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Effect of manufactured diets on the yield, biochemical composition and sensory quality of *Evechinus chloroticus* sea urchin gonads

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ABSTRACT

Sea urchins (*Evechinus chloroticus*) were held in sea cages and fed either one of two manufactured diets, or seaweed. Physical (gonad index, maturity index and colour $L^*a^*b^*$), biochemical (lipids, glycogen, soluble protein, moisture and free amino acids) and sensory characteristics of the gonads from caged and wild sea urchins were measured at the end of a 13- week trial. The gonad index of sea urchins fed the manufactured diet which was highest in protein, was approximately double that of wild sea urchins, and significantly ($p \leq 0.05$) higher than sea urchins fed seaweed. Gonads from sea urchins fed the manufactured diet highest in protein had significantly ($p \leq 0.05$) higher glycogen and total free amino acid concentration, and a lower triglyceride concentration than gonads from sea urchins fed seaweed or collected from the wild. Ovaries had a significantly ($p < 0.01$) higher soluble protein concentration than testes, and there were significant ($p \leq 0.05$) differences in the concentration of individual free amino acids between genders and diet. Descriptive analysis carried out using a trained sensory panel reported differences in gonad sensory quality. Ovaries from wild sea urchins were significantly higher for sweet and umami taste, than ovaries from sea urchins fed the manufactured diets, which had a higher rating for bitter taste. Testes from wild sea urchins had a significantly harder texture than testes from sea urchins fed manufactured diets or seaweed. Sweet taste was significantly ($p \leq 0.05$) positively correlated to glycine concentration in testes but not ovaries, while bitter taste was positively correlated to valine, leucine, and isoleucine concentration in both testes and ovaries. This study demonstrated that diet had a significant influence on the physical, biochemical and sensory characteristics of *E. chloroticus* gonads, and highlighted the importance of analysing *E. chloroticus* testes and ovaries separately.

1. Introduction

The increasing demand for sea urchin gonads has in many cases resulted in an overexploitation of natural stocks. This decline has resulted in worldwide interest in both the development of methods for sea urchin aquaculture to increase gonad yield and quality (Liyana-Pathirana et al., 2002) and in the utilisation of previously unfished wild stocks of sea urchins. There is an abundant supply of the native species, *Evechinus chloroticus* Valenciennes (Echinoidea: Echinometridae), in New Zealand (Booth and Cox, 2003). However, attempts to develop an export market have been unsuccessful as the gonads are variable in size and colour, and have been reported to have an intermittent bitter taste (McShane et al., 1994). Both the sensory quality and consistency of *E. chloroticus* gonads need to be enhanced in order to develop a successful export industry in New Zealand (Goebel and Barker, 1998). Methods of gonad enhancement include growing out from juveniles or the enhancement of wild-caught sea urchins, with the latter being faster and less cost intensive. Many enhancement projects conducted in New Zealand are laboratory-based as it allows greater control of physical parameters and measuring of feeding rates. However, sea-based holding systems for gonad enhancement tend to be more economical than land-based facilities, due to lower capital, operating and maintenance costs, especially if existing mussel farms can be used (James, 2006a).

It is important to appreciate that even though food is the strongest driver of an increased gonad size, other environmental factors are also known to influence the gonad size and gametogenic cycle of *E. chloroticus* (James and Heath, 2008b). Gonad enhancement trials have been carried out for *E. chloroticus* in sea-based holding systems (Fell, 2002; James et al., 2004; James, 2006b; a), with no significant

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difference being reported for the gonad index (GI) or gonad colour between sea urchins from sea- and land-based experiments (James, 2006a). As the price of gonads is strongly influenced by their appearance, colour, texture and flavour (Unuma et al., 2002; McBride et al., 2004), it is important to measure these quality attributes as well as yield, in diet enhancement trials. Studies on other sea urchin species have compared the gonad yield and quality of sea urchins from the wild with those fed kelp/seaweed or manufactured diets (Bureau et al., 1997; Vadas et al., 2000; McBride et al., 2004; Pearce et al., 2004; Senaratna et al., 2005; Siikavuopio et al., 2007).

Manufactured diets have been shown to affect the yield, biochemical composition and sensory quality of gonads. Fernandez (1997) reported that a manufactured diet rich in protein favoured the storage of reserves in the gonad in the form of lipids and/or carbohydrates. However, high levels of protein (% dry weight) in diets have been associated with bitter tasting gonads (Pearce et al., 2002b). Manufactured diets have also been shown to influence the free amino acid (FAA) composition of *Strongylocentrotus droebachiensis* gonads (Liyana-Pathirana et al., 2002), and the authors recommended that sensory evaluation be carried out to confirm if taste quality is affected by diet. Previous studies reporting differences in the quality of *E. chloroticus* gonads caused by diet (Barker et al., 1998; Goebel and Barker, 1998; James et al., 2004; James et al., 2007; Woods et al., 2008) did not consider gender differences. As gender has been shown to strongly influence gonad quality (Phillips et al., 2009, 2010a,b), an equal gender split within each diet treatment was recommended by Phillips et al. (2009) for future diet studies.

The aim of this trial was to compare the gonad yield, gender specific biochemical composition differences and sensory quality of gonads from *E. chloroticus* fed either one of two manufactured diets or seaweed, with wild sea urchins. Relationships between biochemical composition and sensory quality of gonads were explored to increase understanding of the influence of husbandry practices on gonad quality. The trial was carried out in winter because at this time gonads actively increase in size in response to feed intake. Furthermore this allowed sea urchins to be harvested before they reached the mature and partly spawned stages in their reproductive cycle.

2. Methods

2.1. Sea urchin samples and diet composition

Sea urchins were collected from Dieffenbach Point at the Queen Charlotte Sound entrance to the Tory Channel (41° 14′ 10 S, 174° 08′ 65 E), off the northeast coast of the South Island of New Zealand on 5th June 2006 and placed in 20 L plastic buckets filled with seawater. Sea urchins were then transported to D'Urville Island (40° 49′ 60 S, 173° 52′ 0 E) and placed in metal-framed cages covered in plastic mesh (600 mm × 600 mm × 250 mm) on arrival. Thirty sea urchins were placed in each cage (nine cages in total) and the cages were suspended along a mussel line in Catherine Cove, 60 m off the coast at a depth of approximately 2 m. The trial commenced on 5th June 2006 and ran for a total of 13 weeks.

Sea urchins within the cages were fed either manufactured diets, prepared at the Product Development Research Centre, University of Otago (Table 1), or wild-collected seaweed as a natural diet. Manufactured diets were a semi-moist product that had been cooked in sealed plastic sausage casings for 25 min at an internal temperature of 75 °C, and then stored at 4 °C until required. Species of wild seaweeds found at both the collection site and the cage location included *Macrocystis pyrifera*, *Carpophyllum maschalocarpum*, *Ulva* sp., *Sargassum* sp. *Marginariella* sp., and *Xiphophora* sp (Brewin et al., 2000). At weekly intervals the cages were hauled to the surface and the urchins were fed either unwashed seaweeds harvested locally or manufactured diets ad libitum. Small amounts of uneaten diet were almost always present at the subsequent feedings. Sea urchins were collected from the Tory Channel on September 20th 2006 as a post experiment comparative sample. Following the feeding trial, sea urchins were packed in polystyrene bins, covered in damp sacking and transported (18 h) at 6 °C to the Department of Food Science at the University of Otago, where they were held at 4 °C prior to analysis. Groups of between 10 and18 sea urchins per diet treatment were used for physical and biochemical analyses (one gonad from each sea urchin). A random subset of 10 sea urchins (five males and five females where possible) per treatment was subsequently used for sensory analysis (four gonads from each sea urchin).

Table 1
Composition of the two manufactured diets.

	Percent in diet	
	Diet 1	Diet 2
Ingredients		
Arcon SM (concentrate)	19.0	11.0
Wheaten cornflour	27.9	35.0
Flaxseed oil	5.4	5.9
Water	22.3	20.2
Kelp (wet)	19.5	22.1
Vitamin premix	0.3	0.3
Mineral premix	0.3	0.3
Algro	0.3	0.3
Glycine	0.0	1.2
MSG	0.0	1.2
CaCO3	0.0	0.4
Sodium alginate	0.0	2.0
GLA acid	0.0	0.1
Glycerol	5.0	0.0
Proximate composition		
Total protein (dry basis)	22.8	17.8
Total carbohydrate (dry basis)	51.7	60.3
Total fat (dry basis)	10.8	11.4
Total water	42.5	42.8

2.2. Physical analysis

Dissection protocol was carried out according to Phillips et al. (2009). A small sample of gonad from each sea urchin was preserved in 10% formalin for histological analysis to determine gender and the sexual maturity index (MI) of the gonads according to the method of Byrne (1990). Colour analyses were carried out on one of the five gonads removed from each sea urchin using a HunterLab Miniscan XE™ at D65/10°. Three replicate measurements of L^*, a^* and b^* were taken for each individual gonad. The individual gonad was then homogenized in preparation for biochemical analysis using an Ultra-Turrax T25 with an 18G blending head at 8000 rpm for 1 min (Janke & Kunkel IKA®-Labortechnik) in plastic tubes (30 mm × 100 mm). The homogenate was divided into aliquots of approximately 1 g, which were placed into microcentrifuge tubes and stored at −80 °C until required.

2.3. Biochemical analysis

Lipid determination was carried out according to Sewell (2005) with modifications. A known amount of roe homegenate (16.5 to 40.0 mg) was diluted with 50 times the volume of nanopure water in a 2 mL Eppendorf tube. Each Eppendorf tube was sonicated three times for 15–20 s at an amplitude of 7–10 μm with an ultrasonicator fitted with an Eppendorf tip, with 1 min intervals on ice in between bursts of sonication. The Eppendorf tube was then vortexed and six aliquots (250 μL each) of the roe homogenate were prepared and frozen at −80 °C until further lipid analysis. For each roe sample, two of the 250 μL aliquots were used for lipid extraction. Lipids were extracted with 50 μL of HPLC grade chloroform added to the N_2-dried sample before spotting. The amount of each lipid class (triglycerides,