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Publication details
Postprint of: Li, Y, Qin, JG, Li, X & Benkendorff, K 2009, 'Monthly variation of condition index, energy reserves and antibacterial activity in Pacific oysters, Crassostrea gigas, in Stansbury (South Australia)', Aquaculture, vol. 286, nos. 1/2, pp. 64-71.  
Publisher's version of this article is available at http://dx.doi.org/10.1016/j.aquaculture.2008.09.004
Accepted Manuscript

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PII: S0044-8486(08)00670-4
Reference: AQUA 628410

To appear in: Aquaculture

Received date: 26 November 2007
Revised date: 2 September 2008
Accepted date: 2 September 2008

Please cite this article as: Li, Yan, Qin, Jian G., Li, Xiaoxu, Benkendorff, Kirsten, Monthly variation of condition index, energy reserves and antibacterial activity in Pacific oysters, Crassostrea gigas, in Stansbury (South Australia), Aquaculture (2008), doi: 10.1016/j.aquaculture.2008.09.004

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Monthly variation of condition index, energy reserves and antibacterial activity in Pacific oysters, *Crassostrea gigas*, in Stansbury (South Australia)

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Abstract

This paper investigates the temporal responses of 2-year old *Crassostrea gigas* to environmental changes in Stansbury, South Australia from September 2005 to October 2006. A total of 360 oysters were grown in one-line baskets on the farm using six replicates that were sampled monthly. A range of environmental parameters were assessed and correlated against biological indicators for oyster condition, metabolism and antimicrobial activity. Food availability by chlorophyll $a$, was low throughout the study period (0.5~1.5 µg L$^{-1}$) and was significantly correlated to phosphorus concentrations. The condition index and shell weight of oysters significantly increased over the year, with the condition index dropping after spawning but then recovering within one month. Significant temporal variation in energy storage and utilization were observed in different tissues over the year. Glycogen in the mantle tissue was influenced by reproduction and correlated to chlorophyll $a$ levels, but not in the gill or adductor muscle. The mantle glycogen and gill protein reached the lowest level in February when spawning occurred and presented evidence for seasonal variation in oyster metabolic activity. However, mantle and adductor muscle proteins did not drop after spawning indicating that these proteins contribute little to gametogenesis. Hemolymph protein was negatively correlated to water temperature and chlorophyll $a$, reaching the lowest level during summer. Hemolymph antibacterial activity significantly decreased after spawning, implying that the period of post-spawning is critical for oyster health. This study revealed trade-offs in the energy budget between immune resistance, growth, and reproduction. The results indicate that in a lean water environment, spawning events significantly regulate metabolic and immune capacities of oysters and a second year of rearing increased meat.
and shell weight but not the shell length. These findings are applicable to the
management and development of oyster aquaculture within temperate southern
hemisphere.

*Key words:* Oyster growth; Energy reserves; Antibacterial activity; Reproduction;

Aquaculture management
1. Introduction

The Pacific oyster *Crassostrea gigas* (Thunberg) dominates over all other molluscan species with respect to global distribution and aquaculture production. It was introduced to South Australia in 1960s as a farming species, when attempts to farm the Sydney rock oyster (*Saccostrea commercialis*) and native mud oysters (*Ostrea angasi*) were only partially successful (Olsen, 1994). The introduction of Pacific oysters has resulted in significant expansion in production by increasing feasible farming areas in South Australia. Stansbury was one of the earliest places for oyster farming in South Australia, and is also known as Oyster Bay. However, when the same farming practices developed in other regions in South Australia are applied in Stansbury, the oysters typically have a lower meat to shell ratio after two growing seasons (Paul Dee, SA Oyster Growers Association, personal communication). In comparison to other locations around the world, the oyster farming area in Stansbury, South Australia, appears to have marginal growing conditions for the Pacific Oysters, especially with low chlorophyll \( \alpha \) levels (Saxby, 2002) and warm water temperatures (11~37 °C) (Baghurst, 2002). For effective management of Pacific oyster productivity on aquaculture leases, it is important to understand the natural variability in their condition and any factors that may influence their health across the year.

In recent decades, mass summer mortality in Pacific oyster aquaculture has become a widespread concern in the world (Perdue et al., 1981; Cheney et al., 2000; Huvert et al., 2004; Garnier et al., 2007). In France, a 19 °C threshold has been proposed for summer mortality (Samain et al., 2004), whereas temperatures regularly exceed 30 °C in the
shallow waters of Southern Australian Gulfs (Baghurst, 2002). Therefore, the situation becomes more critical when the species is located in shallow waters where the temperature significantly increases (Frechette et al., 2003). To improve the management strategy for thin Pacific oysters grown on the coast of Stansbury, it is necessary to understand the temporal variation in condition index, energy reserves and immune health.

Like many other bivalve species in temperate intertidal areas, Pacific oysters exhibit a seasonal cycle in glycogen synthesis and mobilization (Whyte and Englar, 1982; Kang et al., 2000; Dridi et al., 2007). Glycogen storage has been considered as a bio-indicator for environmental changes and the capacity of oysters to sustain environmental stress (Patrick et al., 2006). In addition to glycogen, protein also serves as a primary energy supply during and after the spawning period in Pacific oysters (Mao et al., 2006). As protein accounts for 60-80% of muscle dry weight (Berthelin et al., 2000a), any fluctuation of somatic proteins may directly influence the oysters’ nutritional condition.

However, in South Australia, the pattern of variation in the biochemical storage in tissues is unpredictable, given that this area has low nutrients and high annual temperature fluctuation. As somatic glycogen and protein levels in bivalves have been considered the main energy reserves to cope with environmental stress (Berthelin et al., 2000a; Kang et al., 2000; Dridi et al., 2007), their monthly variation of storage and mobilization in relation to the reproductive cycle will be an important index for oyster aquaculture management.

Generally, it is believed that the capability of oysters to react to disease, injuries or parasite infections depends upon their immune defense system. Defence mechanisms in bivalves have been shown to be affected by environmental factors (Chu et al., 1995;
Fisher et al., 2000; Oliver et al., 2001). Despite the concomitance between immune and environmental variables, recently evidence has shown that reproduction could be sometimes more important than environmental parameters in regulating the immune status of mollusks (Duchemin et al., 2007; Li et al., 2007). In particular, Duchemin et al. (2007) found less seasonal variation in the immune responses of triploid oyster compared to diploid oysters, even after partial spawning. The high energetic cost of reproductive processes results in immune-depression and thus may contribute to summer mortality events by making oysters more susceptible to opportunistic pathogens and environmental stress (Pouvreau et al., 2003; Soletchnik et al., 2006; Li et al., 2007; Samain et al., 2007).

In another study, decreases in oyster condition after spawning are always coincident with increase in seasonal temperatures and disease outbreaks (Dittman et al., 2001). Antibacterial activity against specific marine pathogens provides a good indicator of the capacity to fight infection (Hooper et al., 2007) and has been shown to decrease in post-spawning Pacific oysters (Li et al., 2007). Consequently, monthly analyses of antibacterial activity would help identify immune critical periods for local oyster populations to help manage potential mass mortality events.

Validation and interpretation of biomarker responses require identification of temporal variations for appropriate characterization of normal ranges (Ringwood et al., 1999). Studies on biochemical indicators are required to assess the normal physiological or biological status of animals in the field and to control deviations from this standard value, related to environmental modifications imposing either chronic or acute stress (Moal et al., 1991). Therefore, the objectives of this study are to determine the temporal variation in biochemical metabolism and antibacterial activity in C. gigas, in attempt to
provide baseline data for the performance of Pacific oysters in southern hemisphere regional aquaculture. Particularly, this study tests two hypotheses: (1) there is monthly variation in the metabolic and immune capacities of oysters, especially in relation to major spawning events, and (2) an additional year of rearing positively affects the meat to tissue ratio in nutrient poor areas. By assessing the physiological impact of reproduction and environmental conditions, this study aims to help improve management strategies to optimize oyster farming and health management in Stansbury and other areas with similar environmental conditions.

2. Materials and Methods

2.1. Study area and sampling

The experiment was conducted on the lease of Southern Yorke Oysters in Stansbury, located on the Western coast of Gulf St Vincent in South Australia (34°54′S,137°48′E) (Fig. 1). In total, 360 two-year old oysters with approximate 9 cm in length were selected from the same cohort of cultivated stock on the farm, then subdivided evenly into 6 replicate baskets and grown in one line among other stock on the farm. Oysters are suspended by rubber slings on a system of wooden racks that lie parallel to the soft substratum below. Samples were taken monthly from September 2005 to October 2006. Oyster spawning was visually monitored by the farmer on a daily basis through visual inspection of the gonadal change for commercial purpose, and in February sampling was undertaken on the second day after spawning occurred. Each month, three oysters were
randomly sampled from each basket, with one for growth measurement and two for tissue
and hemolymph assays (18 oysters in total). The oysters were cleaned of epiflora and
fauna with a toothbrush, and then kept for 1 h in ambient seawater at the same
temperature as where the oysters were collected until various measurements were
conducted.

2.2. Monthly variation of environmental conditions

Water temperature was measured at the oyster basket layer on the same day as oyster
sampling using a thermometer. Seawater samples were collected at the experimental site
using six replicated 1-L dark bottles and stored in ice until nutrient and chlorophyll \(a\)
analysis in the laboratory. The water samples for ammonia, nitrite, nitrate and phosphate
determinations were all filtered through a 300 µm mesh to remove zooplankton and large
debris, and then filtered through a 0.45 µm Millipore filter before putting on an automatic
chemical analyzer (\(\mu\)CHEM MP) to remove any suspended particles. To determine the
chlorophyll \(a\) concentration, each seawater sample (\(n = 6\); 475 mL each) was filtered as
above in the dark. The Millipore filter was wrapped by aluminum foil and frozen at -20
°C until chlorophyll \(a\) analysis. Laboratory extraction of chlorophyll \(a\) was conducted
within one month and chlorophyll \(a\) concentrations were determined using the protocols
of Lorenzen and Beninger (1980). The salinity in Stansbury is relatively stable
(36~38‰), as measured by the South Australian Shellfish Quality Assurance Program in
past years (Jan Lee, personal communication); therefore salinity was not recorded for
analysis in this study.
2.3. Growth measurement

On each sampling day, the shell lengths and widths of six selected oysters were measured with vernier calipers (mm). After drying the shells with blotting paper, the whole weight was measured (on an analytical scale, 0.01 g) before opening. Whole flesh was taken, dried at 60°C for 48 h and then weighed. The shell weight was also recorded. The condition index (CI) of oyster was determined according to Crosby and Gale (1990) by the formula:

\[ CI = \frac{\text{Dry Weight} \times 100}{\text{Total Weight} - \text{Shell Weight}} \]

2.4. Protein and glycogen analysis

Among previous studies, most have used the gonad-visceral mass as sample tissues to monitor seasonal variation in biochemical composition (e.g. Ojea et al., 2004; Dridi et al., 2007). But variations in biochemical composition content have been identified amongst different tissues (Berthelin et al., 2000a, b). Therefore, the analysis of separate body parts is often more informative than the analysis of the whole animal, to relate biochemical compositions to growth and gametogenic development (Berthelin et al., 2000a; Dridi et al., 2007). Consequently, in this study oyster gill, mantle and adductor muscles were selected for somatic biochemical composition analysis and gonad was excluded because of inter-animal variability and difficulty of sampling.
To minimize inter-animal variability, the remaining 12 oysters from each month were randomly grouped into three with four oysters pooled in each replicate. When the oyster was opened, the hemolymph was first withdrawn from the pericardium cavity using a 1 mL sterilized syringe for hemolymph parameter analysis. Then, gill, mantle and adductor were separately dissected and preserved in liquid nitrogen prior to the assay of glycogen and protein. The frozen tissues were ground to a fine powder with a mortar and pestle on dry ice. The results of protein and glycogen measurements are expressed in mg per gram of tissue weight.

Gill, mantle and adductor protein levels were measured according to the method of Li et al. (2000). Briefly, 100 mg of frozen powder was homogenized with 8 mL of 20 mM Tris-HCl buffer containing 2% NaCl and 0.1% NaNO₃ (pH 8.0) and centrifuged at × 1500 g for 30 min at 4 °C. The supernatant protein portion was pipetted into an eppendorf tube on ice. Then 10 μL aliquots were used to determine protein content using the Bio-Rad Protein Assay Kit at 590 nm on a spectrophotometer (Spectra Max 250) with the bovine serum albumin as a standard (Bradford, 1976).

Glycogen content of the gill, mantle and adductor was determined on a spectrophotometer using the method of Kristman (1962). A total of 200 mg frozen powder was extracted with 1 mL of perchloride acid (PCA) (n = 3). Then 0.2 mL of PCA extraction was pipetted into a micro cuvette and added 1.3 mL I₂KI solution (dissolved 0.26 g of iodine and 2.6 g of KI in 10 mL distilled water, then 1.92 mL I₂KI to 500 mL saturated CaCl₂ solution). The samples were incubated at 25 °C for 20 min before analysis at 460 nm with a Unicam UV-Visible spectrometer. Purified oyster glycogen was used to generate standards, containing 0, 0.1, 0.4 and 0.7 mg mL⁻¹.
2.5. Hemolymph protein and antimicrobial activity analysis

After 10 sec vortex, the three pooled hemolymph replicates (four oysters each) were quickly preserved in liquid nitrogen, and then transferred to -80 °C for storage before subsequent analyses within 2 months. The hemolymph protein level was measured by the Bio-Rad Protein Assay Kit (as above) after thawing at 20 °C.

The antibacterial activity in hemolymph was tested using the procedure outlined by Li et al. (2007). Cultures of marine pathogen *Vibrio harveyi* were obtained from the Tasmania Department of Primary Industries and Fisheries. Single bacterial colonies from the culture plates were inoculated into 25 mL sterile nutrient broth (1g NaCl, 2 g yeast extract and 1 g peptone per 100 mL distilled H$_2$O), followed by incubation overnight at 37 °C on an orbital shaker (Ratek) at 200 rpm. The reason we incubated the hemolymph sample at 37 °C was that this temperature was most suitable for checking bacterial viability (K. Benkendorff, unpublished data). This is also a routine protocol in the measurement of hemolymph antimicrobial activity in stress associated immunological studies (e.g., Morvan et al., 1997; Vakalia and Benkendorff, 2005).

The cultures reached exponential growth phase prior to antimicrobial assays. The defrosted hemolymph was centrifuged at × 500 g for 5 min to pellet the hemocytes and then 90 μL aliquots of plasma were pipetted into a 96 well plate in triplicate. Then 10 μL of *V. harveyi* culture was added into each well before incubating for 40 min at 37 °C.

After adding 20 μL of CellTitre 96® Aqueous One Solution (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt reagent,
Promega Co. Madison, WI, USA), the cultures were returned to the incubator (37 °C) for 2 h when control wells developed a red color (formazan), then the absorbance was measured at 492 nm using a 96-well plate reader (Spectra Max 250). The background absorbance from hemolymph broth controls was subtracted from the treatment wells and then the cell viability was calculated as a percentage of the absorbance in positive control cultures. The antimicrobial activity was calculated by the formula:

\[ \text{Antimicrobial activity (\%)} = 100 - \text{Bacterial viability} \]

2.6. Statistical analysis

To test the difference between months, the values of each parameter (except seawater temperature and gill glycogen) were compared using one way ANOVA followed by post hoc comparisons using Duncan test \((a = 0.05)\) on SPSS (v. 14). Prior to performing these analyses, data were transformed by logarithm or square root to meet assumptions of normality and homogeneity of variance, if necessary. Correlations between environmental parameters and biological indices were examined using stepwise multiple regression analysis on SPSS (v. 14) with the mean values for each sampling month. Results are presented throughout the year from September 2005 to October 2006, except for September 2006 for which no data were available.

3. Results
3.1. Environmental parameters

Chlorophyll $a$ levels were generally concomitant with seawater temperature fluctuation over the year, except during winter months (June and July) (Fig. 2A). From September 2005, seawater temperature gradually increased and reached a maximum of $31^\circ$C in February 2006, when oyster spawning was observed in the field. In the same month, the chlorophyll $a$ reached the highest level of $1.52 \mu g \text{ L}^{-1}$ which was significantly higher than the values in other months ($P < 0.05$). Temperature then decreased after March 2006, concomitant with the reduction in chlorophyll $a$ levels, but chlorophyll $a$ increased again in July 2006. Partial correlation analysis indicated that chlorophyll $a$ level was significantly correlated to seawater phosphate in the most significant regression model ($P < 0.05$). Phosphate accounted for $60.2\%$ of the variation in chlorophyll $a$ (Table 1).

The four seawater nutrients (ammonia, nitrite, nitrate and phosphate) all varied over the year ($P < 0.05$, Fig. 2B). The mean concentration of nitrate was the highest and this was the most variable nutrient recorded between months. The second highest nutrient was phosphate, which was significantly higher in the warmer month of February ($P < 0.05$) and lower from April to October 2006 (excluding July when chlorophyll $a$ peaked). Nitrite and ammonia had consistently low values ($< 10 \mu m \text{ L}^{-1}$), although significant temporal variation was found, with higher NO$_2$ values during spring (September-November) 2005 and in June and October 2006, whereas ammonia peaked in winter (June-July 2006).
3.2. Oyster growth

Mortality was less than 2% during experimental period. The monthly variation in oyster condition index (CI) and tissue dry weight (DW) was significant ($P < 0.001$, Fig. 3A). The CI and DW increased dramatically from October to December in 2005 and from February to May in 2006 ($P < 0.001$). In February 2006, they both significantly dropped, concomitant with oyster spawning in the field, to the levels in September and October 2005, which were significantly lower than that in all other months ($P < 0.05$). After spawning, the CI and DW increased in March 2006, reaching levels similar to later months (April–October 2006), but significantly higher than the initial value in early spring (September–October 2005) ($P < 0.05$). Multiple regression analysis indicated that PO$_4$ and NO$_2$ were negatively correlated to CI ($P < 0.05$, Table 1), accounting for 74.5% of the variation in the model. The PO$_4$ levels also significantly contributed to the most significant regression model for DW. Neither CI nor DW was correlated to temperature or chlorophyll $a$ ($P > 0.05$).

The size of oyster shell (length and width) did not change significantly over time ($P > 0.05$), but the shell weight did ($P < 0.05$, Fig. 3B). At the end of this study (October 2006) the oyster shells were about 25 g (~ 35%) heavier than those at the beginning ($P < 0.05$). Both CI and DW also approximately doubled after the year of additional grow out (Fig. 3A).

3.3. Monthly variation of biochemical parameters
ANOVA analysis indicated that the glycogen content in the adductor muscle did not change over the year ($P > 0.05$, Fig. 4A). However, in the mantle tissue, there was a significant temporal variation in glycogen levels ($P = 0.001$). Although the glycogen level in the mantle tissue was generally higher than that in the adductor muscle or gill tissue, it dropped dramatically in summer with the lowest value recorded in February 2006 ($P < 0.05$), coinciding with the oyster reproduction season. The mantle glycogen increased again from March to October 2006, reaching values similar to the spring 2005 (September-November) ($P > 0.05$). Although the data of gill glycogen were not all represented, the glycogen levels were intermediate between the mantle and adductor glycogen contents. Multiple regression analysis indicated that none of the environmental parameters measured were significantly correlated to either gill or adductor glycogen.

Mantle glycogen showed a significant negative correlation to chlorophyll $a$ ($P < 0.05$), but was not correlated to temperature or any of the seawater nutrients ($P > 0.05$, Table 1).

Tissue protein was measured in the gill, adductor and mantle tissues and showed some temporal fluctuations (Fig. 4B). The protein in the adductor muscle was not statistically variable over the season ($P > 0.05$), but was maintained at a higher level than in other tissues. On the other hand, the protein in the mantle and gill showed significant monthly variations ($P = 0.018$ and $P = 0.002$, respectively). In the mantle tissue, the protein levels peaked in January, before spawning, then dropped between February and May 2006, before rising again to the significantly highest level in July 2006 ($P < 0.05$), then dropped in October to a similar level as recorded in the previous October ($P > 0.05$). The protein levels in the gill steadily rose to a maximum value in February then gradually dropped to the lowest value in October 2006 ($P < 0.05$). In February 2006, oyster gills
turned to algal green color, which was quite different from the appearance in other months. The chlorophyll $a$ was positively correlated to gill protein, whereas there was a negative correlation to the seawater ammonia levels ($P < 0.05$, Table 1). Conversely, the mantle protein was positively correlated to the seawater ammonia, which accounted for 61.1% of mantle protein variation ($P < 0.05$, Table 1). No significant correlations were found for the adductor protein against any environmental variables ($P > 0.05$).

3.4. Monthly variation of hemolymph protein and antibacterial activity

Hemolymph protein also showed significant monthly variation ($P = 0.02$, Fig. 5A). In general, the protein level declined from September 2005 and reached a minimum in January 2006. The recovery started in February and reached a maximum in August 2006 ($P < 0.05$). Multiple regression analysis revealed that the hemolymph protein was negatively correlated to chlorophyll $a$ and positively correlated to seawater temperature. In the most significant regression model, these two environmental factors accounted for 69.4% of the variability in hemolymph protein ($P < 0.01$, Table 1).

The antibacterial activity in hemolymph was significantly different between months ($P = 0.036$, Fig. 5B). The antibacterial activity was always high during the gametogenesis period, but reduced sharply in February when spawning occurred ($P < 0.05$). It then recovered quickly in March ($P < 0.05$). However, the antibacterial activity gradually declined in winter, then increased in August 2006 to the initial level in spring 2005. The activity levels over winter were similar to that in February ($P > 0.05$). There was a
significant negative correlation between hemolymph antibacterial activity and seawater ammonia ($P < 0.05$, Table 1).

4. Discussion

This study investigated the monthly variation in environmental conditions and biological responses of Pacific oysters in Stansbury, South Australia, and revealed that the principal fluctuations in oyster biological parameters are concomitant with the reproductive cycle and influenced by environmental parameters, such as temperature, chlorophyll $a$ and phosphate in the seawater. The biological responses observed in oysters with respect to reproduction and high temperature conditions are consistent with the findings from controlled laboratory experiments (e.g. Cronin et al., 2001; Li et al., 2007), as well as previous field studies from the Northern Hemisphere (e.g. Mao et al., 2006; Soletchnik et al., 2006; Samain et al., 2007). This study further validates the use of energy reserves and antimicrobial measurements to monitor temporal changes due to abiotic and biotic stressors on Pacific oysters.

Pacific oysters have a broad adaptation to environmental temperatures, with an optimal range being at 15~18 °C in South Australia (Paul Dee, personal communication). The seawater temperature measured in Stansbury, South Australia, was high in summer (up to 31°C) and above 12°C in winter. This suggests the growing conditions for oysters in this area could be suitable during winter, although the condition index, tissue weight and shell weight were only observed to increase slightly over this period (Jun-Aug), with maximum growth occurring in the milder months of spring and autumn. Oyster tissue
growth and condition index were not found to be correlated to the chlorophyll $a$ levels in Stansbury, which may be due low variation in primary productivity, as chlorophyll $a$ remained less than 1 $\mu$g L$^{-1}$ throughout most time of the year. Although the chlorophyll $a$ levels were highest in summer, the values were still lower than measurements taken from other bays in South Australia, such as Ceduna and Smoky Bay (Baghurst and Mitchell, 2002; Saxby, 2002) and were much less than the chlorophyll levels found in northern hemisphere oyster growing regions (1~20 $\mu$g L$^{-1}$) (Berg and Newell, 1986; Deslous-Paoli and Héral, 1988; Field et al., 1998). This difference is due in part to low levels of terrestrial nutrient input, a consequence of the close proximity of deserts to oyster growing regions in South Australia (Shepherd, 1987). Nevertheless, in this nutrient poor environment, Pacific oysters can still have significant somatic tissue growth (i.e. doubled in one year).

Interestingly, the shell sizes of these two-year old oysters did not significantly increase, rather the shell became thicker, which may be a response to the warm temperature condition in South Australia (Wheaton, 2007). However, increases in their condition index and tissue dry weight suggests that an additional year of holding on the farm can also make the market-sized oysters much plumper. Although improvement in flesh fullness (condition index) would increase oysters’ market value, the potential benefits of extending the farming period of two-year old oysters in Stansbury would require further bioeconomic analysis.

As a suspension feeder, oysters could obtain nutrition from algae, organic particles, ciliates and other zooplanktons (Nell et al., 1983; Dupuy et al., 1999; Dupuy et al., 2000; Trottet et al., 2008). However, in this study, only chlorophyll $a$ was used as a
conventional measure to facilitate the comparison of food availability with other studies. Chlorophyll $a$ in Stansbury was significantly correlated to the change in seawater phosphate concentration, consistent with a previous study in South Australia (Ganf, 1982). The sharp increase in chlorophyll $a$ level in winter was associated with a peak of phosphate in the seawater, suggesting phosphate levels could be an important factor limiting primary productivity, and therefore oyster food availability, in nutrient poor areas such as Stansbury. Negative correlations between condition index and the seawater nutrient concentrations of phosphate and nitrite were detected. The condition index varied across the seasons and was significantly reduced after spawning in February, concomitant with a peak in phosphate in February. The similar pattern was also observed on mussels in Georgia intertidal salt marsh (Edward, 1961). The condition index then recovered within a month, when the chlorophyll $a$ level was concurrently high.

The monthly variation of biochemical composition in Pacific oysters clearly differs among various tissues. In agreement with Perdue et al. (1981) and Berthelin et al. (2000a, b), we found that the mantle glycogen storage was reduced in summer and reached a minimum during the spawning period. This indicated that mantle glycogen was the main energy source for gonadal production, consistent with previous studies (de Zwaan and Mathieu, 1992; Berthelin et al., 2000a). Berthelin et al. (2000a) further demonstrate that the seasonal variation of glycogen in mantle is the same as palps and gonad, confirming that the mantle glycogen presents valid information for seasonal variation in energy metabolism, especially regarding the reproduction cycle. Similar to previous observations on $M. edulis$ (Gabbott, 1983), we did not find a significant fluctuation in adductor glycogen over the year. Accordingly, it appears that glycogen reserves are selectively
depleted and deposited among tissues. Physiological control of the storage and mobilization of glycogen is a major energy metabolism in bivalves (Berthelin et al., 2000b), therefore, the quick increase of mantle glycogen after spawning indicates robust energy anabolism in the recovery process.

Although the seasonal variation in glycogen reserves is strongly influenced by food availability, in addition to reproductive demands (Li et al., 2000; Patrick et al., 2006), we found no biologically meaningful correlation between glycogen and chlorophyll $a$ levels in this study. The mantle glycogen content was actually negatively correlated to chlorophyll $a$, which is most likely explained by the warm water conditions in February causing a peak in primary productivity and inducing spawning, which then leads to a drop in mantle glycogen. With respect to the minimal glycogen storage after spawning, Mori et al. (1965) point out that oysters in areas of high mortality exhibit extremely low glycogen levels. Consequently, low glycogen storage might lead to stressful conditions in oysters during and after spawning, thus increasing susceptibility to mortality (Cho and Jeong, 2005; Soletchnik et al., 2006; Li et al., 2007; Song et al., 2007). In Stansbury, however, the relatively high food availability (chlorophyll $a > 1.5 \mu g L^{-1}$) during the spawning season would facilitate a quick recovery of post-spawning oysters in energy storage.

The protein level in adductor muscle was relatively stable over the year, in comparison to gill and mantle protein. This is possibly because the oyster adductor is functionally less involved in physiological activities (Evseev et al., 1996). This study showed that high gill protein levels were concomitant with gametogenesis and the duration of the recovery period, whereas lower gill protein was observed in winter. As bivalves demand high aerobic metabolism for reproduction (Bayne, 1973) and reduce
their metabolic activity in winter (Gabbott, 1983; Mao et al., 2006), the gill protein level appears to be synchronized with the seasonal variation of organism metabolic activity. Gill protein was also significantly correlated to chlorophyll $a$ levels, which is consistent with greater metabolic activity when more food is available (Baghurst, 2002). Although the mantle protein was not significantly correlated to the chlorophyll $a$, its increase in winter was possibly influenced by the concurrent second annual peak of chlorophyll $a$.

The significantly positive correlation between mantle protein and seawater ammonia suggests that a nitrogen rich diet may promote protein synthesis, which is consistent with previous studies (Bayne and Svensson, 2006; Mao et al., 2006). However, high ammonia can also be toxic and considering the significant negative correlation between ammonia and gill tissue proteins. Protein anabolism may be selective among various tissues depending on environmental conditions, as previously reported for energy catabolism in the Pacific oysters (Garcia-Esquivel et al., 2002). Mao et al. (2006) reported excess utilization of protein during and after spawning period in Pacific oysters in Sanggou Bay (China), whereas this phenomenon was not observed in our study. The gill and mantle proteins were strongly correlated to environmental parameters, but did not contribute to gametogenesis.

Contrasting to tissue protein, hemolymph protein showed a general decline toward the spawning season and a gradual increase after spawning. Its negative correlation to chlorophyll $a$ and temperature suggests a reduction of hemolymph protein synthesis during the period of high metabolic activity in the oyster. The decrease of hemolymph protein during oyster reproduction is consistent with previous studies by Cronin et al. (2001) and Chu and La Peyre (1989). Low hemolymph protein was also found in highly
pathogen-infected *Ostrea edulis* (Cronin et al., 2001) and *C. virginia* (Barber et al., 1988; Chu and La Peyre, 1993). This implies that the chance of disease infection may be increased during the reproductive season. Similarly, the lowest antibacterial activity was detected after spawning, which was consistent with our previous laboratory study (Li et al., 2007). The negative correlation between seawater ammonia and antibacterial activity in hemolymph in this study provides further evidence that ammonia is a toxic compound that can cause stress to oysters. Although there was no massive mortality in summer during this study, the coupled relationship of low antibacterial activity and hemolymph protein is a strong indication of low immunity in spawned oysters. This result is supported by previous detailed reports on spawning relevant immunosuppression by Samain et al. (2007) and Cartier et al. (2004) in Pacific oysters and blue mussels, respectively.

Interestingly, we found no significant correlation between antibacterial activity and temperature. But the elevated temperature in February was concomitant with a decrease of antibacterial activity, which is coincident with previous studies showing that high temperature inhibits oyster immune systems (Chu and La Peyre, 1993; Gagnaire et al., 2006; Li et al., 2007). Consequently, the synergistic interactions between high temperature and spawning (Li et al., 2007), as well as the high prevalence of pathogens in summer (Harvell et al., 1999; Garnier et al., 2007) is a management concern for the oyster aquaculture industry globally.

Overall, this study shows that market-sized Pacific oysters need a month recovery after spawning to become plump in the lean farming area of Stansbury, South Australia. Although glycogen and protein are selectively deposited and depleted among tissues, only mantle glycogen and gill protein provide a good indication of energy storage and
metabolic activity in Pacific oysters. Our results illustrate that while growing oysters in a site with different environmental parameters to the optimal growing areas used overseas, especially regarding food availability and warm temperature, energy reserves and immunity of Pacific oysters behave in the same manner. These results provide more evidence that gametogenesis and spawning are main driving forces of oyster physiology, at least during summer. By providing information on the range of variation in environmental parameters in the field, this study provides a reference point for understanding the stressful conditions imposed on local oysters. It is anticipated this information could be used to improve oyster management strategies in Stansbury and other similar growing or farming regions.

Acknowledgement

We are grateful to Dr. Jeremy Carson from the Fish Health Unit, Department of Primary Industry and Fisheries, Tasmania for kindly providing the strain of marine bacteria and Mr. Paul Dee and his company Southern Yorke Oysters in Stansbury, South Australia for the generous contribution to this project. This work was supported by International Postgraduate Research Scholarship (IPRS) and Flinders University Research Scholarship (FURS) to Y. Li.
References


Baghurst, B.C., 2002. Biological response of the Pacific oyster *Crassostrea gigas* to the South Australian environment, Ph.D. thesis, School of Biological Sciences, Flinders University, Adelaide, pp. 188.


Dridi, S., Salah Romdhane, M., Elcafsi, M.h., 2007. Seasonal variation in weight and biochemical composition of the Pacific oyster, *Crassostrea gigas* in relation to the
gametogenic cycle and environmental conditions of the Bizert lagoon, Tunisia.

559 Aquaculture 263, 238-248.

561 parameters in diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg).
562 Aquaculture 264, 73-81.

563 Dupuy, C., Le Gall, S., Hartmann, H.J., Bréret, M., 1999. Retention of ciliates and
564 flagellates by the oyster *Crassostrea gigas* in French Atlantic coastal ponds,
565 protists as a trophic link between bacterioplankton and benthic suspension-
566 feeders. Mar Ecol Prog Ser 177, 165-175.

567 Dupuy, C., Vaquer, A., Lam-Hoai, T., Rougier, C., Mazouni, N., Lautier, J., Collos, Y.,
568 Le Gall, S., 2000. Feeding rate of the oyster *Crassostrea gigas* in a natural
569 planktonic community of the Mediterranean Thau Lagoon. Mar Ecol Prog Ser
570 205, 171-184.

571 Edward, J.K., 1961. Phosphorus budget of a mussel population Limnol Oceanogr 6, 400-
572 415.

573 Evseev, G.A., Yakovlev, Y.M., Li, X., 1996. The anatomy of the Pacific oyster,
574 *Crassostrea gigas* (Thunberg) (Bivalvia: *Osteridea*). Publ Seto Mar Biol Lab 37,
575 239-255.

577 of the biosphere: integrating terrestrial and oceanographic components. Science
578 281, 237-240.

580 *Crassostrea virginica* from Tampa Bay, Florida: associations of internal defense

582 Frechette, M., Goulletquer, P., Daigle, G., 2003. Fluctuating asymmetry and mortality in
583 cultured oysters (*Crassostrea gigas*) in Marennes-Oleron basin. Aquat Living
584 Resour 16, 339-346.


Shepherd, I.J., 1987. A climatology of Eyre Peninsula, South Australia, School of Sciences. Flinders University, Adelaide, South Australia, pp. 93.


Table 1. Multiple regression analysis between environmental parameters and biological indices of cultured *Crassostrea gigas* in Stansbury, South Australia.

<table>
<thead>
<tr>
<th></th>
<th>R²</th>
<th>Chlorophyll a</th>
<th>Temperature</th>
<th>NH₃</th>
<th>NO₂</th>
<th>NO₃</th>
<th>PO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>0.602</td>
<td>0.165</td>
<td>0.183</td>
<td>-0.401</td>
<td>-0.249</td>
<td><strong>0.776</strong></td>
<td></td>
</tr>
<tr>
<td>Condition index</td>
<td>0.745</td>
<td>-0.318</td>
<td>0.039</td>
<td>0.150</td>
<td><strong>-0.739</strong></td>
<td>0.428</td>
<td><strong>-0.787</strong></td>
</tr>
<tr>
<td>Tissue dry weight</td>
<td>0.413</td>
<td>0.060</td>
<td>-0.016</td>
<td>0.227</td>
<td>-0.533</td>
<td>0.306</td>
<td><strong>-0.642</strong></td>
</tr>
<tr>
<td>Gill glycogen*</td>
<td>0.355</td>
<td>-<strong>0.596</strong></td>
<td>-0.224</td>
<td>-0.195</td>
<td>0.507</td>
<td>0.016</td>
<td>-0.179</td>
</tr>
<tr>
<td>Mantle glycogen</td>
<td>0.061</td>
<td>0.154</td>
<td>-0.002</td>
<td><strong>0.746</strong></td>
<td>-0.556</td>
<td>-0.407</td>
<td>0.252</td>
</tr>
<tr>
<td>Adductor glycogen*</td>
<td>0.694</td>
<td>-<strong>0.666</strong></td>
<td>-0.703</td>
<td>-0.160</td>
<td>0.430</td>
<td>0.233</td>
<td>-0.208</td>
</tr>
<tr>
<td>Adductor protein*</td>
<td>0.362</td>
<td>-0.407</td>
<td>-0.346</td>
<td><strong>-0.602</strong></td>
<td>0.088</td>
<td>-0.068</td>
<td>-0.229</td>
</tr>
</tbody>
</table>

R² value indicates that the proportion of variance in the parameters in the left column is explained by the significant partial correlation(s). The bold font indicates the correlation is significant (P < 0.05) and the variable meets the entry requirement for the most significant regression model analysis (P ≤ 0.05); Asterisk (*) indicates that correlation is insignificant and all variables fail to meet the entry requirement of stepwise multiple regression analysis at 0.05 level.
Figure legends

Figure 1. Map of the Yorke Peninsula, South Australia showing location of the study area in Stansbury. The right panel shows the location of oyster leases in the region and in particular the lease of Southern Yorke Oysters (▲ indicated by the arrow) where samples were taken. Adapted from Atlas of South Australia 2007.

Figure 2. The monthly variation of environmental variables for A) chlorophyll $a$ level ($n = 3$) and water temperature, and B) seawater nutrients: $PO_4$, $NO_2$, $NH_3$ and $NO_3$ ($n = 6$). The values are means ± SE. The asterisk (*) beside each variable indicates significant temporal variation ($P < 0.05$). Different small letters indicate significant difference between months for chlorophyll $a$ ($P < 0.05$). The arrow indicates spawning time.

Figure 3. The monthly variation in *Crassostrea gigas* for A) condition index and tissue dry weight, and B) oyster shells’ length, width and weight (mean ± SE, $n = 6$). The asterisks (*) indicate significant temporal variation ($P < 0.05$) and different small letters indicate significant difference between months using the post hoc analysis ($P < 0.05$). The arrow indicates spawning time.

Figure 4. The monthly changes in biochemical variables measured from the gill, adductor and mantle of *Crassostrea gigas* for A) glycogen, and B) protein (mean ± SE, $n = 3$). Asterisks (*) indicate significant temporal variation ($P < 0.05$). Different capital letters indicate significant difference between months in mantle protein (B); different small
letters indicate significant monthly difference in mantle glycogen (A) and gill protein (B) 
(P < 0.05). The arrow indicates spawning time. (Note: the gill glycogen represents the 
value of the pooled gill samples from 12 oysters)

Figure 5. The monthly variation of A) protein and B) antibacterial activity in the 
hemolymph of *Crassostrea gigas* (mean ± SE, n = 3). Different letters indicate significant 
monthly difference using the post hoc analysis (P < 0.05). The arrow indicates spawning 
time.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.