CSIRO PUBLISHING
www.publish.csiro.au/journals/mr *Molluscan Research*, 2003, **23**, 179–183

Short contribution

Changes in tissue composition during larval development of the blacklip pearl oyster, *Pinctada margaritifera* (L.)

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Abstract

This paper reports on the changes in proximate composition (i.e. protein, lipid and carbohydrate) of tropical blacklip pearl oyster, *Pinctada margaritifera* (L., 1758), larvae throughout development. Protein was the largest component of dried larval tissues. Mean protein, lipid and carbohydrate contents all decreased from Day 1 to Day 4. Lipid loss between Day 1 and Day 4 contributed 56% of the total energy utilised during this period, whereas protein contributed almost 40%. Between Day 18 and Day 21, the accumulation of lipid contributed almost 70% of the total energy gain per larva during this period, suggesting that lipid may be the primary energy reserve utilised during metamorphosis. Patterns of energy reserve composition, utilisation and accumulation within *P. margaritifera* larvae were comparable to those reported for temperate species.

Introduction

Fundamental differences exist between tropical and temperate marine environments that directly influence the organisms living within them. Tropical marine environments are characterised by surface-water temperatures of 20–30°C, relatively low nutrient loads and correspondingly low phytoplankton levels (Nybakken 1982). In contrast, temperate marine environments are characterised by seasonal, but relatively high, nutrient loads, high phytoplankton levels and water temperatures fluctuating between 10 and 20°C in a seasonal fashion (Nybakken 1982). On this basis, the rates of energy metabolism in temperate bivalve molluscs cannot be assumed to pertain to tropical species. However, studies reporting on changes in proximate composition during larval development of bivalves have focused on temperate species (Holland and Spencer 1973; Bayne *et al.* 1975; Gallager and Mann 1986; Gallager *et al.* 1986; Whyte *et al.* 1987, 1990, 1991). The hypothesis tested in this study was that patterns of energy-reserve utilisation and accumulation in larvae of the tropical blacklip pearl oyster, *Pinctada margaritifera* (L., 1758), differ from those reported for temperate species. This was examined by determining changes in proximate composition (i.e. protein, lipid and carbohydrate) during larval development.

Materials and methods

Spawning induction and larval rearing of *P. margaritifera* followed standard procedures (Southgate and Beer 1997). Ten hatchery-conditioned *P. margaritifera* broodstock were induced to spawn using thermal shock. Progeny from each of the females were randomly distributed across larval batches. Larvae were reared in six 500-L fibreglass tanks and water was changed every 48 h. Larvae were fed a diet consisting of the two prymnesiophytes, *Pavlova salina* and *Isochrysis* aff. *galbana* clone T-ISO, and the diatom *Chaetoceros muelleri* (Southgate and Beer 1997). A sample of approximately 15 000 larvae was taken for

 10.1071/MR02018 1323-5818/03/020179

proximate analysis at each water change from each tank and stored in liquid nitrogen to await analysis of protein, lipid and carbohydrate content (Mann and Gallager 1985; Baethgen and Alley 1989). Prior to analysis, larval samples were freeze-dried. The experiment ended after 21 days, when 50% of larvae were 'eyed'. Water temperature was measured at 0800 hours and 1800 hours each day and ranged from 25°C to 29°C with a mean of 27.2°C.

Proximate compositional data were used to calculate total energy values using caloric equivalents of 36.42, 23.86 and 17.16 kJ g^{-1} for lipid, protein and carbohydrate respectively (Brett and Groves 1979).

Results and discussion

Pinctada margaritifera larvae increased in mean (± SEM, $n = 30$) shell length from 76 ± 1 μm on Day 1 (24 h after fertilisation) to 213 ± 5 μm on Day 21. Mean (± SEM, $n = 30$) larval dry weight increased from 556.6 ± 1.1 ng $larva^{-1}$ on Day 1 to 2793.4 ± 40.0 ng $larva^{-1}$ on Day 21. Mean survival of larvae to Day 21 was 13.2 ± 5.0%.

Changes in mean protein, lipid and carbohydrate content of *P. margaritifera* larvae are shown in Fig. 1. Protein was the largest component of dried larval tissues. Mean protein content almost halved from 133.7 ± 5.1 ng $larva^{-1}$ on Day 1 to 67.3 ± 3.1 ng $larva^{-1}$ on Day 4 (± SEM, $n = 6$). The most notable increase in mean protein content of larval tissues occurred between Day 12 (133.5 ± 17.7 ng $larva^{-1}$) and Day 18 (395.2 ± 12.9 ng $larva^{-1}$). Subsequently, the increase in protein content slowed considerably; the tissues of 21-day-old larvae possessed 417.7 ± 16.78 ng $larva^{-1}$ of protein.

Lipid was the second largest component of dried larval tissue during development. Mean lipid content decreased by greater than 75% from 73.3 ± 9.1 ng $larva^{-1}$ to 11.7 ± 1.1 ng $larva^{-1}$ between Day 1 and Day 4. Subsequently, larval lipid content increased to 96.8 ± 11.6 ng $larva^{-1}$ on Day 15, declined to 76.9 ± 5.2 ng $larva^{-1}$ on Day 18, then increased rapidly to 140.6 ± 2.2 ng $larva^{-1}$ on Day 21.

Carbohydrate was the smallest component of dried larval tissues. Mean carbohydrate content decreased by about 80% between Day 1 (13.5 ± 4.9 ng $larva^{-1}$) and Day 4 (2.7 ± 0.8 ng $larva^{-1}$). Subsequently, carbohydrate content increased to 63.1 ± 31.1 ng $larva^{-1}$ on Day 21.

Lipid loss between Day 1 and Day 4 contributed 56% of the total energy utilised during this period, whereas protein contributed almost 40%. The energy contributed by carbohydrate was very small, less than 10% of the amount contributed by lipid. Between Day 18 and Day 21, the accumulation of lipid contributed almost 70% of the total energy gain per larva during this period. The energy available from lipid was more than four times that available from protein and carbohydrate, each of which contributed about 15% of the total energy value at this time.

Patterns of energy reserve composition, utilisation and accumulation within *P. margaritifera* larvae were found to be comparable to those reported for temperate species (Holland and Spencer 1973; Gallager *et al.* 1986; Whyte *et al.* 1987). As reported in all previous studies with bivalves (Holland 1978; Whyte *et al.* 1989), protein was the largest organic component of *P. margaritifera* larvae and showed the largest increase during larval development. Protein forms the bulk of the structural organic components of larval tissues (Holland 1978; Whyte *et al.* 1989). Also in accordance with previous studies, lipid was the second largest organic component of *P. margaritifera* larvae and carbohydrate content was relatively small and changed little during larval development.

The marked decline in protein, lipid and carbohydrate content of *P. margaritifera* larvae between Days 1 and 4 is similar to that reported for the larvae of temperate scallops (*Patinopecten yessoensis* and *Crassadoma gigantea*), where protein, lipid and carbohydrate were reported to be catabolised simultaneously and linearly with time during embryonic

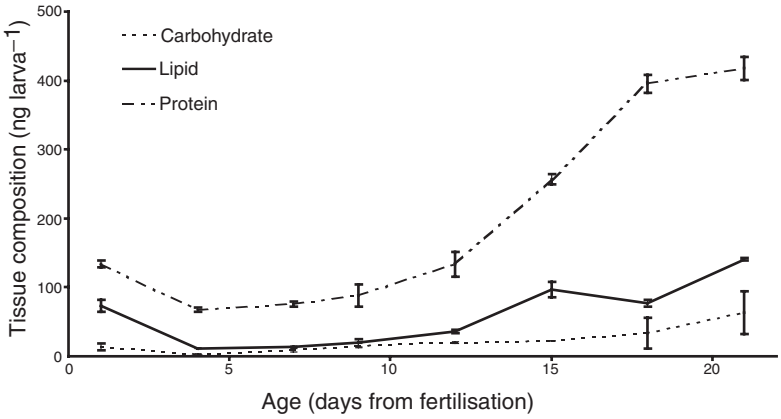

Fig. 1. Changes in mean (± SEM) protein, lipid and carbohydrate content of *P. margaritifera* larvae (ng larva^{-1}) during development ($n = 6$, Days 1 to 9; $n = 3$, Days 12 to 21).

development (Whyte *et al.* 1990, 1991). This decline is thought to result from utilisation of endogenous reserves, provided to the egg by the female parent, to fuel the transition to an exogenous mode of feeding (Bayne *et al.* 1975; Mann and Gallager 1985). Utilisation of protein, lipid and carbohydrate by *P. margaritifera* between Days 1 and 4 shows that larval development during this period is an energetically expensive process; approximately 66% of the total energy content of 1-day-old larvae was utilised by Day 4. Similarly, 56.9% of the energy content of fertilised eggs was reported to be utilised during the first 72 h after fertilisation in scallop (*Crassodoma gigantea*) larvae (Whyte *et al.* 1990).

Over half the energy utilised by *P. margaritifera* larvae between Days 1 and 4 was contributed by lipid, suggesting that lipid is the primary energy reserve during this period. Similarly, embryogenesis of the oyster (*Crassostrea virginica*) and clam (*Mercenaria mercenaria*) has been reported to be fuelled primarily (55–96% and 50–65% respectively) by parentally derived lipid (Gallager and Mann 1986).

A number of studies with temperate bivalves only investigated changes in the lipid content of tissues during larval development and disregarded changes in protein or carbohydrate contents (Gallager and Mann 1986; Gallager *et al.* 1986; Napolitano *et al.* 1988; Delaunay *et al.* 1992, 1993). However, in *P. margaritifera* larvae, protein was found to contribute 40% of the total energy utilised between Days 1 and 4, indicating it to be an important secondary energy source during this period. Similarly, Whyte *et al.* (1990, 1991) reported protein to contribute a significant portion of the total energy expended during the embryogenesis of the scallops, *Patinopecten yessoensis* and *Crassodoma gigantea* (44.9% and 43.5% respectively). Such values were only slightly lower than the energy contribution reported for lipid of 47.6% and 4% respectively (Whyte *et al.* 1990, 1991).

Although the overall energy contribution from carbohydrate in *P. margaritifera* tissues was small between Days 1 and 4, it accounted for an 80% depletion of its initial energy content, which is comparable to the decline in lipid (86%). Similar depletion of carbohydrate has been reported during embryogenesis of scallops (Whyte *et al.* 1990, 1991). In comparison, the decline in protein content of *P. margaritifera* larvae between Days 1 and 4 accounted for only 50% of the initial energy content. This reflects the important structural role of proteins in keeping the larval body intact (Holland 1978).

Larval lipid and carbohydrate contents increased markedly between Days 18 and 21. During the same period, the rate of protein accumulation declined considerably. Although both lipid and carbohydrate contents increased by almost 50% between Days 18 and 21, accumulation of lipid contributed more than four times the total energy gain (per larva) than either protein or carbohydrate. This strongly suggests that lipid may be the primary energy reserve utilised during metamorphosis. Although this assumption is supported by the results of similar studies with temperate species (Holland and Spencer 1973; Whyte *et al.* 1987), further study is required to confirm this role for lipid in *P. margaritifera*.

These results provide a strong basis for future study by clearly indicating changes in proximate composition and energy-reserve utilisation and accumulation during larval development of *P. margaritifera* larvae. This research has increased our understanding of the energetics of bivalve larvae, being the first study of its kind to investigate a tropical species. These findings have significant practical application and will be useful in the development of more efficient hatchery techniques for pearl oysters; for example, in formulating diets that best provide the biochemical requirements of larvae to maximise growth and survival and to minimise the time to metamorphosis.

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