

## Evaluation of the acceptability of diets formulated with hydrolyzed algae for juvenile abalone of *Haliotis fulgens*

### Evaluación de la aceptabilidad de dietas formuladas con algas hidrolizadas para juveniles de abulón *Haliotis fulgens*

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### ABSTRACT

**Background.** Abalones are aquaculture resources of great importance to the international food market. However, abalone cultivation faces a problem of food availability depending on the season, especially in Mexico. **Goals.** This study aimed to formulate diets for juvenile *Haliotis fulgens* from hydrolyzed algae. **Methods.** We evaluated the physicochemical parameters of the diets and their acceptability to juveniles *H. fulgens*. Three diets were formulated from different algae species: 1) *Macrocystis pyrifera* (MP), 2) *Eisenia arborea* (EA) and 3) mixture of both algae (MPEA). The total amount of hydrolyzed alga in each diet was 26%. Fresh *E. arborea* blades and the ABKELP® commercial diet were used as negative and positive control, respectively. Finally, the toughness, stability and acceptability of each diet were determined at 3, 6, 9, 12 and 24 h. **Results.** The toughness and stability of all hydrolyzed algae diets were significantly different ( $p > 0.05$ ) compared to the those of positive and negative controls. The toughness of the MP, EA and MPEA diets ranged from 600-630 g cm<sup>-2</sup>, whereas the ABKELP diet was tougher (700 g cm<sup>-2</sup>) after 24 h. The stability of the formulated diets was > 80%, while the ABKELP control reached 95% at 24 h. Overall ABKELP stability was significantly higher than those of the other diets ( $p < 0.01$ ). The EA, MPEA, and ABKELP diets were more attractive to organisms than fresh *E. arborea*, although no significant differences in food intake were present ( $p < 0.05$ ). **Conclusions.** All formulated diets exhibited physicochemical and acceptability characteristics that are suitable for *H. fulgens*.

**Key words:** attractability, *Eisenia arborea*, food intake, green abalone, *Macrocystis pyrifera*.

### RESUMEN

**Antecedentes.** Los abulones son un recurso acuícola de gran importancia en el mercado alimentario internacional. Sin embargo, el cultivo de abulón enfrenta un problema de disponibilidad de alimento según la temporada, especialmente en México. **Objetivo.** El presente estudio tuvo como objetivo formular dietas para juveniles de *Haliotis fulgens* a partir de algas hidrolizadas. **Métodos.** Se evaluaron los parámetros fisicoquímicos de las dietas y su aceptabilidad para juveniles de *H. fulgens*. Se formularon tres dietas a partir de diferentes especies de algas: 1) *Macrocystis pyrifera* (MP), 2) *Eisenia arborea* (EA) y 3) mezcla de ambas algas (MPEA). La cantidad total de algas hidrolizadas en cada dieta fue del 26%. Se utilizaron laminas frescas de *E. arborea* y la dieta comercial ABKELP® como control negativo y positivo, respectivamente. Finalmente se determinó la dureza, estabilidad y aceptabilidad de cada dieta a las 3, 6, 9, 12 y 24 h. **Resultados.** La dureza y estabilidad de todas las dietas de algas hidrolizadas fueron significativamente diferentes ( $p > 0.05$ ) en comparación con el control positivo y negativo. La dureza de las dietas MP, EA y MPEA varió de 600 a 630 g cm<sup>-2</sup>, mientras que la dieta ABKELP fue más dura (700 g cm<sup>-2</sup>) después de 24 h. La estabilidad de las dietas formuladas fue >80%, mientras que la del control ABKELP alcanzó el 95% a las 24 h. La estabilidad de ABKELP fue significativamente mayor que la de las otras dietas ( $p < 0.01$ ). Las dietas EA, MPEA y AKELP fueron las de mayor atracción para los organismos que *E. arborea* fresca, aunque no hubo diferencias significativas en el consumo de alimentos ( $p < 0.05$ ). **Conclusiones.** Todas las dietas formuladas presentaron características fisicoquímicas y de aceptabilidad adecuadas para *H. fulgens*.

**Palabras claves:** abulón azul, atractabilidad, consumo, *Eisenia arborea*, *Macrocystis pyrifera*.

## INTRODUCTION

Abalones are economically important aquaculture resources in the international market and are highly valued in Asian countries where the demand for these products is increasing (Hartmann *et al.*, 2015). Abalone meat is sold either whole or in pieces and may be canned, fresh, dehydrated or frozen. In addition to the meat, the shell, mantle, viscera and gonad are also commercialized (Cook, 2019).

Since the 1970s, abalone production worldwide has decreased considerably from 20,000 t in 1970 to 6,500 t in 2017 (Vega-García *et al.*, 2015; Cook, 2019). This drop in production has been caused by overfishing, illegal fishing, climatic variations and diseases caused by various pathogens such as *Perkinsus olseni* Lester & Davis, 1981, *Xenohalotis californiensis* Friedman *et al.* 2000 and *Vibrio* spp. (Guzmán del Proó *et al.*, 2013; Dang & Miller, 2016). As an alternative to fishing, abalone crops have been developed in countries such as China, Korea, Australia and Mexico (Cook, 2019). In addition to focusing on abalone growth and crop sales, these operations have directed their efforts towards applying technological advances to improve production. Examples of these operations include those in China with *Haliotis discus* Reeve, 1846, and *Haliotis diversicolor* Reeve, 1846, *Haliotis laevigata* Donovan, 1808 (Zhang *et al.*, 2004) as well as those in Korea with *H. discus* Ino, 1953 (Park & Kim, 2013), Australia with *H. asinina* Linnaeus, 1758 (Amin *et al.*, 2020), and the Philippines with *H. asinina* (Santizo-Taan, 2020).

The main problem facing abalone cultivation is food availability (e.g., fresh *Eisenia arborea* Areschoug, 1876 and *Macrocystis pyrifera* (Linnaeus) C. Agardh, 1820 along the Baja California peninsula) and a reduction in biomass due to seasonal meteorological phenomena (Edwards & Hernández-Carmona, 2005; SAGARPA, 2017). In recent years, formulated foods have been developed and studied to ensure that the necessary nutrients are provided to farmed abalone to optimize growth (Fitzgerald, 2008; Meng *et al.*, 2019). Of note, hydrolyzed ingredients have been found to promote efficient nutrient (peptides, amino acids, monosaccharides, and oligosaccharides) absorption by the digestive system, which favors faster growth (i.e., weight and size) (Benitez *et al.*, 2008; Meng *et al.*, 2019). In addition, hydrolyzed ingredients favor immunostimulant activity and function as attractants that improve food intake (Hou *et al.*, 2017).

A wide variety of attractants that improve food intake and favor development exist for abalone diets and include amino acids, biogenic amines, nucleotides, algae extracts, and carbohydrates (Britz *et al.*, 1994; Dunstan *et al.*, 2002). Currently, the aim of abalone cultivation is to improve quality by providing foods with novel ingredients that benefit development (Lee *et al.*, 2016; Meng *et al.*, 2019; Tan *et al.*, 2020). Among these food items are brown algae, which contain polysaccharides (e.g., alginate, fucoidan, and laminaran) and amino acids that directly benefit the development of farmed organisms, as has been shown in fish and shrimp species (Peso-Echarri *et al.*, 2012; Ringø *et al.*, 2012). Therefore, it is important to evaluate the effects of algae hydrolysates on the feeding parameters of cultivated abalone.

In Mexico, the green abalone *Haliotis fulgens* Philippi, 1845 is an aquaculture product of great economic importance because a substantial portion of the total production is exported to Asian countries (FEDECOOP, 2022; SAGARPA, 2017). In Baja California Sur, green abalone has been successfully farmed in the Laboratory of Abalone Production

of the fishing cooperative Sociedad Cooperativa Progreso de Producción Pesquera S.C. de R.L., which continues to lead the world in *H. fulgens* commercialization (FEDECOOP, 2022). Despite advances that have improved the formulations of abalone diets and the cultivation of *H. fulgens*, thus far it has not been possible to formulate a diet that optimizes *H. fulgens* development in Mexico, where they are currently fed fresh algae. In addition, no information is available (globally) regarding the manner in which brown algae hydrolysates are incorporated either individually or when combined in the feed of farmed abalone. The present study aimed to formulate diets with hydrolyzed algae as the main ingredient and to evaluate the acceptability (i.e., attractability and food intake) of these diets in juvenile *H. fulgens*. Our results can be used to improve culture conditions to ensure the continuous supply of juvenile green abalone from Mexico.

## MATERIALS AND METHODS

### Production of hydrolyzed algae paste

Samples of two species of brown macroalgae (*Macrocystis pyrifera* and *Eisenia arborea*) were harvested in May 2021 from La Bocana in Baja California Sur and subsequently dried. The dried samples were ground, sieved (400 µm), and placed in a water bath in a ratio of 1:2 (macroalgae: water). The algae were hydrolyzed in the water bath (90 °C) by means of an alkaline process for 2 h at pH 10, which was adjusted with 10% sodium carbonate (Hernández-Carmona *et al.*, 2012). The resulting hydrolyzed algae paste was refrigerated until use. The proportion of hydrolyzed algae used for formulating the abalone feed was 26% (260 g Kg<sup>-1</sup>; Table 1).

### Diet formulation

Three diets were formulated from two species of brown algae: 1) *M. pyrifera* (MP), 2) *E. arborea* (EA), and 3) *M. pyrifera* and *E. arborea* (MPEA). The diet were formulated as suggested by Viana (2002), Viana *et al.* (2007), and Durazo-Beltrán *et al.* (2014) using NUTRION v. 10.55 (Table 1). The formulation of the MP, EA, and MPEA diets was adjusted to ensure that all had similar nutritional content. During the experiment, the commercial brand ABKELP (Algas Marinas, S.A., Ensenada, Mexico) was used as a positive control, and fresh *E. arborea* (harvested in May 2021 from La Bocana in Baja California Sur) was used as a negative control.

### Diet preparation

The resulting hydrolyzed algae were mixed with dry ingredients (spirulina, fish meal, polymethyl carbamide, and dried *M. pyrifera* and *E. arborea*, proportion used Table 1) in an MX1200 stand mixer (BLACK & DECKER, New Britain, USA) until the mixture was homogenized. The food mixture was prepared using a manual pasta extruder (Markwind, Walnut, USA). The resulting paste was formed into 5 g square bars (2.5 x 2.5 x 0.5 cm). The bars were dried in a DX312 convection oven (Yamato, Chuo City, Japan) at 55 °C for 48 h and subsequently placed in a desiccator for 24 h. The control diets were not processed in any way.

### Proximal chemical analysis

A proximal chemical analysis was performed in triplicate for the formulated diets (MP, EA, and MPEA), the ABKELP® control, and the negative *E. arborea* control (Table 1) following the guidelines established by the Association of Official Analytical Chemists (AOAC, 2019). The analyses were conducted at the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA, Mexico).

Table 1. Formulations and proximal chemical compositions of the diets evaluated in this study.

	Dietary treatment				
	MP	EA	MPEA	ABKELP (control)	Fresh <i>E. arborea</i> (negative control)
Ingredients (g Kg <sup>-1</sup> )					
<i>M. pyrifera</i> (s) <sup>a</sup>	270.0	---	135	---	---
<i>E. arborea</i> (s) <sup>a</sup>	---	270.0	135	---	---
<i>M. pyrifera</i> (hi) <sup>b</sup>	260.5	---	---	---	---
<i>E. arborea</i> (hi) <sup>b</sup>	---	260.7	---	---	---
Mp-Ea (hi) <sup>b</sup>	---	---	260.7	---	---
Spirulina <sup>c</sup>	100.0	100.0	100.0	---	---
Fish flour <sup>d</sup>	360.4	360.1	360.1	---	---
PMC <sup>e</sup>	9.1	9.1	9.1	---	---
Chemical composition (%)					
Protein	30.0 ± 0.24	30.0 ± 0.13	30.0 ± 0.10	25.0 ± 0.01	11.1 ± 0.12
Ether extract	2.8 ± 0.14	2.7 ± 0.05	2.6 ± 0.18	1.5 ± 0.30	0.2 ± 0.43
Carbohydrates	30.9 ± 0.06	30.1 ± 0.70	30.5 ± 0.45	32.0 ± 0.21	41.4 ± 0.01
Fibre	5.0 ± 0.06	4.1 ± 0.09	4.9 ± 0.32	4.5 ± 0.14	11.9 ± 0.2
Ash	31.3 ± 0.11	33.1 ± 0.71	31.9 ± 0.31	19.2 ± 0.12	22.5 ± 0.67
Moisture	4.99 ± 0.01	5.93 ± 0.01	5.01 ± 0.52	4.89 ± 0.23	4.69 ± 0.38

Abbreviations: Mp (*Macrosystis pyrifera*), Ea (*Eisenia arborea*), hi (hydrolyzed)

--- ingredient not included; ± standard deviation. Seaweeds were dried. <sup>a</sup>*E. arborea* and *M. pyrifera* by Algas Pacific®. <sup>b</sup>Hydrolyzed *E. arborea*, *M. Pyrifera*, and mixture prepared in the Laboratorio de Algas Marinas of CICIMAR-IPN. <sup>c</sup>Spirulina produced by Estrella del Sur. <sup>d</sup>Fish meal produced by PEGUSA, Mexico. <sup>e</sup>Polymethyl carbamide (PMC) product Acuabond® produced by Grupo Norel, Mexico. Amino acids provided by the algae include tryptophan, valine, alanine, leucine, methionine, cysteine, glycine, and threonine. Composition of ABKELP determined by Algas Marinas, S.A. (Ensenada, Baja California, Mexico) and includes wheat flour, seaweed flour, green algae flour, fish meal, soybeans, cereals and by-products, natural algae compactors (NKB), fish oil, oil vegetable, amino acids, minerals and vitamins from seaweed, vitamin E and C, and yeast and fungal inhibitors.

### Toughness and stability

The toughness and stability tests were performed in triplicate using rectangular 10,800 L tanks (6 x 2 x 0.9 m) with aeration and continuous seawater flow (15 water changes per hour). The diet distribution among trials was random.

The toughness (i.e., breaking point) of the food bars (g cm<sup>-2</sup>) was determined using a Nikan-Sui texture analyzer. Three food bars (15 g) of each diet (MP, EA, MPEA, and ABKELP<sup>®</sup>) were placed individually in the center of the plate of the texture analyzer and weight was added until a break was generated in a bar. Measurements were taken every hour over 6 h, and then again at 9, 12 and 24 h.

The stability of the three food bars (three per diet) was individually determined by immersing them in water and subsequently drying them in the DX312 convection oven (Yamato) at 55 °C for 24 h. After which, the bars were weighed. The measurement time was the same as that of the toughness analysis. Stability was determined with the following formula (Pérez-Estrada, 2006):

$$\text{Stability (\%)} = \frac{Fr}{Fp} * 100$$

where, Fr is the food recovered and Fp is the food provided.

The stability of fresh algae was calculated at 24 h to determine food intake.

### Acceptability (attractability and food intake)

The acceptability of the diets was evaluated in 900 *H. fulgens* juveniles (32.8 ± 0.3 mm, 4.67 ± 0.8 g) under commercial culture conditions (temperature: 18.4 ± 0.7 °C, pH: 7.8 ± 0.4, dissolved oxygen: 7.5 ± 0.6 mg/mL, photoperiod: 24 h darkness) at the facilities of the Sociedad Cooperativa Progreso de Producción Pesquera S.C. de R.L. which are located in La Bocana, Baja California Sur (26° 46' N. 113° 42' W). Prior to the acceptability tests, the organisms were acclimated for 30 days under commercial culture conditions during which time they were fed a fresh seaweed mix. After which, the organisms were fasted for two days before initiating the acceptability tests.

The acceptability tests were performed in triplicate with a total of 300 organisms per tank (600 L) based on the methodology of Dunstan *et al.* (2002) with some modifications. The three formulated diets plus the positive and negative controls were placed inside each tank in the devices described in Figure 1a. The device consisted of a metal mesh base (10 x 10 cm) and a plastic cover (20 x 20 cm; Fig. 1a). The devices contained 15 g (three bars) of each diet under evaluation and were randomly yet equidistantly distributed in the tanks. The tests were conducted for 24 h in the dark.

The attractability of each diet was determined by visual and photographic observations based on the frequency of approach and the interactions between the organisms and food bars at 1, 3, 6, 9, 12, and 24 h (Fig. 1b). Only the organisms that were found on top the food were

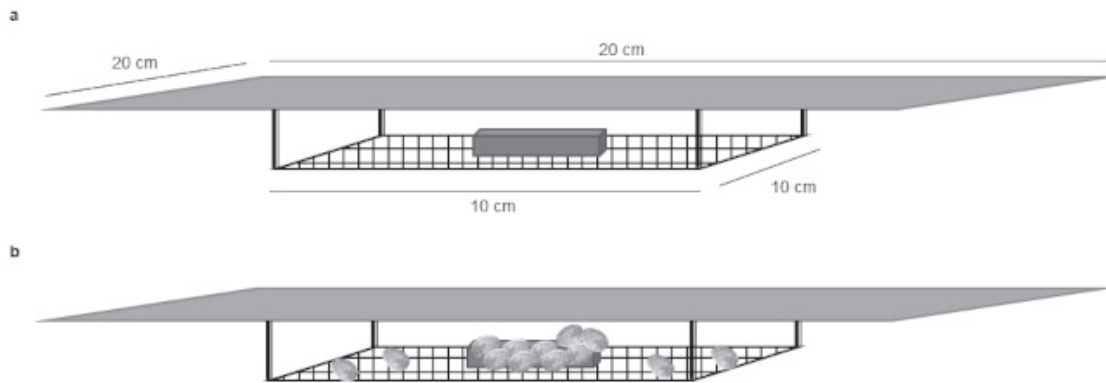


Figure 1. Device used to evaluate diet attractability (a). Device with interaction of abalone juveniles (b).

counted. A device without food was also included in the attractability tests to see if its presence influenced the attraction of the organisms (e.g., the device acting as a refuge).

Food intake was evaluated 24 h after providing the food bars. The excess food was recovered, dried in a convection oven at 55 °C for 48 h, and weighed. Food intake was determined with the following equation (Uki & Watanabe, 1992):

$$\text{Food Intake (g)} = \left( \frac{GS}{100} \right) - R$$

where, G is the weight of the food provided (g), S is stability, and R is the weight (g) of the recovered food.

### Statistical analysis

Comparisons were made between treatments in terms of physical parameters (toughness and stability) and acceptability (attractability) in R (R Core Team, 2021).

Shapiro-Wilk tests for normality and Fligner-Killeen tests for homoscedasticity were applied to the data sets (attractability, toughness, and stability) prior to subsequent analyses. Given the nature of the data, a Kruskal Wallis test was used to identify significant differences between diets. Wilcoxon *post-hoc* tests with Bonferroni-Holm adjustments were used to highlight the differences between diet groups. Comparisons of food intake among the formulated diets and controls were conducted with Student t-tests with a significance level of  $p < 0.05$ .

## RESULTS

The ABKELP control diet exhibited the greatest toughness. During the first four hours, ABKELP show a toughness of 2000 g cm<sup>-2</sup>, which decreased with immersion time (700 g cm<sup>-2</sup> at 24 h). The *post-hoc* test indicated that there were significant differences in toughness ( $p < 0.01$ ) between the ABKELP® control diet and the formulated diets. At 24 h, the toughness of all formulated diet was similar ( $p > 0.01$ ). The MP, EA, and MPEA diet exhibited toughness values of 615.2 g cm<sup>-2</sup>, 620.1 g cm<sup>-2</sup> and 622 g cm<sup>-2</sup>, respectively (Fig. 2).

The stability of the formulated diets (MP, EA, and MPEA) showed similar trends with the values ranging from 94–81% (Fig. 3), although ABKELP® exhibited the greatest stability (99–95%; Fig. 3). After 24 h, the stability values of the MP, EA, MPEA, and ABKELP control diets were  $81.7 \pm 1.3\%$ ,  $83.2 \pm 0.7\%$ ,  $81.8 \pm 0.8\%$ , and  $95.9 \pm 0.6\%$ , respectively. Furthermore, the stability of the ABKELP control diet was significantly higher (i.e., 13.7% more stable,  $p < 0.01$ ) than those of the formulated diets (Fig. 3).

The attractability of fresh *E. arborea* was significantly lower compared to that of the EA ( $p < 0.01$ ), MPEA ( $p < 0.05$ ), and ABKELP ( $p < 0.01$ ) diets (Fig. 4). With the exception of fresh *E. arborea*, the control treatment (i.e., structure without food) was significantly different from those of the other diet treatments ( $p < 0.05$ ). The attractability of the MP diet was also significantly lower when compared to those of the EA and MPEA diets ( $p < 0.05$ ).

The most consumed diets (> 50%) were the ABKELP and EA diets. The abalone consumed more than 7.5 g of these diets. The control treatment of fresh *E. arborea* showed an intermediate food intake value of 45%, which was equivalent to 6.8 g of food consumed. The lowest intake value was recorded for the MP and MPEA (< 40%) diets, with both diets showing consumption values lower than 6 g (Fig. 5). However, the median difference test indicated that no significant differences were present among treatments ( $p < 0.05$ ).

## DISCUSSION

Toughness is critical to elaborating suitable food for abalone. These organisms feed by browsing, and their ability to ingest foods depends on their radula type (i.e., rhipidoglossan in *H. fulgens*) (Fitzgerald, 2008). In their natural environments, abalone feed on algae stipes and thus tend to consume foods that are not overly rigid (Fitzgerald, 2008; Currie *et al.*, 2016). In this study, the ABKELP control diet exhibited the highest toughness (> 700 g cm<sup>-2</sup>) when compared to those of the formulated diets (MP, EA, and MPEA). This toughness value may be considered appropriate for juvenile abalone based on the results reported by McShane *et al.* (1994) with *H. rubra*. Moreover, an appropriate interval of toughness for the juvenile stage of *H. fulgens* has been found to be

between 500–600 g cm<sup>-2</sup> (Rivero & Viana, 1996), which seems to favor attraction and consumption. This could explain why the attractability in this study was highest after 24 h when both the formulated and the control diets were softer in texture.

Stability is a physical property that must be considered when formulating abalone feed. This is because abalones feed slowly, and thus food must remain as stable as possible after being immersed in water to avoid losing its nutritional properties and negatively affecting water quality (Britz *et al.*, 1994; Kinkerdale *et al.*, 2010; Ruff *et al.*, 2014; Ansary *et al.*, 2018). Based on the suggestions by Fitzgerald *et al.* (2008) and Ruff *et al.* (2014), stability results higher than 80% are acceptable. In this study, the stability values of the formulated diets (MP, EA, and MPEA) were above 81%, although lower than that of ABKELP (> 95%). It is important to highlight that the formulated diets (MP, EA, and MPEA) were manually prepared in the laboratory in contrast to the ABKELP® control feed. Additional factors, such as density, binder type, particle size, and the production process, directly influence stability as has been described by Fleming *et al.* (1996), Sales & Jensens (2004), and Ruff *et al.* (2014). In this study, the stability values of the formulated diets were acceptable for *H. fulgens*. If these diets were to be industrially produced, their stability would likely increase (Ruff *et al.*, 2014).

Attractability is the attraction that food generates toward an organism through the release of chemical compounds that are detected by sense organs (Britz *et al.*, 1994). When optimizing abalone cultivation, it is important to develop diets that contain attractive ingredients that stimulate food intake, which improves weight gain, and that favor abalone quality (especially of the foot) (Bansemer *et al.*, 2016; Denti *et al.*, 2016). When compared with the attraction to fresh *E. arborea* (the algae used by cooperatives in northern Baja California Sur), the juvenile *H. fulgens* in this study showed higher attraction at 24 h to the control food ABKELP, followed by their attraction to the EA and MPEA diets. The lower attractability recorded for fresh *E. arborea* may be due to the formulated diets (MP, EA, MPEA, and ABKELP) containing fish and soybean meal and other ingredients (e.g., oils, amino acids, and by-products) that are known to stimulate attraction (O'Mahoney *et al.*, 2014; Bansemer *et al.*, 2016). Our formulated diets contained hydrolyzed algae. Hydrolyzation degrades proteins (amino acids) and carbohydrates (monosaccharides, disaccharides, and oligosaccharides), which makes assimilation more efficient (Cruz-Suárez *et al.*, 2000). The hydrolyzed algae functioned as an attractant and were easily detected by the abalone due to the low molecular weights of the compounds that were released into the water column (Cruz-Suárez *et al.*, 2000; Viana, 2002; Hou *et al.*, 2017).

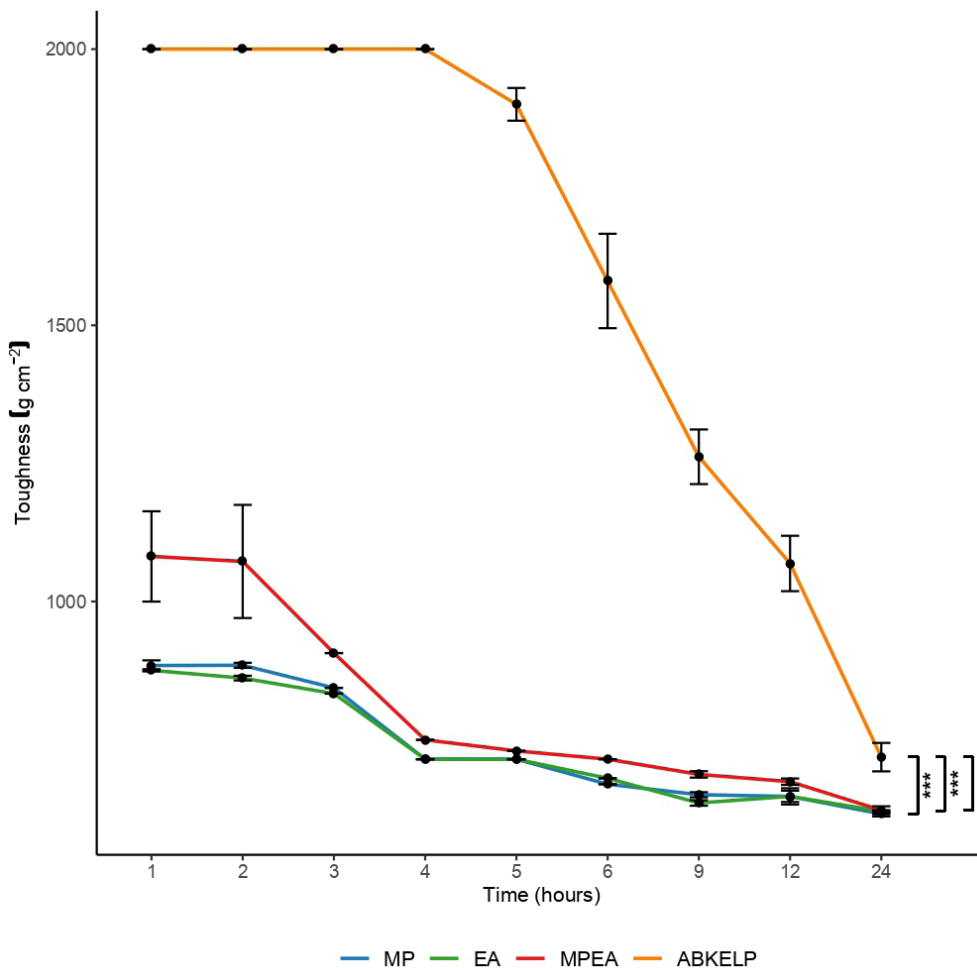


Figure 2. Diet toughness over time. \*\*\*Significant at  $\alpha = 0.01$

Despite the presence of hydrolysates in the formulated diets, attractability was higher with the ABKELP diet, which may be due to the use of compounds (ingredients not reported by the manufacturer) that favor attraction. Fleming *et al.* (1996) indicated that information related to diet formulations and the ingredients used to favor attraction can be extensive, although it may not be reported in some cases.

It is important to consider the food selectivity of abalone. For example, species, such as *H. discus*, *H. laevigata*, and *H. rufescens*, have shown specific attractions to diets composed of certain groups of macroalgae that depend on their geographical distributions (Conrwall *et al.*, 2009; Sakata, 2013). This affinity can influence the attraction of abalones to formulated diets (Sakata, 2013). This was observed in this study among the formulated diets given that the attractability of *H. fulgens* was higher for diets that contained *E. arborea* (EA and MPEA). On the other hand, Avilés & Sherperd (1996) indicated that *H. fulgens* can feed on *E. arborea* or *M. pyrifera* depending on the season and geographic distribution. However, given that *E. arborea* has a higher tolerance to unfavorable weather conditions than *M. pyrifera*, it is consumed more than *M. pyrifera* in some areas of the Baja California peninsula (Zertu-

che-González *et al.*, 2014). This could explain the low attractability that *H. fulgens* juveniles exhibited towards the diets containing *M. pyrifera* (MP) compared to the diet consisting entirely of *E. arborea* (EA). On the other hand, since the algal composition of ALBKELP is not specified, we are unable to explain why it exhibited higher attractability than our formulated diets.

The food intake values of the formulated diets compared to those of the commercial and negative controls were not significantly different. This may be because the MP, EA, and MPEA diets were composed of the algae that *H. fulgens* usually consumes (*E. arborea* grows in the same area in which the study was conducted). Although it is not usual for abalone to consume *M. pyrifera* in the area of La Bocana, it has been observed that consumption can occur due to massive upwellings from the northern coast of La Bocana (Mazariegos-Villarreal *et al.*, 2012; Vegas-García *et al.*, 2015). A similar situation could explain the food intake of the ABKELP control diet, as it is produced in Baja California where *M. pyrifera* and *E. arborea* are also present, and thus these species are likely important components of this product.

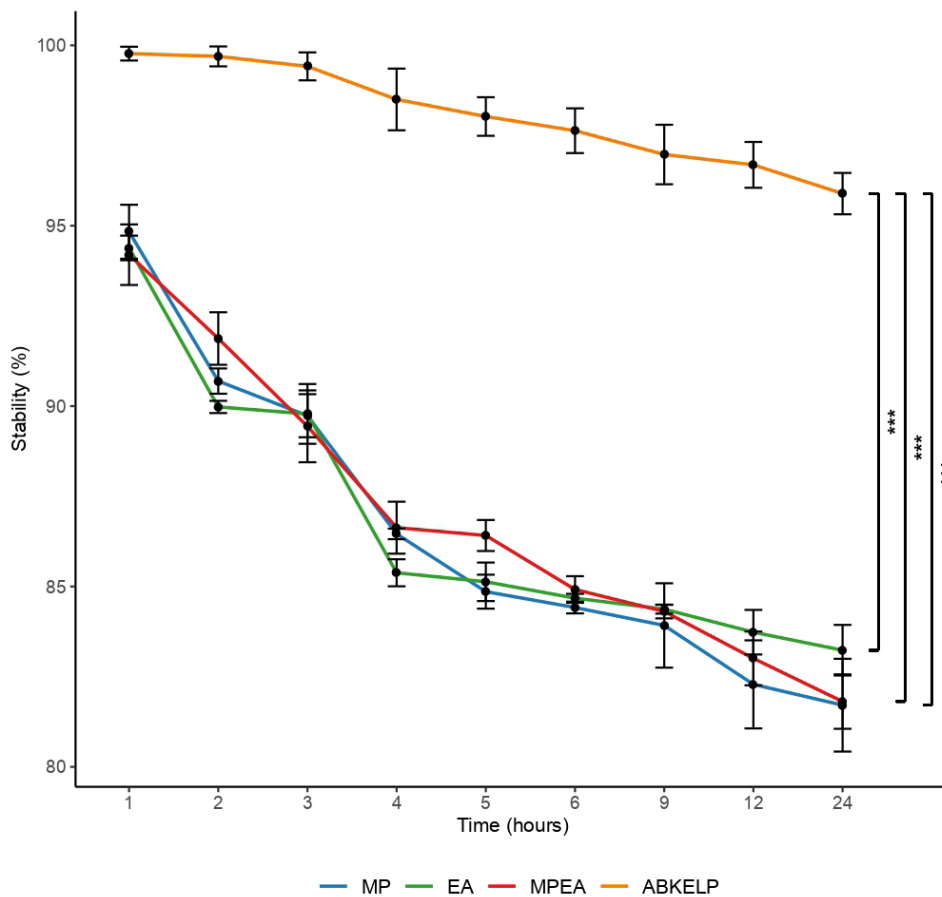


Figure 3. Diet stability over time. \*\*\*Significant at  $\alpha = 0.01$

The formulated MP, EA, and MPEA diets presented toughness values of 600–630 g cm<sup>-2</sup> and stability values > 80%. These values, coupled with the physicochemical characteristics, render the diets appropriate for abalone culture conditions. The EA, MPEA, and ABKELP control diets were more attractive to the abalone than fresh *E. aborea*. Despite this, the food intake values of the formulated diets and controls were not significantly different.

With the evaluation of the physicochemical and acceptability characteristics (attractability and food intake) of the formulated diets, we can conclude that the use of hydrolyzed algae in diets for *H. fulgens* is acceptable and may constitute a viable alternative for feeding under culture conditions. Even so, it is necessary to evaluate the effects of these compounds on the growth, digestive physiology, immunostimulant activity, nutrition, and overall health status of cultured abalo-

ne. This information will greatly contribute to improving the feeding of cultured abalone.

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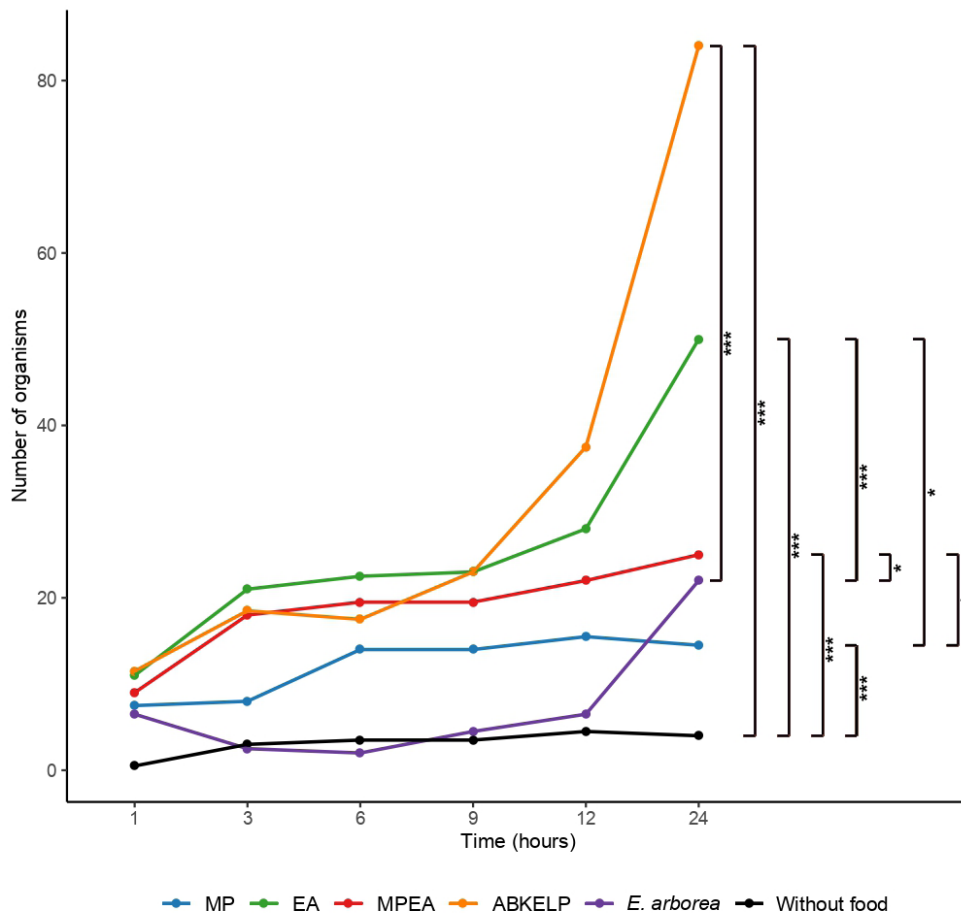


Figure 4. Diet attractability over time. \*Significant at  $\alpha = 0.05$ . \*\*\*Significant at  $\alpha = 0.01$

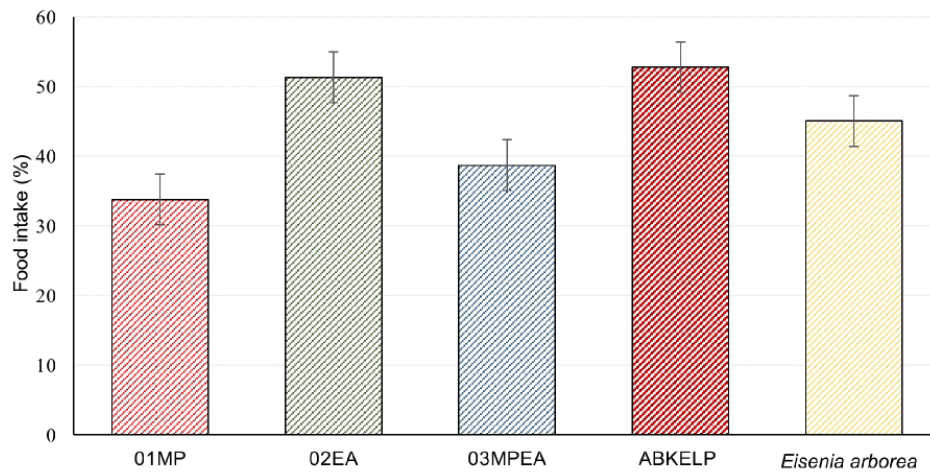


Figure 5. Food intake percentages of the formulated (MP, EA, MPEA) and control (ABKELP® and *Eisenia arborea*) diets. Bars indicate standard error.

## REFERENCES

- AMIN, M., C. J. S. BOLCH, M. B. ADAMS & C. M. BURKE. 2020. Growth enhancement of tropical abalone, *Haliotis asinina* L, through probiotic supplementation. *Aquaculture International* 28: 463-475. DOI: 10.1007/s10499-019-00473-4
- ANSARY, M. W. R., H. S. JEONG, K. W. LEE, H. S. KIM, J. KIM, A. YUN, S. H. CHO, P. Y. KIM & T. KIM. 2018. The effect of substituting *Undaria pinnatifida* in formulated feeds with *Sargassum horneri* on growth and body composition of juvenile abalone (*Haliotis discus*, Reeve 1846). *Journal of Applied Phycology* 31: 2125-2132. DOI: 10.1007/s10811-018-1672-2
- AOAC. 2019. *Official methods of analysis of AOAC international*. AOAC international. 500 pp.
- AVILES, J. G. G. & S. A. SHEPHERD. 1996. Growth and survival of the blue abalone *Haliotis fulgens* in barrels at Cedros Island, Baja California, with a review of abalone barrel culture. *Aquaculture* 140: 0-176. DOI: 10.1016/0044-8486(95)01199-4
- BANSEMER, M. S., J. G. QIN, J. O. HARRIS, G. S. HOWART & D. A. J. STONE. 2016. Nutritional requirements and use of macroalgae as ingredients in abalone feed. *Reviews in Aquaculture* 8: 121-135. DOI: 10.1111/raq.12085
- BENÍTEZ, R., A. IBARZ & J. PAGAN. 2008. Hidrolizados de proteína: procesos y aplicaciones. *Acta Bioquímica Clínica Latinoamericana* 42 (2): 227-236.
- BRITZ, P. J., T. HECHT, J. KNAUER & M. G. DIXON. 1994. The development of an artificial feed for abalone farming. *South African Journal of Science* 90 (1): 7-8.
- COOK, P. A. 2019. Worldwide abalone production statistics. *Journal of Shellfish Research* 38 (2): 401-404. DOI: 10.2983/035.038.0222
- CORNWALL, C. E., N. E. PHILLIPS & D. C. McNAUGHT. 2009. Feeding preferences of the abalone *Haliotis iris* in relation to macroalgal species, attachment, accessibility and water movement. *Journal of Shellfish Research* 28 (3): 589-597. DOI: 10.2983/035.028.0323
- CRUZ-SUÁREZ, L. E., D. RICQUE-MARIE, M. TAPIA-SALAZAR & C. GUAJARDO-BARBOSA. 2000. Uso de la harina de kelp (*Macrocystis pyrifera*) en alimentos para camarón. In: Cruz-Suárez L. E., D. Ricque-Marie, M. Tapia-Salazar, M. A. Olvera-Novoa & R. Civera-Cerecedo (eds.). *Avance en nutrición acuícola V*. Memorias del V Simposium Internacional de Nutrición Acuícola. 227-265 p.
- CURRIE, K.-L., H. DAVIDSON, M. S. BANSEMER, J.O. HARRIS & D. A. J. STONE. 2016. Ventral videographic assessment of the feeding behavior of juvenile greenlip [*Haliotis laevigata* (Donovan, 1808)] and hybrid (*H. laevigata* x *Haliotis rubra*) Abalone in response to dietary and temperature manipulation. *Journal of Shellfish Research* 35(3): 641-651. DOI:10.2983/035.035.0310
- DENTI, G. 2016. Inclusion of seaweed meals in abalone ("*Haliotis tuberculata coccinea*" Reeve) diets: effects on growth performance, body composition and colour. Tesis de Maestría en Cultivos Marinos. Facultad de Ciencias del Mar, Universidad de Las Palmas de Gran Canaria, España. 221 p.
- DUNSTAN, G. A., M. R. BROWN, J. K. VOLKMAN & G. B. MAGUIRE. 2002. Formulated feeds for newly settled juvenile abalone based on natural feeds (diatoms and crustose coralline algae). CSIRO, Hobart, Tasmania, Australia. Available online at: <https://www.frdc.com.au/Archived-Reports/FRDC%20Projects/1996-386-DLD.pdf> (downloaded February 16, 2022)
- DURAZO-BELTRÁN, E. 2014. Los lípidos en la nutrición del abulón azul, molusco con potencial de cultivo. *Entreciencias* 2 (4): 125-135.



- EDWARDS, M. S. & G. HERNÁNDEZ-CARMONA. 2005. Delayed recovery of giant kelp near its southern range limit in the North Pacific following El Niño. *Marine Biology* 147 (1):273-279. DOI: 10.1007/s00227-004-1548-7
- FEDECOOP. 2022. Federación regional de sociedades cooperativas de la industria pesquera de Baja California. Available online at: <https://www.fedecoop.com.mx/> (downloaded September 14, 2022).
- FITZGERALD, A. 2008. *Abalone feed requirements*. South West Abalone Growers Association, UK, 35 p.
- FLEMING, A. E., R. J. VAN BARNEVELD & P. W. HONE. 1996. The development of artificial diets for abalone: A review and future directions. *Aquaculture* 140(1-2): 5-53. DOI: 10.1016/0044-8486(95)01184-6
- FRIEDMAN C.S., K.B. ANDREE, K.A. BEAUCHAMP, J.D. MOORE, T.T. ROBBINS, J.D. SHIELDS & R.P. HEDRICK. 2000. "Candidatus *Xenohaliotis californiensis*" a newly described pathogen of abalone, *Haliotis spp.*, along the west coast of North America. *Int. J. Syst. Evol. Microbiol.* 50: 847-855.
- GUZMÁN DEL PRÓO, S. A., P. DEL MONTE-LUNA & L. J. CARRILLO-LAGUNA. 2013. Reclutamiento del abulón (*Haliotis fulgens*) en Baja California, México, y su relación con la temperatura del mar. *Interciencia* 38 (8): 609-614.
- HARTMANN, K., E. HOSHIMO, C. GARDNER, S. JENNINGS, S. PATTERSON, L. RIPPIN, J. MORISON & D. WORTHINGTON. 2015. Economic management guidance for Australian abalone fisheries. The Seafood CRC Company Ltd. the Fisheries Research and Development Corporation and University of Tasmania. Available online at: <https://www.frdc.com.au/sites/default/files/products/2009-714.30-DLD.pdf> (downloaded 20 November 2021).
- HERNÁNDEZ-CARMONA, G. Y., E. RODRÍGUEZ-MONTESINOS, D. L. ARVIZU-HIGUERA, R. REYES-TISNADO, J. L. MURILLO-ÁLVAREZ & M. MUÑOZ-OCHOA. 2012. Avances tecnológicos en la producción de alginatos en México. *Ingeniería Investigación y Tecnología* 13 (2): 155-168.
- HOLDT S. L. & S. KRAAN. 2011. Bioactive compound in seaweed: functional food applications and legislation. *Journal of Applied Phycology* 23: 543-597. DOI: 10.1007/s10811-010-9632-5
- HOU, Y., Z. WU, Z. DAI, G. WANG & G. WU. 2017. Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. *Journal of Animal Science and Biotechnology* 8: 1-13. DOI: 10.1186/s40104-017-0153-9
- KINKERDALE, L., D. V. ROBERTSON-ANDERSSON & P. C. WINBERG. 2010. *Review on the use and production of algae and manufactured diets as feed for sea-based abalone aquaculture in Victoria*. Report by the University of Wollongong, shoalhaven marine and freshwater center, Nowra, for the department of primary industries, fisheries Victoria. Available online at: <https://ro.uow.edu.au/cgi/viewcontent.cgi?referer=&httpsredir=1&article=1006&context=smfc> (Downloaded 30 November 2020)
- LEE, K. W., H. S. KIM, A. YUN, D. G. CHOI, B. I. JANG, H. J. KIM, S. H. CHO, Y. JOO, B. KIM & B. MIN. 2016. Effect of the formulated diets on performance and resistance of juvenile abalone *Haliotis discus* (Reeve, 1846) subjected to various stress conditions. *Journal of Shellfish Research* 35 (2): 481-491. DOI: 10.2983/035.035.0221
- MAZARIEGOS-VILLARREAL, A., M. CASAS-VALDEZ, D. A. SIQUEIROS-BELTRONES, A. PÍNON-GIMATE & E. SERVIERE-ZARAGOZA. 2012. Changes in the natural diet of green abalone *Haliotis fulgens* during the 1997 to 1998 El Niño event in Baja California Sur, Mexico. *Journal of Shellfish Research* 31 (3): 795-800. DOI: 10.2983/035.031.0325
- MCSHANE, P. E., H. K. GORLINE & L. A. KNUCKEY. 1994. Factors influencing food selection in the abalone *Haliotis rubra* (Mollusca: Gastropoda). *Journal of Experimental Marine Biology and Ecology* 176: 27-37.
- MENG, X., X. YANG, G. LIN, Y. FANG, Z. RUAN, M. LIU & D. YANG. 2019. Mannan oligosaccharide increases the growth performance, immunity and resistance capability against *Vibrio parahaemolyticus* in juvenile abalone *Haliotis discus hannai* Ino. *Fish Shellfish Immunol* 94: 654-660. DOI: 10.1016/j.fsi.2019.09.058
- O'MAHONEY, M., O. RICE, G. MOUZAKITIS & G. BURNELL. 2014. Towards sustainable feed for abalone culture: Evaluating the use of mixed species seaweed meal in formulated feed for the japonese abalone, *Haliotis discus hannai*. *Aquaculture* 430: 9-16. DOI: 10.1016/j.aquaculture.2014.02.036
- PARK, C. J. & S. Y. KIM. 2013. Abalone aquaculture in Korea. *Journal of Shellfish Research* 32 (1): 17-20. DOI: 10.2983/035.032.0104
- PÉREZ-ESTRADA C. J. 2006. Efecto de diferentes dietas naturales en el crecimiento y composición química de juveniles de abulón azul *Haliotis fulgens* Philippi. Tesis de Maestría en Ciencias en Acuicultura. Universidad Autónoma de Baja California Sur, México. 84 p.
- PESO-ECHARRI, P., C. FRONTELA-SASETA, C. A. GONZÁLEZ-BERMÚDEZ, G. F. ROS-BARRUEZO & C. MARTÍNEZ-GRACIÁ. 2012. Polisacáridos de algas como ingredientes funcionales en acuicultura marina: alginato, carragenano y ulvanos. *Revista de Biología Marina y Oceanografía* 47 (3): 373-381.
- RINGØ, E., R. E. OLSEN, J. L. GONZALEZ, S. WADSWORTH & S. K. SONG. 2012. Use of immunostimulants and nucleotides in aquaculture: a review. *Journal of Marine Science: Research & Development* 2 (1): 1-22. DOI: 10.4172/2155-9910.1000104
- RIVERO, L. E. & M. T. VIANA. 1996. Effect of artificial pH, water stability and toughness of diets on the palatability for juvenile abalone *Haliotis fulgens*. *Aquaculture* 144 (4): 353-362. DOI: 10.1016/0044-8486(96)01309-9
- RUFF, N., M. BRANSDEN, B. WYBOURNE & R. HAULER. 2014. Optimized performance and efficiency in abalone feed. World Aquaculture - Adelaide 2014. Available online at: <https://www.was.org/MeetingAbstracts/Index> (Accessed 07 November 2021).
- SAGARPA. 2017. Carta Nacional Pesquera. Diario Oficial de la Federación. México. Available online at: [http://dof.gob.mx/nota\\_detalle.php?codigo=5525712&fecha=11/06/2018](http://dof.gob.mx/nota_detalle.php?codigo=5525712&fecha=11/06/2018) (downloaded 26 November 2021).
- SAKATA, K. 2013. Feeding attractants and stimulants for marine gastropods. In: Scheuer P. J. (ed.). *Bioorganic marine chemistry* 3. Springer, pp 115-127.
- SALES J. & G. P. J. JENSENS. 2004. Use of feed ingredients in artificial diets for abalone: a brief update. *Nutrition Abstracts and Reviews Series* 74: 13-21.

- SANTIZO-TAAN, R., M. BAUTISTA-TERUEL & J. R. H. MAQUIRANG. 2020. Enriched *Ulva pertusa* as partial replacement of the combined fish and soybean meals in juvenile abalone *Haliotis asinina* (Linnaeus) diet. *Journal of Applied Phycology* 32: 741-749. DOI: 10.1007/s10811-019-01977-5
- STONE, D. A. J., M. S. BANSEMER & J. O. HARRIS. 2014. *Development of formulated diets for cultured abalone*. South Australian Research and Development Institute. Available online at: [https://www.pir.sa.gov.au/\\_data/assets/pdf\\_file/0007/262987/CRC\\_Cultured\\_Abalone\\_Report\\_-\\_FINAL.pdf](https://www.pir.sa.gov.au/_data/assets/pdf_file/0007/262987/CRC_Cultured_Abalone_Report_-_FINAL.pdf) (downloaded February 3, 2022)
- TAN, K., H. ZHANG, L. S. LIM, H. MA, S. LI & H. ZHENG. 2020. Roles of carotenoids in invertebrate immunology. *Frontiers in Immunology* 10: 1-10. DOI: 10.3389/fimmu.2019.03041
- UKI, N. & T. WATANABE. 1992. Review of the nutritional requirements of abalone *Haliotis spp* and development of more efficient diets. In: Sheperd S. A., M. J. Tegner & S. A. Guzmán del Proó (eds.). *Abalone of the World: Biology, Fisheries and Culture*, pp. 504-517.
- VEGA-GARCÍA, P. D. 2016. Cambios históricos en las poblaciones de abulón azul y amarillo en la Península de Baja California. Tesis de Doctorado en Ciencias (Biología). Posgrado en Recursos Naturales, Centro de Investigaciones Biológicas del Noroeste, S. C., México. 57 p.
- VIANA M. T. 2002. Avances en la nutrición, fisiología digestiva y metabolismo del abulón. In: Cruz-Suárez L. E., D. Ricque-Marie, M. Tapia-Salazar, M. Gaxiola-Cortés & N. Simoes (eds.). *Avances en nutrición acuícola VI*. Memorias del VI Simposio Internacional de Nutrición Acuícola, pp. 294-308.
- VIANA, M.T., L. R. D'ABRAMO, M. A. GONZÁLEZ, J. V. GARCÍA-SUÁREZ, A. SHIMADA & C. VÁZQUEZ-PELÁEZ. 2007. Energy and nutrient utilization of juvenile green abalone (*Haliotis fulgens*) during starvation. *Aquaculture* 264: 323-329. DOI: 10.1016/j.aquaculture.2007.01.004
- ZERTUCHE-GONZÁLEZ, J. A., M. SÁNCHEZ-BARREDO & J. M. GUZMÁN-CALDERÓN. 2014. *Eisenia arborea* J.E. Areschoug as abalone diet on an IMTA farm in Baja California, México. *Journal of Applied Phycology* 26 (2): 957-960. DOI: 10.1007/s10811-013-0138-9
- ZHANG, G., H. QUE, X. LIU & H. XU. 2004. Abalone mariculture in China. *Journal of Shellfish Research* 23 (4): 947-950.