Aquaculture Reports 10 (2018) 17–22

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Effects of dietary protein to energy ratios on growth performance of yellowfoot limpet (*Cellana sandwicensis* Pease, 1861)

Anthony Mau, Rajesh Jha*

College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, HI 96822, USA

ARTICLE INFO

Keywords:
Diet formulation
Feed utilization
Growth rate
Limpet
Mollusk

ABSTRACT

The aquaculture of yellowfoot limpets (*Cellana sandwicensis)* is a prospect industry in research and development. The effects of dietary protein to energy (PE) ratio on growth performance were evaluated for 180 days in a flow-through system. Replicate animals (5.9 ± 1.72 g and 33.9 ± 2.13 mm) were stocked randomly on individual plates, and four paste diets containing PE ratios ranging from 87.2 to 102.9 mg/kcal were offered once daily (1600 h). A significant increase in daily feed intake ($P < 0.05$) was observed to coincide with seasonal decrease in air temperature. Although dietary treatment had no significant effect on overall growth performance ($P > 0.05$), average daily gain (ADG) and feed conversion efficiency (FCE) improved both linearly and quadratically (ADG $P = 0.03$, $P = 0.08$; FCE $P = 0.05$, $P = 0.04$, respectively). These results indicate potential seasonal growth patterns, which are controlled by environmental cues (i.e. temperature, feed availability, etc.) and must be considered in future trials. Limpets offered higher PE ratio diets did not compensate for lower energy levels with increased feed intake, and specific growth rate increased up to 0.20% BW/d as the dietary PE ratio decreased. A PE ratio of 87.2 mg/kcal produced the best tissue growth and can be recommended as a suitable formulated diet for limpet production.

1. Introduction

A group of mollusks known as limpets (order Patellogastropoda) are important seafood derived from the rocky intertidal environment (Erlandso et al., 2011; McCoy 2008). These mollusks are usually wild harvested for food consumption; however, continual exploitation has pushed some populations to the brink of extinction (Espinosa et al., 2009). Furthermore, declines in wild stocks have pushed governments to intensify management efforts as well as to consider the development of limpet aquaculture (Mau and Jha, 2017). For instance, in South Africa, the government designated multiple "Marine Protected Areas" to preserve the overharvested South African limpet (*Cymbula oculus*) (Branch and Odendaal 2003). And in Portugal (1993–1998), the Regional Government of the Azores implemented a law to ban the wild harvest of two species of limpets, *Patella aspera* and *P. candei* (Ferraz et al., 2001).

In Hawaii, the native group of limpets, referred to as opihi (*Cellana* spp.), are consumed as a staple food during traditional gatherings. Despite management efforts and law prohibiting harvest of Hawaiian limpets less than 31 mm in shell length (SL), there has been a drastic reduction in market availability. Total annual catch landings of Hawaiian limpets decreased from about 68,000 kg to 5000 kg since the early 20th century (Kay and Magruder, 1977; Bird 2006); and population densities have decreased by 99.9% for the island of Oahu since western contact (Personal Communication; CE Bird, 2017). To prevent complete decimation and overcome market deficiencies, optimizing a grow-out diet is required to support aquaculture production of these socioeconomically important limpet.

For yellowfoot limpet (*Cellana sandwicensis*), the first formulated diet was developed following formula and dietary guidelines used for abalone feeds in a study by Cho (2010). Later, Hua and Ako (2016) found optimal protein and carbohydrate requirement for adult yellowfoot limpet to be 35% and 32%, respectively. Based on this study, yellowfoot limpet appears similar to abalone (*Haliotis*) with respect to feeding behaviors, metabolism and nutrition. However, the gross energy levels were not measured and requirements are still unknown.

In other studies, both the South African abalone (*Haliotis midae)* and green abalone (*H. fulgens*) were shown to consume feed based on their energy requirement with respect to dietary protein to energy (PE) ratio, which ranged from 43 to76 mg/kcal (Green et al., 2011) and from 62 to 108 mg/kcal (Gómez-Montes et al., 2003), respectively. Although direct comparisons cannot be made between different aquaculture groups, these results were similar to that of shrimp (*Penaeus monodon*) where an increase in energy with respect to a constant protein improved growth

* Corresponding author at: University of Hawaii at Manoa, 1955 East-West Rd., Honolulu, HI 96822, USA.
E-mail address: rjha@hawaii.edu (R. Jha).

https://doi.org/10.1016/j.aqrep.2018.03.001
Received 18 November 2017; Received in revised form 3 February 2018; Accepted 2 March 2018

performance (Bautista, 1986). These findings led to the hypothesis that feeding and growth performance of yellowfoot limpet would be affected by the dietary PE ratio. To best of our knowledge, there is no such information available for yellowfoot limpet. Therefore, the objectives of this study were to develop, fabricate and implement a novel grow-out system, and to evaluate effect of varying PE ratio diets on growth performance of yellowfoot limpet.

2. Materials and methods

2.1. Animal collection

Altogether 90 wild adult yellowfoot limpets were collected from a non-disclosed intertidal location in Puna, Hawaii. The smallest, legal sized animals (approximately 31 mm SL) were selected and carefully removed using metal putty knifes. Prior to stocking, a standard 45 L cooler was filled with natural seawater, chilled to 15 °C, and supplied with aeration. Upon removal from the rocks, animals were allowed to adhere themselves to black acrylic plates, which were designed to stand vertically in the cooler. Limpets were transported within 48 h without feeding to the research facility. Upon arrival, animals were transferred into circular tubs supplied with overhead irrigation spray for quarantine and acclimation at ambient, outdoor conditions.

The conditioning of animals to a formulated feed (Hua and Ako, 2016) were initiated weeks prior to the start of the trial. Limpets were fed daily to satiation and showed no signs of acceptability or palatability issues; and animals consumed feed effectively without knocking feed off surfaces.

2.2. Diet preparation and analysis

Four experimental diets were formulated (Table 1) to make different PE ratio. To test the effect of PE ratio of diets (Diet1, 87; Diet2; 95; Diet3 97; Diet4, 103) on growth performance of limpet, crude protein was kept constant (40%) and gross energy level was graded (3.85–4.63 kcal/g). Krill meal and *Porphyra* were found to be necessary attractants in the feed and were kept constant. Alginate was used as a binder at 5% in all diets. Diatomaceous earth was used as a filler as whole wheat volume was reduced. Assuming limpets are inefficient users of fat, crude fat was kept constant at 6% for all diets. A vitamin premix (MP Biomedical LLC, Solon, OH) used for previous abalone diets were included at 1% in all diets. All diets were analyzed for their proximate nutrients, amino acid and fatty acid profile (Tables 1–3, respectively).

The four diets were moist feeds that were adhered to vertical surfaces by pressing feed to the substrate surface. To make the diets, dry starch ingredients (whole wheat and alginate) were homogenized in a food mixer for 10 min. Water was boiled and added in a 1:1 ratio (1 mL water: 1 g dry ingredient) along with oils to the mixture. Starches were homogenized for an additional 10 min. The rest of the dry ingredients were homogenized and added to the gelatinous mixture and further homogenized until reaching a dough-like consistency. To dry, the dough was rolled out into 1 cm thick sheets and air dried at room-temperature until cooled (approximately 30 min) and feeds were placed in the freezer until use.

Feed samples were analyzed for proximate composition using methods of AOAC (2006). Moisture content was determined from a 2 g sample using an air-circulated oven at 135 °C for 2 h (method 930.15) followed by ashing in a muffle furnace at 600 °C for 6 h (method 942.05). Crude protein was estimated by determining total nitrogen (N) by dry combustion using a LECO analyzer (LECO CN-2000; Leco Corp., St. Joseph, MI; method 976.05, CP = N × 6.25). Crude fat (lipid) was determined by ethyl-ether extraction (method 920.39) using an Accelerated Solvent Extractor (Dionex Corporation, Bannockburn, IL). Gross energy (GE) was determined using an oxygen bomb calorimeter (Parr Isoperibol Bomb Calorimeter 6200, Parr Instrument Co., Moline, IL) with benzoic acid as the calibration standard. Minerals were analyzed by inductively coupled plasma atomic emission spectroscopy (Thermo Jarrel Ash Corporation, Franlin, MA). Amino acid (AA) contents of diets were determined using a High Performance Liquid Chromatography system (Agilent 1200 HPLC equipped with an Agilent1200 Series diode detector, Santa Clara, CA) following procedures

Table 1
Ingredient composition and analyzed proximate nutrient profile of experimental diets.

Ingredients	Diet (g/100 g diet)			
	1	2	3	4
Wheat flour[a]	28.50	21.55	14.50	7.55
Fish meal[b]	21.00	22.25	23.50	24.75
Soybean meal – defatted[c]	16.60	16.60	16.60	16.60
Porphyra[d]	14.00	14.00	14.00	14.00
Krill meal[e]	11.00	11.00	11.00	11.00
Alginate[f]	5.00	5.00	5.00	5.00
Diatomaceous earth[g]	1.80	7.65	13.60	19.40
Vitamin mix[h]	1.00	1.00	1.00	1.00
Menhaden fish oil[i]	0.45	0.45	0.45	0.45
Corn oil[j]	0.45	0.30	0.15	0.05
Cholesterol[k]	0.20	0.20	0.20	0.20
Analyzed composition (% dry matter basis)				
Dry matter	48.8	48.9	47.1	45.3
Crude protein	40.4	40.9	39.8	39.6
Crude fat	6.1	5.7	5.8	6.1
Ash	11.2	17.1	23.0	28.2
Gross energy (kcal/g)	4.63	4.31	4.10	3.85
Protein to energy ratio (mg/kcal)	87.2	94.9	96.9	102.9

[a] Hawaiian Flour Mill, Honolulu, HI.
[b] RMI Fishmeal, Republic of the Marshall Islands.
[c] Land-o-Lakes, Seattle, WA.
[d] Porphyra yezoensis (powder), Global Maxlink LLC, Antelope, CA.
[e] Florida Aqua Farms Inc., Dade City, FL.
[f] Sigma-Aldrich, Louis, MO.
[g] Hawaiian Hydroponics, Honolulu, HI.
[h] MP Biomedical LLC, Solon, OH. nicotinic acid (3.00 g/kg), D-calcium pantothenate (1.60 g/kg), pyridoxine HCl (0.70 g/kg), thiamine HCl (0.60 g/kg), riboflavin (0.60 g/kg), folic acid (0.20 g/kg), D-biotin (0.02 g/kg), vitamin B12 (0.1% triturated in mannitol) (2.50 g/kg), a-tocopherol powder (250 U/gm) (30.00 g/kg), vitamin A palmitate (250,000 U/gm) (1.60 g/kg), vitamin D3 (400,000 U/gm) (0.25 g/kg), phylloquinone (0.075 g/kg), and powdered Sucrose (959.655 g/kg).
[i] Virginia Prime Gold Menhaden Fishoil, Omega Protein Corporation, Houston, TX.
[j] Local supermarket, Honolulu, HI.
[k] Zeigler Brothers Inc., Gardners, PA.

Table 2
Amino acid composition of the experimental diets and pooled soft body tissue (SB) (% dry matter).

Amino Acid	Diet				SB
	1	2	3	4	
Alanine	7.40	7.84	7.83	7.84	5.70
Asparagine + Aspartate	10.10	10.05	10.32	10.06	15.34
Cystine	3.53	1.79	2.08	3.42	3.25
Glutamate + Glutamine	12.16	12.40	11.91	11.17	12.08
Glycine	6.62	6.95	6.86	7.55	8.72
Proline	5.45	5.36	5.14	5.16	4.18
Serine	4.37	4.52	4.49	4.38	3.96
Tyrosine	3.48	3.55	3.53	3.44	2.09
Taurine	1.05	1.06	1.07	1.04	3.74
Arginine	7.03	7.47	7.39	7.35	8.80
Histidine	2.71	2.73	2.85	2.74	1.30
Isoleucine	4.41	4.43	4.41	4.28	4.05
Leucine	7.26	7.32	7.33	7.10	7.22
Lysine	7.10	7.10	7.34	7.30	8.43
Methionine	3.07	2.99	3.14	3.17	3.29
Phenylalanine	4.69	4.41	4.41	4.34	3.94
Threonine	5.01	5.36	5.30	5.20	3.63
Valine	5.62	5.73	5.69	5.51	4.02

Table 3
Fatty acid composition of the experimental diets and pooled soft body tissue (SB) (% dry matter).

Fatty acid	Diet				SB
	1	2	3	4	
Octanoic acid (C8:0)	0.03	0.03	0.03	0.03	0.03
Decanoic acid (C10:0)	0.26	0.05	0.06	0.05	0.29
Dodecanoic acid (C12:0)	0.44	0.54	0.59	0.39	0.59
Tetradecanoic (Myristic) acid (C14:0)	4.72	4.92	5.79	4.34	3.75
Pentadecanoic acid (C15:0)	0.70	0.73	0.83	0.62	1.55
Palmitic acid (C16:0)	30.42	29.79	32.22	27.96	34.32
Palmitoleic acid (C16:1n-7)	3.60	3.80	4.40	3.43	0.84
Hexadecenoic (trans-Palmitilaidic) acid (C16:1n-9)	0.85	0.88	0.97	0.78	0.76
Heptadecanoic acid (C17:0)	0.27	0.30	0.35	0.28	0.05
Hexadecadienoic acid (C16:2n-4)	0.13	0.16	0.18	0.16	0.12
Hexadecatrienoic acid (C16:3n-4)	0.36	0.36	0.43	0.35	7.41
Stearic acid (C18:0)	4.31	4.29	4.46	3.97	5.65
Oleic acid (C18:1n-9)	10.08	9.64	9.85	10.04	6.05
Octadecenoic acid (C18:1n-7)	3.30	3.22	3.33	3.11	4.32
Linoleic acid (C18:2n-6)	10.04	9.53	8.08	11.93	0.89
Gamma Linolenic acid (C18:3n-6)	0.12	0.13	0.13	0.12	0.00
alpha-Linolenic acid (C18:3n-3)	1.68	1.70	1.67	1.77	0.76
Eicosanoic acid (C20:0)	2.13	2.08	2.20	2.01	0.35
Eicosenoic acid (C20:1n-9)	0.00	0.00	0.00	0.00	0.00
Eicosatrienoic acid (C20:3n-3)	0.84	0.95	0.75	1.09	0.00
Eicosatetraenoic acid (C20:4n-3)	0.21	0.22	0.18	0.22	0.99
Arachidonic acid (C20:4n-6)	0.60	0.62	0.58	0.59	5.85
Eicosapentaenoic acid (C20:5n-3)	13.52	13.68	12.21	13.74	10.40
Docosapentaenoic acid (C22:5n-3)	0.27	0.28	0.22	0.31	0.18
Docosapentaenoic acid (C22:5n-6)	0.14	0.17	0.12	0.21	0.59
Docosahexaenoic acid (C22:6n-3)	3.73	4.16	2.90	4.71	0.00

of AOAC (AOAC, 2006; method 982.30 E, a,b,c). For all AA except cysteine, methionine and tryptophan, dietary samples were hydrolyzed in 6 mol/L HCl for 24 h at 110 °C prior to injection. A β-amino-*n*-butyric acid and ethanol amine mixture was used as the internal standard. Fatty acids were analyzed using gas chromatography (Varian 3800 GC; Varian Analytical Instrument, Walnut Creek, CA) and flame ionization detector. The response factor for each peak was identified using an internal standard composed of 28 fatty acids (462 standard, Nu-Check Prep, Inc., Elysian, MN).

2.3. Growth performance trial

A 180-day growth performance trial was conducted in a semi-indoor facility, similar to a greenhouse with natural venting and airflow. Air temperature in the laboratory (24.6–29.3 °C) was continuously recorded in 6 s intervals using HOBO MX1101 Temperature/Relative Humidity Data Logger (Accuracy ± 0.2 °C, Onset Computers, Bourne, MA). Ambient seawater temperature (25.6–27.4 °C) and dissolved oxygen (4.41–6.37 mg/L) was measured twice daily and averaged using an YSI Pro20 Dissolved Oxygen Meter (Accuracy ± 0.3 °C and ± 0.2 mg/L, YSI Inc., Yellow Springs, OH). The pH (7.89–8.25) was measured once daily using an EcoSense pH10A instrument (YSI Inc., Yellow Springs, OH), and salinity (31 g/L) was measured once daily using a Vital Sine Refractometer (Accuracy ± 1.0%, Pentair, Apopka, Florida). The photoperiod was maintained at 13:11 h (Light:Dark) to simulate a non-spawning environment.

Out of 90 collected yellowfoot limpets, a total of 60 animals with mean initial weight 5.9 ± 1.72 g and length 33.9 ± 2.13 mm were selected and stocked in a completely randomized design. There were a total of 5 tanks (75 cm × 30 cm × 30 cm) assigned to each dietary treatment, 3 replicate (animals) nested in each tank, and a single animal per plate. Each animal was restricted to its own vertically suspended acrylic plate (15 cm length × 15 cm width × 2 sides = 450 cm^2 total surface area) with 50% surface area above and 50% surface area below the water level. Animals were allowed to crawl on either side of the plate. Overhead inlets were plumbed with irrigation nozzles providing spray (1 L/min flow rate) to air-exposed surfaces to prevent desiccation and osmotic stress.

Animals were fed once daily 1600 h by adhering a pre-weighed piece of feed on each plate above the water level to prevent leaching of nutrients. The flow rate was reduced overnight to maintain feed moisture levels and reduce feed loss. Feed intake was assessed the following morning at 0800 h by subtracting the remaining unconsumed moist feed (g) from the offered moist feed ration (g). The daily feed ration started at 0.2 g and increased to 6.5 g; and was increased by 0.1 g when > 50% of animals consumed 100% of ration from the previous night. This criterion prevented excessive waste of feed as feeding intervals reached up to 5 days, especially in the first half of the experiment.

Animals were measured prior to stocking, as well as every 30-day period for shell length (mm), shell width (mm), and total wet weight (g) using a dial caliper (0.1 mm, Empire Level) and electronic scale (0.0001 g, AG104 Mettler-Toledo International, Columbus, OH). Individuals were detached from each plate using a metal putty knife, placed on a towel to remove excess water, and measured. Mortalities were recorded and discounted for respective 30 days and 180 days calculations. At the end of the trial, surviving animals were sacrificed for soft body tissue measurements by removing the shell via dissecting knife.

2.4. Calculations

Recorded data and measurements were used to calculate average daily feed intake (ADFI), average daily gain (ADG), specific growth rate (SGR), shell length increment (SLI), shell width increment (SWI), feed conversion efficiency (FCE), protein efficiency ratio (PER), and soft body to shell ratio (SB/S). Calculations were done using wet masses; and protein intake was based on feed that was consumed by animals with respect to dietary proximate composition analyses.

ADFI = feed intake (wet g)/t (d)
ADG = $Wt_f - Wt_i$/t (d)
FCE = SGR/ADFI
PER = $Wt_f - Wt_i$/protein intake (g)
SGR (%BW d^{-1}) = Ln (Wt_f) − Ln (Wt_i)/t (d) × 100
SLI (μm d^{-1} day) = SL_f (mm) − SL_i (mm)/t (d) × 1000
SWI (μm d^{-1}) = $SW_f - SW_i$/t (d) × 1000
SB/S = Soft body tissue (wet g)/Shell (g)

2.5. Statistical analyses

Statistical analyses were done using SAS (SAS v9.2, SAS Institute Inc., Cary, NC). Treatment effect was analyzed by one-way ANOVA using MIXED procedure of SAS. Differences among individual treatment means were tested by Tukey-Kramer test and means were separated using pdmix macro of SAS. The differences were considered to be significant at a probability level of 0.05.

3. Results

The growth performance measurements (ADFI, ADG, FCE, and PER) are reported in Table 4. Average daily feed intake ranged from 0.07 to 0.10 g at the start of the trial, and ranged from 4.31 to 4.54 g at the end of the trial. There were significant differences ($P < 0.05$) in ADFI in period d91-120 and d121-150. There were also linear effects of dietary treatment on ADG ($P < 0.05$), FCE ($P = 0.05$), and PER ($P < 0.05$) for d0-180, and quadratic effects of dietary treatment on FCE ($P < 0.05$) and PER ($P = 0.05$) for d0-180.

Growth rates (SGR, SLI, and SWI) are presented in Table 5. There was a linear effect ($P < 0.05$) of dietary treatment on SGR for d151-180; and a significant effect ($P < 0.05$) on SGR for overall study period (d0-180). The SGR of Diet 1 (0.12%BW/d) was significantly higher than

Table 4
The average daily feed intake (ADFI), average daily gain (ADG), feed conversion efficiency (FCE), and protein utilization of limpets fed experimental diets.

Variable	Diet				SEM	P-value		
	1	2	3	4		Main	Linear	Quadratic
ADFI (g/d)								
Day0–30	0.07	0.10	0.07	0.07	0.01	0.28	0.73	0.70
Day31–60	0.13	0.13	0.12	0.12	0.00	0.96	0.73	0.82
Day61–90	0.31	0.30	0.28	0.32	0.01	0.46	0.94	0.39
Day91–120	1.35	1.34	1.04	0.90	0.11	0.00	< .0001	0.00
Day121–150	2.23	2.08	1.80	1.51	0.16	< .0001	< .0001	< .0001
Day151–180	4.54	4.31	4.49	4.37	0.05	0.10	0.26	0.36
Day0–180	1.21	0.70	1.04	0.82	0.11	0.15	0.24	0.37
ADG (mg/d)								
Day0–30	−2.14	2.22	−5.00	−6.92	1.99	0.24	0.11	0.13
Day31–60	3.57	−5.56	2.50	−1.54	2.08	0.26	0.62	0.65
Day61–90	1.43	−7.14	5.83	−4.55	2.93	0.36	0.83	0.46
Day91–120	3.33	−2.00	−6.67	4.44	2.57	0.42	0.96	0.36
Day121–150	13.64	2.00	5.00	1.11	2.86	0.55	0.26	0.49
Day151–180	12.73	2.50	−5.00	−10.00	4.94	0.17	0.04	0.17
Day0–180	6.36	5.00	2.50	0.00	1.41	0.16	0.03	0.08
FCE								
Day0–30	114.32	184.94	111.55	84.60	21.44	0.64	0.54	0.49
Day31–60	103.47	108.24	106.58	52.63	13.40	0.66	0.32	0.22
Day61–90	74.40	34.27	69.28	58.11	8.92	0.70	0.90	0.97
Day91–120	16.35	14.67	11.84	10.85	1.27	0.82	0.36	0.54
Day121–150	12.77	16.58	13.34	11.70	1.05	0.89	0.68	0.54
Day151–180	4.94	6.20	4.79	4.14	0.43	0.94	0.63	0.62
Day0–180	8.43	9.22	7.64	3.63	1.24	0.18	0.05	0.04
PER								
Day0–30	4.76	7.78	4.29	4.00	0.87	0.66	0.60	0.61
Day31–60	4.53	5.29	4.32	2.36	0.62	0.67	0.29	0.23
Day61–90	2.87	1.74	3.48	2.64	0.36	0.47	0.75	0.95
Day91–120	0.79	0.76	0.53	0.86	0.07	0.80	1.00	0.53
Day121–150	0.63	0.82	0.63	0.52	0.06	0.72	0.42	0.31
Day151–180	0.25	0.27	0.21	0.24	0.01	0.98	0.79	0.96
Day0–180	2.38	2.63	1.87	1.11	0.34	0.17	0.03	0.05

Table 5
The specific growth rates (SGR), shell length increment (SLI), and shell width increment (SWI) of yellowfoot limpet fed experimental diets.

Variable	Diet				SEM	P-value		
	1	2	3	4		Main	Linear	Quadratic
SGR (% BW/d)								
Day0–30	−0.03	0.07	−0.06	−0.11	0.04	0.14	0.09	0.08
Day31–60	0.03	−0.16	0.03	−0.02	0.04	0.24	0.89	0.87
Day61–90	0.03	−0.19	0.09	−0.09	0.06	0.37	0.85	0.60
Day91–120	0.04	−0.01	−0.13	0.02	0.04	0.61	0.71	0.68
Day121–150	0.20	0.07	0.09	0.05	0.03	0.76	0.40	0.61
Day151–180	0.19	0.08	−0.06	−0.15	0.08	0.12	0.02	0.10
Day0–180	0.12	0.08	0.05	0.01	0.02	0.06	0.01	0.04
SLI (μm/day)								
Day0–30	7.14	7.41	8.33	10.26	0.71	0.96	0.62	0.63
Day31–60	2.38	3.70	0.00	0.00	0.92	0.50	0.20	0.36
Day61–90	21.43	11.90	19.44	13.64	2.28	0.63	0.53	0.56
Day91–120	23.61	10.00	9.72	7.41	3.69	0.27	0.11	0.39
Day121–150	21.21	26.67	20.00	3.70	4.95	0.31	0.12	0.06
Day151–180	9.09	16.67	12.50	2.08	3.08	0.37	0.27	0.10
Day0–180	15.41	14.58	13.54	6.60	2.01	0.15	0.04	0.03
SWI (μm/day)								
Day0–30	21.43	18.52	25.00	25.64	1.66	0.85	0.47	0.57
Day31–60	7.14	0.00	0.00	2.56	1.68	0.18	0.23	0.95
Day61–90	21.43	16.67	22.22	21.21	1.26	0.96	0.87	0.89
Day91–120	12.50	20.00	2.78	11.11	3.53	0.21	0.35	0.92
Day121–150	18.52	26.67	18.33	5.56	4.36	0.30	0.14	0.07
Day151–180	9.09	8.33	4.17	0.00	2.10	0.39	0.09	0.15
Day0–180	14.90	18.06	14.24	10.76	1.50	0.47	0.18	0.14

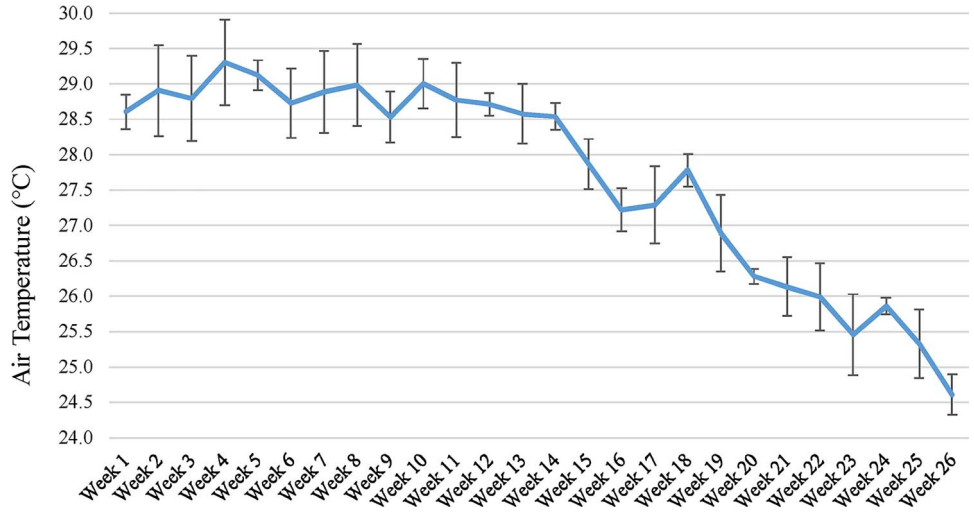

Fig. 1. Weekly air temperature during 180 day study period (mean ± standard deviation).

that of Diet 4 (0.01%BW/d), but not Diet 2 (0.08%BW/d) and Diet 3 (0.05%BW/d). There was no significant difference in SWI ($P > 0.05$); however, there was both linear and quadratic effect ($P < 0.05$) of dietary treatments on SLI for d0-180. SLI decreased from 15.41 µm/d for Diet 1–6.60 µm/d for Diet 4.

The relative softbody tissue to shell mass ratio (g/g) ranged from 0.45–0.55. There was no significant differences ($P > 0.05$) between dietary treatments.

Survival for each 30-day period was good (75–100%) for all dietary treatments, with the exception of Diet 2 during Day0–30 (40%) (Fig. 2).

4. Discussion

The overall design of this grow-out system allowed for successful rearing of yellowfoot limpet with improved survival rates, which was a major issue during past trials of Hua and Ako (2016). In terms of feeding strategy, this design improved the capacity for culturing limpets that graze above sea level in the intertidal.

In the feeding trial, there was notable change in feed behavior with respect to ADFI. Prior to period d91-120, limpets were exhibiting intermittent, non-feeding days of up to four days. However, this pattern shifted to daily feeding, as well as a 3–4 fold increase in ADFI for all treatments from d61-90 to d91-120. It was considered feed acclimation to be a possible explanation, however, these animals were accustomed to the feed weeks prior to the start of the trial. Instead, this change in feeding behavior was observed to coincided with a decrease in air temperature from 28.5 °C to 27.9 °C in week 15 (Fig. 1). This inverse relationship between temperature and ADFI remained for the rest of the study period. Although air temperature was not considered as a main factor, it is reasonable to say ambient air temperatures were not in the optimal range for limpets during the first 3 periods (d0-30, d31-60, and d61-90). Similar to this study, optimal temperatures for abalone (*H. midae)* were found to parallel the range of mean seawater temperature of their natural habitat; and when the water temperature was elevated outside of that range, the feed consumption, growth, and feed utilization decreased (Britz et al., 1997). Additionally, low survival for Diet 2 during the first period suggested hot summer-like temperatures may have negatively impacted performance. Although metabolism was not monitored, it seems that animals showed signs of shifting metabolism as ambient air temperature changed. Mortalities showed what appeared to be oxygen depletion (pale or discolored foot muscles), poor osmoregulation (shrunken mantle tissues), and possible infections (foot atrophy); all similar physiological responses to temperature that were reviewed for abalone (Morash and Alter, 2016). A study by Parry (1978) also found that metabolism of a cousin limpet (*C. tramoserica*) were caused by temperature mediated growth rates, and that these particular snails do not acclimate to seasonal changes in temperature. For yellowfoot limpet, metabolism may have increased uncontrollably during warmer daytime hours, ultimately causing the breakdown of their energy reserves or foot muscle, decreasing feed utilization, and stunting growth early on in the trial. As temperature decreased in the system, animal metabolism shifted favorably, and growth performance improved.

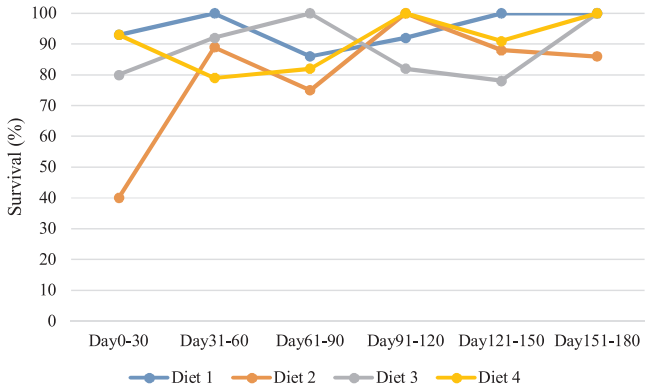

Fig. 2. Percent survival of yellowfoot limpet during 180 day study period.

As far as the effect of energy level on growth performance, there were no significant differences between treatments for ADG, FCE, and PER. In a related PE ratio study, Bautista-Teruel and Millamena (1999) reported best FCE (1.50 ± 0.08) and PER (2.47 ± 0.05) for the highest PE dietary treatment (31.48% CP/3090 kcal/kg ME; 101.88 mg/kcal) for abalone (*H. asinine*); and authors considered that low energy dietary treatment animals had higher feed intake to compensate for dietary energy deficiencies. In this study, all treatments were offered constant dietary protein level (40%), but animals offered lower energy diets did not overcompensate by increasing feed intake.

These results indicate that basal PE ratio demand was met for all diets; however, higher energy diets performed better. This means that protein was spared by increasing carbohydrate energy sources. The SGR across dietary treatments ranged from 0.01 to 0.12%BW/d over 180 days with peak SGR of 0.20%BW/d, which followed a significant increase in ADFI in the fourth period of trial (d91-120).

As far as growth rates, animals on Diet 1 had a significantly higher SGR (0.12%BW/d) compared to Diet 4 (0.01%BW/d). In the last two months of trial, animals on Diet 1 had 0.20%BW/d, which compares to that of abalone (Uki et al., 1985; Nelson et al., 2002). SGR for all treatments increased dramatically from d91-120 to d121-150, which

followed the increase in ADFI. In fact, animals on all treatments appeared to sustain minimal or negative growth during the first four periods of trial when feeding was marginal. The poor growth pattern observed are nearly identical to that in the wild, where yellowfoot limpet exhibited decreased growth rates from May through October (Kay et al., 2006). For all treatments, the SGR increased over a short period of time, which indicates that growth rates for yellowfoot limpet are dynamic. Shell length increment for the entire study period decreased linearly from Diet 1 (15.41 μm/d) to Diet 4 (6.60 μm/d), and followed the other growth measurement trends.

The slight decrease in SGR, SLI, and SWI for the last month are indicative of a possible shift in growth from somatic to reproductive, though these are not mutually exclusive. Although we did not investigate maturation, it can be noted that natural spawning season of limpet (October to January) coincided with last half of this study period. Also, 51.7% of the study population had gonads at the end of the study. Kay et al., (1982) reported the earliest onset of maturation for yellowfoot limpet to be 20–25 mm SL; and in terms of optimizing somatic growth rates for limpet production. Based on these information, it can be suggested to use juvenile animals < 20 mm to ensure that there is no influence of reproductive growth. However, due to local fishing laws, wild limpet collected below 31 mm SL would require permitting. Henceforth, the production of captive reared juveniles for this type of research would benefit our understanding of limpet nutritional demands.

Moreover, according to Kay et al. (2006), yellowfoot limpet gonad constitutes up to 46.5% of soft body weight in spawning season. The relative soft body to shell mass ratio found in this study indicates that nearly half of the total limpet weight come from shell mass. Thus, it can be suggested that the market value of limpet sold by weight may be drastically impacted by these factors (i.e. gonad development and relative soft body to shell mass). And local preference is for limpets with gonads due to the rich flavor of these fat-rich tissues, which supports understanding both somatic and reproductive growth for this species.

5. Conclusions

Overall, yellowfoot limpets performed indifferently with respect to diets with constant 40% protein level and staggered energy levels. Although there were no significant effects of diet on growth performance across the 180 day period, the highest energy diet (Diet 1, 87.2 mg/kcal) maintained the best overall growth. A diet with increased non-protein sources of energy may improve growth performance by freeing protein sources of energy for muscle growth. Growth rates also appear to change seasonally, dependent on the temperature regime. Temperature should be controlled/maintained in the optimal thermal range in future growth performance studies.

Acknowledgments

Thank you to Shauna Kehaunani Tom and Pat Springer for helping in the collection of the experimental animals. Special thanks to Kathleen Ramm and Spencer Davis for routine maintenance of the experiment. Thank you to the Aquatic Feeds and Nutrition Department of the Oceanic Institute, Hawaii for providing the facility, feed resources, and biochemical analyses. This work was supported by the Center for Tropical and Subtropical Aquaculture through a grant from the USDA/National Institute of Food and Agriculture (grant number 2014-38500-22241).

References

AOAC, 2006. Official Methods of Analysis, 18th edition. Association of Official Analytical Chemists, Gaithersburg, MD, USA.
Bautista, M.N., 1986. The response of *Penaeus monodon* juveniles to varying protein/energy ratios in test diets. Aquaculture 53 (3), 229–242.
Bautista-Teruel, M.N., Millamena, O.M., 1999. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: protein/energy levels. Aquaculture 178 (1), 117–126.
Bird, C.E., 2006. Aspects of Community Ecology in Wave-Exposed Rocky Hawaiian Coasts. Doctoral Thesis. University of Hawaii, Manoa, Hawaii.
Branch, G.M., Odendaal, F., 2003. The effects of marine protected areas on the population dynamics of a South African limpet Cymbula oculus, relative to the influence of wave action. Biol. Conserv. 114 (2), 255–269.
Britz, P.J., Hecht, T., Mangold, S., 1997. Effect of temperature on growth: feed consumption and nutritional indices of *Haliotis midae* fed a formulated diet. Aquaculture 152 (1), 191–203.
Cho, S.H., 2010. Effect of fishmeal substitution with various animal and/or plant protein sources in the diet of the abalone *Haliotis discus hannai Ino*. Aquacult. Res. 41 (10), e587–e593.
Erlandso, J.M., Braje, T.J., Rick, T.C., Jew, N.P., Kennett, D.J., Dwyer, N., Watts, J., 2011. 10,000 years of human predation and size changes in the owl limpet (*Lottia gigantea*) on San Miguel Island California. J. Archaeolog. Sci. 38, 1127–1134.
Espinosa, F., Rivera-Ingraham, G.A., Fa, D., García-Gómez, J.C., 2009. Effect of human pressure on population size structures of the endangered ferruginean limpet: toward future management measures. J. Coast. Res. 25 (4), 857–863.
Ferraz, R.R., Menezes, G.M., Santos, R.S., 2001. Limpet (Patella spp.) (Mollusca: Gastropoda) exploitation in the Azores, during the period 1993–1998. Arquipelago. Life Marine Sci. 59–65 Supplement 2 (Part B).
Gómez-Montes, L., García-Esquivel, Z., D'Abramo, L.R., Shimada, A., Vásquez-Peláez, C., Viana, M.T., 2003. Effect of dietary protein: energy ratio on intake, growth and metabolism of juvenile green abalone *Haliotis fulgens*. Aquaculture 220, 769–780.
Green, A.J., Jones, C.L., Britz, P.J., 2011. The protein and energy requirements of farmed South African abalone *Haliotis midae L.* cultured at optimal and elevated water temperatures. Aquacult. Res. 42 (11), 1653–1663.
Hua, N.T., Ako, H., 2016. Dietary protein and carbohydrate requirement of juvenile Hawaiian limpet (*Cellana sandwicensis* Pease, 1861) fed practical diet. Int. Aquat. Res 8 (4), 323–332.
Kay, E.A., Magruder, W., 1977. The Biology of ‘Opihi. Department of Planning and Development, State of Hawaii, Honolulu, Hawaii, USA.
Kay, E.A., Corpuz, G.C., Magruder, W.H., 1982. ‘Opihi. Their Biology and Culture. Report of the Aquaculture Development Program. Department of Land and Natural Resources, State of Hawaii, Honolulu, Hawaii, USA.
Kay, E.A., Bird, C.E., Holland, B.S., Smith, C.M., 2006. Growth Rates, Reproductive Cycles, and Population Genetics of ‘Opihi from the National Parks in the Hawaiian Islands.
Mau, A., Jha, R., 2017. Aquaculture of two commercially important molluscs (abalone and limpet): existing knowledge and future prospects. Rev. Aquacult. http://dx.doi.org/10.1111/raq.12190.
McCoy, M.D., 2008. Hawaiian limpet harvesting in historical perspective: a review of modern and archaeological data on Cellana spp. from the Kalaupapa Peninsula, Moloka‘i Island. Pacific Sci. 62, 21–38.
Morash, A.J., Alter, K., 2016. Effects of environmental and farm stress on abalone physiology: perspectives for abalone aquaculture in the face of global climate change. Rev. Aquacult. 8, 342–368.
Nelson, M.M., Leighton, D.L., Phleger, C.F., Nichols, P.D., 2002. Comparison of growth and lipid composition in the green abalone, *Haliotis fulgens*, provided specific macroalgal diets. Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol. 131 (4), 695–712.
Parry, G.D., 1978. Effects of growth and temperature acclimation on metabolic rate in the limpet, *Cellana tramoserica* (Gastropoda: patellidae). J. Anim. Ecol. 47 (2), 351–368.
Uki, N., Kemuyama, A., Watanabe, T., 1985. Nutritional evaluation of several protein sources in diets for abalone *Haliotis discus hannai*. Bull. Japan. Soc. Sci. Fish. 51, 1835–1839.