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Cannibalism contributes significantly to the diet of cultured sand crabs, *Portunus pelagicus* (L.): A dual stable isotope study

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ABSTRACT

The significance of cannibalism in the diet of juvenile pond-cultured blue swimmer crabs (*Portunus pelagicus* (L.)) was investigated using dual stable isotope analysis of carbon and nitrogen. In a laboratory feeding experiment, $\delta^{15}N$ demonstrated a constant trophic shift ($\Delta\delta^{15}N \approx +1.6‰$), and therefore seemed to be a reliable indicator for assessing trophic position for *P. pelagicus*. This agrees with previously reported trends. Difference in growth rate did not seem to influence $\delta^{15}N$ values. In contrast, $\delta^{13}C$ did not display consistent shifts between trophic levels (range of $\Delta\delta^{13}C$: +1 to +1.7‰). The results from the pond experiment showed that larger individuals had a more enriched $\delta^{15}N$ than smaller individuals, which, when compared to the results from the laboratory experiment, indicates that larger individuals were at a higher trophic level. This is most likely due to cannibalism prevailing in the pond rather than a direct result of faster growth rate. Cannibalistic behaviour might further increase growth, resulting in the observed positive correlation between size and $\delta^{15}N$.

1. Introduction

Cannibalism is a common phenomenon amongst crustaceans (Jormalainen and Shuster, 1997; Moksnes et al., 1997; Luppi et al., 2001; Marshall et al., 2005). Irrespective of the size of the species, the intraspecific predation by fitter or larger animals upon smaller or more vulnerable individuals is a factor influencing the dynamics of the population, e.g. recruitment patterns, age structure, microhabitat use (Lovrich and Sainte-Marie, 1997; Luppi et al., 2001). Jormalainen and Shuster (1997) hypothesized that specific conditions encouraged cannibalistic behaviour: i) an unusually small habitat that prevents dispersal, ii) a high local population density, iii) apparent food shortages during peak densities, and iv) structural simplicity of the habitat, which resulted in little or no shelter from conspecifics. Cannibalism is a primary source of mortality in many animals (Elgar and Crespi 1992) and is the major cause of juvenile crab mortality in both natural and culture environments (Luppi et al., 2001; Zmora et al., 2005), affecting population dynamics by altering the distribution of individuals, recruitment patterns, and population size. Such effects have been observed in many brachyuran species, including *Cancer magister* (Lovrich and Sainte-Marie, 1997), *Callinectes sapidus*, *Chionoecetes opilio* and *Carcinus maenas* (Luppi et al., 2001, see review by Wahle 2003).

Crab aquaculture is a growing industry with great potential for high economic gains in Australia and other parts of the world. However, due to the lack of necessary biological information required for the development of appropriate grow-out techniques, the industry is still in the developing phase (National Aquaculture Development Committee, 2002). Although some problems have been solved, the incidence of cannibalism within the culture ponds is still a major constraint on the industry (Fielder and Allan, 2004). Extensive information on the nature of cannibalism is still required to develop suitable production practices that mitigate cannibalism (Fielder, 2004).

In all areas of its wide geographical range in the Indo-west-Pacific, the blue swimmer crab *Portunus pelagicus* supports important commercial and recreational fisheries (Sukumaran, 1997; Kangas, 2000) targeted at production of processed crab meat. Current fishery yields may be unsustainable with heavy fishing pressure, loss of natural habitats and reduced water quality due to urbanisation implicated as threatening the wild populations of *P. pelagicus*. Sustained production of this species is therefore increasingly more dependent on aquaculture. However, the economics of production depends greatly upon efficient intensive production of large numbers of juvenile crabs.

This study aims to provide, with the use of stable isotope analysis, a novel approach to studying cannibalistic behaviour of the blue swimmer crab in an aquaculture setting. A laboratory experiment was conducted to ascertain the applicability of stable isotope analysis,

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particularly δ^{15}N signature, to identifying cannibalistic individuals. The results were then applied to a population of *P. pelagicus* in a semi-intensive pond to investigate the relationship between cannibalistic behaviour and animal size.

2. Materials and method

The study consisted of two components: i) a laboratory manipulative experiment, and ii) a pond (natural) experiment. Both experiments were conducted at Bribie Island Aquaculture Research Centre (BIARC), located on the northern limit of Moreton Bay, southeast Queensland, Australia, in 2004. All crabs utilized in the study were obtained from a single female with post-larvae produced in the hatchery of BIARC. Hatchery feeds consisted of rotifers, *Artemia* nauplii and commercial Penaeid prawn micro-particulate diets. Larvae were harvested from the hatchery tanks at the megalopa stage and stocked directly into an outdoor pond as well as 2 m×1 m×0.8 m (LxWxD) 'hapa' nets floating within the same pond. After stocking the post-larvae in the pond and nets, the crabs were fed to excess a commercial prawn diet (Ebistar, Higashimaru, Japan) 3 times per day.

2.1. Laboratory experiment

A laboratory-based feeding experiment investigated whether the stable isotope approach was an applicable technique for identifying cannibalistic animals within a population of pond-reared crabs.

Juvenile crabs in the C3 stage (3rd post-larval crab instar) were collected from "hapa" nets. Seventy-two crabs were randomly assigned to two treatment groups: cannibals and non-cannibals, 36 per treatment, and were subsequently housed individually in small containers. The cannibal group was fed specially prepared 'food' crabs from the same cohort and the non-cannibal group was fed the same particulate prawn diet as provided to crabs in the pond. To ensure that the cannibal crabs would only be one trophic level above the non-cannibal crabs (i.e. the food crabs would not prey upon other crabs) the food crabs were also maintained individually in containers. Ten food crabs were randomly selected for measurements of initial nitrogen and carbon isotopic signatures. Food crabs were chilled and then halved or quartered prior to feeding to the cannibals. All crabs were fed 2x per day with a quantity expected to be slightly in excess of that consumed during the feeding period. This amounted to 1/4 crab twice a day (1/2 crab per day) for the cannibal crabs. Left over food was removed using a small scoop net prior to the next feeding.

The experiment was conducted in 200 litre trays within a partial recirculating system. Each tray drained to a sump where mechanical fitration and biological filtration, aeration and heating occurred prior to being pumped back through the trays. High water quality was maintained by water exchange with fresh, 10 μm filtered seawater at 200% of volume per day. Within the trays the treatment crabs were kept in individual containers (100×100×150 mm). Each container received an inflow of seawater from an overhead distribution pipe and a mesh covered slit in the container allowed water to flow out. The water level in the containers was set to 75 mm, leaving adequate space (75 mm) above the water to prevent the crabs from escaping while allowing enough water for moulting to occur. Continuous flow of water into each container ensured sufficient oxygen levels as well as flushing out waste material and the unidirectional flow also prevented cross contamination of food among treatments. Water temperature was maintained above 24 °C for the entire experimental period, with temperatures varying between 24.5° − 32.0 °C. Flow rate of new seawater into the system was initially set to 3.7 L min^{-1} (260% day^{-1}), however this was reduced to 2.0 L min^{-1} (140% day^{-1}) after two weeks.

Once a day, large particles such as faeces and left over food were removed by rapidly draining the tray and allowing the recirculating water to pass through a 10 μm filter bag.

2.1.1. Sample preparation

In weeks three, five, seven and nine of the experimental period, 6 crabs from each treatment were randomly sampled for analyses of δ^{15}N and δ^{13}C signatures. Their body mass and carapace width (CW, exclusive of the lateral spines or "notch to notch") were also recorded. After removal from the water the crabs were put on ice for a 1.5 h transport time before being frozen at −20 °C prior to analysis.

Tissues for stable isotope analysis were extracted from the crabs before they were completely thawed. The cheliped muscle tissues were then removed and dried at 60°C for ~24 h. Due to the small size of the crabs obtained in the first two sampling times (<25 mm CW, weeks 3 and 5), additional tissues (gills and body muscle) were used to get sufficient amounts of tissue for the stable isotope analyses. Only cheliped muscle tissue was used for stable isotope analysis in the last two sampling times. The samples for the pre-experimental nitrogen and carbon isotopic composition were composite samples of gills and body muscle tissue of eight individuals as the size of the crabs (<10 mm CW) prevented extraction and analysis of sufficient tissue from single crabs.

Dry crab tissue was ground and homogenised with a mortar and pestle, and a sample of ~0.6 mg was weighed to 0.01 mg and kept dry in a desiccator before being analysed for carbon and nitrogen isotopic composition using a continuous flow Isoprine isotope ratio mass spectrometer (G.V. Instruments, Manchester, U.K.).

2.1.2. Data analyses

Exploratory data analysis was performed using SigmaPlot 2000. Student's t-tests were used to compare the mean body mass and CW of the two treatments each sampling week. Two-way ANOVAs were applied to test the effects of treatment (Fixed factor, 2 levels: cannibals and non-cannibals) and time (Fixed factor, 4 levels: weeks 3, 5, 7 and 9) on δ^{13}C and δ^{15}N, after checking the assumptions. In order to test whether a relationship between crab size and δ^{15}N could be detected, Pearson correlations were performed on data pooled across all sampling weeks, and for individual weeks for the two treatments. In the cases where the assumptions of the Pearson's correlation were violated, Spearman's rank correlation was applied. A Pearson's correlation was run to test the relationship between δ^{13}C and δ^{15}N. The level of significance, α was set at 0.05 for all analyses. All statistical tests were performed using SPSS for Windows, version 11.5.

2.2. Pond experiment

This part of the study focused on whether a correlation between cannibalistic tendency and growth rate in a high-density aquaculture pond could be identified. The pond (15 m×15 m×1.8 m) was stocked with ~12,000 megalopa (~50 m^{-2}). Twice a day, both prior to and during the 9-week experimental period, 80 g of a commercial shrimp pellets was broadcast over the surface of the pond. The crabs were also feeding opportunistically on the algae and small invertebrates that made up the epibenthic layer.

On the first day of the experiment, ten crabs were sampled for baseline carbon and nitrogen isotopic composition. Additionally, possible natural food sources (i.e. other invertebrates and macroalgae) were sampled from the trial pond and adjacent ponds being managed in the same manner to provide a reference of the signatures of alternative food items. Ten crabs were collected from the nursery pond every 2 weeks over a 9 week period however due to the difficulty extracting tissue from very small crabs, the first sampling was conducted at week 3. At each sampling time, about 40 crabs were collected using a butterfly net pulled through the water and along the pond side. Ten crabs representing the spread of the size spectrum were then sampled randomly from this initial catch. Their mass, carapace width and carapace lengths were measured and recorded and the crabs were then frozen for storage individually. Triplicate plankton samples were also taken by filtering 300 mL of the pond water through a pre-combusted GF/C glass fibre filter paper with a