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Interactions between haemolymph chemistry and condition in the southern rock lobster, Jasus edwardsii

Abstract A haemolymph colour index is developed in an attempt to improve the resolution of serum protein data in the characterisation of temporal and spatial changes in the condition and growth of a wild population of Jasus edwardsii. The index can be used as an indicator of nutritional condition if combined with conventional moult staging techniques. Lobsters and haemolymph samples were collected from two high- and two low-growth sites over two fishing seasons. Haemolymph samples were analysed for serum protein and astaxanthin level and categorised according to colour, that is, “pigment stage” (PS). Moulting stage data were collected and abdominal and hepatopancreatic tissue analysed for percent dry weight. Haemolymph colour changes from light blue-grey, through beige, to deep orange during the moult cycle. These changes were explained with reference to the major pigment, astaxanthin, which increased from 0.135 mg/l (± 0.054, n = 38) at PS 1 (early intermoult) to 2.670 mg/l (± 0.599, n = 12) at PS 4.5 (late premoult). There were significant increases in percent abdominal and hepatopancreatic dry tissue weight over the moult cycle (Kruskal–Wallis non-parametric ANOVA, $P < 0.05$), especially during intermoult. Serum protein levels increased concomitantly and were significantly correlated with percent dry weight of both tissues (abdomen: $r^2 = 0.78$, $n = 871$, $P < 0.001$; hepatopancreas: $r^2 = 0.64$, $n = 864$, $P < 0.001$).

There were also significant differences between sites in both PS-specific serum protein and percent dry tissue. Lobster condition differed significantly between sites, probably as a result of temperature-mediated effects on growth rate. The addition of haemolymph pigment to the serum protein index allows the differentiation of lobsters at the beginning, middle and end of intermoult.

Introduction

The study of condition involves investigation of an organism’s growth response to the biotic and abiotic features of its habitat. Such a study may examine change in body fluid or tissue components or the relationships between body dimensions such as length and weight (Suthers 1991). As lobsters do not retain hard parts against which changes in weight can be measured, assessment must be made of some fraction of the body’s composition. The picture is further complicated by the linkage between condition and the moult cycle. The moult cycle in crustaceans includes the moult (ecdysis) itself, the development of a cuticle to harden the new shell, gonadal and somatic growth, and the development of the next shell under the old in preparation for the subsequent cycle (Passano 1960). Five general stages or phases have been superimposed on this continuous process for ease of description. These are postmoult (subdivided into stages A, B, and C−C3), intermoult (C4), premoult (D), and ecdysis (E) (Drach 1939; Aiken 1973).

Stages A−D can be identified by reference to the state of the cephalothorax integument and development of setae on the appendages using light microscopy (Aiken 1973; Musgrove 2000).

In the periodic assessment of condition in lobsters, one is really obtaining a measure of progression through the moult cycle with its attendant changes in somatic or reproductive tissue mass. Just prior to ecdysis the animal begins to take up water, diluting the haemolymph. This is accompanied by some tissue catabolism in preparation for the moult and to supply metabolic needs associated with the period of inanition during late premoult and ecdysis itself (Depledge and Bjerregaard 1989; Mykles and Skinner 1990; Musgrove and Geddes 1995). Some haemolymph components reach a minimum concentration during postmoult (e.g. protein, haemocyanin, Ca$^{2+}$, Mg$^{2+}$). Levels then increase slowly to peak in early premoult (Mercaldo-Allen 1991). Such changes are more the result of variation in haemolymph space than in haemolymph components per se (Depledge and Bjerregaard...
1989). For example, rising serum protein and haemocyanin concentrations are concomitant with decreasing blood volume (Smith and Dall 1982) and a corresponding increase in tissue mass (Stewart and Li 1969).

Condition assessment in rock lobsters is problematic because of the long intermoult stage (MUSGroVE 2000). Although serum protein concentration may be useful as an index because of its correlation with tissue weight (Stewart et al. 1967), there has been no way of distinguishing between lobsters at the beginning, middle, or end of intermoult (Dall 1974).

This study has three objectives: firstly, to investigate the use of haemolymph pigmentation as a means of subdividing the moult cycle for use in condition assessment; secondly, to explore the relationship between serum protein and percentage weight of dry tissue in the abdomen and hepatopancreas and to examine the nature and degree of change in these factors with change in the colour of the haemolymph during the moult cycle; and thirdly, to look at the capacity of these factors to distinguish between high- and low-growth sites in the fishery as a means of testing the index.

Materials and methods

Field collection of lobsters for haemolymph and tissue analysis

Approximately 50 lobsters of either sex and 80–101.9 mm carapace length (CL) were collected from fishers from each of two low-growth (Robe and Cape Jaffa, Fig. 1) and two high-growth sites (Marion Bay and Kangaroo Island) each month from November 1997 to March 1998. Estimates of growth rates were obtained from Prescott et al. (1997). During the last three months (January–March) of the following season (1998/1999) haemolymph samples were also taken from similar-sized lobsters at Marion Bay and Cape Jaffa.

Initial processing of the lobsters and data collection took place adjacent to the study site. After noting latitude, longitude, and water depth as supplied by the fishers, each animal was sexed, weighed (nearest 0.1 g), and measured (CL, to the nearest 0.1 mm). The state of the lobster’s exoskeleton was also assessed in terms of hardness and fouling to assist with moult staging. Any damage to shell or appendages was also noted. Pleopod samples were taken during the 1997/1998 season for moult stage determination by examination of setal development (MUSGroVE 2000). Pleopod collection was not possible during the 1998/1999 season.

Haemolymph pigmentation of lobsters in the field

A haemolymph sample (1-ml syringe, 22-gauge needle) was taken by pericardial puncture. Colour assessment was standardised as follows. During the first month, photographs were taken of about 150 haemolymph samples to be sure of covering the range of haemolymph colours. The photographs were taken of syringes full of freshly extracted, uncoagulated haemolymph placed on a small light box. All photographs were compared and nine “pigment” stages (PS 0.5 to PS 4.5) identified, representing visually separate points along the continuum of increasing colour intensity (Fig. 2) from light blue, through beige, to deep orange. The photographs were then used to standardise interpretation of the pigment stage for subsequent haemolymph samples using the same light box.

Haemolymph protein analysis

An aliquot of the sample was also placed in a hand-held refractometer (Model UR-2, Industrial and Scientific Supply Co.) to measure the refractive index (RI) (Leavitt and Bayer 1977). The remainder of the sample was snap-frozen at −196°C for later serum protein analysis.

At the laboratory, a range of whole haemolymph samples was randomly selected from the field collection for serum protein analysis. The clotted haemolymph was broken up gently with a glass stirring rod and the sample centrifuged (15 min at 17,280 g) to extract the serum. One aliquot of serum was then taken for measurement of RI and another analysed for serum protein using the Biuret method (Sigma Aldrich test kit 542) on a Cobis Mira Autoanalyser with bovine serum protein as the standard. Accuracy was maintained at ±1 g/l using commercially available quality controls (Nycomed Farmer). The resulting linear regressions were used to convert field whole haemolymph RI to serum RI and the resulting data converted to serum protein (grams per litre).

Fig. 1 Sites for monthly rock lobster collection and sampling of haemolymph during the 1997/98 fishing season: Marion Bay (mb), Kangaroo Island (ki), Cape Jaffa (cj), and Robe (ro). During the 1998/1999 fishing season only Marion Bay and Cape Jaffa were used for monthly surveys of lobster haemolymph

Fig. 2 Haemolymph pigment stages (PS) for the southern rock lobster Jasus edwardsii. Stage PS 0.5 occurs during postmoult but is not included because it is highly variable and it is more practical to assess carapace rigidity at this stage