Standard metabolic rate of spiny lobster (*Sagmariasus verreauxi*) pueruli determined by intermittent flow-through respirometry

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**Abstract**

An improved understanding of the metabolic physiology of larval spiny lobsters will aid the development of spiny lobster propagation. The study describes an intermittent flow-through respirometer system for larval crustaceans and discusses its benefits over typical static respirometry methods. The technique is used to determine the standard metabolic rate of the nektonic puerulus stage of the spiny lobster, *Sagmariasus verreauxi*. Respiration rates of intermoult pueruli (n=5) were determined every 20 min for 16 h. Pueruli metabolism was elevated for 2 h after introduction to the respirometer chamber due to the effects of transfer stress. Thereafter, metabolic rates fluctuated due to spontaneous activity. *S. verreauxi* pueruli mean standard metabolic rate, determined as the lowest 10% of metabolic recordings, was $0.39 \pm 0.03 \text{ mg h}^{-1} \text{ gDW}^{-1}$. This is substantially lower than previous recordings of *Panulirus cygnus* pueruli metabolism using static methodologies. The lower recorded metabolism of *S. verreauxi* appears due to the use of improved methods which allowed the exclusion of measurements elevated by stress or activity. The results of the current study demonstrate that the standard metabolism of pueruli is substantially reduced to previous recordings of pueruli routine metabolism. Pueruli may have reduced metabolism due to lower maintenance costs associated with lecithotrophy which may be a mechanism of preserving energy during the non-feeding stage. Lower maintenance costs would help pueruli achieve the energetic challenge of swimming from open-ocean to find suitable benthic substrate.

**Keywords:** Oxygen consumption, respiration, metabolic physiology, energetics, packhorse lobster, respirometry, puerulus, decapod crustacean larvae

**Introduction**

Spiny lobsters are recognized as a premium seafood product attracting high prices in international seafood markets. Their high value, coupled with fully (or over) exploited wild fisheries, has been the impetus for substantial research effort into their larval rearing. Sustainable increase in worldwide spiny lobster production is only possible through the commercial production of hatchery-reared seedstock. Although steady progress has been made over more than a century of research, larval rearing success rate is still poor, with only small numbers of individuals able to be cultured through the numerous and long larval stages (Kittaka, 1997; Ritar *et al.*, 2006; Matsuda and Takenouchi, 2007). The transition between laboratory and commercial production remains a substantial research challenge. Over the last decade spiny lobster propagation research effort has focused on new species and husbandry protocols (Kittaka *et al.*, 2001; Matsuda and Takenouchi, 2005; Matsuda *et al.*, 2006; Smith *et al.*, 2009a), health management (Ritar *et al.*, 2006; Bourne *et al.*, 2007) and larval nutrition (Cox and Johnston 2004; Johnston *et al.*, 2004; Johnston *et al.*, 2008; Matsuda *et al.*, 2009; Smith *et al.*, 2009b). A few studies have examined larval physiology, however, these have been restricted to the early larval stages (Bermudes and Ritar, 2004; Bermudes...
and Ritar, 2005; Bermudes et al., 2008). The difficulties of culture and wild capture means that very little is known about the physiology of larval spiny lobsters, especially the late stages. The long larval development of spiny lobsters, which can take up to two years for some species in the wild, is quite different to clawed lobsters and all other Decapoda (Anger, 2001). Consequently, spiny lobster larval physiological ontogeny is likely to be distinct. A solid understanding of larval physiology will support the other key areas of spiny lobster propagation research towards the goal of commercial seedstock production.

Respiration rate is the most commonly used method for determining rate of metabolism of aquatic organisms, and when converted to its energy equivalents is referred to as indirect calorimetry (Brett and Groves, 1979; Brafield, 1985). Studies of metabolic physiology are particularly important for gaining a better understanding of cultured organisms physiological energetics or rates of energy expenditure, losses, gains and efficiencies of transformation (Brett and Groves, 1979). Furthermore, metabolic studies provide insight into the effects of intrinsic factors such as phylogenetic differences, ontogeny, activity, mass, moult cycles, nutrition and physiological status, and extrinsic factors such as temperature, respiratory gasses, toxins, and pressure (Anger, 2001). Respiration rates of larval crustaceans have almost exclusively been measured within static respirometer systems (Anger, 2001), where oxygen level is measured only at the start and end of an experiment. Measurements are commonly integrated over long periods where it is impossible to isolate periods of rest from episodes of high activity. Consequently, measurements made in static respirometer systems are at an intermediate “routine” state of metabolism ($R_r$), which is defined as the mean rate observed in specimens whose metabolic rate is influenced by random activity under experimental conditions (Fry, 1971). The routine metabolic rate of zooplankton can vary depending on the animals’ state of activity (Halcrow and Boyd, 1967; Torres and Childress, 1983) and thus can be a source of considerable experimental error (Anger, 2001). To eliminate this source of variation, physiologists commonly make substantial effort to measure better defined standardized metabolic states. In teleost research, the standard metabolic rate ($R_s$) or minimum intact metabolic rate is most frequently employed for inter- and intra-specific comparisons. For active fish, $R_s$ can be determined by the extrapolation of metabolism at various levels of forced activity to zero activity using systems such as a water tunnel respirometer (Brett, 1964; Fry, 1971). Alternatively, $R_s$ can be determined as the lowest observed values from multiple repeat measurements over short measuring intervals, thereby excluding measurements elevated by spontaneous activity (Steffensen, 1989; Herrmann and Enders, 2000; Ohlberger et al., 2007). This can be achieved through the use of automated intermittent flow-through respirometry which allows high temporal resolution of measurements of respiration rates over short time intervals for indefinite periods (Forstner, 1983; Meskedahl et al., (in press) Steffensen, 1989; Steffensen, 2002). Further benefits of intermittent flow-through respirometry are that it permits adequate acclimation and avoids the accumulation of excretory products as well as large changes in oxygen concentrations (Steffensen, 1989). Intermittent flow-through respirometry has been employed to examine the metabolism of adult spiny lobsters (Crear and Forteath, 2000; Kemp et al., 2009) and may have potential to improve the standardization of metabolic states in larval crustacean respirometry.

The present study describes an intermittent flow-through respirometer system for larval crustaceans which is simple and economical to construct. It is used to determine the $R_s$ of cultured spiny lobster Sagmariasus verreauxi pueruli. Pueruli are the transitional nektonic stage between pelagic phyllosoma and benthic juveniles (Booth and Phillips, 1994). The process of metamorphosis from phyllosoma to pueruli occurs off the continental shelf (Phillips and McWilliam, 2009). Pueruli then face the great energetic challenge of being required to swim vast distances from open ocean to find suitable benthic substrate (Phillips and McWilliam, 1986). This is made even more difficult because they are solely reliant on accumulated energy reserves as they are incapable of feeding (lecithotrophy) (Lemmens, 1994a). Although
substantial scientific effort has focused on understanding the energetic demands of pueruli (Lemmens, 1994a; Jeffs, et al., 1999; Jeffs et al., 2001a; b; Jeffs et al., 2002; Phillips et al., 2006; Limbourn et al., 2008; Limbourn and Nichols, 2009), little is known about their metabolism. Only a single study has employed indirect calorimetry to investigate the energy requirements of the pueruli on a spiny lobster species, *Panulirus cygnus* (Lemmens, 1994b). However, this study employed static respirometry over a measurement period of just 30 min without allowing for chamber acclimation. The present study benefits from recent advances in larval culture to make the first report of cultured pueruli metabolism and the $R_s$ of a spiny lobster larvae.

**Material and Methods**

**Experimental animals:** Broodstock *S. verreauxi* were collected from the wild and held in 4,000 l fibreglass tanks at the Tasmanian Aquaculture and Fisheries Institute, Marine Research Laboratory, Hobart under a regime of simulated ambient photoperiod. They were fed a combination of fresh blue mussel (*Mytilus edulis*) and commercial prawn pellet (Higashimaru, Vital No 12, http://www.k-higashimaru.co.jp/), and weighed approximately 2.5 kg at spawning. Mating and egg extrusion occurred spontaneously within tanks and hatching occurred from three females between 15th January and the 2nd February, 2008. Phyllosoma were mass-cultured to the final instar (instar 17) and fed 2-6 mm juvenile *Artemia* supplemented with mussel gonad. *Artemia* were cultured in 800 l cones, fed live microalgae (*Chaetoceros mulleri*, *Isochrysis galbana* and *Pavlova lutheri*), bakers yeast, *Dunaliella salina* paste and rice bran as described by Ritar et al. (2006), and disinfected before feeding to phyllosoma with concentrated *C. mulleri* (Tolomei et al., 2004). Immediately after metamorphosis, pueruli were placed into translucent 2 l flat bottom plastic jugs described by Smith et al. (2009a) where they remained for 7 days before respiration measurement to ensure that they were in the intermoult period of the moult cycle.

**Intermittent flow-through respirometer:** Intermittent flow-through respirometer system composed of a custom made 14.5 ml Perspex chamber (ID = 30 mm and length = 40 mm) where dissolved oxygen level was logged every minute by a dissolved oxygen optode (optic-oxygen sensors, Hach HQ40, www.hach.com) (Fig. 1). The optode was sealed into the chamber by an O-ring that could be compressed by a screw fitting to provide an air tight seal. Larvae were introduced into the chamber through another screw fitting at the opposite end of the chamber. Water was continuously circulated through the chamber at a rate of 12 ml min$^{-1}$ by a peristaltic pump (Harvard Apparatus MP11, harvardapparatus.com) using 1.6 mm laboratory tubing (Tygon R3603, www.tygon.com/). Shifts between closed and flow-through (from external water bath) cycles were controlled by a solenoid valve (Burkert 330, www.buerkert.com) connected to a recycler timer set to shift between cycles every 10 min. This allowed a pueruli oxygen consumption rate measurement every 20 min. When in flow-through cycle, water was expelled from the system through a medical grade one-way valve which restricted water uptake during the closed cycle. The respirometer chamber was submerged within a water bath receiving filtered (1 μm bag filter) and disinfected (ozonated and UV sterilized) temperature controlled (21°C) sea water at a rate of six exchanges h$^{-1}$. A gentle trickle of air, delivered through an aquarium air stone, maintained dissolved oxygen in the water bath at 100 to 105 % saturation. Dissolved oxygen in the chamber never fell below 85% saturation during oxygen consumption rate measurements. Following each experiment, the respirometer system was sterilized with a 1 mg l$^{-1}$ solution of sodium hypochlorite, rinsed with fresh water and air dried for a minimum of 12 h before subsequent experiments.

**Blank testing:** Intermittent flow-through respirometer system was first tested without experimental specimens to determine the level and pattern of background respiration due to microbial and other extraneous influences over an experimental period (16 h). Respirometer chamber was sealed in the late afternoon and oxygen consumption logged for 16 h (n=4).

**Pueruli oxygen consumption rate measurement:** Individual pueruli (n=5) were placed into the respirometer chamber in the late afternoon.
Standard metabolic rate of spiny lobster pueruli and oxygen consumption logged for 16 h (Fig. 2).

Standard metabolic rate ($R_s$) was defined as the mean of the lowest 10% of the oxygen consumption rate recordings (Herrmann & Enders, 2000). Pueruli were then removed, carapace length ($L_c$) and wet mass ($WW$) recorded, before being frozen in liquid nitrogen for later dry mass determination. Dry mass ($DW$) was determined by rinsing puerulus in distilled water and drying at 60°C for 24 h before being weighed to the $10 \mu$g on a precision balance (AT261 DeltaRange, Mettler-Toledo, Switzerland). After removing the puerulus from the respirometer chamber, the system was then sealed again without cleaning and oxygen uptake measured for 1 to 2 h (3 to 6 measurement cycles) as a measure of background respiration for that experiment.

**Data Analysis:** Oxygen consumption rates of pueruli were determined by applying linear regressions to the rate of decline of dissolved oxygen concentration over the final 8 min of each 10 min respirometer closed cycle period. Only measurements with regression coefficients ($R^2$) greater than 0.96 were used to calculate pueruli oxygen consumption rates. The mean level of background respiration measured for that experiment was then subtracted from pueruli respiration rates before dividing by $DW$ to express pueruli respiration rate as mg h$^{-1}$ g DW$^{-1}$. Linear regression was fitted to background respiration measurements from blank trials and ANOVA was used to test the significance of regression. Statistics were performed with the SPSS 16.0 for Window software. Values are given as means ±SE and significance level was set at $P<0.05$.

**Results**

Rate of background respiration of the respirometer system without specimens (blank trials) was low with mean levels ranging from approximately 0.03 to 0.04 mg h$^{-1}$ (Fig. 2). Linear regression analysis showed no relationship between background respiration and time, over the 16 h experimental period (ANOVA; d.f.=1,46; $F=2.08$; $P=0.16$). Respirometer dissolved oxygen during puerulus experiments typically oscillated depending on the water exchange cycle (closed or flow-through cycles) (Fig. 3). During the flow-through cycle, dissolved oxygen would rapidly increase for the initial 5 to 7 min and stabilize at external sump dissolved oxygen level (approximately 100% saturation). During the closed cycle, dissolved oxygen would decrease linearly by approximately
1 mg l\(^{-1}\) due to the oxygen demand of the pueruli. Study mean regression coefficients of linear regressions applied for pueruli oxygen consumption measurement was \(R^2 = 0.98 \pm 0.01\) (Table 1). Only 4.8\% of the regressions were assessed to be unsatisfactory for puerulus oxygen consumption rate measurement.

Approximately fifty oxygen consumption rate measurements were made with each pueruli (Table 1). Pueruli oxygen consumption rates fluctuated significantly during the 16 h measurement period, ranging between 0.29 and 1.01 mg h\(^{-1}\) gDW\(^{-1}\) (Fig. 4, Table 1). Measurements were often elevated immediately after introduction to the respirometer and generally fall within a few hours of transfer. Thereafter, pueruli oxygen consumption rate could oscillate considerably above a minimum plateau. Pueruli \(R_s\) was determined at this plateau as the lowest 10\% of oxygen consumption rate measurements. Pueruli were inactive during these periods and were observed to remain motionless within the respiratory chamber. There was little variation in calculated \(R_s\) between replicate pueruli, averaging 0.39 ±0.03 mg h\(^{-1}\) gDW\(^{-1}\) (Table 1).

Table 1. Details of puerulus and oxygen consumption rate (MO\(_2\)) measurements

<table>
<thead>
<tr>
<th>Rep.</th>
<th>(L_c) (mm)</th>
<th>WW (mg)</th>
<th>DW (mg)</th>
<th>MO(_2) (n)</th>
<th>(R^2)</th>
<th>MO(_2) range (mg h(^{-1}) gDW(^{-1}))</th>
<th>(R_s) (mg h(^{-1}) gDW(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.0</td>
<td>370</td>
<td>90.1</td>
<td>50</td>
<td>0.98±0.01</td>
<td>0.29-0.78</td>
<td>0.31</td>
</tr>
<tr>
<td>2</td>
<td>10.5</td>
<td>280</td>
<td>66.9</td>
<td>52</td>
<td>0.97±0.05</td>
<td>0.42-0.97</td>
<td>0.46</td>
</tr>
<tr>
<td>3</td>
<td>11.5</td>
<td>353</td>
<td>79.5</td>
<td>49</td>
<td>0.98±0.06</td>
<td>0.46-0.93</td>
<td>0.48</td>
</tr>
<tr>
<td>4</td>
<td>10.5</td>
<td>305</td>
<td>77.0</td>
<td>48</td>
<td>0.99±0.01</td>
<td>0.32-0.74</td>
<td>0.35</td>
</tr>
<tr>
<td>5</td>
<td>9.7</td>
<td>257</td>
<td>62.0</td>
<td>50</td>
<td>0.99±0.02</td>
<td>0.33-1.01</td>
<td>0.36</td>
</tr>
<tr>
<td>Mean±se</td>
<td>10.8±0.4</td>
<td>313±21</td>
<td>75.1±4.9</td>
<td>49.8±0.7</td>
<td>0.98±&lt;0.01</td>
<td></td>
<td>0.39±0.03</td>
</tr>
</tbody>
</table>

\(L_c\); carapace length, WW; wet weight, DW; dry weight, MO\(_2\); number of oxygen consumption rate measurements, \(R^2\); mean ± se of regression coefficients of linear regression use to determine MO\(_2\), MO\(_2\); range of oxygen consumption rate measurements MO\(_2\), \(R_s\); standard metabolic rate

**Discussion**

Automated intermittent flow-through respirometry system described in the present study was effective in making accurate repeat measurements of respiration over extended periods facilitating an advanced examination of pueruli metabolism. Levels of background respiration of respiratory system were low and remained stable over the blank testing experimental periods. Maintaining low levels of background respiration is important for respiratory studies with larval crustaceans given their low biomass and oxygen demand. It is especially critical with intermittent flow-through respirometry because the tubing connecting respirometer components can develop biofilm, potentially increasing the system oxygen demand. Low microbial levels are best maintained by rigorous cleaning between experiments and the supply of high quality filtered water (Cech, 1990). Further to the more commonly used mechanical and
UV water filtration, the present study used ozonation for water sterilization. Ozonation disinfects directly through oxidation by ozone or through the production of more stable oxidation by-products such as bromine and bromate (Tango and Gagnon, 2003; Ritar et al., 2006). Low levels of these oxidation by-products act to limit microbial proliferation. The stable levels of background respiration over the 16 h blank trials suggest that bacteria proliferation did not occur, or was greatly reduced. This justifies the use of a background respiration measurement taken at the end of an experiment to represent the level throughout the trial.

The decline of dissolved oxygen during respirometer closed (measurement) cycles was strongly linear (study mean $R^2 = 0.98$) providing evidence that the respirometer system was completely sealed and homogeneously mixed. High regression coefficients also demonstrate the accuracy and stability of the optodes used for dissolved oxygen measurement. Optodes rely on the dynamic quenching of a fluorescent indicator by oxygen (Klimant, et al., 1995; Gouin, et al., 1997) and are a relatively new tool in aquatic biology. They offer several significant advantages over traditional Clark-type sensors (electrodes) including that they do not consume oxygen, are not dependant on stirring and have far superior short and long term stability (Klimant et al., 1995).

This study clearly demonstrates that *S. verreauxi* puerulus metabolism is elevated for at-least two hours after transfer to the respirometer chamber (Fig. 4). Elevated metabolism due to transfer stress is commonly observed in aquatic respirometry (Cech, 1990) and the period required for metabolism to stabilize is referred to as the adaptation phase (Herrmann and Enders, 2000). Respirometry studies should be designed to exclude metabolism measurement during the adaptation phase which can vary considerably between species and individuals (Herrmann and Enders, 2000; Steffensen, 2002). Previous measurements of *P. cygnus* pueruli metabolism were made within 30 min of transfer without a period for adaptation (Lemmens, 1994b). These measurements would clearly represent a heightened state of pueruli metabolism. Following the adaption phase, *S. verreauxi* pueruli metabolism could fluctuate widely and often reach maximum levels recorded during the adaptation phase. These fluctuations were likely due to spontaneous activity, in particular swimming. Nektonic pueruli are well adapt for swimming either forward in a linear manner propelled by the pleopods or backwards by rapid flexing of the abdomen (retreat swimming) (Calinski and Lyons, 1983). Forward swimming is considered to be used for active transport whilst retreat is an escape response. In the present study, pueruli were observed to attempt retreat swimming soon after introduction to the chamber which would likely have resulted in elevated metabolism during the adaptation phase. Following the adaption phase they would generally remain motionless or would attempt forward swimming by pulsation of the pleopods.

![Fig. 4. Oxygen consumption rate of a) an individual pueruli (replicate 1) and b) puerulus study mean (±se, n=5) over the 16 h trials. Dashed line indicates the evaluated standard metabolic rate (Rs, mean of 10% lowest recordings)](image)
The fact that some measurements were similar to maximum levels during the adaptation phase suggests that for some periods pueruli swam very actively, possibly at their maximum aerobic capacity. These results demonstrate how variable metabolic measures made in static respirometer can be depending on the specimens’ state of activity.

Lemmens (1994a) demonstrated that the metabolism of *P. cygnus* pueruli preparing for moult to 1st instar juvenile is greater than that of newly-settled pueruli. Increased metabolic activity in the post and to a lesser degree during the pre-moult has commonly been observed in both larval (Anger, *et al*., 1989; Dawirs, 1984; Anger and Schuh, 1992; Anger, 1996) and adult crustaceans (Hagerman, 1976; Penkoff and Thurberg, 1982; Carvalho and Phan, 1998). Enhanced metabolism around the moult is thought to be due to energy-consuming reconstruction processes associated with the moult (Anger, 2001). Due to this, it is important that studies of crustacean metabolism are conducted at a well-defined point of the moult cycle, typically during the stable intermoult period. In the present study, *S. verreauxi* metabolism was recorded 7 days after metamorphosis to pueruli. The duration of the puerulus stage of *S. verreauxi* in cultured is approximately 18 to 20 days (Q. Fitzgibbon, unpublished data) which indicates that respiratory measurements were made within the intermoult period and thus representative of minimum levels for the stage.

When normalized to the current study temperature of 21°C (using a temperature coefficient, $Q_{10} = 2$), the routine metabolic rate of *P. cygnus* pueruli recorded by Lemmens (1994a) ranged from 0.66 mg h$^{-1}$ gDW$^{-1}$ for newly-settled animals to 1.46 mg h$^{-1}$ gDW$^{-1}$ for pueruli preparing to moult. This is at the higher end or greater than the routine metabolism of *S. verreauxi* recorded in the present study and considerably greater than the standard metabolism (0.39 ±0.03 mg h$^{-1}$ gDW$^{-1}$) (Fig. 4b). It is possible that some of the discrepancy between studies could be related to species variation. However, species variation would be unlikely to explain variation of this magnitude. Instead it is more likely that this variation was associated with the different methodologies employed (intermittent flow-through versus static respirometry). As discussed in previous paragraphs, the metabolism of *P. cygnus* was likely elevated due to activity, transfer stress, or physiological processes associated with the moult.

The results of the present study demonstrate that the standard of resting metabolism of pueruli is substantially reduced to previous recordings of pueruli routine metabolism. Comparisons of pueruli metabolic requirements with other larval decapods is difficult because they are considerably larger (generally orders of magnitude larger) than most other larval crustaceans. However, it has been hypothesized that lecithotrophic larvae have relatively low metabolic rates when compared to feeding counterparts (Dawirs, 1984; Lemmens, 1994b; Anger, 2001). Low metabolic rates of lecithotrophic larvae is likely a result of reduced maintenance costs associated with negative growth and sluggish behaviour, and has been suggested as a mechanism for preserving energy during the non-feeding stage (Anger, 2001). Reduced maintenance costs would make pueruli particularly energy efficient which would assist them to cover the long distance in the search for suitable benthic substrate. However the actual metabolic state of pueruli during onshore transport would likely rarely represent the standard metabolism. Precisely how pueruli return to shore is poorly understood however active swimming is likely to be important. Pueruli are negatively buoyant and therefore must actively swim to either maintain position in a current or swim inshore. They are competent swimmers capable of sustained swimming velocities of 15 cm s$^{-1}$ (Philips and Olsen, 1975; Calinski and Lyons, 1983; Jeffs and Holland, 2000). Metabolic rate of pueruli during active swimming would likely to be significantly greater than the standard metabolism. Buskey (1998) found the active metabolism of swarming copepod, *Dioithona oculata*, to be three times routine metabolism and six times greater than standard metabolism. Mean maximum routine metabolic recording of pueruli were 2.3 times that of standard metabolism in the present study. Further respiratory research is required to precisely determine the active metabolism of pueruli which will improve our
understanding of pueruli energy utilization by allowing the determination of metabolic scopes for activity and the assessment of cumulative energy requirements of the stage. Improved understanding of pueruli energy utilization will provide insights into how pueruli achieve the great energetic challenge of finding suitable habitat and potentially factors that affect wild recruitment and survival in culture.

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