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Effects of Dietary Protein Source and Amount on Shell Morphology of Juvenile Abalone *Haliotis iris*

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ABSTRACT

The aim of this study is to investigate the effect of dietary nutritional value (protein source and amount) and temperature on shell morphology of cultured abalone juveniles. Morphological variations have long been used by ecologists and evolutionary biologists to identify physiological and/or environmental conditions associated with the growth and health of organisms. Two important factors affecting morphological plasticity are food supply and temperature conditions. Two different experiments were conducted to test the effect of dietary protein (source and amount) and water temperature on shell morphology (length, width, height, thickness and weight) of juvenile abalone (*Haliotis iris*). In the first experiment, nine different dietary protein sources (white fish and red fish meals, blood meal, meat and bone meal, casein, soybean concentrate, wheat gluten, maize gluten and *Spirulina* powder) resulted in wider, higher and heavier shells with casein diets and flatter shells with red fish meal. Abalone fed blood meal produced significantly narrower, thinner and lighter shells compared to animals fed the other diets. In the second experiment, six different dietary protein amounts (0, 10, 20, 30, 40 and 45%) and two temperature regimes (13-21 and 8-16°C) resulted in wider and heavier shells with increasing protein content. However, shell height and thickness were not affected by different protein amounts. Significant differences between temperatures were observed only for shell height and thickness. The results suggest that diet and temperature may be used to manipulate abalone shell morphology in aquaculture environments and they may be potential factors in shell variations of wild populations.

Key words: Black-footed abalone, amino acid profile, temperature, larval nutrition

INTRODUCTION

Morphological variations provide an important index for understanding the distribution of species and diversification processes. Thus, an entire field of eco-morphology has been dedicated to investigating the relationships between an organism's morphology and environmental conditions and changes over different time scales (Wainwright and Reilly, 1994). A number of studies have illustrated how specific marine environmental conditions result in animals having distinct morphological characteristics (Lively, 1986; Shadrin and Lezhnev, 1990; Saunders and Mayfield, 2008). For example, Lively (1986) reported that barnacles (*Chthamalus anisopoma*) may grow with different morphologies depending on the level of predation at a given site. Other studies have identified plastic responses (short temporal scales) to local environmental stressors (Lively, 1986;

Johannesson *et al*., 1990; Robles and Robb, 1993; Trussell, 1996; Steffani and Branch, 2003). For instance, the shell height and width of *M. galloprovincialis* has been shown to vary by 4-9% depending on the level of sewage contamination in the environment (Shadrin and Lezhnev, 1990). Furthermore, juvenile trochiid snails (*Trochus niloticus* L.) are known to change their shell morphology (smooth *versus* rough) depending on environmental conditions (wild versus hatchery) (Purcell, 2002). Within longer temporal scales, morphological variability has been associated with genetic differences resulting from historical selective pressures for a particular morphotype (Etter, 1996; Luttikhuizen *et al*., 2003; Swain *et al*., 2005).

Abalone have been shown to have high morphological variability among populations inhabiting different coastal sites (Worthington *et al*., 1995; Prince *et al*., 2008; Saunders and Mayfield, 2008). It has been suggested that morphological variation in abalone is a result of plastic response to food availability, such as drift algae (Day and Fleming, 1992; Shepherd and Steinberg, 1992; McShane and Naylor, 1995; Saunders *et al*., 2009). Shepherd and Hearn (1983) reported that *H. rubra* have rounder shells when the animals are found in high densities (and experiencing shortages of food) compared to the oval shell shape of animals growing under low densities. In addition, Breen and Adkins (1982) found that slow-growing *H. kamatsakana* populations had taller shells compared to fast-growing individuals, while Worthington *et al*. (1995) reported that slow-growing *H. rubra* populations in New South Wales had significantly wider shells than those of fast-growing abalone. Studies on shell morphology for the New Zealand black-footed abalone (*H. iris*) are lacking, although this species inhabits similar subtidal rocky shore habitats and feeds on similar algal food sources as *H. rubra* (Australia) and *H. kamatsakana* (British Columbia). Thus, *H. iris* would be expected to have similarly high morphological variability as its counterparts throughout the world. If different types and amounts of protein produce abalone with different shell morphologies, then cultured species may be manipulated to produce specific shell shapes for the jewellery market. In addition, shell morphology may be used as a proxy for animal health within farms and in the wild. Thus, the aims of this study are to identify the shell variations of *H. iris* juveniles grown with formulated diets containing different protein sources and amounts and different temperature regimes.

MATERIALS AND METHODS

Experimental design: Abalone (*H. iris*) were obtained from Seahorses Australasia Limited, Warrington, Dunedin, New Zealand in 2005. Two sets of animals were used in two different experiments. For experiment 1, abalone of about 1.5 to 2 years old (20 to 22 mm in maximum shell length) were used to test the effect of different dietary protein sources on shell growth parameters. This experiment was conducted at Seahorses Australasia Limited. The experimental animals first were acclimatized in a flow-through tank for one month, during which time they were fed a commercial diet (Adam and Amos abalone food, Australia). After acclimatization, the animals were carefully removed from the holding tank with a flat, blunt blade and placed in the experimental tanks. Twenty randomly selected animals were placed in each of 27 experimental containers (1 L in volume with square PVC lids, semi-transparent in color). Three out of the 27 containers were randomly assigned one of nine dietary protein sources, which were fed to the animals daily for a five-month period. All the tanks were maintained with semi-recirculated (50% water exchange day^{-1}), filtered (10 μm filter) seawater and the temperature was kept constant at 18±0.3°C throughout the experimental period.

For experiment 2, another set of animals (2 to 2.5 years old, 30 to 33 mm in maximum shell length) were used to test the effect of six different dietary protein levels and two temperature regimes on shell growth parameters. This experiment was conducted at the Portobello Marine Laboratory, University of Otago, Dunedin, New Zealand in 2006. For this experiment, five randomly selected animals were respectively placed in each of 36 experimental containers (1 L in volume with square PVC lids, semi-transparent in color) with flow-through seawater. The containers were arranged into two temperature regimes (18 containers per temperature regime). The high (13 to 21°C) and low (8 to 16°C) temperature regimes were maintained throughout the four-month experimental period. For each temperature, three replicate tanks were assigned one of the six dietary protein levels.

For both experiments, water quality always was kept in good condition (pH between 8.3-8.5, dissolved oxygen > 10 mg L^{-1}). The daily feeding rates were 5% of the total body weight per tank and the water flow rate was 500 mL h^{-1} (total water exchange of 12 times daily). Faeces and uneaten food residues were swept away every other day.

Diet formulation: For experiment 1, nine diets were formulated to contain about the same nutritional profile, but using different sources of protein (white and red fish meals, blood meal, meat and bone meal, casein, soybean concentrate, wheat gluten, maize gluten and *Spirulina* powder) (Table 1). For experiment 2, six experimental diets were formulated to contain different levels of red fish meal protein (about 0, 10, 20, 30, 40 and 45%), while maintaining all other nutritional components similar (Table 2).

The formulation of both sets of diets involved the mixing of dried ingredients in a blender without the starch and cellulose. The starch was first mixed with boiling water in order to activate its binding properties (gelatinize). Then, the activated starch and cellulose were added to the mixture of all other dry ingredients. Finally, lipid oil was added to make a dough, which was spread on a flat, square-shaped board to a 3 mm thickness. Small pieces (1 cm^2) were cut with a knife just

Table 1: Recipes for nine experimental diets with different protein sources. The nine protein sources were white fish meals (WF), red fish meals (RF), blood meal (BL), meat and bone meal (MB), casein (CA), soybean concentrate (SO), wheat gluten (GL), maize gluten (MZ) and *Spirulina* powder (SP). Units are % dry matter

	Diet								
Composition	White fish meal[a] (WF)	Red fish meal[a] (RF)	Blood meal[a] (BL)	Meat and bone meal[a] (MB)	Casein[a] (CA)	Soybean concentrate[a] (SO)	Wheat gluten[a] (GL)	Maize gluten[a] (MZ)	*Spirulina*[a] (SP)
Protein	42.40	40.50	29.70	52.00	31.90	54.90	35.80	42.50	49.60
Cellose[b]	14.10	16.50	22.40	-	20.2	-	16.7	11.9	7.40
Starch[c]	40.0	40.00	40.00	40.00	40.0	40.0	40.0	40.0	40.00
Lipid[d]	0.50	-	4.90	-	4.90	4.10	4.50	2.60	-
Vitamine[e]	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Minerial Mix[f]	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Average pellet weight (g)	0.18	0.25	0.23	0.34	0.19	0.25	0.39	0.26	0.26

[a]White and red fish meal supplied by Sealord, Nelson (NZ) LTD. Casein supplied by Anchar. Meat and bone meal and blood meal supplied by Affco (NZ) Limited. Wheat gluten, maize gluten and soybean concentrates from J. C. Sherratt and Co. Ltd. *Spirulina* from Superfood Spirulina Ltd; [b]From commercial product Just-Fiber ™; [c]Modified starch (Firm-MIX ™) used for binding pellets from New Zealand Starch Ltd; [d]Cold-filter cod liver oil (Healther™, NZ); [e]Vitamin mix (Shin- Chan™, Taiwan) g^{-1} content: Riboflavin 10 mg, PABA 40 mg, Pyridoxine HCl 4 mg, Niacin 80 mg, Ca pantothenate 20 mg, Inositol 400 mg, Ascorbic acid 400 mg, Biotin 1.2 mg, Vitamin E 45 mg, Menadione 8 mg, Vitamin B_{12} 18 mg, Vitamin A 10000 I.U., Vitamin D 200 I.U., Ethoxyquin 40 mg, Folic acid 3 mg, Thiamin HCl 12 mg; [f]Mineral mixture g kg^{-1} mixture: NaCl (BDH) 10 g, $MgSO_4 \cdot 7H_2O$ (BDH) 10 g, $NaH_2PO_4 \cdot 2H_2O$ (BDH) 150 g, KH_2PO_4(BDH) 250 g, K_2HPO_4(BDH) 320 g, $Ca(H_2PO_4)_2 \cdot H_2O$ (Merck) 200 g, Fe-lactate (SIGMA) 25 g, Ca-lactate (SIGMA) 35 g, $ZnSO_4 \cdot 7H_2O$ (BDH) 3.53 g, $MnSO_4 \cdot 4\ H_2O$ (BDH) 1.62 g, $CuSO_4 \cdot 5H_2O$(BDH) 310 mg, $CoC1_2 \cdot 6H_2O$ (Merck) 10 mg, KIO_3 (Merck) 30 mg

Table 2: Recipes for diets with different amounts of protein. Data are presented as means of three replicate samples. Six diets were formulated with different protein amounts (1 = 0%, 2 = 10%, 3 = 20%, 4 = 30%, 5 = 40% and 6 = 45%). Units are % dry matter

	Diet No.					
Composition	1	2	3	4	5	6
Protein level	0%	10%	20%	30%	40%	45%
Red fish meal[a]	-	9.6	19.6	29.6	39.6	44.6
Cellose[b]	49.8	41.5	32.8	24.1	15.4	11.7
Starch[c]	40.0	40.0	40.0	40.0	40.0	40.0
Lipid[d]	7.2	5.9	4.6	3.3	2.0	0.7
Vitamine[e]	1.5	1.5	1.5	1.5	1.5	1.5
Minerial Mix[f]	1.5	1.5	1.5	1.5	1.5	1.5
Average pellet weight (g)	0.19	0.22	0.23	0.25	0.28	0.31

[a]Red fish meal supplied by Sealord, Nelson (NZ) Ltd.; [b]from commercial product Just-Fiber ™. [c]modified starch (Firm-MIX ™) used for binding pellets from New Zealand Starch Ltd. [d]Cold-filter cod liver oil (Healther™, NZ). [e]Vitamin mix (Shin-Chan™, Taiwan) g^{-1} content: Riboflavin 10 mg, PABA 40 mg, Pyridoxine HCl 4 mg, Niacin 80 mg, Ca pantothenate 20 mg, Inositol 400 mg, Ascorbic acid 400 mg, Biotin 1.2 mg, Vitamin E 45 mg, Menadione 8 mg, Vitamin B_{12} 18 mg, Vitamin A 10000 I.U., Vitamin D 200 I.U., Ethoxyquin 40 mg, Folic acid 3 mg, Thiamin HCl 12 mg. [f]Mineral mixture g kg^{-1} mixture: NaCl (BDH) 10 g, $MgSO_4 \cdot 7H_2O$ (BDH) 10 g, $NaH_2PO_4 \cdot 2H_2O$ (BDH) 150 g, KH_2PO_4 (BDH) 250 g, K_2HPO_4 (BDH) 320 g, $Ca(H_2PO_4)_2 \cdot H_2O$ (Merck) 200 g, Fe-lactate (SIGMA) 25 g, Ca-lactate (SIGMA) 35 g, $ZnSO_4 \cdot 7H_2O$ (BDH) 3.53 g, $MnSO_4 \cdot 4\ H_2O$ (BDH) 1.62 g, $CuSO_4 \cdot 5H_2O$(BDH) 310 mg, $CoC1_2 \cdot 6H_2O$ (Merck) 10 mg, KIO_3 (Merck) 30 mg

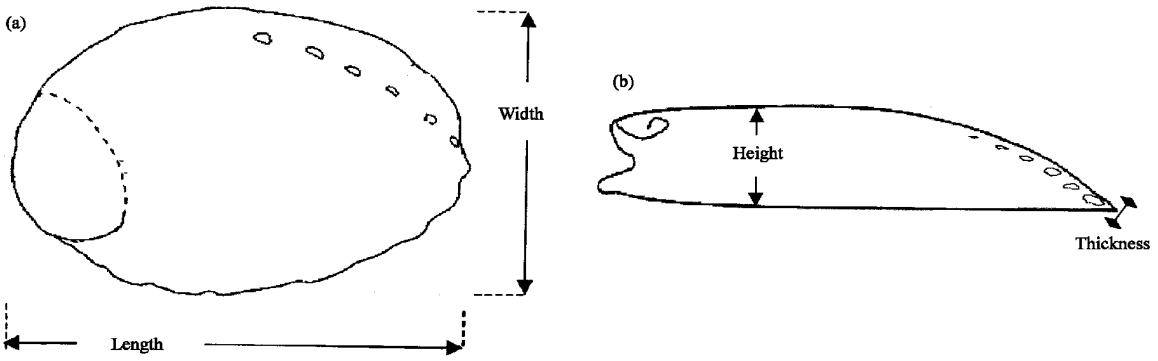

Fig. 1: Diagram indicating the measurements obtained from abalone shells: (a) Top view of shell and (b) side view of shell

prior to drying in a McGreger hot-air oven at 45°C for about 12 h. The diets were then stored in a -20°C freezer until they were used.

Shell analysis: Prior to the start of the experiments, measurements of maximum shell length and width were made for each individual animal (Fig. 1a, b). At the end of each experiment (five and four months, respectively), all surviving animals were stripped of their shells with a flat, blunt blade. Broken shells were excluded from the analysis. A total of 16 to 33 shells per diet treatment were analyzed for the first experiment and 3-9 shells per treatment combination (diet and temperature) were analyzed for the second experiment. Individual shells were kept in 5% NaOH for 7 h to remove all organic material. Then, the shells were rinsed thoroughly with de-ionized water three times. After drying at 60°C for 48 h, all the shells were measured to obtain their maximum shell lengths, widths and heights (Fig. 1) with a digimatic caliper with accuracy of 0.01 mm (Mitutoyo Co., Japan). Shell thickness was measured at the edge of the first respiratory pore. Shell weight was measured with digital balance AB104-S with accuracy to 1 mg (Mettler Toledo Co., Japan).

Statistical analyses: Linear regressions were calculated between shell width, height, thickness and weight, against maximum shell length to test for linearity between the paired parameters. After corroborating linearity, shell width, height and weight measurements were standardized by dividing each parameter by the shell length. Shell thickness was not divided by shell length because these two parameters did not follow a linear relationship. All data were checked for and met parametric assumptions. The shell growth parameters obtained from experiment 1 were analyzed with one-way ANOVAs (protein source as fixed factor), followed by Tukey *post-hoc* tests. The data obtained from experiment 2 were analyzed with two-way ANOVAs (diet and temperature as fixed factors) and Tukey *post-hoc* tests. All analyses were conducted using a MiniTab version 14 software package.

RESULTS

Results from the initial shell measurements (prior to the start of the experiments) indicated that there were no significant differences in growth parameters (maximum shell length and width) among diets for experiment 1 and among diets, temperatures and interaction for experiment 2 (Table 3). Thus, it was assumed that all growth differences observed at the end of the experiments were a product of treatment effects. For both experiments, linear relationships were found between shell width, height and weight and shell length, but not between shell thickness and shell length (Table 4).

Experiment 1: The shell morphology varied significantly among the diet treatments after the five-month experiment (Fig. 2a, b and 3a, b). The mean (±SD) shell length, width, height, thickness and weight across all treatments were 27.22±2.11, 18.60±1.58, 6.29±0.45, 0.66±0.07 mm and 0.76±0.20 g, respectively, with ranges from 24.39 to 29.70 mm, 16.46 to 20.29 mm, 5.73 to 7.00 mm, 0.53 to 0.72 mm and 0.46 to 1.00 g, respectively. Significant linear regressions were found between shell length and all other shell parameters except for thickness (Table 4).

The highest means (±SD) width/length, height/length and weight/length ratios were 0.696±0.002 (casein), 0.241±0.003 (casein) and 0.034±0.004 (white fish meal), respectively. The lowest means (±SD) width/length, height/length and weight/length ratios were 0.673±0.003 (meat and bone), 0.217±0.004 (red fish meal) and 0.019±0.002 (blood meal), respectively. One-way

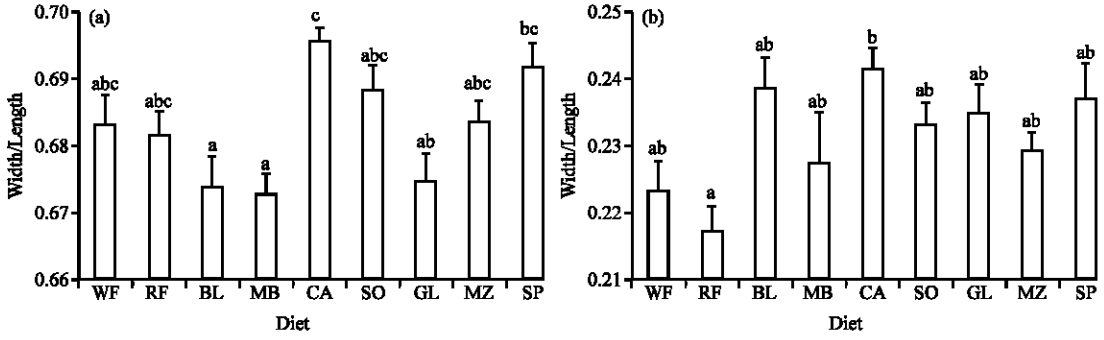

Fig. 2: Mean (±SD) shell (a) width/length and (b) height/length of abalone after five-months cultivation with nine different dietary protein sources (WF: White fish meals, RF: Red fish meals, BL: Blood meal, MB: Meat and bone meal, CA: Casein, SO: Soybean concentrate, GL: Wheat gluten, MZ: Maize gluten and SP: *Spirulina* powder. Different letters on top of the error bars denote significant differences resulting from Tukey post-hoc tests ($p<0.05$)

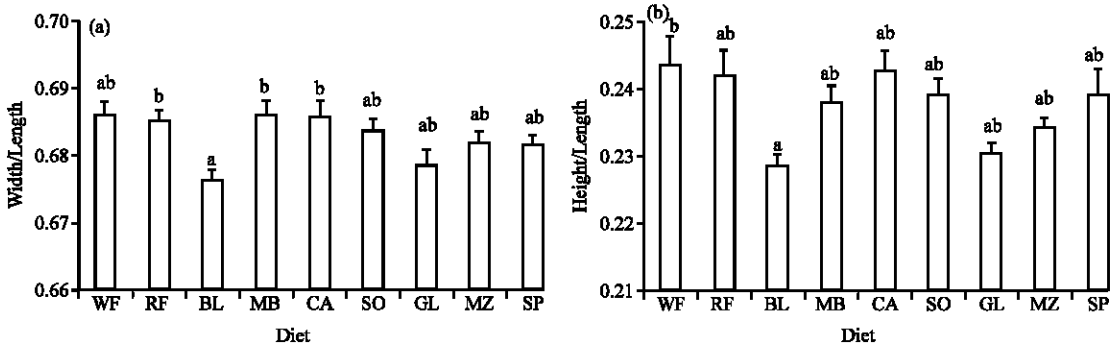

Fig. 3: Mean (±SD) shell (a) thickness and (b) weight/length of abalone after five-months cultivation with nine different dietary protein sources (WF: White fish meals, RF: Red fish meals, BL: Blood meal, MB: Meat and bone meal, CA: Casein, SO: Soybean concentrate, GL: Wheat gluten, MZ: Maize gluten and SP: *Spirulina* powder. Different letters on top of the error bars denote significant differences resulting from Tukey post-hoc tests ($p<0.05$)

Table 3: (a) One-way ANOVA analyses of nine diets in experiment 1 for initial shell length and width measurements and (b) two-way ANOVA analyses of six diets and temperatures in experiment 2 for initial shell length and width measurements

	Length				Width			
Source	df	MS	F-value	p-value	df	MS	F-value	p-value
Experiment 1								
Diet	8	0.43	1.11	0.359	8	0.11	1.11	0.366
Error	18	0.38			18	0.10		
Total	26				26			
Experiment 2								
Temperature	1	0.27	0.18	0.673	1	0.13	0.18	0.674
Diet	5	0.72	0.47	0.795	5	0.34	0.47	0.796
Interaction	5	0.50	0.32	0.897	5	0.23	0.32	0.900
Error	24	1.52			24	0.72		
Total	35				35			

Table 4: Linear regression results of shell length to four shell parameters (width, height, thickness and weight) for experiments 1 and 2

	Experiment 1			Experiment 2		
	Standard coefficient	t-value	p-value	Standard coefficient	t-value	p-value
Width	0.74	19.77	**0.001**	0.71	8.86	**0.001**
Height	-0.04	-2.13	**0.030**	-0.02	-0.39	0.700
Weight	0.29	6.53	**0.001**	0.28	2.99	**0.001**
Thickness	-0.20	-1.15	0.250	0.03	0.78	0.440

Bold values denote significant differences ($p<0.05$)

ANOVAs for these ratios resulted in significant differences among the diets (Table 5). Since, shell thickness did not follow a linear relationship with shell length, shell thickness alone was used in the analysis. After the five-month experimental period, abalone fed both fish meal, casein and meat and bone meal had significantly thicker shells and abalone fed blood meal had significantly thinner shells than animals fed the other diets (Fig. 3).

Table 5: One-way ANOVA analyses among diets for shell width/length, height/length, weight/length and thickness

	Width/Length				Height/Length			
Source	df	MS	F-value	p-value	df	MS	F-value	p-value
Diet	8	0.00	5.10	**0.002**	8	0.00	2.82	**0.032**
Error	18	0.00			18	0.00		
Total	26				26			
	Thickness				Weight/Length			
Source	df	MS	F-value	p-value	df	MS	F-value	p-value
Diet	8	0.02	4.00	**0.007**	8	0.02	3.36	**0.016**
Error	18	0.00			18	0.00		
Total	26				26			

Bold values denote significant differences

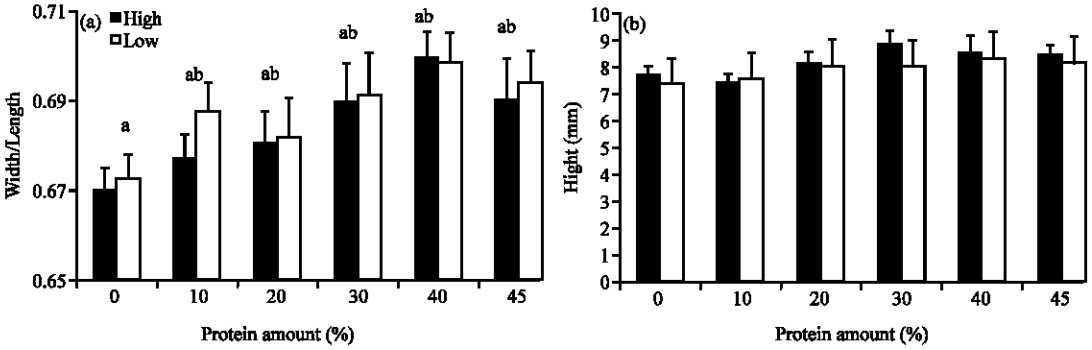

Fig. 4: Mean (±SD) shell (a) width/length and (b) height of abalone after four-months cultivation with six different dietary protein amounts (0, 10, 20, 30, 40 and 45% protein) diets and two temperature regimes. Data are presented as means±SD of all animals in treatments. Different letters on top of the error bars denote significant differences resulting from Tukey post-hoc tests ($p<0.05$)

Experiment 2: Results from the second abalone culturing experiment with six diets with varying amounts of protein and two different water temperatures are shown in Fig. 4a, b and 5a, b. After the four-month experiments, the mean (±SD) shell length, width, height, thickness and weight across all treatments were 35.73±1.64 mm, 24.53±1.38 mm, 8.00±0.47 mm, 0.87±0.05 mm and 1.72±0.29 g, respectively for high temperature treatments were 35.98±2.00 mm, 24.65±1.65 mm, 8.15±0.54 mm, 0.90±0.04 mm and 1.81±0.33 g, respectively for low temperature treatments were 35.48±1.33 mm, 24.41±1.19 mm, 7.85±0.36 mm, 0.83±0.03 mm and 1.63±0.25 g. The ranges for shell length, width, height, thickness and weight of animals cultured at high temperatures were from 33.30 to 37.74 mm, 22.32 to 26.04 mm, 7.36 to 8.81 mm, 0.86 to 0.97 mm and 1.38 to 2.17 g, respectively and from 33.79 to 37.00 mm, 22.99 to 25.84 mm, 7.32 to 8.24 mm, 0.79 to 0.88 mm and 1.33 to 1.90 g, respectively, for those grown at low temperatures. Significant linear regressions were found between shell length and all other shell parameters except for height and thickness (Table 4).

The highest means (±SD) width/length, height and weight/length ratios were 0.700±0.005 (40% protein at high temperature), 8.81±0.48 mm (30% protein at high temperature) and 0.575±0.022 (30% protein at high temperature), respectively, while the lowest means (±SD) values were

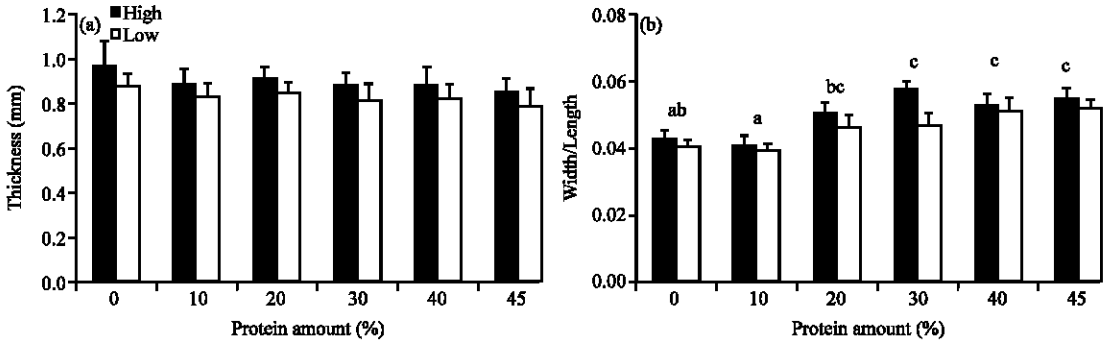

Fig. 5: Mean (±SD) shell (a) thickness and (b) weight/length of abalone after four-months cultivation with six different dietary protein amounts (0, 10, 20, 30, 40 and 45% protein) diets and two temperature regimes. Data are presented as means±SD of all animals in treatments. Different letters on top of the error bars denote significant differences resulting from Tukey post-hoc tests ($p<0.05$)

Table 6: Two-way ANOVA analyses among diets and temperatures for shell width/length, height, thickness and weight/length

	Width/Length				Height			
Source	df	MS	F-value	p-value	df	MS	F-value	p-value
Temperature	1	0.00	0.48	0.494	1	0.80	1.32	0.262
Diet	5	0.00	3.52	**0.016**	5	1.11	1.83	0.145
Interaction	5	0.00	0.15	0.977	5	0.16	0.27	0.926
Error	24	0.00			24	0.61		
Total	35				35			
	Thickness				Weight/length			
Source	df	MS	F-value	p-value	df	MS	F-value	p-value
Temperature	1	0.05	5.01	**0.035**	1	0.00	6.19	**0.020**
Diet	5	0.01	0.75	0.591	5	0.00	7.84	**0.001**
Interaction	5	0.00	0.04	1.000	5	0.00	0.67	0.647
Error	24	0.01			24	0.00		
Total	35				35			

Bold values denote significant differences

0.670±0.005 (0% protein at high temperature), 7.32±0.50 mm (0% protein at low temperature) and 0.039±0.002 (10% protein at low temperature), respectively. Two-way ANOVAs for these ratios resulted in significant differences among the diets for shell width/length and weight/length and between temperatures for weight/length ratios only (Table 6). Shell thickness again was analyzed alone and resulted in significant differences between temperatures only (Table 6).

DISCUSSION

In this study, different diets (sources and amounts of protein) had significant effects on most shell morphology parameters, but temperature had little effect on shell morphology. Specifically, shell width varied greatly with the different protein sources and amounts in the feed, while shell height and weight were highly variable with protein source and shell weight varied among diets with different protein amounts. Conversely, shell thickness did not appear to vary much with

different sources and amounts of protein. It is possible that such dietary differences may reflect morphological variations observed among wild abalone populations and that shell morphology may be regulated in aquaculture environments by adjusting the source and amounts of protein in the diets.

The relationship between food and shell morphology: Abalone have been shown to have strong morphological variability in different coastal habitats (Breen and Adkins, 1982; Shepherd and Hearn, 1983; Worthington *et al*., 1995; Wells and Mulvay, 1995). It is believed that differences in food supplies regulate abalone growth, especially of shells (Day and Fleming, 1992; Saunders *et al*., 2009). For example, nutritional experiments on *H. discus hannai* have shown that feeding animals with different seaweed species results in distinctive shell growth variations (Uki *et al*., 1986). Moreover, increase in growth with high food supplies may indicate that the animal has more energy to allocate for shell growth and/or that a more balanced nutritional supply is needed to produce an "optimal" shell shape. However, the relationship between diet and shell morphology still needs further investigation.

Effect of dietary protein source on shell morphology: In the present study, shell morphology was shown to be affected by the type of dietary protein in the five-month experiment. Among the nine different sources of dietary protein, the casein diet produced animals with the widest, highest and heaviest shells, while the white fish meal diet yielded a heavier shell and the red fish meal diet produced the flattest shell shape. Similar results were found by Uki *et al*. (1986), where *Haliotis discus hannai* increased shell length with casein diets compared with fish protein. In the present study, all nine diets had similar nutritional values (e.g., similar protein, lipid and carbohydrate contents) and it is suggested that the shell differences are a result of the animals incorporating different amino acids from the different food sources (i.e., different protein sources contain different amino acid profiles). For example, Mai *et al*. (1994) found that different amino acids produced different shell growth rates in *Haliotis discus hannai*. In the present study, animals fed blood meal produced significantly narrower, thinner and lighter shells compared to those of abalone fed the other diets. These results indicate that blood meal is a poor diet for *H. iris* and that these animals may have experienced negative growth during the five-month experimental period. While there is no prior information about the effect of blood meal on growth of abalone, blood meal has been shown to be a poor protein source for prawns, since it is difficult to digest (Brand and Colvin, 1977).

A change in shape to a wider and taller shell may increase the volume to accommodate the soft tissues; including muscles used for attachment and movement and may indicate good growing conditions. McCarthy *et al*. (2004) found that an increase in shell width of the periwinkle *Littorina saxatilis* resulted in a 12% increase in the total volume inside the shell. In addition, Bertness and Cunningham (1981) reported that snails with wider shells experienced lower mortality rates than those with thinner shell. Thus, allocating more resources to the shell may indicate better growing conditions and may enhance survival rates.

Effect of dietary protein amount on shell morphology: Results from the second experiment indicate that high protein content (40%) in the diet tends to produce wider and heavier shells than foods with less protein. However, shell height and thickness do not appear to be affected by different dietary protein amounts. In this study, a wider and heavier shell, as a response to high protein diets, may be associated with the development of a stronger (bigger) muscle (Trueman and

Brown, 1989) and may result in stronger shell resistance against predators (Bertness and Cunningham, 1981). Thus, the findings of this research support the idea that high protein diets enhance shell growth and improve the health and survival of abalone. These findings also are in agreement with Mai *et al.* (1995) who found an increase in shell growth with increasing dietary protein level for *Haliotis tuberculata* and *Haliotis discus hannai*. In addition, Worthington *et al.* (1995) found that wild populations of *H. rubra* in New South Wales, Australia, had wider shells in areas of low drift seaweed abundance compared to areas with high drift algal abundance. The reason for this discrepancy may be due to the fact that in areas of low macroalgal abundance, abalone tend to feed on microalgae which have higher protein contents (Thail and Juinino-Menez, 1999). For example, drift seaweed normally contain 7-24% protein (Emmanuel and Corre, 1996; Barbarino and Lourenco, 2005), whereas many microalgal biofilms contain 41-50% protein (Nagarkar *et al.*, 2004).

Effect of temperature on shell morphology: Temperature also has been shown to affect the shell growth of many marine invertebrates. For example, the shell thickness of gastropod *Cypraea annulus* at Okinawa Island was found to be positively correlated with temperature, while the surface sea temperature increased from 18-30°C (Irie, 2006). In the present study, *H. iris* juveniles exposed to high temperatures resulted in animals with thicker and heavier shells across different diets, but temperature did not affect shell width and height. It has been shown that at low temperatures, calcium carbonate solubility decreases, thereby increasing both shell deposition and shell thickness. In addition, under low temperature, the precipitation of calcium carbonate increases on shell surfaces (Graus, 1974; Clarke, 1983). Thus, this passive shell deposition as a response to environmental temperature may explain the increase in shell thickness and weight, while active growth may be required for directional shell width and height increases. While snail shell morphological variations have been reported to occur as a response to different environmental temperatures, such studies have not been conducted for abalone until now. These findings may be useful in aquaculture situations where shell morphology needs to be manipulated (i.e., shell jewellery products) and/or to monitor wild abalone growth and health across different environmental conditions.

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