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Growth and biochemistry of the spiny lobster *Sagmariasus verreauxi* cultured at low and high density from hatch to puerulus

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ABSTRACT

Stocking density is an important factor affecting the competency of later stage lecithotrophic spiny lobster larvae, yet its influence on biochemical composition has rarely been considered. Biochemical analysis of phyllosoma during ontogeny provides information on the energy storage requirements of late instar phyllosoma and their ability to survive metamorphosis and achieve the energetic demands of the puerulus stage. The current study is the first to examine biochemical composition of spiny lobsters through the entire larval phase. Survival, growth, development, and biochemical composition were measured in *Sagmariasus verreauxi* phyllosoma that were cultured at High Density (HD) and Low Density (LD) from hatch to puerulus. Protein measured directly by Lowry was considerably lower than crude protein as calculated from nitrogen (N) content using N×6.25, suggesting that a conversion factor of 6.25 was too high. Survival of phyllosoma was significantly higher in the HD treatment after instar 9 due to high mortalities of LD phyllosoma caused by high ozonation during instar 9. However, HD phyllosoma were less susceptible to the high ozonation event possibly due to the larger biomass in HD tanks. Phyllosoma growth and development were more advanced in LD phyllosoma after 108 d. Instar 17 LD phyllosoma were also significantly larger than instar 17 HD phyllosoma. The C:N ratio confirmed proportionally more lipid than protein was accumulated during larval development before a significant decrease in lipid reserves between instar 17 and the puerulus stage by over 21% to fuel the process of metamorphosis and the non-feeding puerulus stage. The study demonstrated the larval phase of *S. verreauxi* is important for accumulating lipid reserves to fuel metamorphosis and the puerulus stage and provides a more complete picture of the culture requirements of spiny lobsters during ontogeny, particularly for the rarely studied late phyllosoma instars.

1. Introduction

The survival and recruitment success of spiny lobster species depends on the efficient utilisation of energy from both food and body reserves accumulated during earlier larval stages (Capuzzo and Lancaster, 1979; Sasaki et al., 1986). In *Sagmariasus verreauxi*, ontogeny consists of lecithotrophic and planktotrophic stages, and a shift from a planktonic to a benthic lifestyle following metamorphosis. This is accompanied by many changes in anatomy, morphology, and biochemical composition, which often result in different strategies of energy utilisation (Dall et al., 1990; Lovett and Felder, 1989). However, while the substrates for energy utilisation have been well documented for several decapod species during ontogeny (Anger and Harms, 1990; Anger et al., 1989; Lemos and Phan, 2001b), such studies are uncommon in spiny lobsters due to the difficulty of culturing phyllosoma through their long and complex larval phase (Ritar et al., 2003) or capturing undamaged individuals from the wild (Ikeda et al., 2011).

After hatching, phyllosoma progress through a series of planktonic larval instars which are characterised by changes in morphology and increased size, and each instar is clearly separated by moulting (Anger, 2001). The planktonic larval phase may take up to 12 months for *S. verreauxi* in the wild (Booth and Phillips, 1994) or 8 months in the laboratory, with 11 distinct morphological stages and 17 moults (Kittaka et al., 1997). Since larvae undergo intermittent growth and development achieved by each successive moult, changes in energy utilisation and biochemical composition may result from shifts in feeding behaviour and diet (Ikeda, 1984, 1985; Matsuda et al., 2009; Omori, 1979). Accordingly, shifts in energy substrate utilisation may provide information on changes in the nutritional requirements of phyllosoma (Sasaki et al., 1986; Jensen et al., 2013). The high metabolic rates of crustacean larvae and rapid biochemical turnover (Anger, 2001) means they are particularly susceptible to any nutritional deficiencies caused by food deprivation (Jones et al., 1997) incurred at high stocking densities.

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At high stocking densities, increased competition for available food may restrict feed intake (Mikami, 1995; Minagawa and Murano, 1993; Jensen et al., 2013). Reduced feeding success at high density may also affect the viability of phyllosoma in later instars and their ability to survive metamorphosis (Smith and Ritar, 2006; Jensen et al., 2013). Larval rearing of crustaceans often uses high densities but without testing the effects on physiological parameters (Emmerson and Andrews, 1981). High stocking density may also contribute to excessive expenditure of energy caused by increased physical interactions with prey and other phyllosoma such as, entanglement or physical damage of phyllosoma, which may impair their ability to actively capture prey (Mikami, 1995; Smith and Ritar, 2006; Jensen et al., 2013). Larvae cultured under optimal conditions should develop through the shortest possible progression of instars, whereas weak or stressed larvae may have extended moult increments (Anger, 2001). Moult cycle duration and moult increment are genetically set at an optimal level, which is then modified by nutrition and other extrinsic factors (Freeman, 1990). Because larvae increase in biomass during their development, successive instars also require higher amounts of energy per individual (Anger, 2001).

Factors affecting biochemical composition of crustacean larvae has been studied extensively in a large number of species (Anger, 2001; Conover, 1978; Lemos and Phan, 2001b; Ritar et al., 2003). Most available information is based on biochemical (protein, lipid, and carbohydrate) and elemental (carbon; C, hydrogen; H, and nitrogen; N) composition of decapod crustaceans (Childress and Nygaard, 1974; Ikeda and Bruce, 1986; Ikeda and Skjoldal, 1989). However, very few studies have analysed biochemical (Anger, 2001; Harms et al., 1991) and elemental (Ikeda et al., 2011) composition during the entire larval period. Variations in individual biochemical components during ontogeny reflect the dynamic balance between nutrient acquisition and metabolism, and identifying shifts in substrate utilisation are essential when determining the components conserved as energy reserves and those catabolised (Olsen, 1998). Changes in the relative proportions of lipids, proteins and carbohydrates in the overall biomass have been frequently used as indicators of larval viability, which may be correlated with the overall survival (Ferron and Leggett, 1994; Suthers, 1998). Furthermore, it is often useful to determine changes in the relative chemical composition of organic biomass by comparing mass proportions between single elements (Anger, 2001). Since carbohydrates only play a minor role in crustaceans (Anger and Harms, 1990), the C:N ratio reflects changes in the relative proportions of lipids and proteins, respectively (Anger, 2001).

Lipids are essential nutrients (Kattner et al., 1994); they provide essential fatty acids, cholesterol and a large amount of energy. Lipids are the primary energy reserve accumulated during larval development of crustaceans (Heras et al., 2000) and are metabolised by the non-feeding puerulus stage in spiny lobsters (Jeffs et al., 2001). The relationship between reduced growth and reduced lipid retention (Cockcroft, 1997; Jeffs et al., 1999; Ward et al., 2003) highlights the importance for further studies on factors that affect lipid content of spiny lobsters. Protein accounts for the largest proportion of biomass in crustacean larvae (Anger, 1988; Anger et al., 1983; Capuzzo and Lancaster, 1979; Pandian, 1967). Whilst metabolism of protein as an energy source has been well documented in many aquatic species (Claybrook, 1983; Finn et al., 1995; Lemos and Phan, 2001a), it has not been investigated in *S. verreauxi* phyllosoma. The efficiency with which protein is assimilated into new tissues is influenced by a number of factors, including quality and quantity of dietary protein, the presence of other energy substrates in the diet and the efficiency of nutrient digestion (Carter and Houlihan, 2001; Ward et al., 2003; Wilson, 1989).

Changes in biochemical composition during the entire course of phyllosoma development have rarely been measured (Ikeda et al., 2011). Also, simultaneous measurements of protein (measured directly) and crude protein (calculated from N), have not been investigated previously in phyllosoma. The aim of the present study was to investigate the instar-specific changes in biochemical composition, with regard to lipid and protein content, during ontogeny of *S. verreauxi* in order to evaluate energy storage and utilisation through larval development and better understand the energy storage requirements of late instar phyllosoma prior to metamorphosis. We investigated the effects of stocking density on biochemical composition and growth and development, measured as dry mass (DM), total length (TL), carapace width (CW), and instar, testing the null hypothesis that these parameters vary between phyllosoma cultured at Low Density (LD) and High Density (HD).

2. Materials and methods

2.1. Experimental animals

Broodstock were held in captivity in a 4,000 l fibreglass tank year round under a regime of ambient photoperiod and water temperature (11–19 °C), 33–35 psu salinity, pH approximately 8.1, and 90–100% oxygen saturation at the Institute for Marine and Antarctic Studies (IMAS), Taroona, Hobart. Broodstock were fed a combination of fresh whole blue mussels (*Mytilus edulis*) and commercial prawn pellet (Higashimaru, Vital No. 12, http://www.k-higashimaru.co.jp/) twice a week. Phyllosoma used in this experiment were hatched on the 8th of February 2009 from one female weighing approximately 2.0 kg.

2.2. Larval culture

Triplicate 7 ml sub-samples of newly-hatched phyllosoma were counted from a 20 l bucket to estimate density and then stocked into 50 l cylindrical tanks with flow-through seawater filtered to 1 μm and treated with ozone and ultra-violet irradiation according to Jensen et al. (2011) and maintained at 21–23 °C using an industrial heat/chill unit (Carrier, C010PHH7AA, Australia). Four replicate tanks were initially stocked at LD (20 phyllosoma l^{-1}) and HD (60 phyllosoma l^{-1}). However, due to high mortalities in the LD treatment caused by an acute high ozonation event during instar 9, this treatment was reduced to three replicates in order to maintain experimental treatment densities. Phyllosoma from the LD tank with the highest mortality were randomly distributed into the remaining three tanks to maintain densities. Because phyllosoma increased in size with successive instars, the densities were progressively reduced and the density of the HD treatment was three times greater than the LD treatment throughout the experiment. Stocking densities of the LD treatment were reduced to 10, 5, 2.5, 1.25, and 0.5 l^{-1} at instars 3, 6, 9, 12, and 15, respectively, by randomly culling phyllosoma. Stocking densities of the HD treatment were reduced to 30, 15, 7.5, 3.75, and 1.5 l^{-1} at equivalent instars. The stocking densities of the LD treatment were based on the densities of mass culture tanks used at IMAS at the respective instars and are in the density range recommended by Smith and Ritar (2006). The survival of phyllosoma was calculated between instars prior to reducing densities.

Phyllosoma were fed a combination of freshly prepared blue mussel (*M. edulis*) gonad (~5 mm diameter) once daily and on-grown *Artemia* of 5.0–8.0 mm in length three times a week. Despite extensive water treatment, the daily addition of food increased bacterial levels and tanks (50 l) were cleaned every 14 days to reduce accumulation of bacteria and fouling.

2.3. Feed production

Artemia nauplii were stocked in 670 l tanks at 5 *Artemia* ml^{-1} and on-grown to approximately 8.0 mm in flow-through tanks receiving a diet of blended brine shrimp food (consisting of rice pollard, soyflour and wheat flour; Eyre Peninsula Aquafeeds Pty Ltd, South Australia) and algae (T. *Isochrysis* and *Chaetoceros muelleri*). Prior to