2008

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Publication details

Postprint of: Woodcock, SH & Benkendorff, K 2008, 'The impact of diet on the growth and proximate composition of juvenile whelks, *Dicathais orbita* (Gastropoda : Mollusca)', *Aquaculture*, vol. 276, nos. 1/4, pp. 162-170.
The publisher's version of this article is available at http://dx.doi.org/10.1016/j.aquaculture.2008.01.036

The impact of diet on the growth and proximate composition of juvenile whelks, *Dicathais orbita* (Gastropoda: Mollusca).

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Abstract

Dicathais orbita is a predatory marine whelk of interest as a new source of protein for potential production in aquaculture. Establishment of a successful aquaculture industry based on this species will require optimisation of their diet for fast growth and survival, whilst maintaining a good proximate composition in the flesh. Here we compare the effects of four diets fed to juvenile *D. orbita* over a 12 week period, to evaluate consumption, feed conversion, growth rates, mortalities and proximate composition. It was found that diet impacts significantly on the growth and survival rates of this whelk, with bivalve feeds resulting in significantly higher consumption ($P < 0.0001$) and growth rates ($P < 0.0001$) compared to artificial pellets, despite similar feed conversion ratios ($P = 0.359$). The artificial pellets were found to have significantly less moisture, but higher protein, glycogen and lipid content than the bivalve feeds ($P < 0.05$) and resulted in whelk flesh with significantly higher energy and ash content ($P < 0.05$). *D. orbita* showed a preference for scavenging frozen bivalves over predation on live molluscs in captivity, which could reflect an optimal foraging strategy to minimise the energy required to subdue prey. Overall, juvenile *D. orbita* display similar growth (up to 0.8g/month) and high survival (>90%) compared to other gastropods in culture. Their flesh has a high calorific value (~19kj/g), with significantly higher protein (>26 mg/g) and glycogen (>35 mg/g) content than their bivalve prey ($P < 0.05$). Consequently, this species has a promising future as a new species for molluscan aquaculture in Australia.

Key Words: Feeding Preference; Growth Rate; Muricidae; Nutritional Quality**;** *Dicathais orbita*.

1. Introduction

The demand for protein rich food is increasing, especially in developing countries, stimulating exploration of underutilised or non-traditional resources. Whelks are predatory marine gastropods that are highly favoured for their high protein meat (Xavier Ramesh and Ayyakkannu, 1992). Whelks in the family Muricidae are currently fished throughout Asia (Nugranad et al., 1994; Xavier Ramesh et al. 1994), Europe (Marin et al., 1995; Ramon and Amor, 2002), Central and South America (DiSalvo, 1994; Leiva and Castilla, 2002; Naegel, 2004a). These whelks are primarily exported to Europe, Japan, China and Korea where their meat is considered a delicacy (Marin et al., 1995; Ramon and Amor, 2002). In Australia, the collection of whelks only occurs at a recreational level (Kingsford et al., 1991), with no consistent supply through established fisheries or aquaculture. Consequently, the commercial potential of locally common species, such as the Dogwhelk *Dicathais orbita* (Gmelin 1791) remains to be investigated.

Murcid whelk fisheries can provide a valuable commodity for local communities (e.g. FOA, 2002; Leiva and Castilla, 2002), with additional resources including the natural dye Tyrian purple (Naegel, 2004a), operculum and shell (Patterson and Ayyakkannu, 1993; Patterson Edward et al., 1994), greatly adding to their meat value. However, fishery pressure on some whelks has led to serious stock depletion and localised extinction (e.g. Cadee et al. 1995; Uyan and Aral, 2003), thus instigating controlled harvest as part of an active management regime in some places (e.g. Central and South America, DiSalvo, 1994; Naegel, 2004a). Excessive harvesting has also led to the development of several research programs aimed at reseeding natural populations and reducing fisheries pressure through the aquaculture of valuable Muricidae species (DiSalvo and Carriker, 1994; Nugrand et al., 1994; Xavier Ramesh et al., 1994; Naegel, 2004b).

On-growing efficiency (growth rate and feed conversion) is one of the three principle biological criteria that must be considered for the production of a new species by aquaculture (Webber and Riordan, 1976; Le Francois et al., 2002). Feed can represent 40 - 50% of overall production costs in aquaculture (Webber and Riordan, 1976; Craig and Helfrich, 2002). Therefore it is important to understand the nutritional requirements of a species, to devise optimal, physiologically-efficient and economic feeds (Webber and Riordan, 1976). Previous laboratory studies on predatory whelks (Neogastropods) indicate that providing food in excess can lead to increased growth rates and survival (Morton, 1986; Chaitanawisuti and Kritsanapuntu, 1999; Chaitanawisuti et al., 2001). Prey type has been shown to influence growth rates both in laboratory experiments (Gutiérrez and Gallardo, 1999; Nasution and Roberts, 2004) and in the field (Moran et al., 1984; Etter, 1995). Diet has also been shown to have an impact on the nutritional composition and taste of the Muricidae *Chorus giganteus* (Carrasco et al., 2006). Consequently, proximate (nutritional) analyses of whelks fed on different diets could be useful for optimising the diet to obtain a high quality muricid product.

The Muricidae are dominant gastropod predators, feeding opportunistically on a range of prey (Etter, 1996; Gutiérrez and Gallardo, 1999; Morton, 1999; Carrasco et al., 2006; Peharda and Morton, 2006). Prey selection can be influenced by the detectability, accessibility and nutritional content, as well as the ease of prey capture and time required for processing (Hughes and Dunkin, 1984; Gutiérrez and Gallardo, 1999). Feeding studies on *Chicoreus ramous* in culture have revealed that they will only eat live food, contributing greatly to the cost and logistical difficulties of culturing this species (Nugrand and Kerdppoom, 1995). However, some Muricidae including *D. orbita*, have been observed to scavenge fresh carrion (Phillips, 1969; Morton, 1999). This behaviour provides opportunity to explore the potential use of frozen or artificial feeds in Muricidae culture.

The aim of this research was to study the dietary preference, growth rates and nutritional quality of the Australian muricid *Dicathais orbita*, to assess the grow-out prospects for using this whelk as a future aquaculture species. *D. orbita* is distributed along the temperate coast of Australia and New Zealand, within the intertidal and shallow subtidal zones of rocky shores (Phillips et al., 1973). Philips and Campbell (1974) have established that the size at sexual maturity is approximately 38mm for this species. The current investigation involved two separate experiments; the first compared growth and nutritional quality parameters of *D. orbita* fed four different diets; and the second looked at preference between two diets. The null hypotheses tested in these experiments were: (1) diet will have no impact on the growth rate of *D. orbita*; (2) food type will have no impact on the proximate composition of *D. orbita* and (3) *D. orbita* will display no preference for food type.

2. Materials and methods

2.1 Animal collection and maintenance

Dicathais orbita juveniles were sourced from sub-populations along the Fleurieu and Eyre Peninsulas, South Australia. All whelks were combined in the laboratory and juveniles less than 30 mm (maximum shell length dimension mean 22.4 ± 3.19 mm and fresh weight mean 1.78 ± 0.728 g) were marked (using coloured nail polish) then randomly allocated to one of 12 feeding tanks. Each tank contained a total of 15 individually marked whelks that were left to acclimatise without feed for two weeks prior to the commencement of feeding trials. Dead whelks were removed as soon as noticed and replacement whelks were uniquely marked and added in exchange to ensure consistency in the number of whelks consuming prey between treatments. Replacement whelks were excluded from the growth and nutritional analyses.

Feeding and growth experiments took place in a saltwater recirculation system attached to a bio-filter and protein skimmer, with a constant mean temperature of 18^{o}C and salinity of 41 ‰ (natural salinity reaches as high as 46 ‰ towards the head of the Spencer Gulf, S.A. where *D. orbita* occurs). Twelve aquaria (395 mm x 295 mm) were used with a constant flow rate (Pan World Magnet Pump HH-200ps STD point flow rate 50L/minL), whereby the water outlet was situated halfway up the aquarium to allow whelks to migrate in and out of the water (tank height 300 mm and water depth 135 mm, 16 litres).

2.2 Effect of diet on growth rates

Four feeding treatments were replicated in three separate tanks, randomly distributed in the aquarium system. Feed treatments included: (1) live mussels, (2) frozen mussels, (3) frozen cockles, and (4) pellets. Mussels (*Xenostrobus pulex*, Lamarck 1819) were collected from rocky reefs along the Fleurieu Peninsula. Frozen cockles (*Katelysia* spp.) were obtained from a commercial supplier (Born to Fish, O'Halloran Hill, South Australia). Hikari Tropical Sinking Wafers, designed for bottom feeding fish, were employed as artificial feed. Several other pellet types were tested in a pilot study, however the Tropical Sinking Wafers were the only artificial feed ever observed to be consumed by the whelks.

Whelks were fed to satiation by providing each feed treatment in excess throughout the 12 week growth experiment. All food was replaced three times a week, except for pellets, which were replaced more regularly (morning and night 5 days per week) due to the relatively rapid loss of structural integrity. Feed was weighed on a bench top scale (Mettler Toledo, Mono Bloc inside PB 602-S) before and after the feeding period to determine consumption. Uneaten pellets were removed from aquaria and dried in a drying oven at 60^{o}C for 24 h before weighing. Consumption was adjusted to account for autogenic changes in the weight of feed due to tank conditions (Table 1). This was achieved by recording the weight of replicate batches of each feed placed in aquaria without whelks for the same time period as the feeding trial for each diet.

Growth measurements of weight (g) and shell length (mm) were undertaken at the beginning of the feeding experiments (t_0) (Table 1), then again at weeks three (t_3), six (t_6), nine (t_9) and 12 (t_{12}). Shell length was measured using SON TAX 150 mm digital calipers to the nearest 0.01 mm, from the apex of the shell spire to the anterior (siphon) canal. Wet weight was measured to the nearest 0.01 g on bench top scales (Mettler Toledo, Mono Bloc inside PB 602-S) after drying with paper towel. Growth increments were calculated as the change in shell length and weight over time according to Nasution and Roberts (2004).

2.3. Nutritional proximate analysis

Proximate analysis was conducted on all distinct feed types used in the feeding trials, with data collected from frozen mussels used to represent both the live and frozen mussel diet. Analysis was also carried out on 30 randomly-selected whelks from each treatment, at the end of the 12-week growth trial, to determine if feed type influenced the proximate composition of *D. orbita* flesh. Analysis of proximate composition included amounts of moisture, lipid, protein, glycogen, ash and total energy of flesh. Five whelks were randomly selected from the frozen 30 for each diet treatment and allocated to one of the six proximate measurements. Samples were pre-frozen and shells and opercula were removed for analysis of the whole wet flesh. Flesh from the five whelks was combined and ground using a mortar and pestle, then split into three replicate samples and weighed for testing. For the proximate composition analysis of diets, three individual cockles were used as separate replicates for each test, whereas for the mussels and pellets several were combined to achieve the minimum weight required for the test (1g). All weights recorded for proximate composition analysis were obtained using an analytical scale (Mettler Toledo AB204-S).

For moisture content, samples were weighed then placed in a drying oven set to 60^{o}C until a constant weight was obtained and then re-weighed (24 - 48 h). Lipids were extracted using the Bligh and Dyer (1959) method, in 1:2 chloroform methanol ratio. Samples were soaked in 10 - 20 mL of solvent for 1.5 h, then decanted and the solvent replaced until no further colour was extracted from the tissue. The decanted solvent extractions for each sample were combined and allowed to evaporate in a fume hood until a consistent weight was achieved for the dry lipid extract. For protein determination, samples were placed into 10 mL of 0.5M sodium hydroxide and left for two weeks before the top liquid layer was removed (approximately 5 mL) and used to test protein content using the procedure of Bradford (1976), with Coomassie Blue Protein Assay (Bio-Rad, Laboratories Inc.). Absorbance was measured on a spectrophotometer (Metertech UV/VIS SP 8001) at 595 nm, at 30 min, then converted to protein concentration by comparison to a standard curve prepared using Bovine Serum Albumin (Sigma). Glycogen was assessed using a method adapted from Krisman (1962) by homogenizing the flesh with 200 mL of 0.06M perchloric acid (PCA). After centrifugation (Amscorp PTY LTD, ALC Centrifugette 4206) at 6,000 rpm for 30 min, the supernatant (0.4 mL) was decanted into a macro-cuvette with 2.6 mL of iodide (I_2KI) reagent and left for 40 min. Absorbance was read on a spectrophotometer at 460 nm then converted to glycogen concentration by comparison to a standard curve prepared with Oyster Glycogen (Sigma). To determine the ash content, samples were dried in an oven at 60^{o}C for 24 h, weighed and then transferred into the muffle furnace for 4 h at 550^{o}C then reweighed. Energy was determined with a ballistic bomb calorimeter using benzoic acid as the reference standard.

2.4. Preference trial

Trials were conducted to determine if whelks showed a preference between different diets. All cockles (*Katelysia* spp. mean size = 34 mm) were dug up by hand at Section Bank, Outer Harbour, South Australia. Half were kept alive in an aquarium and the other half frozen. Greenlip abalone (*Haliotis laevigata* Donovan, 1808), were sourced from Southern Australian Seafood Pty Ltd (Eyre Peninsula), with mean size of 20 mm (small) and 50 mm (medium). All preference trials ran for one week. Thirty whelks were selected and randomly allocated into one of six tanks (n= 6, five whelks per tank). Whelks were starved for one week before each trial.

Preference trials included feeding one of each of the following diet combinations to whelks: (1) frozen cockles versus live cockles; (2) live cockles versus the artificial pellet used in the growth experiment; (3) frozen cockles versus medium- sized live abalone (50 mm); (4) frozen cockles versus small abalone (20 mm); (5) live cockles versus live abalone (20mm) and (6) small abalone (20 mm) versus medium-sized abalone (50 mm).

2.5. Statistical analyses

Statistical analysis was undertaken on each set of growth measurements and the feeding preference trial using SPSS 14.0 with significant differences recorded for $P \leq 0.05$.The assumption of normality was tested by examining histogram and Q-Q plots and homogeneity of variance was assessed using Levenes test. Log transformations were applied to weight and length data in the growth trial. A linear regression model was used to determine the relationship between log-length and log-weight. Differences between the mean size of whelks between treatments at each time point were assessed using a repeated measures ANOVA with post hoc Tukey analysis to determine where significant differences occurred. Growth rate data was analysed in the same way with time as the repeated measure, and replicate tanks nested within feeding treatment. The number of mortalities, amount consumed and feed conversion ratios were tested using one-way ANOVAs at the end of the twelve week feeding trial.

Due to violations of the assumption of normality or equal variance, which could not be corrected using data transformations, a non-parametric Kruskal Wallis test was used to examine the difference in nutritional parameters between feeds. Comparisons were also made for whelks fed different diets, as well as whelks and their respective diet, for each proximate analysis test. Whelks feed on both live and frozen mussels were compared to the nutritional composition of frozen mussels in this analysis. Multidimensional scaling (MDS) and analysis of similarities (ANOSIM) was produced using Primer, Version 5, to determine overall differences between diets, whelks and whelks with their respective diets. All proximate components except moisture were used in this analysis. Data was treated by standardising to give equal weighting between each proximate component; a similarity matrix was then conducted using normalised Euclidean distance.

For the feeding preference trials, statistical analysis was carried out using paired sample t-test or non-parametric Kruskal Wallis tests (if assumptions of normality and equal variance were not meet) for both the number of prey items consumed, as well as the amount of prey item consumed, per tank at the end of the week.

3. Results

3.1. Effect of diet on growth rates

Whelks were observed to consume all diet options (Table 1). The 12-week feeding trial revealed that the maximum change in growth occurred in whelks fed on frozen cockles, followed by frozen mussels, live mussels and pellets (Table 1, Fig. 1). Changes in final weight and length were substantially lower in the artificial diet compared to other treatments (Table 1). As there was a positive linear relationship between the log-weight and log-length of whelks ($R^2 = 0.951$, $P < 0.0001$), only analyses for weight are presented. There was no significant differences between tanks within treatments over time, for both weight ($P = 0.739$)

and growth increment (P = 0.586) (Table 2). However, repeated measures ANOVA displayed a significance difference in weight between treatments over time (Table 2a). Post hoc Tukey analysis revealed that a significant divergence in weight commenced in week six between whelks fed on frozen mussels and frozen cockles (P = 0.028). By week nine, whelks fed on frozen cockles weighed significantly more than all other treatments (live mussels P = 0.015, frozen mussels P = 0.018 and pellets P < 0.001; Fig. 1a). The significantly heavier weight obtained by whelks fed on frozen cockles was maintained through to the end of the growth trial (week 12, Table 1 and 2a) and in addition, there were significant differences in weight between whelks fed on pellets and frozen mussels (P = 0.023). However, there was no difference between whelks fed on frozen and live mussels (P = 1.000), or live mussels and pellets (P = 0.108).

Over the 12 weeks, whelks fed on frozen cockles displayed the highest overall growth rate with respect to both length (mm/week) and weight (g/week; Table 1). Whelks fed on pellets displayed very little growth. Repeated measures ANOVA revealed that growth significantly varied between feed treatments over time (Table 2b). Whelks feeding on both live and frozen mussels had a similar growth increment, except during weeks six to nine (Fig. 1b), where the growth for whelks fed on live mussels was significantly lower (P = 0.005). In weeks six to nine, whelks fed on frozen cockles were not significantly different to whelks fed frozen mussel (P = 0.084), but with the exception of this time, whelks fed on frozen cockles had a significantly higher growth than any other treatment (P < 0.020). Whelks fed on pellets displayed a significantly lower growth after the first three weeks (P < 0.008), with weight decreasing up until week nine, followed by an increase in growth between weeks nine and 12 (Fig. 1b).

The mean amount of feed consumed for each treatment per week is shown in Figure 2, after adjustment for autogenic changes (Table 1). After the 12-week period, the overall amount of feed consumed was significantly different between treatments ($P < 0.001$, Table 3a), with whelks fed on frozen cockles consuming the most, followed by frozen and live mussels (Fig. 2). Intake of pellets was five times lower than all other feeds (Table 1, Fig. 2). No significant differences occurred between treatments for the feed conversion ratios ($P = 0.359$) (Table 3b). There was a strong positive correlation between the change in weight and amount of feed consumed by whelks ($R^2 = 0.925$, $P < 0.001$).

Mortalities occurred in three of the four treatments across the 12 weeks (Table 1, Fig. 3). The highest number of accumulated deaths occurred in the pellet diet treatment (Fig. 3) with more than 30% of the original population dying (several due to cannibalism). No mortalities occurred in the frozen mussel treatment tanks (Fig. 3) and over 90 % survival was recorded for frozen cockle and live mussel treatments (Table 1). At the end of the 12 week period, ANOVA showed a significant difference in the mean number of deaths between treatments ($P < 0.001$, Table 3c). Post hoc Tukey analysis revealed that significantly more whelks died when fed on the pellet diet compared to live mussels ($P = 0.001$), frozen mussels ($P = 0.001$) and cockles ($P = 0.02$). There was no significant difference between the number of deaths between the other treatment pairs ($P > 0.05$), which were all bivalve molluscs.

3.2. Nutritional (proximate) analysis

Overall, the moisture content of the juvenile whelk flesh was found to vary between 67-87% of the fresh weight, which was a bit lower than the bivalve diets

(Fig. 4). The ash content (3.5 ± 0.4%) of juvenile whelks was also lower than the two bivalves tested, where as protein (2.8 ± 0.3%), glycogen (4 ± 0.8%) and lipids (9.7 ± 0.7%) were higher (Fig. 4, Table 4). On average whelk flesh was found to provide 19 ± 2 kj/g.

Proximate analysis for the various diets fed to whelks showed there were fewer differences between cockles and mussels than between these bivalves and the pellets (Table 4a). The only significant difference between cockles and mussels was for inorganic content (ash, $P = 0.050$) and energy ($P = 0.050$), which were both higher in mussels (Table 4a). Pellets were similar to mussels for these two parameters ($P = 0.273$ and 0.513, respectively). However, pellets contained a significantly lower amount of moisture compared to mussels and cockles ($P = 0.050$) and consisted of higher levels of lipid, protein and glycogen when compared to both mussels and cockles ($P < 0.05$, Table 4a).

For whelks fed on different diets there was no significant difference in the proximate composition of protein ($P > 0.127$) or glycogen ($P > 0.127$, Table 4b). Whelks fed pellets displayed a significantly higher energy content than all other whelks ($P = 0.05$), a significantly greater moisture content than whelks fed on frozen and live mussels ($P = 0.046$) and a significantly larger ash content than whelks fed on frozen mussels ($P = 0.046$). However, there was no difference in ash between other treatments (Table 4b). A significantly higher lipid composition was recorded for whelks feeding on live mussels compared to frozen cockles ($P = 0.050$). The highest moisture content occurred in whelks fed frozen cockles, which was similar to pellets and live mussels ($P = 0.507$ and 0.513, respectively). Whelks fed on frozen mussels contained significantly less moisture than all other treatments ($P < 0.050$).

Comparisons between whelks and their corresponding diets (Fig. 4) show that there is a consistency between whelks and their diet with respect to lipids, which showed no significant difference between all whelks and their associated diets ($P < 0.121$, Fig. 4c). Whelks fed mussels were significantly different from mussels for all other proximate components ($P < 0.05$, Fig. 4). Pellets had significantly less moisture than whelks fed on pellets ($P = 0.046$, Fig. 4a), but the pellets were significantly higher in ash ($P = 0.050$, Fig. 4b), protein ($P = 0.05$, Fig. 4e) and glycogen ($P = 0.050$, Fig. 4f). Whelks feeding on cockles were most similar to their diet (Fig. 4), but with significantly more protein ($P = 0.050$) and glycogen ($P = 0.050$) than cockles (Fig. 4e-f).

The multivariate ordination plot (Fig. 5) shows the overall similarity in nutritional properties between each replicate whelk and diet sample, based on the distance between data points. Tighter clustering is evident between all whelks, suggesting these are more similar in composition to each other than are the different diets, which are widely dispersed. ANOSIM produced a Global $R = 0.695$ with $P = 0.001$ for the overall differences between the various whelk and diet sample groups. However, pair-wise comparisons revealed no significant differences between any groups at $P = 0.05$, most likely due to low power in the analysis ($n = 3$). Nevertheless, the whelks fed cockles clearly have a nutritional composition more similar to their diet than the other whelks and their corresponding diets (Fig. 5).

3.3. Preference trial

Whelks displayed a preference for frozen cockles in all trials (Fig. 6), with respect to both the amount they consumed (Fig. 6a) and the number they ate (Fig. 6b).

Paired T-tests revealed that whelks ate significantly more frozen cockles than live cockles (P < 0.001), medium abalone (P = 0.005) and small abalone (P < 0.001). No significant differences were observed when comparing live abalone and live cockles or between different-sized abalone. There is a trend towards a higher number, but a lower weight of small abalone consumed (Fig. 6).

4. Discussion

This study revealed that juvenile *D. orbita* can be successfully maintained in artificial culture tanks. Depending on diet, whelks effectively ate, grew and displayed high survivorship (Table 1) in densities of 15 whelks per 16 L. The maximum growth rate achieved in this study was over 0.8 g and 2 mm in length per month, which compares favourably to other growth rate trials on juvenile whelks (e.g. *Nucella lapillus* Etter, 1996; *Babylonia areolata* Chaitanawisuit et al., 2001; *Buccinum undatum* Nasution and Roberts, 2004). Considering the initial size of whelks in this study, this equates to nearly a doubling of the body mass during a three month period for the most optimal diet and over 20% increase in shell length. This growth rate greatly exceeds that reported from *D. orbita* in the field, where shells lengths of 10 mm were recorded at 18 months and only increasing to 20 mm after 2-5 years (Philips and Campbell, 1971). The growth rate of most gastropods will decelerate with increasing size/age (Hughes, 1986). For example, Morton (1986) reported a period of dramatic shell growth from 62-72 weeks post hatching for the whelk *Hemifusus tuba*, followed by a period of reduced growth rate. Extended studies on the growth phases of *D. orbita* in captivity would be useful, although our results are nevertheless very promising for the establishment of this species as a new aquaculture species in Australia.

Over the 12-week growth trial, diet was found to have a significant impact on the growth and survivorship of *D. orbita*. Similar impacts of diet on growth have been found in other whelk species (e.g. *Nucella lapillus* Etter, 1996; *Morula marginalba* Moran et al., 1984; *Buccinum undatum* Nasution and Roberts, 2004), highlighting the importance of optimising diets to achieve maximum growth in culture. In our study, whelks fed on frozen bivalves had reduced mortality and higher growth rates compared to those maintained on live or artificial diets. The higher growth and survivorship of whelks fed frozen bivalves than live mussels was surprising, since *D. orbita* is typically considered an active predator, although their scavenging activity has been occasionally observed in the field (Philips, 1969). Previous studies on the Muricidae *Chorus giganteus* reported that cultured whelks grow faster when feeding on a diet endemic to their natural collection habitat (Carrasco et al., 2006). Interestingly, this was not found in our study, as whelks consumed significantly more and grew faster on a diet of soft-sediment cockles, compared to mussels collected from the same rocky reefs where *D. orbita* resides. The lower consumption rate on mussels in our study was possibly due to their smaller size, requiring higher handling times for the same mass of feed consumed than the cockles.

It appears likely that *D. orbita* is using an optimal foraging strategy to select its prey. Results from the feeding preference trials demonstrate that whelks consume frozen feed significantly more often than all live mollusc diets. In frozen bivalves, the shell always opens during defrosting, allowing whelks to consume the meat without exerting too much energy. By comparison, live mussels and cockles typically maintain a closed shell in the predator tanks, requiring shell drilling or ambush strategies for effective predation. Similar results were found by

Carrasco et al. (2006), whereby a higher consumption rate was recorded for *C. giganteus* fed razor clams compared to mussels (razor clams are not totally enclosed allowing whelks to feed on the soft tissue without expending energy in penetrating the shell). These findings are consistent with predictions from the 'optimal foraging theory' (Hughes, 1980), whereby predators choose a diet which yields the most resource and maximises energy per unit of foraging time. An exception to this was seen in the artificial pellet trials, where apparently "easy prey" was not consumed readily by the whelks. However, these pellets may not be an optimal diet for whelks, as they are designed for tropical bottom-dwelling fish.

Proximate composition varied among the different diets. The two natural (bivalve) diets were more similar to one another, with the preferred cockles being most distinct from the artificial diet. The cockles and mussels only differed with respect to ash and energy, which were both higher in mussels. The higher proportion of inorganic material in mussels may explain the slightly lower consumption and growth rates on this diet compared to cockles, although noticeably both these diets had similar feed conversion ratios. The significantly higher moisture, lipid, protein and glycogen content of pellets compared to the bivalve flesh, may explain why whelks show a significantly lower inclination towards consuming this artificial diet. For example, Qi-Cun et al. (2007) have demonstrated that growth performance of juvenile ivory shell (*Babylonia areolate*) significantly decreased when dietary lipid level increased from 7.8 to 11.7%. The lipid content of the pellets in this study was approximately 11% compared to 7% in the preferred cockle diet. Consequently, future research to develop a suitable artificial diet for scavenging whelks should model the nutritional content more closely to their nature bivalve diets.

The different nutritional composition of the diets was found to affect the proximate composition of whelks. *D. orbita* fed on pellets displayed a higher content of ash, lipids, protein, glycogen and energy, relative to the other diets. This has also been reported for abalone, where those fed on an artificial diet displayed a higher percentage of protein and glycogen compared to those fed on red algae (*Gracilaria* sp.) (Choiu et al., 2001). Previous research on the impact of starvation on composition analysis of *Chicoreus virgineus* and *Rapana rapiformis* has shown prolonged starvation results in a drastic depletion of glycogen stores (Patterson Edwards et al., 1996). Consequently, glycogen levels could provide an indicator to determine whether whelks can effectively derive nutrition from a specific (i.e. artificial) diet. As the whelks fed on artificial pellets in this study displayed no significant difference in glycogen to whelks fed bivalves (Table 4), it could be assumed that they were eating enough of the pellet diet to support their weight and nutrient requirements. However, these values may be confounded due to cannibalistic behaviour observed in this treatment contributing to relatively high mortality rates. Whelks fed on the three bivalve diets in this study displayed similar compositions, with few significant differences in nutritional parameters. In the proximate composition comparison of whelks fed on frozen and live mussel diets, there was only a significant difference in the amount of moisture. This shows that freezing a diet for ease of storage in an aquaculture facility will not impact the overall growth or nutritional quality of *D. orbita*.

Overall, *D. orbita* appears to have relatively good nutritional qualities compared to other molluscs. The juveniles tested in this study display significantly higher protein and glycogen content, as well as less variability in their lipid content when compared to their bivalve prey. Comparison to the literature indicates they also have higher glycogen and lipid than abalone, but lower protein (Choiu et al., 2001). Nevertheless, in other studies on proximate composition of adult whelks, protein levels in the foot muscle have been reported to exceed those of abalone (i.e. *Chicoreus ramosus*, Xavier Ramesh and Ayyakkannu, 1992; *Chorus giganteus*, Carrasco et al., 2006). Furthermore, preliminary studies indicate that the protein content of adult *D. orbita* is substantially higher than the juveniles (~3% vs. 20% w/w, Noble and Benkendorff, unpublished data). Holland et al. (1975) also found a lower amount of protein in young *Littorina* spp. compared to adults. This indicates that proximate composition and nutritional requirements differ over the life stage of gastropods.

Conclusion

This research has investigated some of the biological criteria required for establishing *Dicathais orbita* as a new aquaculture species. Monthly growth rates of *D. orbita* fed on frozen bivalve diets were found to be similar to those reported for *Buccinum undatum* (Naustion and Roberts, 2004), with *D. orbita* displaying a higher survival rate. This study also confirms that *D. orbita* can provide an alternative source of energy, marine lipids and protein. High glycogen levels are likely to contribute a relatively sweet taste to the meat at the juvenile stage. The juvenile whelks were found to have a significantly higher protein and glycogen content than their bivalve diets and although their protein levels remain below that reported from other cultured gastropods (Xavier Ramesh and Ayyakkannu, 1992; Choiu et al., 2001; Carrasco et al., 2006), this may increase with a longer grow-out phase. In South Australia, *D. orbita* are concurrently distributed on oyster leases and abalone farms, which may provide options for the poly-culture of whelks alongside commercial aquaculture stock. Preference feeding trials suggest they will avoid abalone if offered an easier target prey, such as frozen cockles. Further research into the optimal conditions for culture and grow-out of *D. orbita* will prevent the over harvesting of this species for food and fine chemicals, as well as providing novel opportunities for Australian aquaculture.

Acknowledgments

We are grateful to the Flinders University of South Australia and a philanthropic research foundation for providing the funds and facilities to support this research. We appreciate assistance from a number of students and staff from Flinders University, in particular Warwick Noble, Glenn Solly, Peter Pfeiffer, Leslie Morrison, Agnes Catin and Stephanie Duong for technical advise and Krystal Jay, Rebecca Forder, John Mathews, Mark Ellis, Warwick Noble, Chantel Westley, Cassandra McIver and Stephan Caddy-Retalic for useful discussions and assistance during field collections.

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Table 1

Mean values (± S.D.) for initial and final whelk measurements, growth rate, food intake, feed conversion ratio and survival rate for *Dicathais orbita* fed on four diets over a 12 week period.

Growth parameters	**Feeding regime**			
	Artificial diet	**Live mussels**	**Frozen mussels**	**Frozen cockles**
Initial shell length (mm)	22.34 ± 0.27	22.74 ± 0.71	21.63 ± 0.43	22.81 ± 0.46
Final shell length (mm)	23.53 ± 1.30	25.35 ± 0.91	26.18 ± 0.70	29.75 ± 0.96
Total increase in length (mm)	1.19 ± 1.17	2.60 ± 0.69	4.32 ± 0.15	6.95 ± 0.52
Growth rate (mm / wk)	0.04 ± 0.04	0.23 ± 0.08	0.36 ± 0.01	0.57± 0.05
Initial body weight (g)	1.17 ± 0.08	1.87 ± 0.20	1.68 ± 0.13	1.86 ± 0.10
Final body weight (g)	2.16 ± 0.46	2.76 ± 0.19	2.94 ± 0.22	4.14 ± 0.33
Total increase in growth (g)	0.44 ± 0.41	0.89 ± 0.18	1.26 ± 0.09	2.28 ± 0.28
Growth rate (g / wk)	0.02 ± 0.02	0.07 ± 0.01	0.10 ± 0.01	0.19 ± 0.02
Total food intake (g)	18.37	237.83	301.96	433.91
Food conversion ratio	6.22	6.34	5.35	4.66
Survival rate	64%	98%	100%	93%
Autogenic change in feed	11.5%	1.0%	4.3%	1.2%

Table 2

Repeated measure ANOVA results for the difference in: a) weight (log transformation); and b) growth of *Dicathais orbita* during a 12 week feeding trial on four different diets.

a)		df	Mean square	F	P value
Treatment	Between groups	3	0.226	15.326	0.001
	Within groups	8	0.015		
	Total	11			
Time	Greenhouse-Geisser	1.451	14.147	359.142	0.000
Time * Diet	Greenhouse-Geisser	4.352	1.464	37.167	0.000
Time * Tank (Diet)	Greenhouse-Geisser	11.605	0.028	0.707	0.739

b)		df	Mean square	F	P value
Treatment	Between groups	3	0.015	47.93	0.000
	Within groups	8	0.000		
	Total	11			
Time	Greenhouse-Geisser	2.433	14.147	15.019	0.000
Time * Diet	Greenhouse-Geisser	7.298	1.464	11.837	0.000
Time * Tank (Diet)	Greenhouse-Geisser	19.462	0.028	0.9	0.586

Table 3

ANOVA results for the difference in: a) consumption rates; b) feed conversion ratios; and c) mortality rates for *Dicathais orbita* at the end of a 12 week growth trial with four diets.

		df	Mean square	F	P value
a) Amount consumed	Between groups	3	10033.66	200.767	0.000
	Within groups	8	49.977		
	Total	11			
b) Feed conversion ratio	Between groups	3	1.868	1.236	0.359
	Within groups	8	1.512		
	Total	11			
c) Mortalities	Between groups	3	18.444	20.121	0.000
	Within groups	8	0.917		
	Total	11			

Table 4

Mean (± S.D.) values per gram fresh weight for the nutritional proximate components of: a) three diets fed to whelks; and b) the whelks (*Dicathais orbita*) fed on four diets. Different superscript letters represent significant differences ($p < 0.05$) between diets for each proximate component (Kruskal and Wallis comparison tests).

a) Feed	Moisture g/g	Ash g/g	Crude lipid g/g	Crude protein g/g	Glycogen g/g	Energy kj/g
Mussels	0.828 ± 0.004^{a}	0.059 ± 0.005^{a}	0.086 ± 0.015^{a}	0.009 ± 0.002^{a}	0.018 ± 0.001^{a}	21.27 ± 0.77^{a}
Cockles	0.843 ± 0829^{a}	0.036 ± 0.007^{b}	0.071 ± 0.023^{a}	0.014 ± 0.006^{a}	0.020± 0.002^{a}	18.47 ± 0.45^{b}
Pellets	0.073 ± 0.033^{b}	0.077 ± 0.015^{a}	0.117 ± 0.003^{b}	0.126 ± 0.020^{b}	0.331 ± 0.026^{b}	21.94 ± 1.51^{a}

b) Whelks	Moisture g/g	Ash g/g	Crude lipid g/g	Crude protein g/g	Glycogen g/g	Energy kj/g
Live mussels	0.698 ± 0.006^{a}	0.031 ± 0.002^{ab}	0.100 ± 0.001^{a}	0.027 ± 0.004	0.041 ± 0.007	19.07 ± 0.55^{a}
Frozen mussels	0.682 ± 0.007^{b}	0.030 ± 0.001^{a}	0.094 ± 0.05^{ab}	0.028 ± 0.002	0.037 ± 0.004	18.90 ± 0.97^{a}
Frozen cockles	0.779 ± 0.089^{ac}	0.028 ± 0.008^{ab}	0.095 ± 0.002^{b}	0.026 ± 0.004	0.035 ± 0.004	19.07 ± 1.17^{a}
Pellets	0.717 ± 0.002^{c}	0.033 ± 0.001^{b}	0.100 ± 0.014^{ab}	0.031 ± 0.002	0.046 ± 0.013	22.89 ± 1.55^{b}

Figure Legends

Figure 1: Mean weight (a) and growth increment (b) of *Dicathais orbita* (± S.D.) fed on four different diets over a 12 week period with weight recorded every three weeks. ** represents significant differences at alpha ≤ 0.01 between treatments at the represented time period. Whelks fed on; –◇– live mussels, –□– frozen mussels, –△– frozen cockles and –×– pellets.

Figure 2: Mean amount of feed (± S.D.) consumed (grams) by *Dicathais orbita* for four different diets over a 12 week period. Whelks fed on; –◇– live mussels, –□– frozen mussels, –△– frozen cockles and –×– pellets.

Figure 3: Cumulative mortalities of *Dicathais orbita* feed different diets over a 12 week period. Whelks fed on; –◇– live mussels, –□– frozen mussels, –△– frozen cockles and –×– pellets.

Figure 4: Comparison of the nutritional composition of *Dicathais orbita* and their corresponding diet for (a) moisture, (b) ash, (c) crude lipid, (d) energy, (e) crude protein and (f) glycogen, per gram fresh weight. * represents significant differences between whelks (▨) and corresponding diet (■) (<0.05).

Figure 5: MDS ordination for the proximate composition of *Dicathais orbita* and diet. Triangles represent diet, ▲ mussels, △ cockles, △ pellets and circles represent whelks fed on individual diets, ● mussels, ○ cockles and ○ pellets.

Figure 6: Preference trials for (a) the mean weight (± S.D.) of prey consumed and (b) mean number (± S.D.) of prey consumed by *Dicathais orbita* over a week. ▧ Live cockles, ■ frozen cockles, □ abalone (20 mm), ◫ abalone (50 mm) and ⊟ pellets.

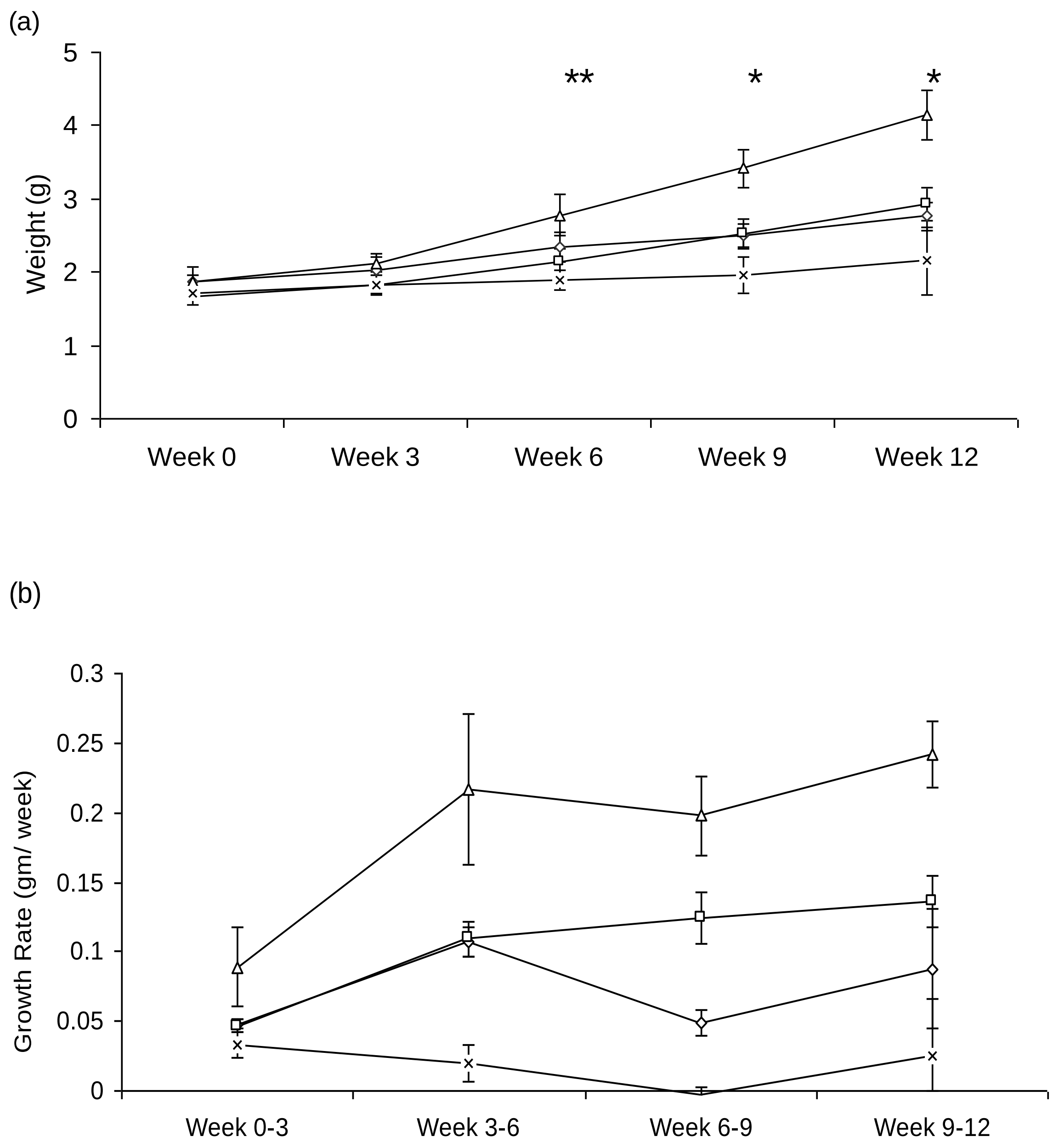
(a)
Weight (g)
5
4
3
2
1
0
**
*
*
Week 0
Week 3
Week 6
Week 9
Week 12
(b)
Growth Rate (gm/ week)
0.3
0.25
0.2
0.15
0.1
0.05
0
Week 0-3
Week 3-6
Week 6-9
Week 9-12

Figure 1.

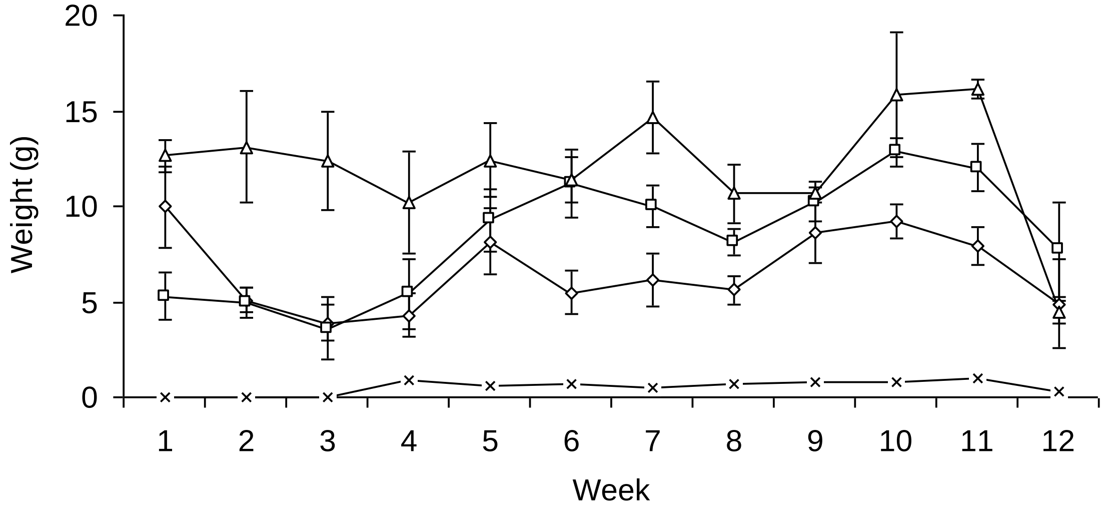

Figure 2.

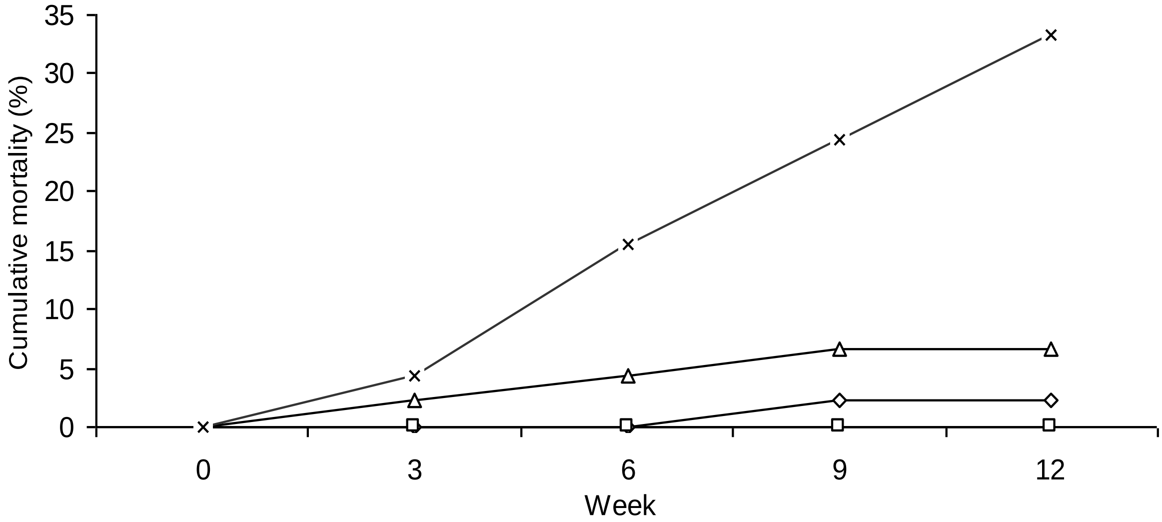

Figure 3.

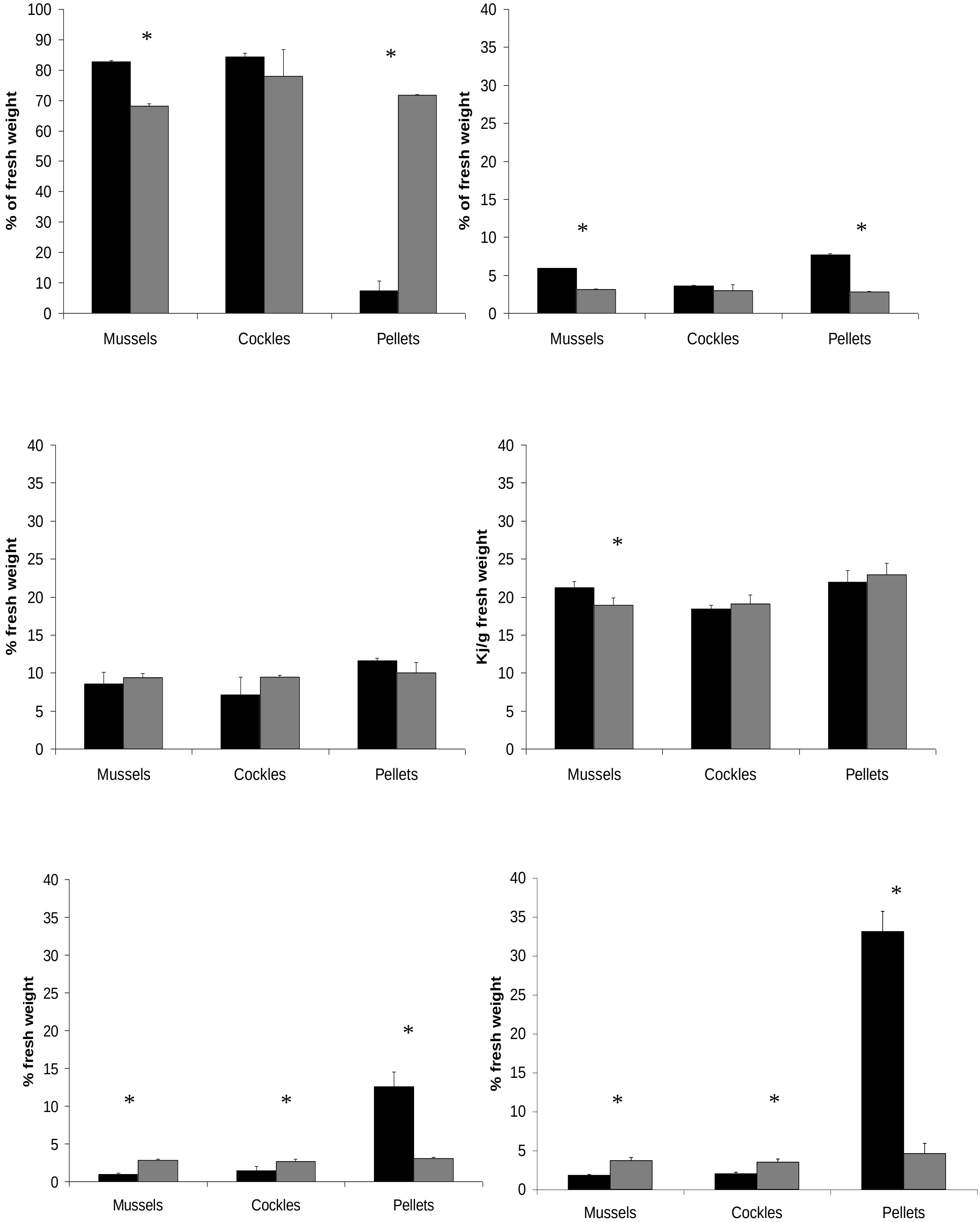
100
90
80
70
60
50
40
30
20
10
0
% of fresh weight
Mussels
Cockles
Pellets
*
*
40
35
30
25
20
15
10
5
0
% of fresh weight
Mussels
Cockles
Pellets
*
*
% fresh weight
Kj/g fresh weight
*
*
*
*
*
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Figure 4.

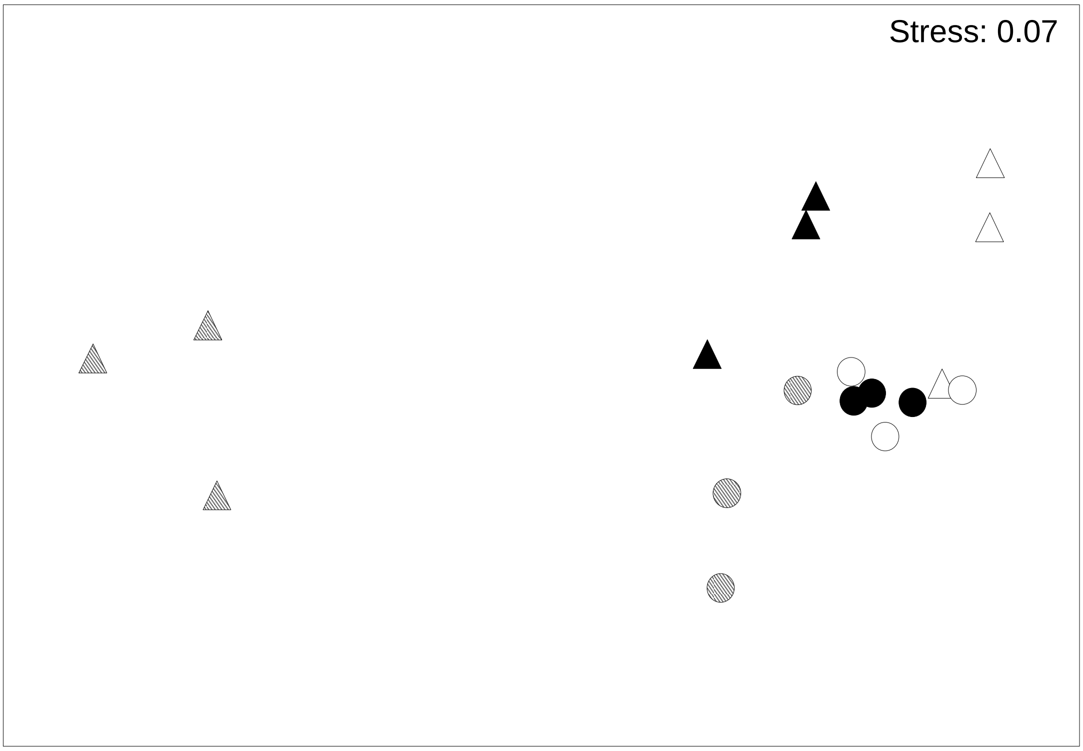

Figure 5.

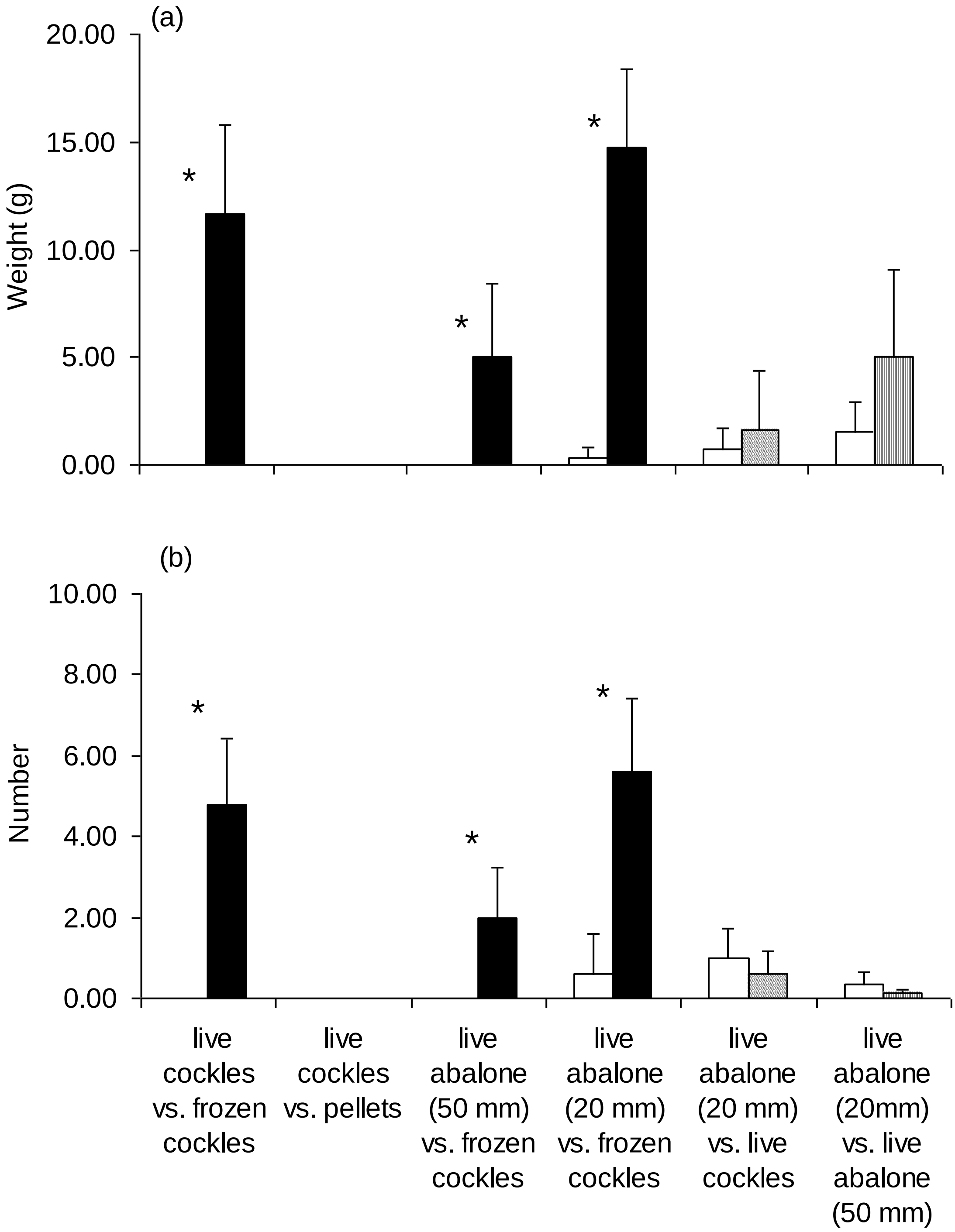
(a)
Weight (g)
20.00
15.00
10.00
5.00
0.00
*
*
*
(b)
Number
10.00
8.00
6.00
4.00
2.00
0.00
*
*
*
live cockles vs. frozen cockles
live cockles vs. pellets
live abalone (50 mm) vs. frozen cockles
live abalone (20 mm) vs. frozen cockles
live abalone (20 mm) vs. live cockles
live abalone (20mm) vs. live abalone (50 mm)

Figure 6