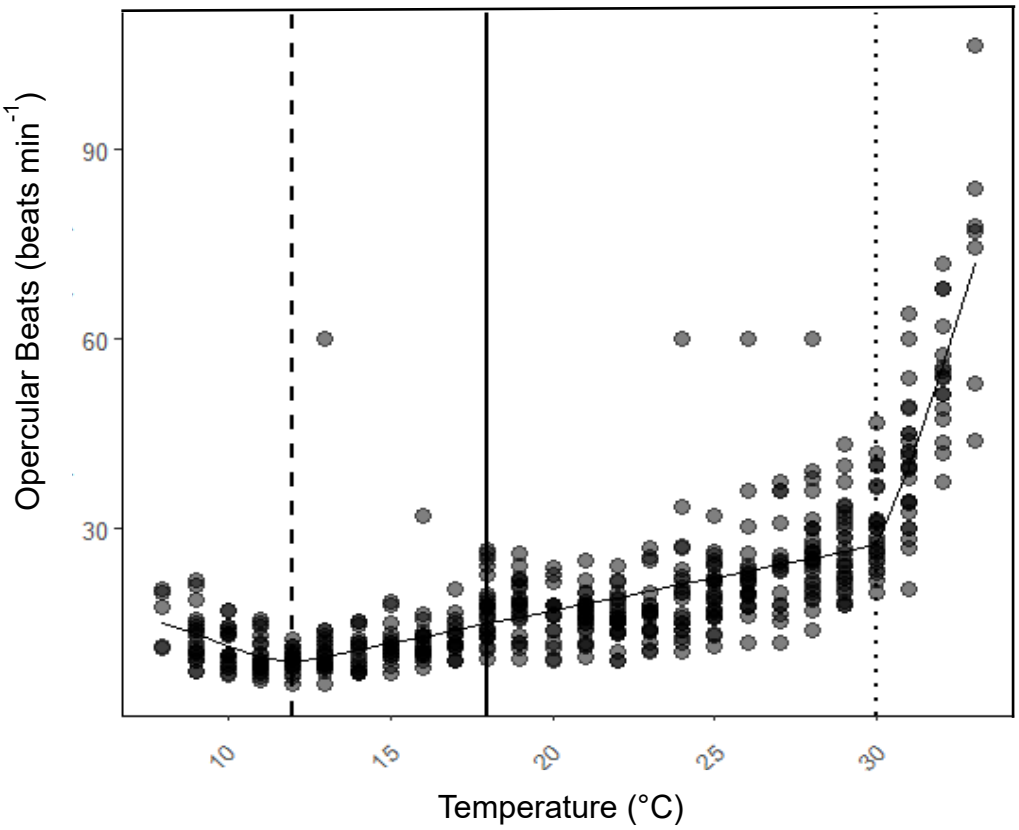



**Figure 3.1** Opercular beats rates (beats $min^{-1}$) recorded on twenty-nine *Argyrosomus japonicus* individuals during the critical-thermal-limit tests at each temperature change (increase or decrease by 1 °C), including linear regression (black line) and breakpoint for the maximum (dotted line), and minimum (dashed line) critical thermal limit tests. The centre line indicates the acclimation and starting temperature at (18 °C).

**Table 3.2** Linear regression analyses examining the relationship between temperature and log-transformed opercular beats of *Argyrosomus japonicus* with significant p-values in bold.

| | **lnOB** | | | |
|---|---|---|---|---|
| **Predictors** | **Estimates** | **SE** | **t-value** | **P** |
| (Intercept) | 1.61 | 0.05 | 34.92 | **<0.001** |
| temperature | 0.06 | 0.00 | 28.72 | **<0.001** |
| Observations | 509 | | | |
| $R^2$ / $R^2$ adjusted | 0.619 / 0.619 | | | |
| AIC | 326.779 | | | |

**Table 3.3** Individual phenotypic classification of *Argyrosomus japonicus* based on the percentile ranking of critical thermal upper ($CT_{max}$) and lower ($CT_{min}$) end points. A rank of 4 denotes a high-ranked individual (high performer), a rank of 2 and 3 denotes intermediate-ranked individuals (intermediate performers), and a rank of 1 denotes a low-ranked individual (low performer). Ranking classification adopted from Bailey 2023. Fish selected for respirometry are indicated by an asterisk.

| Fish ID | Phenotypic ranking at $CT_{min}$ | Phenotypic ranking at $CT_{max}$ | Phenotype Ranking |
|---|---|---|---|
| 1* | 3 | 3 | 3 |
| 2* | 1 | 1 | 1 |
| 3* | 2 | 4 | 3 |
| 4* | 3 | 2 | 2 |
| 5* | 4 | 2 | 3 |
| 6* | 4 | 1 | 2 |
| 7* | 4 | 4 | 4 |
| 8* | 4 | 2 | 3 |
| 9 | 4 | 3 | 4 |
| 10 | 2 | 4 | 3 |
| 11 | 4 | 1 | 2 |
| 12 | 1 | 2 | 1 |
| 13 | 1 | 2 | 1 |
| 14 | 4 | 3 | 4 |
| 15 | 1 | 1 | 1 |
| 16 | 4 | 3 | 4 |
| 17 | 1 | 4 | 2 |
| 18 | 2 | 2 | 2 |
| 19 | 1 | 2 | 1 |
| 20 | 4 | 4 | 4 |
| 21 | 4 | 4 | 4 |
| 22 | 2 | 2 | 2 |
| 23 | 1 | 2 | 1 |
| 24 | 1 | 2 | 1 |
| 25 | 4 | 4 | 4 |
| 26 | 4 | 1 | 2 |
| 27 | 2 | 1 | 1 |
| 28 | 4 | 1 | 2 |
| 29 | 3 | 3 | 3 |

### 3.2 Respirometry

*3.2.1 Mass correcting $MO_2$ data*

There was limited variation in the non-mass-corrected SMR data (Figure 3.2a). There was no significant relationship between the natural logarithm of temperature-corrected, non-mass corrected SMR and mass for the sampled population ($R^2 = 0.02$, p-value < 0.001) (Figure 3.2b). Consequently, SMR for the sampled population was assumed to scale linearly with mass, eliminating the need for an allometric scaling correction (Figure 3.2c).

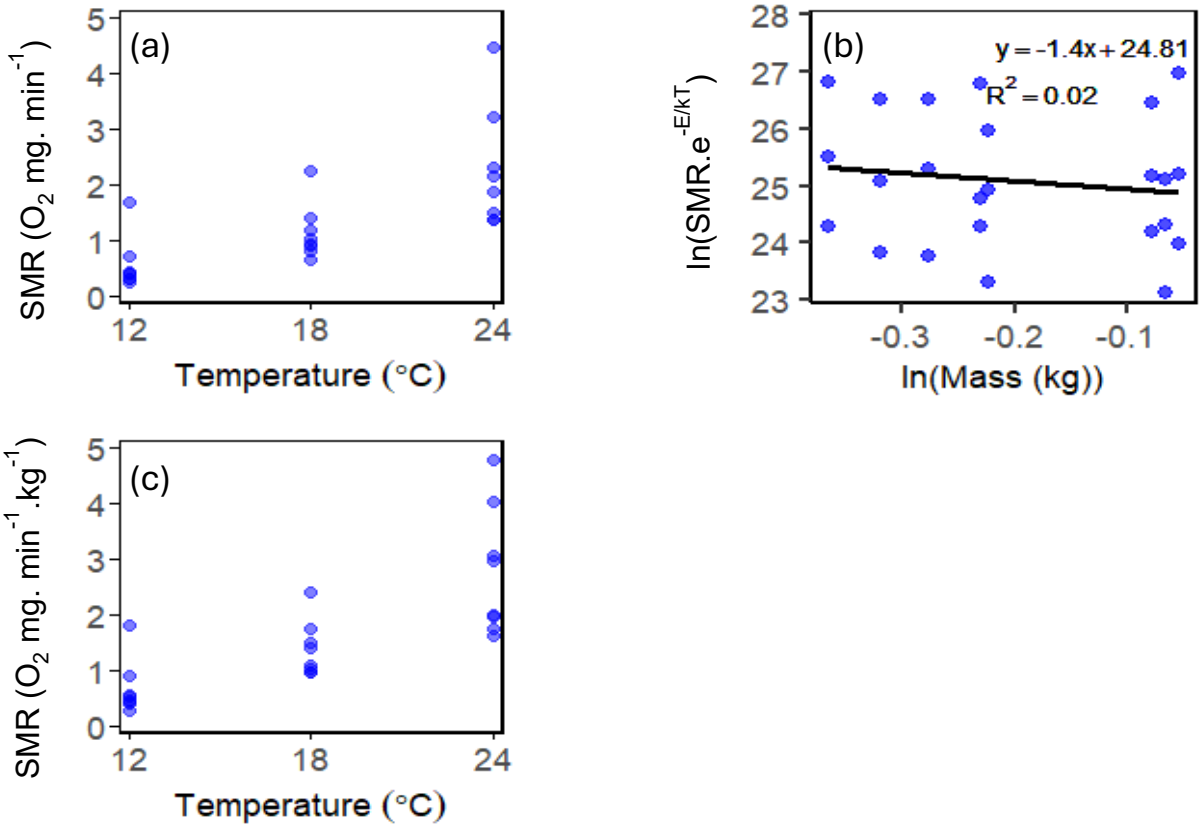



**Figure 3.2** Mass correcting standard metabolic rate (SMR) data process, showing raw SMR data (SMR ($O_2$ mg.min$^{-1}$)) per temperature (a), regression of the natural logarithm of temperature-corrected SMR (ln(SMR.eE/kT)) against the natural logarithm of mass (ln(Mass(kg)) (b), and mass-corrected SMR data (SMR($O_2$ mg.min$^{-1}$.kg$^{-1}$)) per temperature (c).

temperature-corrected non-mass corrected MMR and mass for the sampled population ($R^2$ = 0.05, p-value < 0.01) (Figure 3.3b). Consequently, MMR for the sampled population

was assumed to scale linearly with mass, eliminating the need for an allometric scaling correction (Figure 3.3c).

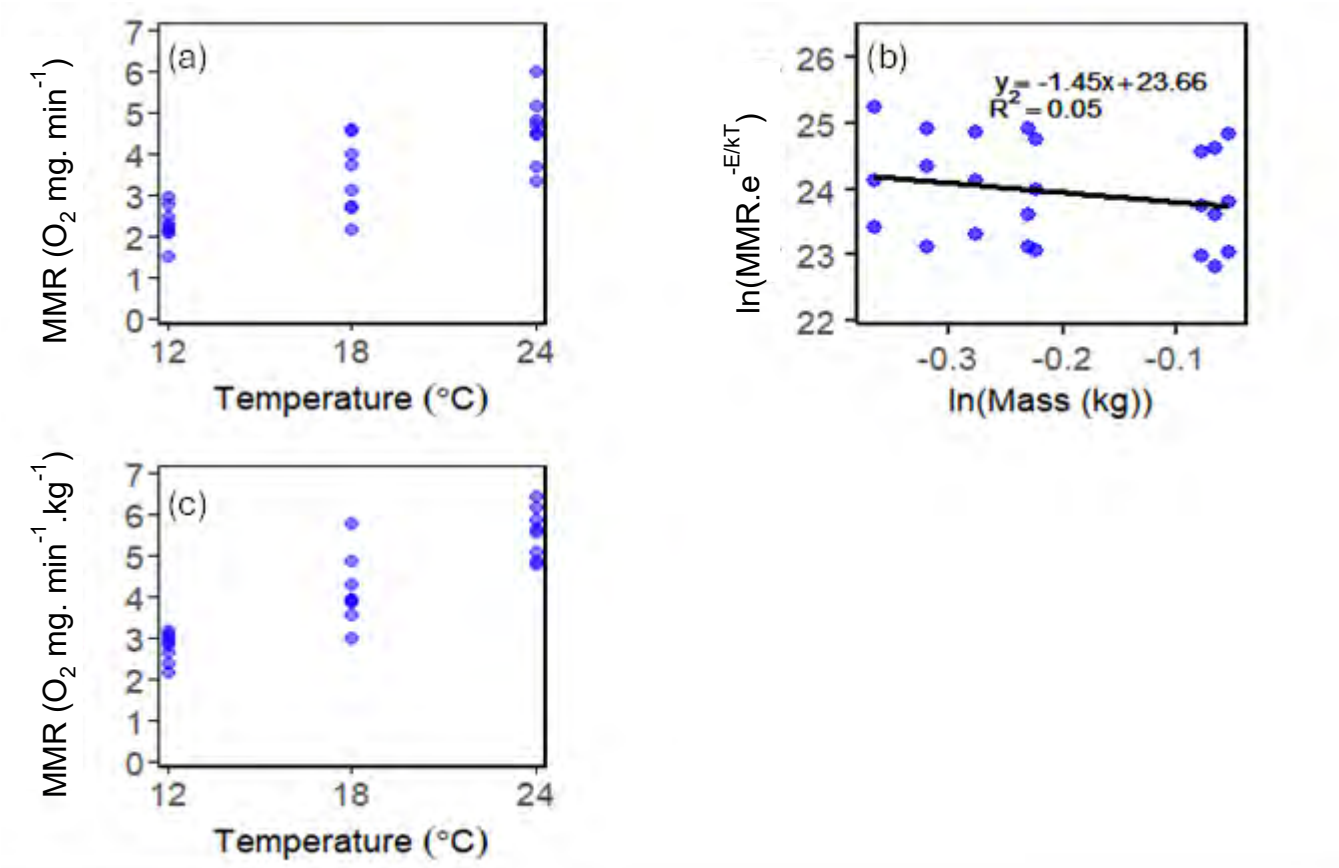



**Figure 3.3** Mass correcting maximum metabolic rate (MMR) data process showing raw MMR data (MMR($O_2$ mg.$min^{-1}$)) per temperature (a), regression of the natural logarithm of temperature-corrected MMR (ln(MMR.eE/kT)) against the natural logarithm of mass (ln(Mass(kg)) (b), and mass-corrected MMR data (MMR($O_2$ mg.$min^{-1}$.$kg^{-1}$)) per temperature used by the analysis (c).

*3.2.2 Effect of temperature on standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS)*

Standard metabolic rate (SMR) increased with temperature and ranged from 0.28 to 1.82, 0.97 to 2.41, and 1.63 to 4.77 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ at 12, 18 and 24 °C, respectively (Figure 3.4a, Table 3.4). The linear relationship between temperature and SMR was significant (p-value < 0.001, Table 3.5), yet the polynomial relationship between SMR and temperature was not significant (p-value = 0.131, Table 3.5). There was notable individual variation in mass-corrected SMR across temperatures (Figure 3.5a)

**Table 3.4** Aerobic scope (AS), standard metabolic rate (SMR), and maximum metabolic rate (MMR) measured at three temperature conditions (12 °C, 18 °C, and 24 °C) for eight individual fish measured in $O_2$ mg. $min^{-1}$ .$kg^{-1}$.

| Fish ID | AS12 | AS18 | AS24 | SMR12 | SMR18 | SMR24 | MMR12 | MMR18 | MMR24 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2.24 | 1.55 | 3.20 | 0.59 | 1.42 | 2.99 | 2.83 | 2.97 | 6.19 |
| 2 | 2.24 | 4.01 | 3.90 | 0.40 | 1.77 | 1.75 | 2.64 | 5.78 | 5.65 |
| 3 | 2.17 | 2.41 | 1.83 | 0.93 | 1.50 | 4.03 | 3.10 | 3.91 | 5.86 |
| 4 | 1.70 | 2.86 | 2.80 | 0.45 | 0.98 | 1.98 | 2.16 | 3.84 | 4.78 |
| 5 | 2.36 | 2.48 | 1.82 | 0.55 | 1.10 | 3.06 | 2.91 | 3.58 | 4.88 |
| 6 | 1.14 | 2.47 | 1.65 | 1.82 | 2.42 | 4.78 | 2.97 | 4.88 | 6.43 |
| 7 | 2.69 | 3.26 | 3.94 | 0.49 | 1.04 | 1.63 | 3.17 | 4.30 | 5.57 |
| 8 | 2.11 | 2.98 | 3.10 | 0.28 | 0.97 | 2.00 | 2.40 | 3.95 | 5.10 |

Maximum metabolic rate (MMR) also increased with temperature and ranged from 2.16 to 3.17 $O_2$ mg. $min^{-1}$ .$kg^{-1}$, 2.97 to 5.78 $O_2$ mg. $min^{-1}$ .$kg^{-1}$, and 4.78 to 6.43 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ at 12, 18 and 24 °C respectively (Figure 3.4b, Table 3.4). The linear relationship between temperature and MMR was significant (p-value < 0.001, Table 3.5), while the polynomial relationship between MMR and temperature was not significant (p-value = 0.957, Table 3.5). There was notable individual variation in mass-corrected MMR across temperatures (Figure 3.5c).

The aerobic scope (AS) of *A. japonicus* ranged from 1.14 to 2.69, 1.55 to 4.01, 1.65 to 3.94 $O_2$ mg.$min^{-1}$ .$kg^{-1}$ at 12, 18, and 24 °C, respectively (Table 3.4). There was an increase in AS of *A. japonicus* with temperature (Figure 3.4c). The linear relationship between temperature and AS was significant (p-value = 0.022, Table 3.5) but, the polynomial relationship between temperature and AS was not significant (p-value = 0.201, Table 3.5). There was notable individual variation in mass-corrected AS across temperature (Figure 3.5b)

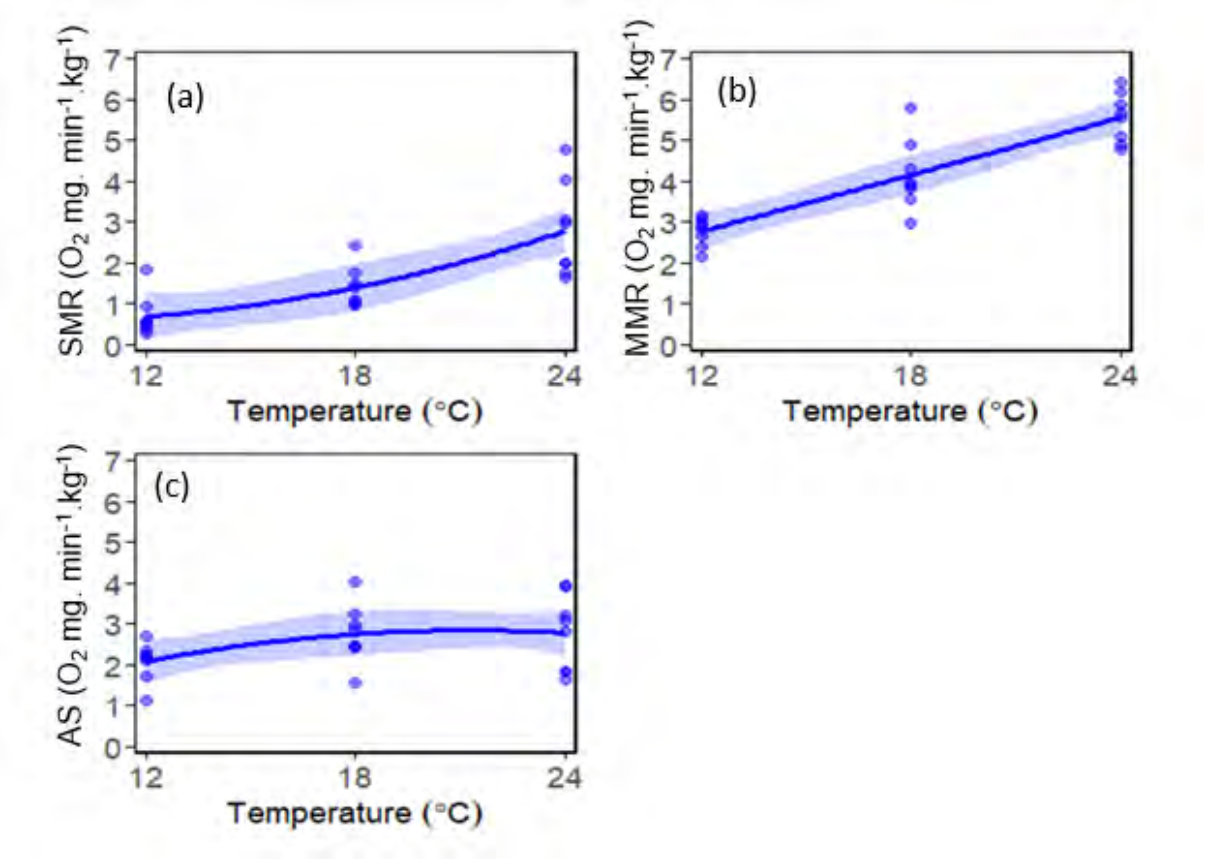



**Figure 3.4** Linear mixed effects models for the standard metabolic rate (SMR) (a), maximum metabolic rate (MMR) (b), and aerobic scope (AS) (c), for *Argyrosomus japonicus* population across different temperatures (12, 18 and 24 °C) with second order polynomial regression and shaded regions representing 95% confidence intervals.

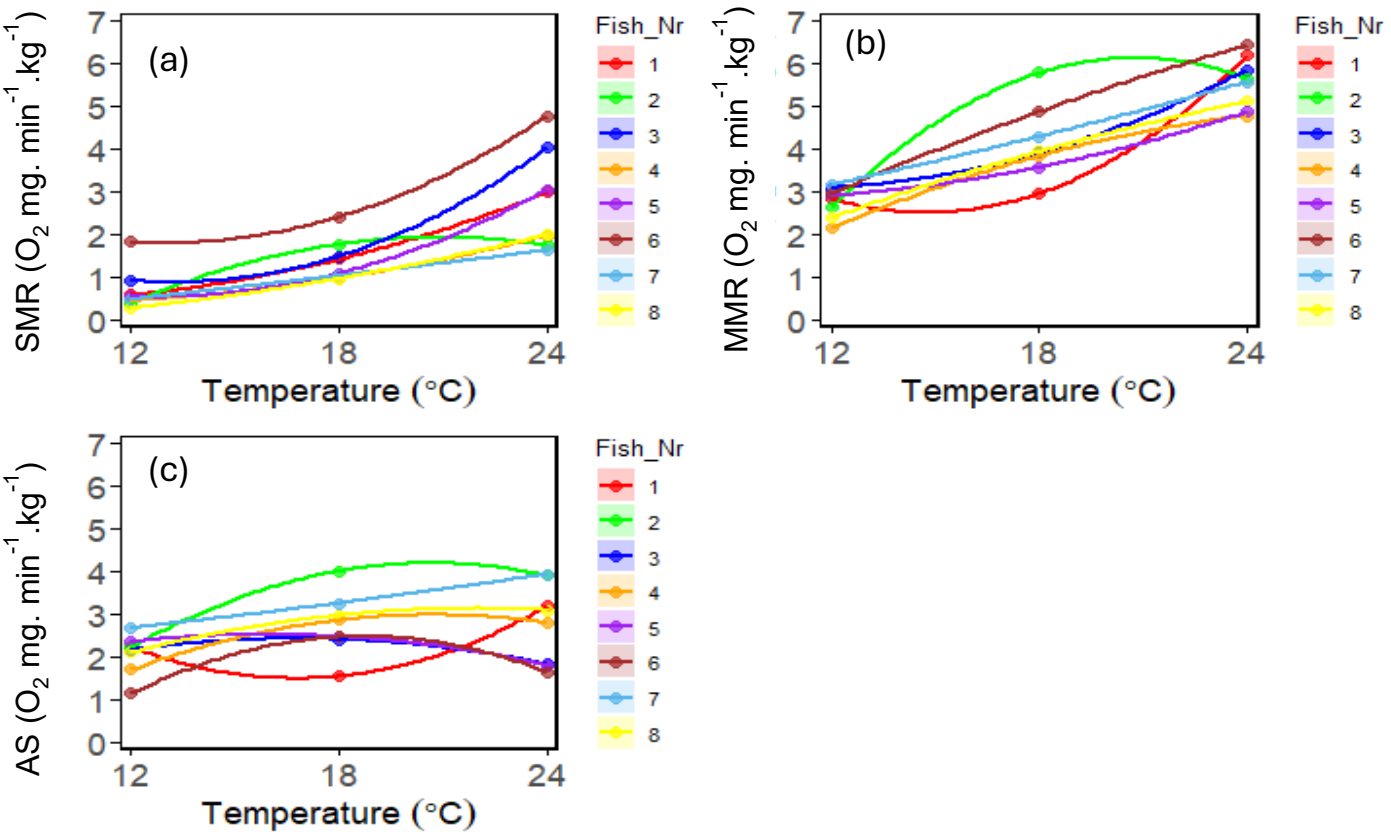



**Figure 3.5** Individual variation in mass-corrected standard metabolic rate (SMR) for ($SMR(O_2.min^{-1}.kg^{-1})$) (a), maximum metabolic rate (MMR) ($MMR(O_2\,mg.min^{-1}.kg^{-1})$) (b), and aerobic scope (AS) ($AS(O_2\,mg.min^{-1}.kg^{-1})$) (c) for *Argyrosomus japonicus*.

**Table 3.5** Linear mixed effects model modelling the effect of temperature on standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) of *Argyrosomus japonicus* with significant p-values in bold.

| Parameter | Predictor | Estimate | SE | t-value | P |
|---|---|---|---|---|---|
| **SMR** | Intercept | 1.62 | 0.24 | 6.85 | **<0.001** |
| | Temp (1st degree) | 4.18 | 0.49 | 8.55 | **<0.001** |
| | Temp (2nd degree) | 0.77 | 0.49 | 1.58 | 0.131 |
| **Random Effects (SMR)** | | | | | |
| | $\sigma^2$ | 0.24 | | | |
| | τ00 Fish.Nr | 0.37 | | | |
| | ICC | 0.61 | | | |
| | $R^2$ (Marg./Cond.) | 0.563 / 0.829 | | | |
| | AIC | 54.793 | | | |
| **MMR** | Intercept | 4.16 | 0.15 | 27.28 | **<0.001** |
| | Temp (1st degree) | 5.57 | 0.57 | 9.69 | **<0.001** |
| | Temp (2nd degree) | 0.03 | 0.57 | 0.05 | 0.957 |
| **Random Effects (MMR)** | | | | | |
| | $\sigma^2$ | 0.33 | | | |
| | τ00 Fish.Nr | 0.08 | | | |
| | ICC | 0.19 | | | |
| | $R^2$ (Marg./Cond.) | 0.769 / 0.812 | | | |
| | AIC | 53.194 | | | |
| **AS** | Intercept | 2.54 | 0.20 | 12.66 | **<0.001** |
| | Temp (1st degree) | 1.40 | 0.56 | 2.50 | **0.022** |
| | Temp (2nd degree) | -0.74 | 0.56 | -1.32 | 0.201 |
| **Random Effects (AS)** | | | | | |
| | $\sigma^2$ | 0.31 | | | |
| | τ00 Fish.Nr | 0.22 | | | |
| | ICC | 0.41 | | | |
| | $R^2$ (Marg./Cond.) | 0.170 / 0.510 | | | |
| | AIC | 56.258 | | | |

*3.2.3 Respirometry phenotypic ranking*

Using the percentile method, of SMR, MMR and AS recorded at 12, 18, and 24°C, two individuals were classified as high performers (Rank 4), four as intermediate performers (Rank 2 and 3), and two as low performers (Rank 1) for each metabolic metric measured at each temperature (Table 3.6). Considerable variation was observed in the rankings of metabolic rates within and between individuals (Table 3.6).

Standard metabolic rate at 12°C ranged between 0.28 and 0.40 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ for high performers, 0.45 and 0.59 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ for intermediate performers, followed by 0.93 and 1.82 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ for low performers (Table 3.6). Standard metabolic at 18°C ranged between 0.97 and 0.98 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ for high performers, 1.04 and 1.50 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ for intermediate performers, followed by 1.77 and 2.42 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ for low performers (Table 3.7). Standard metabolic rate at 24°C ranged between 1.63 and 1.75 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ for high performers, 1.98 and 3.06 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ for intermediate performers, followed by 4.03 and 4.78 $O_2$ mg. $min^{-1}$ .$kg^{-1}$ for low performers (Table 3.7). There was limited plasticity in SMR across temperatures for most individuals (Table 3.6). The majority (87.5%, n = 7) maintained relatively consistent rankings across all temperatures, indicating stable metabolic responses. In contrast, a single individual (12.5%, n = 1) exhibited high SMR performance at thermal extremes but not at the optimal temperature (Table 3.6).

For MMR at 12, 18, and 24°C, two individuals were classified as high performers, four intermediate performers, and two as low performers (Table 3.6). MMR at 12°C ranged between 3.10 and 3.17 $O_2$ mg. $min^{-1}$.$kg^{-1}$ for high performers, 2.91 and 2.83 $O_2$ mg. $min^{-1}$.$kg^{-1}$ for intermediate performers, followed by 2.16 and 2.40 $O_2$ mg. $min^{-1}$.$kg^{-1}$ for low performers (Table 3.7). MMR at 18°C ranged between 4.88 and 5.78 $O_2$ mg. $min^{-1}$.$kg^{-1}$ for high performers, 3.95 and 3.91 $O_2$ mg. $min^{-1}$.$kg^{-1}$ for intermediate performers, followed by 2.97 and 3.58 $O_2$ mg. $min^{-}$

$^{1}$.kg$^{-1}$ for low performers (Table 3.7). MMR at 24°C ranged from 6.19 and 6.43 $O_2$ mg. min$^{-1}$.kg$^{-1}$ for high performers, 5.65 and 5.57 $O_2$ mg. min$^{-1}$.kg$^{-1}$ for intermediate performers, followed by 4.78 and 4.88 $O_2$ mg. min$^{-1}$.kg$^{-1}$ for low performers (Table 3.7). There was considerable inter-individual plasticity in MMR, with some individuals exhibiting high performance at thermal extremes, while others maintained consistent rankings across all temperatures. While the majority of fish (75% n = 6) maintained relatively stable MMR rankings across all temperatures, two individuals (25%, n = 2) exhibited high performance at thermal extremes but not at the optimal temperature (Table 3.6).

For AS at 12, 18, and 24°C, two individuals were classified as high performers, four individuals as intermediate performers, and two as low performers (Table 3.6). AS at 12°C ranged between 2.36 and 2.69 $O_2$ mg. min$^{-1}$ .kg$^{-1}$ for high performers, 2.237 and 2.17 $O_2$ mg. min$^{-1}$ .kg$^{-1}$ for intermediate performers, and between 1.14 and 1.70 $O_2$ mg. min$^{-1}$ .kg$^{-1}$ for low performers (Table 3.7). AS at 18°C ranged between 3.26 and 4.01 $O_2$ mg. min$^{-1}$ .kg$^{-1}$ for high performers, 2.86 and 2.48 $O_2$ mg. min$^{-1}$ .kg$^{-1}$for intermediate performers, and between 1.55 and 2.41 $O_2$ mg. min$^{-1}$ .kg$^{-1}$for low performers (Table 3.7). AS at 24°C ranged between 3.90 and 3.94 $O_2$ mg. min$^{-1}$ .kg$^{-1}$ for high performers, 3.10 and 2.80 $O_2$ mg. min$^{-1}$ .kg$^{-1}$ for intermediate performers, followed by 1.65 and 1.82 $O_2$ mg. min$^{-1}$ .kg$^{-1}$ for low performers (Table 3.7). The observed plasticity in AS across temperatures displayed varying individual responses. Most of individuals (n = 6) maintained relatively stable rankings across temperatures. However, one individual (12.5%, n = 1) ranked high at the low temperature but low at the high temperature, one individual (12,5% n = 1) ranked relatively high at the extreme temperatures but low at the optimal temperature (Table 3.6).

### 3.3 Linking thermal tolerance and respirometry

The comparison (link) between thermal tolerance and respirometry metrics of the eight sampled fish exhibited variability between methods and temperatures within each method, with the metrics of some fish aligning well and others not (Table 3.6, Table 3.7).

Fish 1 scored an overall sum rank of 2, suggesting that this fish was an overall low-intermediate performer (Table 3.6, Figure 3.6). There was alignment in the ranking of its physiological metrics at low temperatures ($CT_{min}$ and MMR 12, Table 3.6 and 3.7, Figure 3.6), as well as its thermal breadth (TB), SMR across all temperatures including Total SMR and Total MMR (Table 3.8, Figure 3.8). This fish was the only fish to exhibit an inverse AS curve (Figure 3.6).

Fish 2 received an overall sum rank of 3, suggesting that it is an overall high-intermediate performer (Table 3.6, Figure 3.6). However, this fish was ranked as a low performer for the thermal tolerance metrics (Rank 1) and a high performer for aerobic scope (Rank 4). Only SMR18 aligned with the low ranked thermal tolerance metrics (Table 3.6, Figure 3.6).

Fish 3 received an overall sum rank of 1, indicating that it was a low performer overall (Table 3.6 and 3.7, Figure 3.6). This fish exhibited consistent low rankings for $CT_{min}$, AS across all temperatures, total AS, SMR across all temperatures and total SMR. Despite this, it showed high performance in $CT_{max}$, TB, and MMR12.

Fish 4 received an overall sum rank of 2, suggesting that this fish was an overall low-intermediate performer (Table 3.6 and 3.7, Figure 3.6). This individual displayed relatively consistent rankings across most physiological metrics, with low-intermediate performance in $CT_{min}$, $CT_{max}$, and TB (Table 3.6 and 3.7, Figure 3.6). Its AS, SMR, and MMR fluctuated across temperatures, performing better at optimal temperatures and slightly reduced performance at extreme temperatures (Table 3.6, Figure 3.6).

Fish 5 received an overall sum rank of 2, suggesting that it is a low-intermediate performer overall (Table 3.6 and 3.7, Figure 3.6). This individual showed relatively stable rankings across most physiological metrics, with $CT_{max}$ and TB aligning with AS18, Total AS, SMR at extreme temperatures, and Total SMR (Table 3.6, Figure 3.6). This individual appeared to perform better at lower temperatures scoring high in $CT_{min}$, AS12, and MMR12. Notably its MMR and AS scored extremely low at higher temperatures (Table 3.6, Figure 3.6).

Fish 6 received an overall sum rank of 1, indicating it is a low performer (Table 3.6 and 3.7, Figure 3.6). This individual consistently ranked low, exhibiting weak performance in $CT_{max}$ and TB, aligning with AS at 12 and 24 °C, total AS, SMR across all temperatures and total SMR. However, despite its generally low performance, this fish exhibited high MMR across all temperatures.

Fish 7 received an overall sum rank of 4, suggesting an overall high performer (Table 3.6, Figure 3.6). This individual displayed exceptional high performance across most of the physiological metrics. This individual consistently ranked high in all thermal tolerance metrics ($CT_{min}$, $CT_{max,}$ and TB), AS across all temperatures, and total AS. Its SMR remained relatively stable across temperatures, ranking moderately high, while its MMR ranked high at low temperatures its rank declined at the high temperature (Table 3.6) (Figure 3.6).

Fish 8 received an overall sum rank of 3, suggesting an overall high-intermediate performer (Table 3.6 and 3.7, Figure 3.6). This individual displayed relatively balanced performance across most physiological metrics, with $CT_{min}$ and $CT_{max}$ aligning with AS at 18 and 24 °C, SMR 24, and MMR18 (Table 3.6, Figure 3.6). This individuals SMR was consistently high at 12, and 18 °C, while its MMR showed moderate performance ranking slightly lower at low temperatures (Table 3.6, Figure 3.6).

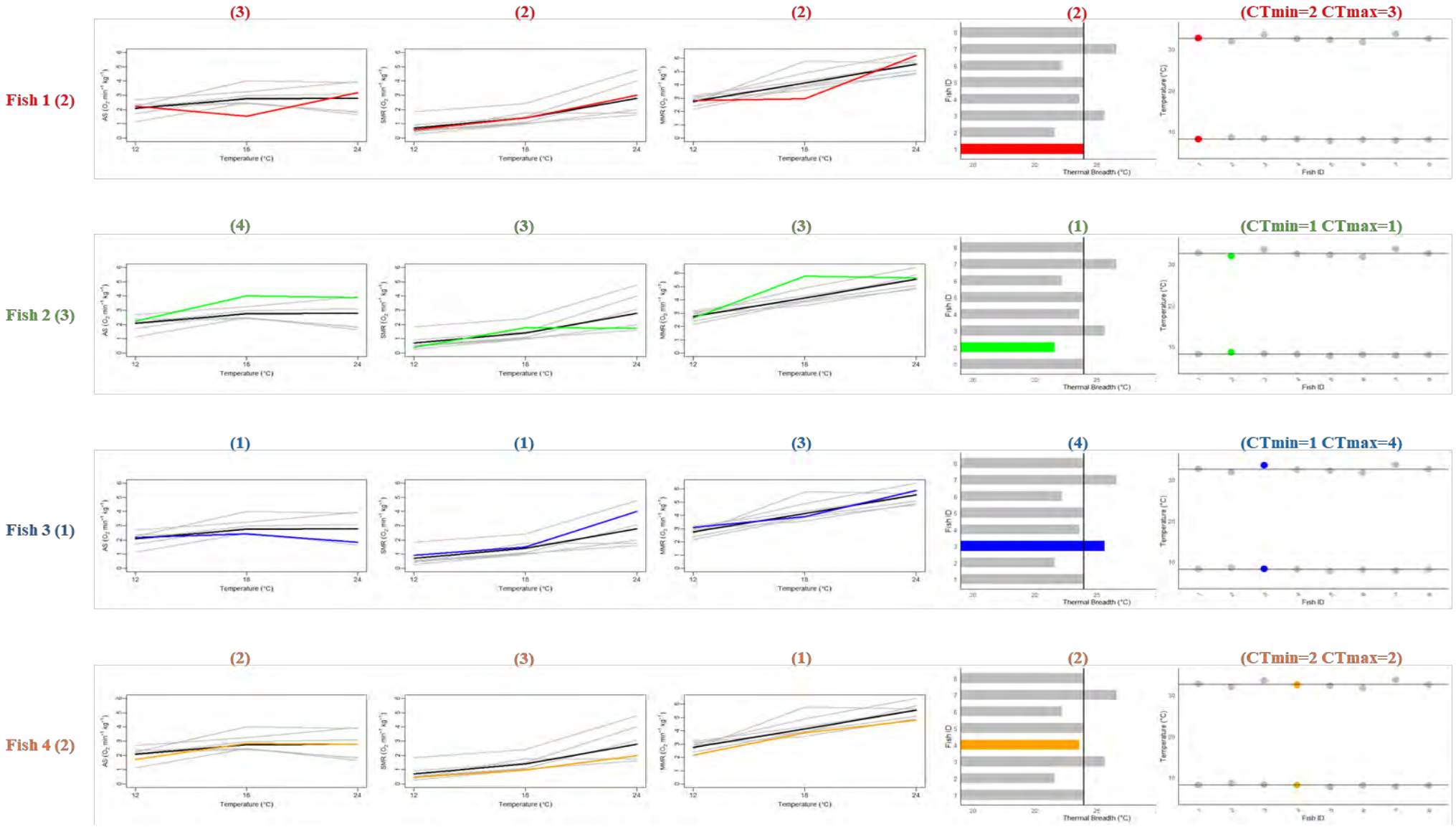
Among the physiological metrics analysed, total AS exhibited the highest accuracy score (6) suggesting it may be the most reliable metric for determining physiological phenotypes (Table 3.6). $CT_{min}$, $CT_{max}$ and thermal breadth (TB) followed closely, each with an accuracy score of 5, indicating reliability in assessing physiological phenotypes. Total SMR also scored 5, suggesting its potential utility at determining physiological phenotypes (Table 3.6). AS 18, AS 24, and SMR-related metrics (SMR 12, SMR 18, and SMR 24) had low accuracy scores (2-4) (Table 3.6). Collectively, these results suggest that total AS, $CT_{min}$, $CT_{max}$, and TB are the most accurate at determining physiological phenotypes.

**Table 3.6** Individual phenotypic classification of *Argyrosomus japonicus* based on percentile ranking of critical thermal minima ($CT_{min}$), maxima ($CT_{max}$), thermal breadth (TB), aerobic scope (AS) at 12, 18, and 24 °C, total AS, standard metabolic rate (SMR) at 12, 18, and 24 °C, total SMR, maximum metabolic rate (MMR) at 12, 18, and 24 °C, total MMR, and sum rank. A rank of 4 denotes a high ranked individual (i.e., high performer), rank 2 and 3 denotes intermediate ranked individuals (i.e., intermediate performer), a rank of 1 denotes low ranked individual (i.e., low performer). Ranking classification adapted from Bailey 2023.

| Fish ID | $CT_{min}$ | $CT_{max}$ | TB | AS12 | AS18 | AS24 | Total AS | SMR12 | SMR18 | SMR24 | Total SMR | MMR12 | MMR18 | MMR24 | Total MMR | Sum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 2 | 3 | 1 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 1 | 4 | 2 | 2 |
| 2 | 1 | 1 | 1 | 3 | 4 | 4 | 4 | 4 | 1 | 4 | 3 | 2 | 4 | 3 | 3 | 3 |
| 3 | 1 | 4 | 4 | 2 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 4 | 2 | 3 | 3 | 1 |
| 4 | 2 | 2 | 2 | 1 | 3 | 2 | 2 | 3 | 4 | 3 | 3 | 1 | 2 | 1 | 1 | 2 |
| 5 | 4 | 2 | 2 | 4 | 2 | 1 | 2 | 2 | 3 | 2 | 2 | 3 | 1 | 1 | 1 | 2 |
| 6 | 3 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 4 | 4 | 4 | 1 |
| 7 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 3 | 4 | 3 | 2 | 3 | 4 |
| 8 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 3 | 4 | 1 | 3 | 2 | 2 | 3 |
| **Accuracy** | 5 | 5 | 5 | 3 | 4 | 4 | 6 | 4 | 2 | 4 | 5 | 2 | 2 | 1 | 2 | |

**Table 3.7** Details of individual *Argyrosomus japonicus* exposed to thermal tolerance and respirometry trials, Including fish ID, critical thermal minima ($CT_{min}$), critical thermal maxima ($CT_{max}$), thermal breadth (TB), aerobic scope (AS) at 12, 18, and 24 °C measured as $O_2$ mg. $min^{-1}$ .$kg^{-1}$, standard metabolic rate (SMR) at 12, 18, and 24 °C, measured as $O_2$ mg. $min^{-1}$ .$kg^{-1}$, and maximum metabolic rate (MMR) at 12, 18, and 24 °C, measured as $O_2$ mg. $min^{-1}$ .$kg^{-1}$.

| Fish ID | $CT_{min}$ | $CT_{max}$ | TB | AS12 | AS18 | AS24 | SMR12 | SMR18 | SMR24 | MMR12 | MMR18 | MMR24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8.2 | 32.7 | 24.5 | 2.24 | 1.55 | 3.20 | 0.59 | 1.42 | 2.99 | 2.83 | 2.97 | 6.19 |
| 2 | 8.6 | 31.9 | 23.3 | 2.24 | 4.01 | 3.90 | 0.40 | 1.77 | 1.75 | 2.64 | 5.78 | 5.65 |
| 3 | 8.3 | 33.6 | 25.3 | 2.17 | 2.41 | 1.83 | 0.93 | 1.50 | 4.03 | 3.10 | 3.91 | 5.86 |
| 4 | 8.2 | 32.5 | 24.3 | 1.70 | 2.86 | 2.80 | 0.45 | 0.98 | 1.98 | 2.16 | 3.84 | 4.78 |
| 5 | 7.8 | 32.3 | 24.5 | 2.36 | 2.48 | 1.82 | 0.55 | 1.10 | 3.06 | 2.91 | 3.58 | 4.88 |
| 6 | 8.1 | 31.7 | 23.6 | 1.14 | 2.47 | 1.65 | 1.82 | 2.42 | 4.78 | 2.97 | 4.88 | 6.43 |
| 7 | 7.9 | 33.7 | 25.8 | 2.69 | 3.26 | 3.94 | 0.49 | 1.04 | 1.63 | 3.17 | 4.30 | 5.57 |
| 8 | 8.1 | 32.6 | 24.5 | 2.11 | 2.98 | 3.10 | 0.28 | 0.97 | 2.00 | 2.40 | 3.95 | 5.10 |


(3)
(2)
(2)
(2)
(CTmin=2 CTmax=3)
Fish 1 (2)
(4)
(3)
(3)
(1)
(CTmin=1 CTmax=1)
Fish 2 (3)
(1)
(1)
(3)
(4)
(CTmin=1 CTmax=4)
Fish 3 (1)
(2)
(3)
(1)
(2)
(CTmin=2 CTmax=2)
Fish 4 (2)
Temperature (°C)
Thermal Breadth (°C)
Fish ID

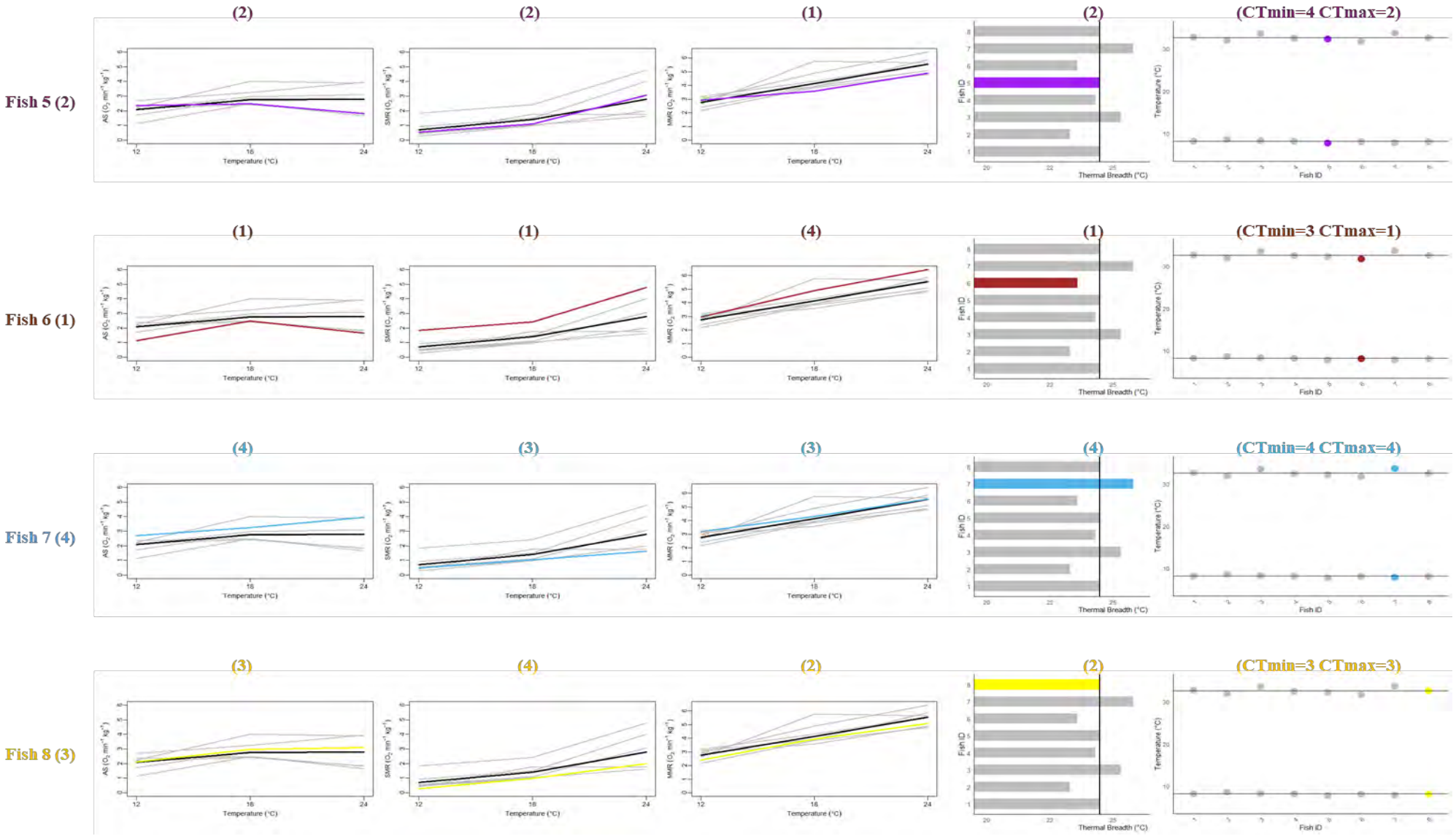

(2)
(2)
(1)
(2)
(CTmin=4 CTmax=2)
Fish 5 (2)
(1)
(1)
(4)
(1)
(CTmin=3 CTmax=1)
Fish 6 (1)
(4)
(3)
(3)
(4)
(CTmin=4 CTmax=4)
Fish 7 (4)
(3)
(4)
(2)
(2)
(CTmin=3 CTmax=3)
Fish 8 (3)
Temperature (°C)
Thermal Breadth (°C)
Fish ID

**Figure 3.6** Phenotypic performance profiles across total aerobic scope (AS), total standard metabolic rate (SMR), total maximum metabolic rate (MMR), thermal breadth (TB), and critical thermal limits ($CT_{min}$ and $CT_{max}$) for the eight sampled fish. The left panels display the thermal performance curves for total AS, total SMR, and total MMR for each fish, with their respective ranks indicated above each panel in brackets. The horizontal bar graphs depict the thermal breadth for each fish, with the rank displayed above the bar plot. The right panels illustrate critical thermal limits ($CT_{min}$ and $CT_{max}$) for each fish, with rankings for these metrics shown above the scatter plot. A black line representing the mean is included in each plot for reference. Each fish is represented by a unique colour corresponding to its ID: Fish 1 (red), Fish 2 (green), Fish 3 (blue), Fish 4 (orange), Fish 5 (purple), Fish 6 (brown), Fish 7 (teal), and Fish 8 (yellow). The sum rank for each fish, based on the overall phenotypic assessment, is displayed in brackets next to the fish ID in the left-most panel. Together, these plots highlight variability in metabolic and thermal performance metrics among individual fish and their alignment with rank-based phenotypic assessments.

### 3.4 Determining the optimal method to assess individual physiological performance based on accuracy, stress, and time scaling.

To determine the optimal method to assess individual physiological performance based on accuracy, stress, and time scaling, accuracy, overall handling (Table 3.8), confinement, and thermal stress were summed to generate a total score to indicate metrics that are more accurate, minimally invasive, time-efficient, and induce less stress (Table 3.9).

Based on accuracy, stress, and time scaling, $CT_{min}$ and $CT_{max}$ scored the highest overall (12 total points), driven primarily by its accuracy (5 points), handling (3 points), and time (3 points) (Table 3.9). Thermal breadth and SMR18 followed closely (10 total points) (Table 3.9). The metrics with the lowest total scores were total MMR (4), and AS12 (5), this was due to their low accuracy scores, increased time demands, and increased stress associated with these metrics (Table 3.9). These results indicate that $CT_{min}$ and $CT_{max}$ may be the least stressful and time-consuming metric while maintaining relative accuracy.

**Table 3.8** Summary of handling stress for *Argyrosomus japonicus* based on four stress-inducing factors: netting (number of times an individual was netted during the experiment), handling (number of times an individual was physically manipulated with hands), air exposure (instances of air exposure ≥30 seconds per trial), and chasing (number of times an individual was chased during trial). The table includes handling scores for critical thermal minima ($CT_{min}$), maxima ($CT_{max}$), thermal breadth (TB), aerobic scope (AS) at either 12, 18, and 24 °C, total AS, standard metabolic rate (SMR) at either 12, 18, and 24 °C, total SMR, maximum metabolic rate (MMR) at either 12, 18, and 24 °C, and total MMR. The total column represents the sum of all stress-inducing factors, while the rank column denotes the percentile-based stress score representing overall handling.

| Metric | Netting | Handling | Air exposure | Chasing | Total | Rank |
|---|---|---|---|---|---|---|
| $Ct_{min}$ | 2 | 0 | 0 | 0 | 2 | 3 |
| $CT_{max}$ | 2 | 0 | 0 | 0 | 2 | 3 |
| SMR | 2 | 1 | 0 | 0 | 3 | 2 |
| TB | 4 | 0 | 0 | 0 | 4 | 2 |
| MMR | 2 | 1 | 1 | 1 | 5 | 1 |
| Total SMR | 6 | 3 | 0 | 0 | 9 | 1 |
| Total MMR | 6 | 3 | 3 | 3 | 15 | 0 |
| Total AS | 12 | 6 | 3 | 3 | 24 | 0 |

**Table 3.9** Evaluation of metric performance in accuracy[1], handling[2], confinement[3], thermal stress[4], and time[5] scaling, to optimise stress minimization, accuracy, and efficiency. The metric performance is based on thermal tolerance trials (critical thermal minima ($CT_{min}$), critical thermal maxima ($CT_{max}$), difference in $CT_{max}$ and $CT_{min}$ i.e., thermal breadth (TB)) and respirometry trials (standard metabolic rate (SMR), maximum metabolic rate (MMR), difference in MMR and SMR i.e., aerobic scope (AS) measured at 12, 18, and 24 °C) of *Argyrosomus japonicus.* Total is the sum of all individual scores for each metric, providing a composite ranking of their overall suitability. Higher total scores indicate metrics that are more accurate, minimally invasive, time-efficient, and induce less stress.

| Metric | Accuracy | Overall Handling | Confinement | Thermal stress | Time | Total |
|---|---|---|---|---|---|---|
| $CT_{max}$ | 5 | 3 | 1 | 0 | 3 | 12 |
| $CT_{min}$ | 5 | 3 | 1 | 0 | 3 | 12 |
| TB | 5 | 2 | 1 | 0 | 2 | 10 |
| SMR24 | 4 | 2 | 0 | 2 | 2 | 10 |
| MMR18 | 2 | 1 | 0 | 3 | 3 | 9 |
| SMR12 | 4 | 2 | 0 | 1 | 2 | 9 |
| SMR18 | 2 | 2 | 0 | 3 | 2 | 9 |
| AS18 | 4 | 0 | 0 | 3 | 1 | 8 |
| AS24 | 4 | 0 | 0 | 2 | 1 | 7 |
| Total AS | 6 | 0 | 0 | 1 | 0 | 7 |
| MMR24 | 1 | 1 | 0 | 2 | 3 | 7 |
| MMR12 | 2 | 1 | 0 | 1 | 3 | 7 |
| Total SMR | 5 | 1 | 0 | 1 | 0 | 7 |
| AS12 | 3 | 0 | 0 | 1 | 1 | 5 |
| Total MMR | 2 | 0 | 0 | 1 | 1 | 4 |

[1] Accuracy reflects on how precise the metric was at identifying phenotypes, a score of 1 indicates low accuracy, whereas a score of 8 indicates high accuracy.
[2] Handling assesses the amount of physical manipulation required during trials, a score of 3 indicates minimal handling, while a score of 0 represents high levels of handling during trials.
[3] Confinement evaluates whether the fish was confined or not, a score of 1 signifies that the fish was not confined, whereas a score of 0 indicates confinement during trials.
[4] Thermal stress quantifies the level of thermal exposure imposed on the fish, a score of 0 represents high thermal stress, whereas a score of 3 corresponds to minimal thermal stress.
[5] Time evaluates the total duration required to complete the measurement, a score of 3 indicates a short procedure, whereas a score of 0 indicates a lengthy procedure.

# Chapter 4: Discussion

The key physiological metrics confirmed that juvenile *A. japonicus* are eurythermal and adapted to the highly dynamic thermal environments that they inhabit. Despite the general pattern of eurythermy, there were substantial differences in the key physiological metrics, including $CT_{min}$, $CT_{max}$, aerobic scope (AS), standard metabolic rate (SMR), and maximum metabolic rate (MMR) between individual fish in this study. Nevertheless, the rank-sum approach (Bailey 2023) could be used to classify fish into a thermal performance phenotype, although some of the nuance around the phenotype was not incorporated. Despite the intraspecific variability in the various metrics, some metrics, such as $CT_{min}$ and $CT_{max}$, were more accurate in predicting the overall phenotypic classification. When handling, thermal stress confinement stress, and time taken during the measurement of each metric was also considered, $CT_{max}$ and $CT_{min}$ appeared to be the most appropriate metrics to link the physiological and wild behavioural phenotypes of fishes. Despite this conclusion, the considerable intraspecific variability observed in this study highlights the complexity of linking multiple physiological traits and emphasizes the need for further investigation into individual variability.

The thermal tolerance ($CT_{min}$ and $CT_{max}$) data indicated that juvenile *A. japonicus* generally exhibits a broad thermal tolerance, typical of a eurythermal species (Figure 3.6). This is not surprising given that this life stage generally occupies highly dynamic estuarine and nearshore environments, where rapid thermal fluctuations are commonplace (Griffiths 1996). Despite the general eurythermy, there was variability in the $CT_{min}$, $CT_{max}$ and thermal breadths with "warm-adapted" (high $CT_{min}$ and high $CT_{max}$, e.g. ID 17, Table 3.3), "cold-adapted" (low $CT_{min}$ and low $CT_{max}$, e.g. ID 6, Table 3.3), "broadly-adapted" (low $CT_{min}$ and high $CT_{max}$, e.g. ID 7, Table 3.3) and "narrowly-adapted" (high $CT_{min}$ and low $CT_{max}$, e.g. ID 2, Table 3.3) individuals

identified. Similarly, Stitt et al. (2014) found that brook trout (*Salvelinus fontinalis*), expressed differences in thermal tolerances amongst populations from different thermal environments. The high phenotypic diversity of *A. japonicus* in this study was somewhat surprising, especially given that the sampled (aquaculture spawned) population are from a limited adult pool (> eight parents) and the fish were reared in a relatively warm and stable thermal environment. These findings suggest that this species may have considerable adaptive potential to the changing thermal environment.

Individual-level information is critical for understanding the phenotypic diversity of thermal tolerance and may be important for understanding the potential resilience of fish populations to changing thermal conditions (Seebacher et al. 2015, Dunn et al. 2022). This information can only be achieved by implementing a repeated measures approach. While this experimental design is complicated, the characterisation of individual phenotypes allows for the assessment of how individuals may tolerate a range of temperatures (Mckenzie et al. 2020). Previous studies employing a repeated measures approach have also demonstrated substantial individual variability in thermal tolerance (Healy and Schulte 2012, Antilla et al. 2013), further supporting the idea that not all individuals within a population respond uniformly to temperature stress (Kerr et al. 2011). Understanding these individual-level variations in thermal tolerance could play a major role in predicting how populations may respond to climate change (Seebacher et al. 2015, Dunn et al. 2022). For example, identifying these individual phenotypes and how they will likely respond to thermal extremes is crucial for improving predictions of species' responses to climate change (Ward et al. 2016). Traditional models that assume homogeneity within populations risk overlooking critical differences in resilience among individuals (Ward et al. 2016). This understanding is particularly relevant for species experiencing range shifts such as the west coast dusky kob (*Argyrosomus coronus*) in Angola (Potts et al. 2014), as

differences in physiological performance may determine which individuals are more likely to migrate or persist under changing thermal regimes (Childs et al. 2015).

While the $CT_{min}$ and $CT_{max}$ metrics provide information on thermal tolerance, they do not provide insight into the response of fish to suboptimal thermal conditions. In this study, the breakpoint analyses of ventilation rate (Figure 3.1) identified sub-lethal limits at the population level, which indicated that fish may experience physiological stress well before reaching their upper and lower thermal limits. The average breakpoints in ventilation rate (12 and 30°C Figure 3.1) were therefore most likely a representation of the species thermal niche. Indeed Childs (2013) using acoustic telemetry in an Eastern Cape estuary found that juvenile *A. japonicus* moved into the nearshore marine environment in summer months when estuarine temperatures rose above 26°C, which aligned with the upper breakpoint. Similarly, fish moved back into the estuarine environment in response to upwelling events, where the coastal temperatures declined rapidly (Childs 2013), likely attaining the lower breakpoint. Therefore, metrics such as ventilation rate break points can provide useful information from which to predict the wild behaviour of fishes.

Although individual break points in respiration rate were not obtained in this study, the identification of individual thermal niches (by estimating lower and upper respiration rate breakpoints) has potential for studies linking physiology and behaviour of fishes. Given that the breakpoints occur at sub-lethal temperatures and the methodology is associated with reduced handling and the fish are not subjected to considerable thermal stress or confinement stress, it is likely that this method and metric is highly suited for this kind of research and should be investigated.

Like the $CT_{min}$ and $CT_{max}$ data, the respirometry results, which indicated high performance across a broad thermal gradient (Figure 3.6), supported the notion that juvenile *A. japonicus*

were eurythermal. There was also no noticeable decline in the AS of *A. japonicus* at the highest test temperature, suggesting that the species was capable of performance at high temperatures. However, it must be recognised that these fish were reared in an aquaculture facility at temperatures that are warmer (5 – 7 °C) than their natural environment. This may suggest that the sampled population may have been acclimated to warm temperatures through their lives and that there may have been some selection towards warm water performance phenotypes. A comparative study using wild fish would be necessary to better understand the likely changes in performance as the thermal conditions in their natural environment.

In addition to the similar population level results for the endpoint and respirometry experiments, there was also high intraspecific variability in the respirometry data (see Figure 3.5). While standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) generally increased with temperature, individual fish exhibited a range of metabolic performance profiles. Some individuals (e.g. Fish 2 and 7, Figure 3.6, Table 3.6) had consistently high AS across all test temperatures, while others (e.g. Fish 4, Figure 3.6, Table 3.6) showed reduced performance (AS) at the upper and lower test temperatures. Norin et al (2014) found similar phenotypic variability the SMR, MMR and AS of barramundi (*Lates calcarifer*) when faced with thermal and osmoregulatory stress. They concluded that this phenotypic diversity in key physiological traits facilitated plasticity to environmental change in the species.

When the individual thermal performance curves were examined (Figure 3.5), it was clear that some species performed better at warmer temperatures (e.g. Fish 2), others at cooler temperatures (e.g. Fish 5), some performed the best at extreme temperatures (e.g. Fish 1), while others performed across the test temperatures (e.g. Fish 7). This variation in thermal performance is positive as it suggests that at least some individuals in the population may be able to perform under most climate change scenarios. Ultimately, understanding what

proportion of the population can adapt to a predicted climate change scenario will be an essential component of fish conservation and management in the Anthropocene (Schulte and Healy 2022). To predict likely future for juvenile *A. japonicus* and to develop appropriate management and conservation strategies, it is essential to extend this research to include the wild population of this species.

It is possible that these discrepancies in the alignment of metrics with the phenotypic classification can be attributed to physiological trade-offs, where optimizing one physiological function may come at the expense of another (Pörtner and Knust 2007, Verberk et al. 2015). For example, the high AS of fish 2 at 18° and 24° C indicates that the individual could perform (e.g. grow, swim, Pörtner and Farrel 2008, Clark et al. 2013, Bates & Morley 2020) well at normal and warm conditions, but its low ranking for $CT_{min}$, $CT_{max}$ and thermal breadth suggests that its ability to tolerate thermal extremes would be poor (Figure 3.6). This individual phenotype, despite a relatively high sum rank, may be vulnerable to climate change impacts, such as an increase in the frequency and intensity of marine heatwaves and cold upwelling events, which are increasingly predicted along the South African coastline (Goschen et al. 2012, van der Walt 2021). Phenotypes with high aerobic capacity, which supports energy-demanding activities such as swimming, growth, and reproduction, often have low thermal breadths, which will ultimately limit their ability to survive at extreme temperatures (Sunday et al. 2012, Pörtner et al. 2017, Neubauer and Anderson 2019).

In contrast to the high aerobic capacity, low thermal breadth phenotypes, some fish may exhibit lower aerobic capacity but possess greater thermal tolerance, allowing them to withstand extreme temperatures despite having reduced overall metabolic performance. For example, the thermal breadth of Fish 3 was ranked high and was particularly tolerant of high temperatures, its AS rank was low across all test temperatures (Figure 3.6, Table 3.6). This phenotype may therefore persist in a warming environment; however, its performance (activity, growth,

reproduction) may be relatively poor. Similarly, Fish 5 was particularly tolerant of cold temperatures and may survive an increasingly variable thermal environment, but its performance (AS) was relatively poor at cool and warm temperatures (Table 3.6, Figure 3.6). Somero (2010) reviewed the physiological trade-offs associated with thermal adaptation and suggested that species with broad thermal tolerance often exhibit reduced aerobic capacity, which may limit their overall performance in stable environments.

The variation observed between individual fish amongst the different physiological metrics raises a few questions about the underlying drivers of these trade-offs (Pörtner and Knust 2007). For example, could acclimation history influence the discrepancies observed between respirometry and thermal tolerance (Zhou et al. 2019)? This could challenge the selection of traits that reliably predict performance. Studies have shown that extended acclimation periods to elevated temperatures alter metabolic traits, including AS and critical thermal endpoints (Nyboer & Chapman 2017, Kır et al. 2017, Moyano et al. 2017, Li et al. 2021). In this study, an example may be Fish 4 (Table 3.6, Figure 3.6), had the highest ranked AS only at 18 °C, which was the acclimation temperature, yet performed relatively poorly for most other metrics. While this fish will likely be successful in environments with constant temperatures (e.g. in an aquaculture environment), it is unlikely to be competitive in the thermally dynamic natural environment.

Given the links between fish physiology, behaviour, fitness and environmental conditions (Horodysky et al. 2015, Bailey et al. 2022), understanding physiological phenotypic plasticity across temperatures is critical. However, a range of repeated measures experiments, while necessary for categorising the physiological phenotype is complex, time consuming and challenging. For example, the mortality of some individuals during a highly stressful experiment can derail the entire phenotypic classification process. To simplify the methodology, it may be necessary to understand the relationships between the various

physiological metrics, so that a reduced experimental procedure can be implemented to assess the physiological phenotypes to link this to the behaviour in the wild of a fish in a follow-up experiment.

In this study, some individuals exhibiting strong alignment across the different metrics whereas others showed little to no alignment. When examining the relationships between all the metrics at an individual level (Table 3.6), the sum rank (which was a composite representing the overall physiological phenotype) aligned with some metrics, but not for all individuals. For example, Fish 7 had a high sum rank (4), and this aligned with its ranking for $CT_{min}$, $CT_{max}$, TB, AS, SMR24, and MMR12. In contrast, Fish 3 had a low sum rank (1) but the highest ranking for and $CT_{max}$, TB and MMR12 (Table 3.6). However, when one examined the overall patterns (Table 3.6) it was clear that total AS (followed by $CT_{min}$, $CT_{max}$ and thermal breadth) was the best predictor of the overall physiological performance of *A. japonicus.* The efficacy of total AS is not surprising as it is increasingly being used as a tool to address questions relating to fish physiology and the effects of climate change (Clarke et al 2013) and this has given rise to the idea that the physiological capacity of fishes has evolved so that AS is maximised over a specific thermal range. This is termed the Oxygen- and Capacity-Limited Thermal Tolerance (OCLTT) theory (Baktoft et al. 2016, Pörtner et al. 2017) and it proposes that as temperatures deviate from an optimal range, an organism's ability to supply oxygen to tissue becomes increasingly constrained, limiting overall performance and survival. However, while total AS (i.e. AS across a thermal gradient) may be a good predictor of the physiological phenotype of an individual, its utility in research that links the physiological and behavioural phenotype may be limited.

The assessment of physiological performance prior to an assessment of behaviour in the wild, for example, using acoustic telemetry, would require a balance between accuracy, limiting stress, and being of short duration, to ensure that the individual's behaviour in the wild is not

compromised. One of the key findings of this study was that $CT_{min}$, $CT_{max}$ and thermal breadth metrics were effective in predicting the physiological phenotype, were relatively benign and were quick to conduct, while the total AS was highly stressful and required extended acclimation and testing periods (Table 3.9). Thermal tolerance experiments, particularly when combined with ventilation rate (given that OB provides insights into thermal stress prior to lethal limits) therefore emerge as a potential and promising assessment for determining physiological performance, particularly when prioritizing minimally invasive and time-consuming techniques.

From the perspective of the application of the physiological phenotype information, the observed intraspecific variability in thermal tolerance and the mean thermal breakpoints in ventilation rate (i.e., thermal niche rather than thermal tolerance) may provide insight into the partial migration strategy observed in fishes (Johnson and Johnson 1993, Kerr et al. 2009, Chapman et al. 2012b, 2012a, Maggs et al. 2019). For *A. japonicus*, Childs et al. (2015) found that some individuals migrate in response to environmental changes (movers) while others remain within their existing habitats (stayers). Such a behavioural strategy has been attributed to conditional strategy where an individual's genetic make-up allows for the adoption of resident or migratory behaviour based on the interaction between an individual's physiology and its environment (Kerr et al. 2009). Freitas et al. (2021) and Dunn et al. (2022) suggested that the divergence in thermal breadth and resilience for the individuals with a broad thermal range could play a key role in shaping movement patterns, habitat use, and overall population dynamics in response to climate change. Indeed, in the case of *A. japonicus*, based on the diversity in the thermal performance (AS) across the thermal gradient, it is likely that those that only perform well across a narrow range are most likely move to more favourable thermal conditions. Thus, the intrapopulation physiological diversity is the most probable explanation for the "movers" and "stayers".

There were several limitations to this study, which may have impacted the findings. Unlike wild populations that face constant environmental challenges, such as varying oxygen levels, marine upwellings and marine heatwaves, aquaculture-reared fish are raised in relatively stable and predictable conditions (Tidwell 2012). These conditions may result in less phenotypic variability or reduced adaptive potential (Hallerman 2008). Nyboer and Chapman (2017) found that the metabolic performance of Nile perch (*Lates niloticus*) in Lake Victoria exhibited changes in metabolic traits, including aerobic scope (AS) and critical thermal maxima ($CT_{max}$) when exposed to elevated acclimation temperatures for extended periods. These findings suggest that acclimation duration can influence the physiological responses of fish to temperature, which may have implications for accurately predicting thermal performance in both wild and aquaculture populations. In the case of this study, the *A. japonicus* were all raised under stable thermal conditions (~ 23°C). These stable conditions most likely promoted the development of physiological trade-offs, less phenotypic variability, and generalist traits. This contrasts with wild fish that may develop specialised traits to deal with fluctuating environmental conditions. Therefore, the results of this study cannot fully reflect the physiological performance of wild populations of *A. japonicus*.

Another limitation of this study was the small sample size. Due to the extensive experimental procedure, with each fish subjected to five different experiments, it was not feasible to conduct this study using a larger number of fish. Only eight fish were used for the respirometry trials given the time constraints of the repeated measures approach, each fish being exposed to each temperature and logistical issues. While the study provides valuable insights and observations between the physiological metrics, a larger sample size could enhance the statistical power of this study, providing significant conclusions.

The methodology employed in this study, particularly the rank-sum approach, may not have fully captured the complexity of physiological phenotypes and the relationships between the

various metrics. The rank-sum method uses a conceptually simple analysis to develop comparability in the metrics and classify the physiological phenotypes but may overlook subtle interactions between the different traits (Bailey 2023). For example, in this study, we encountered difficulty in distinguishing between certain phenotypes and inconsistencies in the rankings, largely due to the limited scoring scale (1–4) used to rank individuals. This constraint may have reduced the resolution of the rankings, making it harder to capture subtle differences in physiological traits.

Due to time constraints and methodological limitations, certain metrics that were measured in this study, such as individual breakpoints and P crit were not included in the rank sum metric assessment. The opercular beats were filmed using GoPro cameras, which was the first time this methodology was implemented. Specific issues arose utilising this approach, such as fish swimming in and out of frame and fish swimming too quickly to observe opercular beats, even when the footage was slowed down to a quarter of the original speed. Due to the objective nature, it was then decided to remove this component from the metric assessment. Due to the repeated measures approach and the invasiveness and extreme stress associated with P crit, it was decided only to do P crit at the end of the respirometry trials to reduce excess stress on the fish. Therefore, P crit could only be captured for four fish at each thermal extreme reducing the sample size for inclusion and comparison. These metrics have the potential to provide valuable insights into physiological performance, their exclusion in this study is a missed opportunity to assess how they may align with the other key metrics used in this study.

In conclusion, thermal tolerance tests (with repeated measures) may be the most appropriate method to providing rapid insights into an individual's physiological (high or low performance) phenotype, without compromising fish health, for experiments planning to link fish physiology and behaviour in the wild. However, if there is a need to use physiology to predict movement patterns in the wild, methods that can estimate sub-optimal temperatures (thermal niche), such

as break points of respiration rate should be explored. Ultimately, this kind of research will enhance our understanding of how physiological traits influence fish survival and adaptation, therefore informing conservation and fisheries management strategies in a rapidly changing climate.

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