Journal of Shellfish Research, Vol. 35, No. 3, 695–701, 2016.

INCREASED DIETARY PROTEIN IMPROVES THE COMMERCIAL PRODUCTION OF HYBRID ABALONE (*HALIOTIS LAEVIGATA* × *HALIOTIS RUBRA*)

DAVID A. J. STONE,[1,2,3,4,6]* MATTHEW S. BANSEMER,[3,6] KRISHNA-LEE CURRIE,[3] LUCY SAUNDERS[5] AND JAMES O. HARRIS[3,4,6]

[1]*South Australian Aquatic Sciences Centre, South Australian Research and Development Institute, 2 Hamra Avenue, West Beach, SA 5024, Australia;* [2]*School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, SA 5371, Australia;* [3]*School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia;* [4]*Marine Innovation Southern Australia, 2 Hamra Avenue, West Beach, SA 5024, Australia;* [5]*Great Southern Waters Pty. Ltd., 366 The Esplanade, Indented Head, VIC 3223, Australia;* [6]*Australian Seafood CRC, Science Park, Bedford Park, SA, 5042, Australia*

ABSTRACT Over the past decade, Australian abalone were produced using a range of commercially formulated grow-out diets with crude protein (CP) levels ranging from 27% to 30% throughout their production cycle. Recent research identified higher optimal dietary CP levels of greater than or equal to 35% for younger than 1-y-old abalone at higher water temperatures (20–22°C). To validate the results of the laboratory-based research, the current trial was designed to investigate the effect of feeding two different commercially manufactured diets (standard-protein diet, 32% CP, versus a high-protein diet, 39% CP) to hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*; initial weight: 3.10 g) on growth performance, feed utilization, and sales revenue under on-farm conditions. During this 18-mo trial, abalone were cultured using normal commercial practices over two summer periods at Great Southern Waters abalone farm (Indented Head, VIC, Australia). A significant improvement in specific growth rate (SGR) led to a 9% improvement in biomass gain for abalone fed with the high-protein diet. This improvement was achieved with no differences in survival, and minimal difference in feed input between diets. In addition, the feed conversion ratio of abalone fed with the high-protein feed was 7.1% superior to that of animals fed with the standard-protein diet. On the basis of a farm gate value of AUD35/kg abalone, for an additional feed input cost of AUD2/m^2 slab tank/y, a 9.5% increase in basic annual sales revenue (AUD44/m^2 slab tank/y) was achieved feeding the high-protein diet. In addition, due to an increased SGR by feeding the high-protein diet, the duration of a typical 3-y production cycle for hybrid abalone may be shortened by up to 3.4 mo. By adopting the high-protein diet, farmers may also harvest abalone sooner, and reduce exposure to one less summer. This may reduce heat-related mortalities and further improve productivity, and when combined with savings made with biomass and feed efficiency gains, a more than 10% improvement in productivity across the entire grow-out period for hybrid abalone may be achieved.

KEY WORDS: abalone, *Haliotis laevigata* × *Haliotis rubra*, nutrition, commercial production, high-protein diets

INTRODUCTION

Recently, there has been an increased demand by Australian abalone farmers to optimize the dietary protein level for the commercial production of abalone in Australia (Stone et al. 2014a). There is evidence that demonstrates marked difference in the growth performance and feed utilization of a range of abalone species in relation to changes in dietary protein level (Mai et al. 1994, 1995, Britz 1996, Britz & Hecht 1997, Bautista-Teruel & Millamena 1999, Stone et al. 2013, Bansemer et al. 2015, 2016). Temperature has also been demonstrated to have a significant effect on growth rate of abalone (Britz et al. 1997, Steinarsson & Imsland 2003, Stone et al. 2013, Bansemer et al. 2015). These differences suggest that feeding diets containing optimal protein levels could improve abalone growth and farm productivity. In addition, optimum levels of dietary protein may also provide other critically important outcomes, such as minimized nitrogenous effluent outputs and other associated culture unit and environmental pollution impacts (National Research Council 1993, Hardy 2002).

*Corresponding author. E-mail: david.stone@sa.gov.au
DOI: 10.2983/035.035.0316

Prior to this study, Australian abalone producers did not routinely alter the protein content of the commercial grow-out diets in response to changing animal sizes or water temperature (Stone et al. 2013). Several feed companies supplied grow-out diets to Australian abalone growers. The ingredient composition of the grow-out feeds differed between manufacturers, but typically contained similar crude protein (CP) levels ranging from 27% to 30%, which was adopted as the Australian industry standard (Stone et al. 2013). These levels were based on research by Coote (1998) and Coote et al. (2000), which used juvenile greenlip abalone of one size class [25-mm shell length (SL)] at one water temperature (20°C).

More recently, investigations into the interactive effects of age and water temperatures on the dietary protein requirements of greenlip abalone *Haliotis laevigata* (Donovan, 1808) by Stone et al. (2013) and Bansemer et al. (2015) demonstrated differences in the growth performance and feed efficiency of post-weaned subjuvenile (6-mo-old; 0.91 g), juvenile (1-y-old; 1.8 g), and subadult (2-y-old; 22.9 g). In summary, very little growth occurred for each size class of abalone at the lower water temperature of 14°C, regardless of the dietary protein level. In contrast, the growth performance and feed utilization of 1-y-old greenlip abalone at warmer water temperatures were improved by feeding 35% dietary protein (Stone et al. 2013).

Previous research focused on optimizing dietary protein levels for Australian abalone have focused primarily on greenlip abalone. Hybrid abalone *Haliotis laevigata* × *Haliotis rubra* (Leach, 1814) is also commercially cultured in southern Australia. The optimum protein requirements of hybrid abalone are unknown, but may be similar to greenlip abalone as they are hybrid between greenlip abalone and blacklip abalone (*H. rubra*). Compared with greenlip abalone, however, hybrid abalone are reported to exhibit higher growth rates and meat yields and also grow better at lower water temperatures (Freeman 2001, Guo 2009, Lafarga de la Cruz & Gallardo-Escaìrate 2011). Results obtained from the laboratory experiments with greenlip abalone from Stone et al. (2013) and Bansemer et al. (2015) suggest that there was considerable scope to improve productivity of hybrid abalone farming by incorporating higher protein diets into the production cycle in land-based farms in Australia. The aim of this study was to investigate potential improvements in growth performance, feed utilization, and sales revenue of hybrid abalone fed with a high-protein versus a standard-protein diet under commercial culture conditions.

MATERIALS AND METHODS

Experimental Design and Culture System

The farm-based trial was designed to evaluate the growth performance and feed utilization of hybrid abalone fed with a standard-protein commercial diet (Halo abalone diet, Skretting Australia, Cambridge, TAS, Australia) or a newly formulated high-protein commercial test diet (Table 1) across the normal commercial production cycle. The trial ran from November 15, 2012 to May 30, 2014 (18.3 mo, 561 days), and covered two summer growing periods. The trial was conducted in a commercial land-based slab tank system at Great Southern Waters (GSW) abalone farm (Indented Head, VIC, Australia). A total of 16 concrete slab tanks were used in the trial. Eight replicate slab tanks were used for each dietary protein level treatment. The slab tanks were 16.1×2.5-m wide, with a laminar flow water depth of 4.6 cm at the inlet end and 1.5 cm at the outlet. Tanks were cleaned daily using tipper flushers that delivered regular pulsed flows down each slab tank.

Experimental Diets

The CP level of the standard-protein commercial diet (32.6%; Table 1) was based on the protein and energy requirements of juvenile greenlip abalone reported by Coote et al. (2000), and was fed to the control treatment group of abalone throughout the trial. The CP level of the high-protein commercial test diet (39.8% CP; Table 1) was selected based on information pertaining to the dietary protein and energy requirements of juvenile greenlip abalone reported by Stone et al. (2013) and Bansemer et al. (2015). The diet chips (4-mm diameter × 2-mm thick) were manufactured by Skretting Australia using extrusion processing technology. The ingredient formulations of the commercial diets used in this trial are confidential. The analyzed biochemical composition of the diets is provided in Table 1.

TABLE 1.

Analyzed biochemical composition of the two commercial test diets used in the on-farm trial.

Item (as fed)*	Standard-protein diet	High-protein diet
Proximate composition		
Moisture (g/100 g)	8.4	8.3
CP (g/100 g)	32.6	39.8
Crude lipid (g/100 g)	5.7	6.2
Ash (g/100 g)	9.1	7.5
Carbohydrate (g/100 g)†	44.0	38.0
Gross energy (MJ/kg)	15.10	15.50
Amino acids (g/100 g)		
Alanine	1.11	1.36
Arginine	1.76	2.21
Aspartic acid	2.49	2.94
Glutamic acid	5.14	6.35
Glycine	1.06	1.30
Histidine	0.71	0.83
Isoleucine	1.13	1.37
Leucine	1.95	2.46
Lysine	1.49	1.78
Methionine	0.32	0.45
Phenylalanine	1.29	1.60
Proline	1.53	1.90
Serine	1.23	1.50
Threonine	0.99	1.19
Tyrosine	0.70	0.94
Valine	1.28	1.57
Minerals (mg/kg)		
Calcium	14,000	18,000
Copper	16	21
Magnesium	2,100	2,300
Manganese	61	64
Phosphorus	8,800	12,000
Potassium	12,000	12,000
Zinc	150	190

* Diets were formulated, manufactured, and provided by Skretting Australia.

† Carbohydrate (g/100 g) = 100% – [moisture (g/100 g) + protein (g/100 g) + lipid (g/100 g) + ash (g/100 g)].

Experimental Animals

Great Southern Waters bred the hybrid Jade Tiger Abalone within their selective breeding program on-site. Before the commencement of the trial, juvenile abalone from the same spawning cohort were cultured indoors and were fed the standard-protein diet under low light conditions, in slab tanks provided with flow-through seawater at ambient temperatures.

Stocking, Feeding, and Sampling of the Trial

The trial was stocked, sampled, and harvested using commercial methods practiced at GSW. The trial was stocked with postweaned juvenile hybrid abalone (initial weight 3.2 g, initial SL 29 mm) at 88 kg abalone/slab tank (25,000 abalone/slab tank) (Table 2). It later became apparent that during stocking, one slab tank from the abalone fed with the high-protein diet treatment received too many animals. The data from this slab tank were excluded from the trial. As a result, the high-protein

TABLE 2.

Growth performance and feed and nutrient utilization of hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*) fed with the standard- or high-protein diet at the completion of the 18.3-mo trial.*

Item	Standard-protein diet (n = 8)	High-protein diet (n = 8)	P value
Survival (%)	98.73 ± 0.27	98.68 ± 0.27	0.897
Initial weight (g/abalone)	3.11 ± 0.02	3.10 ± 0.03	0.800
Final weight (g/abalone)†	90.36 ± 1.37[b]	95.05 ± 1.30[a]	0.029
Weight gain (g/abalone)	87.25 ± 1.38[b]	91.94 ± 1.31[a]	0.030
SGR (%/day)	0.600 ± 0.004[b]	0.610 ± 0.003[a]	0.021
Initial SL (mm)‡	29.49 ± 0.19	28.81 ± 0.63	–
Final SL (mm)†	80.51 ± 0.58	80.42 ± 0.51	0.916
Shell growth rate (μm/day)	80.45 ± 0.58	80.37 ± 0.52	0.917
CF	1.01 ± 0.02[b]	1.07 ± 0.01[a]	0.032
Initial biomass (kg/tank)	77.78 ± 0.59	77.56 ± 0.63	0.800
Final biomass (kg/tank)	1,159.5 ± 37.8	1,259.5 ± 28.5	0.060
Biomass gain (kg/tank)	1,081.7 ± 38.2	1,181.9 ± 28.6	0.062
Total feed offered (kg/tank)	1,065.3 ± 10.5	1,088.8 ± 7.21	0.096
Apparent FCR	0.99 ± 0.08	0.92 ± 0.05	0.082
Apparent PER	3.38 ± 0.11	3.11 ± 0.07	0.051
Apparent energy efficiency ratio	5.34 ± 0.17	5.54 ± 0.12	0.379

* Means (±SE) for each variable in each row that do not share the same superscript are significantly different ($P < 0.05$, one-factor ANOVA, Student–Newman–Keuls test).

† The initial SL was determined from batches of 50 abalone from each of the 11 holding tanks, which were used to stock the experimental slab tanks.

‡ Final weight and SL determined from 500 abalone/slab tank.

diet treatment had seven replicate slab tanks. The standard-protein diet treatment had eight replicate slab tanks.

Abalone were fed to excess, and both diets were fed at the same feed rates (1%–3% biomass/day, depending on the season) throughout the trial. Feed rates were adjusted monthly based on the weight of subsamples of 100 abalone/slab tank. To ensure that excessive stocking densities did not interfere with the growth of abalone as abalone grew, all slab tanks were partially harvested, in an identical manner and at the same rate, on four separate occasions throughout the trial.

Abalone growth was monitored regularly. For monthly weight checks, the bulk weight of 100 animals/slab tank was measured. For three monthly weight checks, the bulk weight of 300 animals/slab tank was measured. All abalone from each slab tank were harvested and bulk weighed at the end of the trial. At final harvest, the individual weights and lengths of 500 abalone/slab tank were also measured. Mortalities were counted and recorded daily.

Biochemical Analysis of Diets

Diets were analyzed for moisture, CP, crude lipid, ash, gross energy, and minerals by the Australian Government National Measurement Institute (Port Melbourne, VIC, Australia). Amino acids were analyzed by the Australian Proteomics Facility (Macquarie University, Sydney, NSW, Australia). Nitrogen-free extract (g/100 g) = 100% – [moisture (g/100 g) + protein (g/100 g) + lipid (g/100 g) + ash (g/100 g)].

Variables Measured

All calculations using abalone weight and feed weights were based on wet values:

$$\text{Apparent biomass gain (kg/tank)} = (\text{final weight} + \text{thinning harvest weight}) - (\text{initial weight})$$

$$\text{Specific growth rate (SGR, \%/day)} = \frac{(\ln \text{final weight} - \ln \text{initial weight})}{\text{time (days)} \times 100}$$

$$\text{Apparent feed conversion ratio (FCR)} = \frac{\text{feed consumed}}{\text{biomass gain}}$$

$$\text{Apparent protein efficiency ratio (PER)} = \frac{\text{abalone weight gain}}{\text{protein consumed}}$$

$$\text{Apparent energy efficiency ratio} = \frac{\text{abalone weight gain}}{\text{energy consumed}}$$

$$\text{Shell growth rate } (\mu\text{m/day}) = \frac{(\text{final shell length} - \text{initial shell length})}{\text{time (days)}}$$

$$\text{Condition factor} = \frac{5,575 \times \text{weight (g)}}{\text{length (cm)}^{2.99}} \text{ (Britz \& Hecht 1997)}$$

An economic analysis of the growth performance of abalone was performed to determine the increase in basic sales revenue, after feed costs, on an AUD/m^2 slab tank/y basis. The economic analysis was based on a farm gate value of AUD35/kg abalone (N. Savva, Executive Officer, Australian Abalone Growers' Association, personal communication), and the differences in feed costs (5%) and feed input costs and yields of abalone grown using the standard-protein diet versus the high-protein diet were included.

Water Quality

Slab tanks were supplied with ambient temperature seawater at a varying flow rates ranging from 170 to 180 l/min throughout the year according to the season. Water quality was measured in the slab tanks on a daily basis between 1 and 3 PM using a handheld YSI Professional Plus (Pro Plus) multiparameter instrument (model, 10102030; YSI Australia, Hemmant, QLD, Australia). The instrument was calibrated monthly. The variables measured were dissolved oxygen (±0.2 mg/l), pH (±0.2 units), salinity, and temperature (±0.2°C).

Statistics

Normality of data was assessed using the Shapiro–Wilk test. Homogeneity of variances among mean values was assessed using Levene's test for equality of variances. Data for each variable were analyzed separately using analysis of variance (ANOVA). Comparisons among means were made using Student–Newman–Keuls test. A significance level of $P < 0.05$ was used for all statistical tests. All statistical analyses were done using IBM SPSS, Version 21 for Windows (IBM SPSS Inc., Chicago, IL). All values are presented as means ± SEM unless otherwise stated.

RESULTS

The water temperature in the tanks demonstrated normal seasonal fluctuations throughout the trial. Water temperatures ranged from 10.1°C to 24.7°C with a mean of 17.5°C, and were similar between treatments (Fig. 1). The mean dissolved oxygen levels were 6.12 and 6.31 mg/l for the high-protein diet and standard-protein diet treatments, respectively, and ranged from 4.21 to 9.14 mg/l (Fig. 2). Dissolved oxygen levels were lowest in all slab tanks during the period of midsummer to early autumn. Salinity levels were similar between treatments (standard-protein diet, 34.5, versus high-protein diet, 34.7; Fig. 3) and ranged from 34.5 to 41.1. Initially, salinity levels were 37–38 up until late June 2013 when levels spiked above 40 for a period 1 mo, where they sharply dropped to levels of 36 for the remainder of the trial. The pH level of the tanks was similar between treatments (mean 8.37, minimum 7.97, and maximum 8.74) and tended to increase as the trial progressed. The tendency for the progressive increase was linked directly to the pH level of the incoming seawater supply (mean 8.57, minimum 8.09, and maximum 8.82; Fig. 4) and may have also been linked to the rapid drop in salinity that was also observed over the same period (Fig. 3).

There was a significant effect of diet and time on the mean individual weight of abalone over the course of the on-farm trial ($P < 0.001$, two-factor ANOVA; Fig. 5), and there was no significant interaction between the two factors ($P = 0.055$). There was a significant 6% increase in the mean individual weight of abalone fed with the high-protein diet compared with the standard-protein diet. The individual weight of abalone significantly progressively increased at each sampling time over the duration of the trial (Fig. 5).

Feeding the high-protein diet significantly improved the growth performance of abalone at the end of the trial (Table 2). The final individual weight ($P = 0.029$), final weight gain ($P = 0.030$), and SGR ($P = 0.021$) of abalone fed with the high-protein diet were significantly higher (95.1 g/abalone, 91.9 g/abalone, and 0.61%, respectively) than abalone fed with the standard-protein diet (90.4 g/abalone, 87.3 g/abalone, and 0.60%, respectively) (one-factor ANOVA; Table 2). The

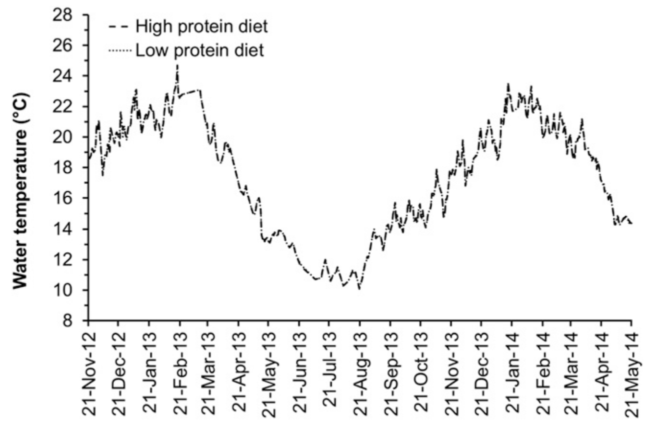

Figure 1. Daily water temperatures (°C) in slab tanks for each treatment during the on-farm trial of two diets of different protein levels fed to hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*).

condition factor (CF) of the abalone fed with the high-protein diet was also significantly higher (5.9%) than for the abalone fed with the standard-protein diet ($P = 0.032$, one-factor ANOVA). There were no significant differences for any of the other variables measured between the standard-protein and high-protein diet for abalone at the end of the trial ($P > 0.05$; Table 2). Nevertheless, compared with the standard-protein diet, the corresponding percent increase for final biomass (8.6%) and biomass gain (9.3%), apparent FCR (7.1%) for abalone fed with the high-protein diet were all numerically superior, with $P < 0.100$, and approaching the 0.05 level of significance (Table 2).

On the basis of a farm gate value of AUD35/kg abalone and a biomass gain of 9.3%, after taking into account an extra AUD2/m^2 of slab tank/y of feed costs (including freight), a substantial 9.5% increase in sales revenue of AUD44/m^2 of slab tank/y was attained by feeding abalone the high-protein diet.

DISCUSSION

Previous research focused on greenlip abalone has provided a foundation to better understand the response of different aged

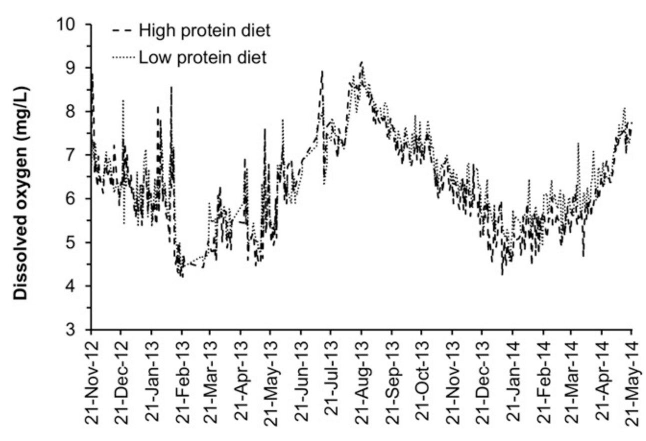

Figure 2. Daily dissolved oxygen levels (mg/l) in slab tanks for each treatment during the on-farm trial of two diets of different protein levels fed to hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*).

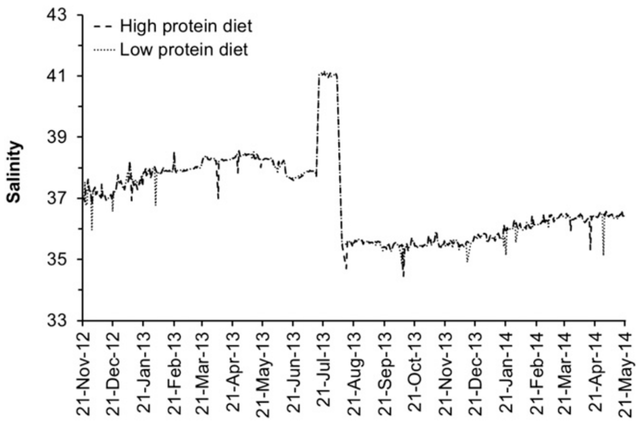

Figure 3. Daily salinity levels in slab tanks for each treatment during the on-farm trial of two diets of different protein levels fed to hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*).

(sized) abalone to differing dietary protein levels during seasonal fluctuations in water temperature (Stone et al. 2013, Bansemer et al. 2015). These studies provided invaluable information for Australian abalone feed companies to better formulate commercial diets, with respect to optimum dietary protein levels, for greenlip abalone and potentially hybrid abalone. In the current study, the aim was to evaluate feeding hybrid abalone a high-protein diet to improve the growth performance, feed utilization, and sales revenue under commercial conditions. Results were positive, and indicated that the optimum dietary protein levels reported for greenlip abalone by Stone et al. (2013) and Bansemer et al. (2015) may be transferable to this closely related hybrid. At the completion of the trial, a significant 5.4% improvement in SGR resulted in the production of an extra 100 kg of abalone per slab tank, which equated to a 9.3% increase in biomass, by using the high-protein diet compared with the abalone fed with the standard-protein diet. In addition, gains were made without an impact on mortality due to high-protein diets. It is important to note that this gain in biomass was obtained with a mere 2.2% increase in feed input weight, as the FCR of abalone fed with the high-protein feed was 7.1% superior to that of animals fed with the

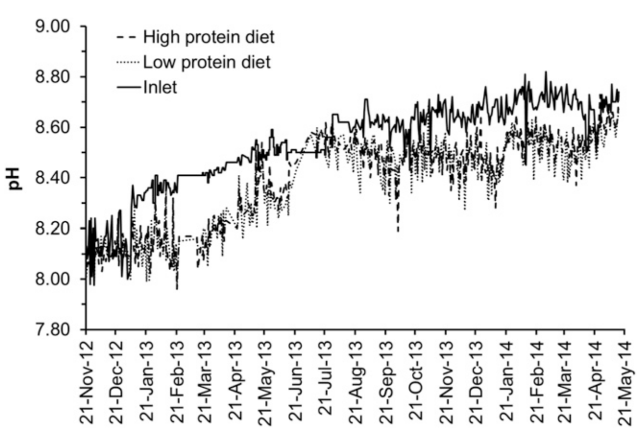

Figure 4. Daily pH levels in the supply inlet water and slab tanks for each treatment during the on-farm trial of two diets of different protein levels fed to hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*).

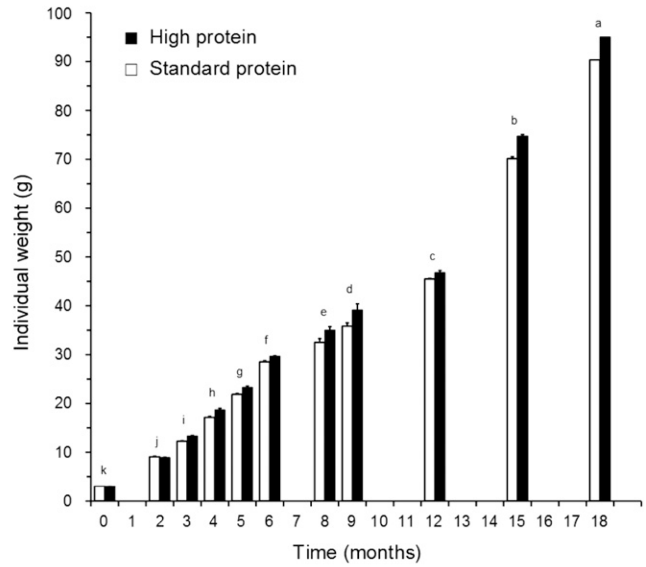

Figure 5. Average individual weights of hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*) fed with the standard- or high-protein diet over the duration of the entire 18-mo trial. Values presented for standard-protein diet treatment data ($n = 8$) and high-protein diet treatment data ($n = 7$) are means ± SE. There were significant effects of diet ($P < 0.001$, response for individual weight of high-protein diet treatment was 6% greater than standard-protein diet treatment) and time ($P < 0.001$, individual weight significantly increased between each sampling time) on the individual weight of abalone over the course of the trial. No significant interactions occurred between the two factors ($P = 0.055$). Different letters denote significant differences between times ($P < 0.001$, two-factor ANOVA, Student–Newman–Keuls test).

standard-protein diet. The high-protein diet, however, had a 5% cost premium compared with the standard-protein diet. Despite this, based on a conservative farm gate value of AUD35/kg abalone (N. Savva, Executive Officer, Australian Abalone Growers' Association, personal communication), there was a 9.5% increase in sales revenue of AUD44/m^2 of slab tank area/y for hybrid abalone produced using the high-protein diet compared with the standard-protein diet. This excellent return was achieved with the minor additional feed cost of AUD2/m^2 of slab tank area/y.

Previous studies have reported superior protein deposition in greenlip abalone fed to apparent satiation with high-protein (>33% CP) diets (Stone et al. 2013, Bansemer et al. 2015). For example, Stone et al. (2013) reported 1-y-old greenlip abalone (initial weight: 1.8 g) to be 17% and 20% more efficient at utilizing diets containing 33% CP compared with 30% protein at 18°C and 22°C, respectively. In the current trial, abalone from both treatments were fed in excess of the requirements at the same feed rate, which may have resulted in a higher proportion of wasted feed from the abalone fed with the high-protein diet. In turn, this may have resulted in reduced apparent PER for abalone fed with the high-protein diet (Table 2). It may be possible to reduce feeding rates and still end up with the same improvements in growth rates. Reducing feed rates when feeding abalone high-protein diets may also result in additional improvements in FCR, apparent PER, and savings in costs related to freight, feed storage, handling, and use. Further research in this area is needed.

Reducing the time for abalone to attain harvest size is of high commercial importance. The growth performance of different aged (sized) abalone fed with the high versus standard-protein diets, weight gains using a high-protein diet were more pronounced with younger 1-y-old greenlip abalone (initial weight: 1.8 g), compared with 2-y-old greenlip abalone (initial weight: 22.9 g; Stone et al. 2013). In the current study, this growth phenomena, although less distinct, was also evident with hybrid abalone. Britz and Hecht (1997) similarly reported younger *Haliotis midae* (initial weight: 0.2–1.0 g) grew significantly faster, on a percentage body weight basis, compared with older *H. midae* (initial weight: 7.0–14.0 g). The improvements in growth and biomass gain in abalone fed with high-protein diets were accompanied by a significant (6%) improvement in the CF (Table 2). The improved CF was obtained without any additional shell growth, which suggests that abalone deposit protein as new tissue rather than shell growth. Any advancement in growth rates captured early in the production cycle, assuming that all things are equal during the latter stages of the production cycle, should result in an earlier arrival at harvest (Britz & Hecht 1997, Stone et al. 2013). In addition, further refinements in dietary protein feeding strategies to improve feed utilization and growth rates for older hybrid abalone during seasonal fluctuations in water temperatures are warranted, which would likely lead to further improvements in hybrid abalone production in Australia.

High data variability, commonly observed with commercial on-farm abalone trials, may create difficulties in discerning statistical differences between treatments responses. This problem has been reported as typical of on-farm trials with low replication (Festing & Altman 2002, Vandepeer 2006). In the current study, the loss of one replicate tank from the high-protein diet treatment reduced statistical power and the ability to discern significant differences for some parameters between treatments, particularly biomass gain. In the current trial, seven (high-protein diet) and eight replicate (standard-protein diet) slab tanks/treatment provided sufficient power (0.62) to discern a statistical significant difference of 5.2% between feeding strategies for weight gain (Table 2). Although, for biomass gain, the power (0.47) was insufficient to detect a statistical difference between treatment means of 8.9%. For future on-farm studies, a minimum of eight replicate slab tanks per dietary treatment is recommend to observe a 10% significant difference in biomass gain. This number will depend on the variability of the response being measured, and will also vary between farms. Allocating eight replicate slab tanks per treatment may be difficult to achieve given the restrictions of staffing, combined with production demands, placed on commercial operators. Regardless, investment in research and development is a serious proposition, where often gains may only be realized in small percentages. If trials are run with insufficient statistical power they may be doomed from the start, and would not only result in a waste of time, resources, and money in the present, they may also cost the industry well into the future, as cost savings procedures may be missed and are often not revisited until the turn-over of management occurs.

On the basis of the results for individual weight gain (5.4%) and biomass gain (9.3%), the overall duration of a typical 3-y production cycle may be shortened by up to 3.5 mo by feeding high-protein diets, which would result in reduction in risk of losing stock from summer mortalities and other problems experienced during production, and also a significant saving in both fixed and variable costs. There would also be considerable advantages to be gained from reduced mortality of stock during the shortened production cycle. Larger abalone are more prone to summer mortality (Vandepeer 2006, Lange et al. 2014, Stone et al. 2014b). Harvesting the faster growing hybrid abalone by up to 3.5 mo sooner may reduce exposure of the valuable larger stock to one less summer. This factor alone could result in significantly improved productivity and cost savings, and when combined with savings made with increased growth rates and biomass gain, significant improvements in productivity across the entire grow-out period for hybrid abalone may be achieved.

In conclusion, feeding hybrid abalone the high-protein diet under commercial conditions accelerates growth rates. There are numerous benefits of producing larger abalone in a shorter time. Hybrid abalone fed with the high-protein diet produced an extra 100 kg of biomass per slab tank. This biomass gain may be achieved with a 7.1% improvement in FCR. The high-protein diet (AUD2.20/kg) had 4.8% cost premium, compared with the standard-protein diet (AUD2.10/kg). This would show a substantial financial return on the use of the high-protein diet. The gains in growth rate and FCR resulted in a AUD44/m^2 slab tank area/y increase in sales revenue for hybrid abalone produced using the high-protein diet, which was achieved with an extra AUD2 of feed input costs/m^2 tank area/y. In addition, as abalone fed with the high-protein diet grow significantly faster, the overall duration of a typical 3-y production cycle may be shortened by up to 3.5 mo. This would result in reduction in risk of losing stock to summer mortality and also lead to a significant saving in both fixed and variable costs.

ACKNOWLEDGMENTS

This research was supported by the Fisheries Research and Development Corporation, Australian Seafood Cooperative Research Centre (AS-CRC, 2010/736) and the Australian Abalone Growers' Association. We would also like to acknowledge the support of The South Australian Research and Development Institute and Marine Innovation Southern Australia for their financial support of Assoc. Prof. David Stone. Dr. Matthew Bansemer held an AS-CRC PhD scholarship (2011/251). We would also like to thank Anton Krsinich and Adam Clark of Great Southern Waters for their input into the experimental design and provision of commercial facilities and technical support to run the trial and Dr. Rhys Hauler, Dr. Matthew Bransden, and Dr. Nicole Ruff of Skretting Australia (Cambridge, Tasmania, Australia) for their input into the experimental design and diet formulations and manufacture. Nick Savva (Australian Abalone Growers' Association), Dr. Tom Coote, and Kym Heidenreich of Eyre Peninsula Aquafeeds (Lonsdale, South Australia, Australia) and Joel Scanlon of Aquafeeds Australia (Formerly Adam and Amos Abalone feeds, Mt Barker, South Australia, Australia) are also acknowledged for their input into the experimental design.

LITERATURE CITED

Bansemer, M. S., J. O. Harris, J. G. Qin, D. N. Duong & D. A. J. Stone. 2015. Growth and feed utilisation of juvenile greenlip abalone (*Haliotis laevigata*) in response to water temperatures and increasing dietary protein levels. *Aquaculture* 436:13–20.

Bansemer, M. S., J. G. Qin, J. O. Harris, G. S. Howarth & D. A. J. Stone. 2016. Nutritional requirements and use of macroalgae as ingredients in abalone feed. *Rev. Aquacult*. 8:121–135.

Bautista-Teruel, M. & O. Millamena. 1999. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: protein and energy levels. *Aquaculture* 178:117–126.

Britz, P. J. 1996. Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. *Aquaculture* 140:55–61.

Britz, P. J. & T. Hecht. 1997. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. *Aquaculture* 156:195–210.

Britz, P. J., T. Hecht & S. Mangold. 1997. Effect of temperature on growth, feed consumption and nutritional indices of *Haliotis midae* fed a formulated diet. *Aquaculture* 152:191–203.

Coote, T. A. 1998. Optimising the nutrient specifications of manufactured feeds for farmed juvenile greenlip abalone (*Haliotis laevigata* Donavan). PhD thesis, Department of Aquaculture, University of Tasmania, Australia. 143 pp.

Coote, T. A., P. W. Hone, R. J. Van Barneveld & G. B. Maguire. 2000. Optimal protein level in a semipurified diet for juvenile greenlip abalone *Haliotis laevigata*. *Aquacult. Nutr*. 6:213–220.

Festing, M. F. & D. G. Altman. 2002. Guidelines for the design and statistical analysis of experiments. *ILAR J*. 43:244–250.

Freeman, K. A. 2001. Aquaculture and related biological attributes of abalone species in Australia—a review. Fisheries Research Report No. 128. Perth, Western Australia: Fisheries Western Australia. 48 pp.

Guo, X. 2009. Use and exchange of genetic resources in molluscan aquaculture. *Rev. Aquacult*. 1:251–259.

Hardy, R. W. 2002. Rainbow trout, *Oncorhynchus mykiss*. In: Webster, C. D. & C. E. Lim, editors. Nutrient requirements and feeding of finfish for aquaculture. New York, NY: CABI Publishing. pp. 184–202.

Lafarga de la Cruz, F. & C. Gallardo-Escaìrate. 2011. Intraspecies and interspecies hybrids in *Haliotis*: natural and experimental evidence and its impact on abalone aquaculture. *Rev. Aquacult*. 3:74–99.

Lange, B., K.-L. Currie, G. S. Howarth & D. A. J. Stone. 2014. Dietary inclusion of grape seed extract and dried macroalgae improves the survival of greenlip abalone (*Haliotis laevigata*) at high water temperatures. *Aquaculture* 433:348–360.

Mai, K., J. P. Mercer & J. Donlon. 1994. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* lno II: amino acid composition of abalone and six species of macroalgae with an assessment of their nutritional value. *Aquaculture* 128:15–30.

Mai, K., J. P. Mercer & J. Donlon. 1995. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. IV. Optimum dietary protein level for growth. *Aquaculture* 136:165–180.

National Research Council. 1993. Nutrient requirements of fish. Washington, DC: National Academy Press. 114 pp.

Steinarsson, A. & A. K. Imsland. 2003. Size dependent variation in optimum growth temperature of red abalone (*Haliotis rufescens*). *Aquaculture* 22:353–362.

Stone, D. A. J., M. S. Bansemer & J. O. Harris. 2014a. Final report. Development of formulated diets for cultured abalone. CRC Project No. 2010/736 (ISBN: 978-1-921563-68-3). Adelaide, South Australia: South Australian Research and Development Institute (Aquatic Sciences). 131 pp.

Stone, D. A. J., M. S. Bansemer, B. Lange, E. N. Schaefer, G. S. Howarth & J. O. Harris. 2014b. Dietary intervention improves the survival of cultured greenlip abalone (*Haliotis laevigata* Donovan) at high water temperature. *Aquaculture* 430:230–240.

Stone, D. A. J., J. O. Harris, H. Wang, G. J. Mercer, E. N. Schaefer & M. S. Bansemer. 2013. Dietary protein level and water temperature interactions for greenlip abalone, *Haliotis laevigata*. *J. Shellfish Res*. 32:119–130.

Vandepeer, M. 2006. Abalone aquaculture subprogram: preventing summer mortality of abalone in aquaculture systems by understanding interactions between nutrition and water temperature. SARDI Publication Number RD02/0035-2. Adelaide, South Australia: South Australian Research and Development Institute (Aquatic Sciences). 85 pp.