

Effects of six diets on the growth and survival rates of postlarvae of red abalone (*Haliotis rufescens*) and its hybrid (*H. rufescens* ♀ × *H. fulgens* ♂)

Francesco Cicala ¹, Miguel A. Tripp-Valdez ², Vincent Montes-Orozco ²,
Gesem S. Cervantes-Vazquez ², Fabiola Lafarga-De la Cruz ²

¹ Department of Biomedical Innovation, Centre for Scientific Research and Higher Education of Ensenada (CICESE). Carretera Tijuana-Ensenada 3918, Fraccionamiento Zona Playitas, 22860 Ensenada, Baja California, Mexico. (FC) E-mail: cicala@cicese.edu.mx. ORCID iD: <http://orcid.org/0000-0003-1116-4310>

² Aquaculture Department, Centre for Scientific Research and Higher Education of Ensenada (CICESE). Carretera Tijuana-Ensenada 3918, Fraccionamiento Zona Playitas, 22860 Ensenada, Baja California, Mexico. (MAT-V) E-mail: miguel.tripp@gmail.com. ORCID iD: <http://orcid.org/0000-0002-1364-6885>
(VM-O) E-mail: vmontes@cicese.edu.mx. ORCID iD: <http://orcid.org/0000-0001-9969-2788>
(GSC-V) E-mail: carlimina@gmail.com. ORCID iD: <http://orcid.org/0000-0002-5980-5378>
(FL-DC) (Corresponding author) E-mail: flafarga@cicese.edu.mx. ORCID iD: <http://orcid.org/0000-0002-5101-9364>

Summary: One of the main bottlenecks in abalone aquaculture is maintaining individuals during the weaning stage, when the natural diet changes from diatoms (postlarvae) to macroalgae (juvenile). During this period, abalone pass through profound morphological and physiological changes, which suitable nutrient requirements must sustain. An inadequate diet can result in adverse effects such as late metamorphosis, starvation, slow growth and even death during this phase. Two strategies have been proposed to increase the growth and survival rates of weaning abalone: (i) extending feeding with benthic diatoms and (ii) abalone hybridization via interspecific crosses. To evaluate the efficiency of the two strategies, we assessed the growth and survival rates of postlarvae of pure red abalone (*Haliotis rufescens*) and a hybrid obtained by crossing red abalone females with green abalone (*H. fulgens*) males. Both crosses were supplied with six different diets consisting of either one macroalgae mono-diet (*Macrocystis pyrifera* or *Ulva ohnoi*) or a mixture with *Navicula incerta*. Overall, cross-specific diets achieved better growth rates, suggesting that each cross may need specific food items (nutrients) during weaning. Moreover, pure red abalone generally showed the highest growth rates, while the hybrid abalone showed the highest survival rates with most tested diets. Hence, hybrids appear to be better at withstanding stressful conditions, and their use in aquaculture could reduce losses and increase commercial production.

Keywords: weaning abalone; *Haliotis rufescens*; *Haliotis fulgens*; hybrid vigour; *Macrocystis pyrifera*; benthic diatom.

Efecto de seis dietas en el crecimiento y supervivencia de poslarvas de abulón rojo (*Haliotis rufescens*) y su híbrido (*H. rufescens* ♀ × *H. fulgens* ♂)

Resumen: Uno de los principales cuellos de botella en la acuicultura de abulón es el mantenimiento de los individuos durante la fase de destete, cuando la dieta natural cambia de diatomeas (postlarvas) a macroalgas (juveniles). Durante este período, el abulón pasa por profundos cambios morfológicos y fisiológicos, que deben ser sostenidos por una alimentación adecuada. Una dieta inadecuada puede provocar efectos adversos como: metamorfosis tardía, inanición, crecimiento lento e incluso la muerte durante esta fase. Por ello, se han propuesto dos estrategias para aumentar las tasas de crecimiento y supervivencia del abulón durante la fase de destete: (i) ampliar el período de alimentación con diatomeas bentónicas y (ii) la hibridación de abulones mediante cruces interespecíficos. Para determinar la eficacia de ambas estrategias, se evaluaron las tasas de crecimiento y supervivencia de postlarvas de abulón rojo puro (*Haliotis rufescens*) y de un híbrido obtenido mediante el cruce de hembras de abulón rojo y machos de abulón azul (*H. fulgens*). A ambos cruces se les suministraron seis dietas diferentes consistentes en una mono dieta de macroalgas (*Macrocystis pyrifera*, *Ulva ohnoi*) o en una mezcla con *Navicula incerta*. En general, con las dietas específicas para cada cruce se obtuvieron mejores tasas de crecimiento, lo que sugiere que cada cruce requiere alimentos (nutrientes) específicos durante el destete. Además, el abulón rojo puro mostró en general las tasas de crecimiento más altas, mientras que en el abulón híbrido se obtuvieron las mayores tasas de supervivencia con la mayoría de las dietas probadas. Por lo que los híbridos parecen soportar mejor las condiciones de estrés, y su uso en la acuicultura podría ayudar a reducir las pérdidas y aumentar su producción comercial.

Palabras clave: destete; *Haliotis rufescens*; *Haliotis fulgens*; vigor híbrido; *Macrocystis pyrifera*; diatomea bentónica.

Citation/Como citar este artículo: Cicala F., Tripp-Valdez M.A., Montes-Orozco V., Cervantes-Vazquez G.S., Lafarga-De la Cruz F. 2023. Effects of six diets on the growth and survival rates of postlarvae of red abalone (*Haliotis rufescens*) and its hybrid (*H. rufescens* ♀ × *H. fulgens* ♂). Sci. Mar. 87(1): e055. <https://doi.org/10.3989/scimar.05300.055>

Editor: M. Ramon.

Received: May 13, 2022. **Accepted:** November 14, 2022. **Published:** February 24, 2023.

Copyright: © 2023 CSIC. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License.

INTRODUCTION

The abalone (*Haliotis* spp.) fishery is one of the oldest in Mexico (Cox 1962). Currently, abalone products are important commodities in national and international markets, and their exploitation is sustained by several socio-economic activities conducted along the Pacific coast of the Baja California peninsula (Searcy-Bernal et al. 2010, Cook 2014). The high demand for abalone has led to intense fishing pressure and dramatic declines in wild populations (Morales-Bojórquez et al. 2008). To compensate for these declines, Mexican abalone aquaculture has rapidly increased (Lafarga-De la Cruz and Gallardo-Escárate 2011). However, several challenges continue to hamper final production outcomes.

Worldwide, a notable bottleneck in abalone aquaculture arises due to the difficulties associated with maintaining individuals through the weaning stage, which encompasses the transition from post-settlement diatom-fed abalone to the juvenile stage macroalgae-fed abalone. The reasons for high abalone mortality during the weaning stage are still poorly understood, although they have been related to ontogenetic development in the morphology of the feeding apparatus (radula development mainly), in addition to the types of digestive enzymes present and their activities (Johnston et al. 2005). Moreover, food consumption in postlarvae abalone initially increases exponentially (up to a maximum point) as they grow. Thus, inadequate diets or an inability of the postlarvae to ingest and assimilate nutrients may result in delayed metamorphosis, starvation, slow growth, and ultimately death (Takami et al. 2002, Johnston et al. 2005, Dyck et al. 2010). To increase abalone survival during the weaning stage, farmers around the world have directed their efforts towards the use of (i) mixed diets to meet the feeding and energetic necessities of developing postlarvae (Correa-Reyes et al. 2001, Parker et al. 2007, Hernández et al. 2009) and (ii) selective breeding and abalone hybridization to generate more resistant lineages (Lafarga-de la Cruz and Gallardo-Escárate 2011).

Under culture conditions, seaweeds and benthic diatoms play pivotal roles in abalone maintenance by inducing larval settlement and serving as the main nourishment sources during the early postlarvae stage. Moreover, postlarval growth rates appear to be modulated by the nutritional value, size, availability and digestibility of the supplied diet (Carbajal-Miranda et al. 2005, Correa-Reyes et al. 2009). Therefore, the diets used in farmed abalone should be composed of different algae to fulfil the nutritional needs of each developmental stage (Hernández et al. 2009). The use of mixed diets has not yet been universally adopted in abalone

aquaculture; for example, farms continue to rely on mono-diets consisting of the macroalgae *Macrocystis pyrifera* because of its availability, ease of harvest and low cost. However, the poor biochemical composition of *M. pyrifera* (5%–12% proteins, 0.5%–1% lipids, and 46%–50% carbohydrates) may be insufficient to meet the nutritional needs of weaning abalone (Simental et al. 2004). In addition, *M. pyrifera* can become scarce or unavailable during winter months and in response to certain environmental changes and conditions such as those associated with the El Niño-Southern Oscillation (ENSO; Edwards 2019).

Moreover, the use of both diatoms (e.g. *Navicula* spp.) and macroalgae (e.g. *Ulva* spp.) in abalone aquaculture systems has generated promising results by promoting the growth and survival of postlarvae (Simental et al. 2004, Strain et al. 2006, Correa-Reyes et al. 2009). Specifically, *Ulva* sp. has been shown to reduce postlarval settlement, although it increases the survival rates of farmed abalone (Muñoz et al. 2012), so it is often supplied along with an easily digestible diatom (Daume et al. 2004, Daume 2006). A potential alternative commercial nutritional source for postlarvae abalone is *Navicula* spp. These benthic diatoms are among those most used in the production of algal films for abalone culture (Siqueiros-Beltrones and Doménico 2000). Moreover, in controlled feeding systems of red abalone (*Haliotis rufescens*), monospecific cultures of *Navicula incerta* have generally been shown to increase both the growth and survival rates of postlarvae (Correa-Reyes et al. 2001). Thus, as proposed by Carbajal-Miranda et al. (2005), the use of mixed diets composed of these algal species could induce higher growth rates in postlarvae abalone.

Hybridization has recently been proved to be a simple and effective way to increase the yields of commercially important molluscs, even after only a few generations (de Melo et al. 2016, Li et al. 2018). Currently, hybridization is a widely used strategy to improve the growth, behaviour and flavour of many commercially important plant and animal species, in addition to their reproductive and processing characteristics (Hamilton et al. 2009). Although hybridization has been successfully applied in abalone breeding, the physiological and molecular mechanisms underlying abalone hybrid superiority remain mostly unknown. Possible explanations of hybrid advantages over pure species may include (i) heterosis, (ii) complementarity and (iii) greater allelic diversity through recombination (Hamilton et al. 2009). Notably, the acquisition of new traits in hybrid offspring relies upon both the morphological and physiological characteristics of the paternal species based on the possible combinations of male and female

gametes, with higher genetic similarity to maternal traits generally observed in the offspring (Lafarga-De la Cruz and Gallardo-Escárate 2011, Liang et al. 2014).

Red abalone is known for its relatively rapid growth (Valenzuela-Miranda et al. 2015), and green abalone (*H. fulgens*) is particularly resistant to low nutrient or starvation conditions (Durazo-Beltrán et al. 2003). Indeed, during both the juvenile and adult stages, green abalone may survive for several weeks under restricted food regimes by successively metabolizing stored carbohydrates first, followed by lipids and finally proteins (Durazo-Beltrán et al. 2004, Viana et al. 2007). In this study, we evaluated abalone growth in terms of shell length (SL) and the survival rates of weaning abalone supplied with six different diets. In addition, physiological performance was compared between pure red abalone (RR) and a hybrid obtained by crossing female red abalone and male green abalone gametes (RG). This combination was chosen to generate a new hybrid cross characterized by a high growth rate that is more suitable for captive maintenance.

MATERIALS AND METHODS

Abalone rearing and maintenance

Our experimental design addressed two main scientific questions: (i) to what extent does each diet affect the growth and survival rates of postlarvae abalone during the weaning phase (<5 mm SL) and (ii) does the growth and survival rates of a hybrid (*H. rufescens* [♀] × *H. fulgens* [♂]; RG) exceed those of pure red abalone (*H. rufescens* [♀] × *H. rufescens* [♂]; RR).

To answer these questions, pure and hybrid crosses were produced at the Aquaculture Department of the Center for Research and Higher Education of Ensenada (CICESE). Competent larvae of pure and hybrid abalone were produced by crossing 12 female red abalone and three red and three green male abalone. Initially, adult spawn was stimulated with the TRIS-H₂O₂ method described by Morse et al. (1977). After 4 h post-fertilization, the oocytes were randomly sampled and collected by sieving to corroborate the presence of fertilized oocytes. For this, each oocyte was observed in a Sedgewick-Rafter counting chamber with the help of a Nikon ECLIPSE E200 optical microscope. Fertilization was confirmed through the presence of oocytes in the first and second mitotic divisions. Pure and hybrid postlarvae abalone were initially fed with a diet of fresh *N. incerta* ad libitum until they reached 3±0.5 mm in SL (~ 7 months). A total of 1440 postlarvae were randomly selected and included in the experiments (up to 13 weeks). After a seven-day acclimation period, 120 abalone from each cross (RR and RG) were randomly selected and collected by sieving and then transferred to three individual 6 L plastic tanks (40 abalone per replicate) with a fine bubble aeration stone under static conditions (no water flow). Tanks were filled using 1 µm filtered and UV-sterilized seawater at 17±0.5°C. A total water change was performed once a week, and feed was provided after each water change. Faeces were removed twice a week, and the water was also re-

filled (up to 20% exchange). Seawater was maintained at 17±0.5°C by setting the laboratory air conditions and monitored daily with HOBO® data loggers (Onset Corp., Bourne, MA, USA). Oxygen and pH were measured with an HI 98193 oximeter (Hanna Instruments, Smithfield, RI, USA) and an HI 98127 pH meter (Hanna Instruments). The experimental systems were maintained with the same photoperiod conditions (12 h light and 12 h darkness), which were regulated through a controller installed inside the laboratory. Light was maintained at an average lux of ~2.2 mE m⁻² s⁻¹ for the tanks. Finally, alkalinity and the concentrations of ammonium, nitrites and nitrates were monitoring using API colorimetric kits, following the instructions of the manufacturer.

Diet composition and algae cultures

Basic diets consisted of two fresh macroalgae of *M. pyrifera* (M) and *Ulva ohnoi* (U) and four mixed diets prepared using different combinations of these macroalgae in equal proportions in terms of weight. The microalgae *N. incerta* (N) was used in a constant volume of 60 mL (10⁵–10⁶ cells mL⁻¹). The mixed diets were named MU, MN, UN and MUN based on their algal combination.

Macrocystis pyrifera

Fresh fronds of *M. pyrifera* were collected weekly by the Abulones Cultivados Company from an intertidal zone near Ensenada (Baja California, Mexico; 31°17'33.00"N; 116°24'34.45"W) and maintained under optimal controlled conditions before being delivered to CICESE. Once in the laboratory, the fronds were rinsed with freshwater to remove fouling to ensure clean fresh fronds for the diets.

Ulva ohnoi

The green algae *Ulva ohnoi* was provided by Dr. José Zertuche from the Instituto de Investigaciones of the Universidad Autónoma de Baja California (IIO-UABC). *Ulva* strains were collected in Bahía San Quintín, Baja California, Mexico, and two different foliar strains of *Ulva ohnoi* were selected to develop its commercial culture (Zertuche-González et al. 2021). The *Ulva ohnoi* used in this study was produced under controlled conditions with a cultivation method consisted of “tumble culture” with aeration from the bottom of the pond (Revilla-Lovano et al. 2021), and fresh and clean fronds were provided once a week. Once in the laboratory of CICESE, the algae were rinsed with freshwater several times and kept in seawater for a few hours prior to being fed to the abalone.

Navicula incerta

The strains of the diatom *Navicula incerta*, a benthic microalga, used in this study were provided from the microalgal collection of the algae repository of CICESE. The microalgae strain was obtained in a volume of

15 mL and scaled to 150 mL non-axenic cultures via a seven-day culture using F/2 Medium (reactive grade) with sodium metasilicate solution (Guillard 1975) and irradiance, temperature and salinity conditions of $100 \mu\text{E m}^{-2} \text{s}^{-1}$, $17 \pm 1^\circ\text{C}$ and 34 ppt, respectively. Once the strain production was stable, the diatom cultures used to feed the abalone were maintained in 150 mL for 4 days. Thereafter, a total volume of 150 mL was inoculated in 15 L of fresh $1 \mu\text{m}$ filtered (cartridge filters) and UV-sterilized seawater (25-L plastic trays) with F/2 commercial medium (ProLine F/2 Algae Food, Pentair) and grown out for four days before being harvested and fed to the abalone. A volume of 60 mL of *N. incerta* (10^5 – 10^6 cells mL^{-1}) that had been sonicated for 2 min was added once a week to each experimental tank with a mixed diet (MN, UN, and MUN) to feed the abalone. Aeration was used to distribute the diatoms throughout the tank for 20 min and then discontinued for 1 hour to enable the diatoms to settle on the tank and macroalgae surfaces depending on the treatment.

Growth and survival

During the 13-week experimental period, we evaluated the relative growth rate (RGR) of postlarvae abalone in terms of the increase in SL from the initial conditions. With the aim of reducing the amount of disturbance to all animals, as handling and/or additional stress are known to negatively affect abalone growth and their overall conditions (Cunningham et al. 2016), SL measurements (mm) were obtained from a random subset of abalone ($n=14$ – 30) from each replicated tank. Abalone were placed on a gridded surface, and digital photographs were taken with a stereo microscope. The images were evaluated with ImageJ v.1.47 (available at <https://imagej.nih.gov/ij/>) with a vertical and horizontal calibration of ± 0.5 mm (Hopkins 1992). To avoid confounding effects due to differences in initial SL between abalone batches, SL increases were compared using the RGR calculated for each experimental unit with Eq. (1):

$$RGR_{length} = (SL_i - SL_0) / SL_0 \times 100 \quad \text{Eq. (1)}$$

where SL_i is the SL at time i , and SL_0 is the initial SL. SL_0 was defined as the mean SL for each replicate tank at week 0 (Hopkins 1992).

The survival of postlarvae abalone was checked each day. Dead individuals were immediately removed from the tanks. Survival was assessed with Kaplan-Meier cumulative survival curves with the survival package in R. Statistical differences in survival curves between diets and abalone crosses were evaluated with the *survdiff* function from the survival package, followed by pairwise comparison with the *pairwise_survdiff* function from the survminer package in R.

Statistical analysis

Statistically significant differences between means with regard to the RGR and abalone survival at the end of the 13-week experimental period were analysed

using a two-way analysis of variance (ANOVA), with “Diet” and “Cross” (RR and RG) included as fixed factors. A Tukey honest significant difference post-hoc test was used to identify the mean differences between diet treatments. Normality and homogeneity of variance for RGR were tested using Shapiro-Wilk and Levene tests, respectively. To consider the time effect on the RGR during the whole experiment, a linear model was fitted using “Diet,” “Cross,” and “Week” as fixed factors. Thereafter, slopes were contrasted with the *emtrends* function from the R package emmeans. All statistical analyses were conducted in R (v. 4.0.0; R Core Team).

RESULTS

Shell growth

All diets successfully weaned abalone, and continuous increases in SL were maintained throughout the 13-week experiment (Fig. 1). At the 13th week, the ANOVA indicated a significant effect of diet ($F_{(5,633)}=13.0$; $P<0.0001$), a non-significant effect of abalone cross ($F_{(1,633)}=1.73$; $P=0.18$) and a significant interaction ($F_{(5,633)}=4.8$; $P=0.002$). The lowest increase in mean SL for both abalone crosses was obtained with the diet consisting of only *M. pyrifera*. Although there was no statistically significant difference between the RGR values of both crosses with this diet ($RR_{RGR}=70.90\% \pm 45.06$ sd; $RG_{RGR}=48.12\% \pm 44.70$ sd; Tukey >0.05 ; Table I), the slopes of the models that considered the weekly values were significantly different, with RR having a steeper slope (slope for RR=6.1; slope for RG=4.4; Tukey $P<0.02$).

The highest increase in the RGR for RR was obtained with the mixed diet MUN ($RR_{RGR}=115.45\% \pm 67.6$ sd; Table I), while the highest RGR value for the hybrid abalone was obtained with the UN diet ($RG_{RGR}=115.34\% \pm 48.2$ sd; Table I). However, it is important to highlight that there were no significant differences in the final RGR values for the MUN diet ($RR_{RGR}=115.45\% \pm 67.6$; $RG_{RGR}=98.7\% \pm 55.2$ sd; Tukey, $P>0.05$; Table I) or in the slopes of weekly growth (slope for RR=8.7; slope for RG=8.8; Tukey, $P=0.88$; Fig. 1) between the two crosses, indicating similar beneficial effects of the MUN diet for the pure and hybrid abalone. By contrast, the beneficial effect of the UN diet occurred only in the hybrid RG, with lower RGR values ($RR_{RGR}=76.15\% \pm 48.96$ sd; $RG_{RGR}=115.35\% \pm 48.22$ sd; Tukey, $P<0.05$; Table I) and a lower slope reflecting growth in RR (slope for RR=6.4; slope for RG=8.4; Tukey, $P=0.01$; Fig. 1). The remaining diets showed no significant differences between the final RGR values (Table I) or in the weekly increases in RGR for the two abalone crosses (Supplementary Material Table S1).

Survival between pure and hybrid crosses

The Kaplan-Meier cumulative survival curves demonstrated significant differences between groups at the end of the 13-week trial (Fig 2). Overall, a higher survival rate for RR was observed with the MUN diet (89.16%), whereas the lowest rate was observed with the U diet (60%). On the other hand, higher survival for RG was observed with

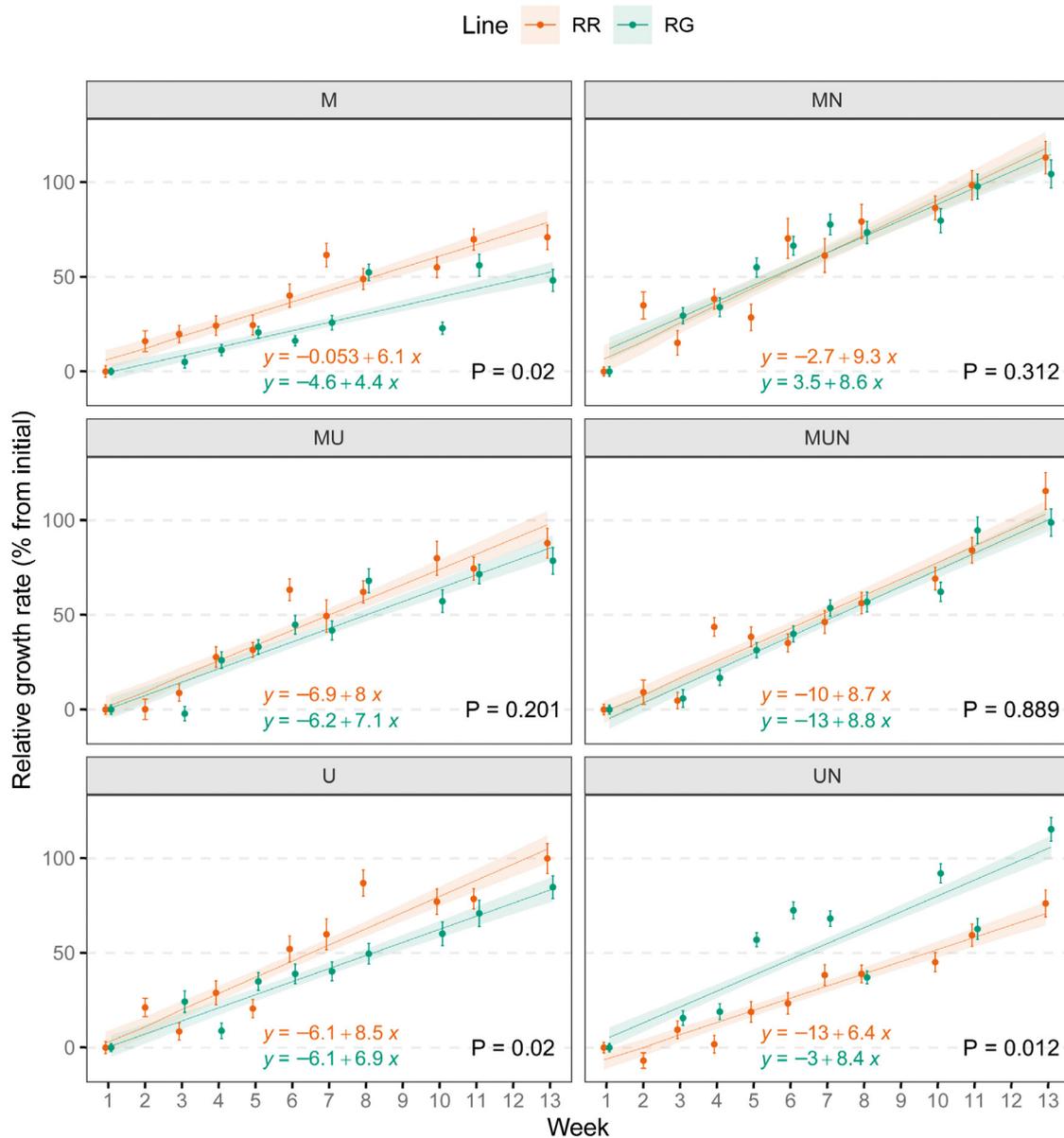


Fig. 1. – Relative growth rate increase in shell length (SL) of RR (red abalone, red line) and RG (hybrid abalone, green line) abalone in each diet treatment at the outset of the experiment. The points and error bars indicate mean \pm SEM. Solid lines and shaded area indicate modelled values \pm 95% CI with the linear model formulae indicated for each abalone cross. For each diet, statistical differences in the slopes are indicated. M, *Macrocystis pyrifera*; U, *Ulva ohnoi*; MU, *M. pyrifera* and *U. ohnoi*; MN, *M. pyrifera* and *N. incerta*; UN, *U. ohnoi* and *N. incerta*; MUN, *M. pyrifera*, *U. ohnoi* and *N. incerta*.

the MN and U diets (86.66% for both diet treatments), while the lowest survival rate for RG was observed with the M diet (65%). The chi square test performed with all pairwise comparisons for all diets revealed significant differences between abalone crosses with only the MUN and U diets (Fig. 2). The MUN diet induced a significantly higher survival rate in RR ($P < 0.001$), whereas the U diet induced a significantly higher survival in RG ($P < 0.0001$). The results from all the pairwise comparisons are shown in Supplementary Material Table S2.

DISCUSSION

Previous studies have evaluated the nutritional value of monospecific cultures of benthic diatoms as food sources for red and blacklip (*H. rubra*) abalone

postlarvae. In both cases, higher growth rates were obtained with abalone supplied with *Navicula* spp. and other diatoms (Correa-Reyes et al. 2001, 2009). Our findings support this observation and suggest that mixed diets including *N. incerta* may increase the growth and survival rates of both the pure RR abalone and RG hybrid cross. Moreover, the use of *M. pyrifera* should be discouraged during this stage due to the nutritional content of this macroalgae, which may not fulfil the nutritional needs of weaning abalone, and the inability of postlarvae abalone (3–5 mm) to properly ingest and assimilate this alga. This was evident in the present study, as the M diet induced the lowest growth increases for both abalone crosses (RR and RG) and also induced the lowest cumulative survival in the hybrid cross (RG).

Table 1. – Mean (\pm sd) shell-length at the beginning (week 1) and end (week 13) of the experiment. * Mean values that do not share superscripts are significantly different (two-way ANOVA; $P < 0.05$).

Diet	Cross	Mean shell length at week 1 (mm \pm sd)	Mean shell length at week 13 (mm \pm sd)	Relative growth rate at week 13 (% from initial SL \pm sd)*
M	RR	3.78 \pm 1.08	6.45 \pm 1.69	70.70 \pm 45.07 ^b ^c
M	RG	4.29 \pm 0.71	6.35 \pm 1.88	48.12 \pm 44.71 ^c
MN	RR	3.90 \pm 1.09	8.07 \pm 1.68	113.02 \pm 59.58 ^a
MN	RG	3.78 \pm 0.98	7.60 \pm 1.75	104.30 \pm 57.05 ^{ab}
MU	RR	3.78 \pm 0.99	6.99 \pm 1.75	87.09 \pm 54.18 ^{ab}
MU	RG	4.11 \pm 1.10	7.29 \pm 2.16	78.57 \pm 53.61 ^{bc}
MUN	RR	3.96 \pm 1.14	8.45 \pm 2.49	115.45 \pm 67.65 ^a
MUN	RG	3.92 \pm 0.88	7.77 \pm 2.10	98.75 \pm 55.28 ^{ab}
U	RR	3.70 \pm 1.10	7.40 \pm 2.05	99.89 \pm 54.56 ^{ab}
U	RG	4.27 \pm 0.97	7.81 \pm 1.74	84.74 \pm 46.55 ^{ab}
UN	RR	4.57 \pm 1.31	7.94 \pm 1.96	76.15 \pm 48.96 ^{bc}
UN	RG	3.98 \pm 0.90	8.58 \pm 1.93	115.35 \pm 48.22 ^a

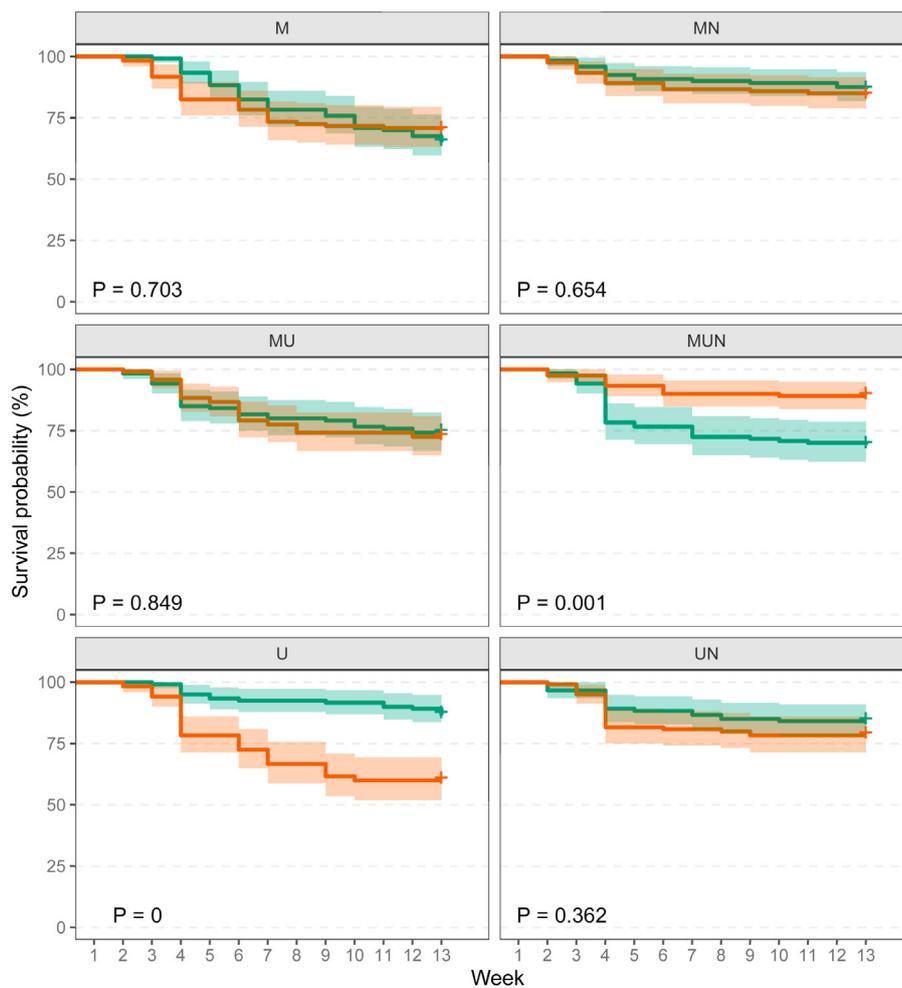


Fig. 2. – Kaplan-Meier cumulative survival curves for pure red abalone (RR, red line) and hybrid abalone (RG, green line) during the 13-week feeding trials. Solid and shaded lines represent the mean survival probability and the 95% confidence intervals. For each diet, significant differences between RR and RG crosses are indicated (chi square test). M, *Macrocystis pyrifera*; U, *Ulva ohnoi*; MU, *M. pyrifera* and *U. ohnoi*; MN, *M. pyrifera* and *N. incerta*; UN, *U. ohnoi* and *N. incerta*; MUN, *M. pyrifera*, *U. ohnoi* and *N. incerta*.

The highest growth and survival rate for RR abalone was observed with the MUN diet. For the hybrid abalone RG, the highest growth rate was observed with the UN diet, whereas the highest survival was observed with the MN and U diets. However, it is important to note that there were no significant differences between the survival curves with the UN diet (chi square $P > 0.05$; Supplementary Material Table S2), although the highest survival values for RG were observed with the MN and U diets. Therefore, we can conclude that the UN diet induced a higher growth rate and high survival rates in RG.

Accordingly, as previously reported, *N. incerta* should be considered one of the most appropriate nourishment sources for recently settled abalone (Martínez-Ponce and Searcy-Bernal 1998, Simental et al. 2004, Carbajal-Miranda et al. 2005). Moreover, the incorporation of this diatom into abalone diets should be encouraged in farmed production scenarios, as it may reduce abalone losses during weaning, which is the most critical stage during abalone cultivation (Viana et al. 1993, Carbajal-Miranda et al. 2005, Hernández et al. 2009). The benefits of *N. incerta* likely reflect its protein and lipid content (up to 30%; Simental-Trinidad et al. 2001, Hernández et al. 2009, Ortiz et al. 2009).

The higher growth rates obtained with *N. incerta*-based diets may also be related to the particular morphologies and digestive enzymes present in postlarvae abalone. Between 80 and 102 days post-settlement, early teeth appear on the radula, while the digestive gland increases in both complexity and enzyme production, suggesting that the abalone is preparing to feed on macroalgae (Johnston et al. 2005). Kawamura et al. (1995) reported that both the radula structure and the ability to digest and assimilate seaweeds are fully developed in *H. discus hannai* measuring 2–4 mm in SL (Kawamura et al. 1995). Thus, the postlarvae abalone used in this experiment (>3 mm) were likely able to feed on seaweed. However, our observations suggest that at this time the reduced cell size of diatoms may still increase the efficiency at which the radula passes food into the mouth; hence, small cells are preferred by immature abalone, as they are easily handled and ingested (Carbajal-Miranda et al. 2005).

The cumulative survival observed during the experimental period (13 weeks) generally ranged from 60% to 89%. These results are particularly encouraging, as survival rates lower than 70% are usually reported in weaning abalone (Takami et al. 2002, Hernández et al. 2009). Moreover, the registered growth rate met the standard growth of commercial postlarvae *Haliotis rufescens* in Mexican farms estimated to be ~2.0 mm/month (Searcy-Bernal et al. 2007). Both observations suggest that the use of mixed diets together with the rearing conditions used in this study should be considered for future experimental studies and farmed production.

Marine mollusc hybrids generally show better growth than their parents (Alter et al. 2017); however, in this study, similar growth rates were obtained between the two crosses. The simplest explanation for such similar growth rates may be the experimental design, as both crosses were maintained within the

optimum temperature conditions for red abalone (17–18°C). However, an alternative or complementary possibility may be proposed. It has been reported that the acquisition of new traits does not rely exclusively on the crossed species but also on the combination of male and female gametes (Cai et al. 2010). Accordingly, the growth rates of the green abalone [♀] × red abalone [♂] combination, the GR hybrid, should be also evaluated.

Finally, previous studies have indicated that hybrid progeny usually share high genetic similarity with their maternal parent (Cai et al. 2010). Hence, in this study, the red abalone [♀] × green abalone [♂] cross was preferred when generating hybrid progeny with the ability to grow in relatively short periods of time, which is a characteristic trait of red abalone (Valenzuela-Miranda et al. 2015). However, the growth and survival of other abalone species and gamete combinations should be evaluated. Notably, although no significant differences between growth rates were obtained between crosses, heterosis was observed in terms of survival rates, suggesting that hybrid abalone may be better able to withstand culture conditions. Similar results have also been reported with an *H. discus hannai* hybrid, with the metamorphosis, growth, and survival rates of the hybrids being superior to those of the pure lineage (Li et al. 2018). Together, these observations should encourage hybrid farming to meet the demands of culture production while limiting abalone losses.

CONCLUSIONS

In conclusion, we assessed the growth and survival rates of postlarvae (4 ± 0.22 mm SL) of pure red abalone and a red abalone [♀] × green abalone [♂] hybrid under culture conditions. Both the hybrid and pure-line abalone were supplied with six different diets. Notably, hybrid abalone did not outperform pure red abalone in terms of survival or growth, as the highest growth rate and survival values were similar for both crosses, but a cross-specific beneficial effect of diet was evident. Overall, a mixture of *M. pyrifera*, *U. ohnoi*, and *N. incerta* induced the highest growth rate and survival in pure red abalone, whereas the mixture of *U. ohnoi* and *N. incerta* induced the highest growth rate and optimum survival in the hybrid abalone. Although the hybrid abalone did not show higher growth rates than those of the pure red abalone, this study sheds light on the different nutritional requirements of hybrid crosses when compared with those of pure lineages. Additionally, it is important to consider that the animals were grown at the optimum temperature of red abalone, so culturing hybrid RG at its specific optimum temperature with its specific diet may result in better outcomes for this cross.

ACKNOWLEDGEMENTS

We are grateful to Andrea Lievana-MacTavish for professional English language editing. Also, we would like to thank Dr. Zertuche and José M. Guzmán-Calderón for providing us with the green alga *Ulva ohnoi*, Claudia Carillo from the Abulones Culti-

vados Farm for providing us with the brown algae *M. pyrifera*, and Jesús Mariscal-Medina for his technical support at the Abalone Lab Production at the CICESE facilities where this study was performed.

FUNDING

The research was funded by the project SEP-CONACYT-CB-2014-238708. CONACYT provided the postdoctoral fellowships granted to MT and FC.

DECLARATION OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

AUTHOR CONTRIBUTIONS

Conceptualization, FLC; Data curation, MT and VMO; Formal analysis, MT and VMO; Funding acquisition, FLC; Investigation, FC, MT and VMO; Methodology, MT, GCV and VMO; Project administration, FLC; Resources, FLC; Software, FLC and MT; Supervision, FC and MT; Validation, FC and MT; Visualization, MT; Writing original draft, FC; Writing review and editing, FC, MT, VMO, GCV, FLC.

REFERENCES

- Alter K., Andrewartha S.J., Morash A.J., et al. 2017. Hybrid abalone are more robust to multi-stressor environments than pure parental species. *Aquaculture* 478: 25-34. <https://doi.org/10.1016/j.aquaculture.2017.04.035>
- Cai M., Wang Z., Ke C., et al. 2010. Allozygotic progeny are produced from a hybrid abalone cross of female *Haliotis diversicolor* and male *Haliotis discus discus*. *J. Shellfish Res.* 29: 725-729. <https://doi.org/10.2983/035.029.0325>
- Carbajal-Miranda M.J., Sánchez-Saavedra M.D.P., Simental J.A. 2005. Effect of monospecific and mixed benthic diatom cultures on the growth of red abalone postlarvae *Haliotis rufescens* (Swainson 1822). *J. Shellfish Res.* 24: 401-405. [https://doi.org/10.2983/0730-8000\(2005\)24\[401:EOMAM-B\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2005)24[401:EOMAM-B]2.0.CO;2)
- Cook P.A. 2014. The Worldwide Abalone Industry. *Mod. Econ.* 5: 1181-1186. <https://doi.org/10.4236/me.2014.513110>
- Correa-Reyes J.G., Sánchez-Saavedra M. del P., Siqueiros-Beltrones D.A., Flores-Acevedo N. 2001. Isolation and growth of eight strains of benthic diatoms, cultured under two light conditions. *J. Shellfish Res.* 20: 603-610.
- Correa-Reyes J.G., Sánchez-Saavedra M. del P., Viana M.T et al. 2009. Effect of eight benthic diatoms as feed on the growth of red abalone (*Haliotis rufescens*) postlarvae. *J. Appl. Phycol.* 21: 387-393. <https://doi.org/10.1007/s10811-008-9381-x>
- Cox K.W. 1962. California Abalones, Family Haliotidae. *Fish Bull.* 118: 1-131.
- Cunningham S.C., Smith A.M., Lamare M.D. 2016. The effects of elevated pCO₂ on growth, shell production and metabolism of cultured juvenile abalone, *Haliotis iris*. *Aquac. Res.* 47: 2375-2392. <https://doi.org/10.1111/are.12684>
- Daume S. 2006. The roles of bacteria and micro and macro algae in abalone aquaculture: A review. *J. Shellfish Res.* 25: 151-157. [https://doi.org/10.2983/0730-8000\(2006\)25\[151:TRO-BAM\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2006)25[151:TRO-BAM]2.0.CO;2)
- Daume S., Huchette S., Ryan S., Day R.W. 2004. Nursery culture of *Haliotis rubra*: the effect of cultured algae and larval density on settlement and juvenile production. *Aquaculture* 236: 221-239. <https://doi.org/10.1016/j.aquaculture.2003.09.035>
- de Melo C.M.R., Durland E., Langdon C. 2016. Improvements in desirable traits of the Pacific oyster, *Crassostrea gigas*, as a result of five generations of selection on the West Coast, USA. *Aquaculture* 460: 105-115. <https://doi.org/10.1016/j.aquaculture.2016.04.017>
- Durazo-Beltrán E., D'Abramo L.R., Toro-Vazquez J.F., et al. 2003. Effect of triacylglycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (*Haliotis fulgens*). *Aquaculture* 224: 257-270. [https://doi.org/10.1016/S0044-8486\(03\)00223-0](https://doi.org/10.1016/S0044-8486(03)00223-0)
- Durazo-Beltrán E., Viana M.T., D'Abramo L.R., Toro-Vazquez J.F. 2004. Effects of starvation and dietary lipid on the lipid and fatty acid composition of muscle tissue of juvenile green abalone (*Haliotis fulgens*). *Aquaculture* 238: 329-341. <https://doi.org/10.1016/j.aquaculture.2004.03.025>
- Dyck M., Roberts R., Jeffs A. 2010. Use of algal diets to aid early weaning in the abalone *Haliotis iris*. *J. Shellfish Res.* 29: 613-620. <https://doi.org/10.2983/035.029.0309>
- Edwards M.S. 2019. Comparing the impacts of four ENSO events on giant kelp (*Macrocystis pyrifera*) in the northeast Pacific Ocean. *Algae* 34: 141-151. <https://doi.org/10.4490/algae.2019.34.5.4>
- Guillard R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith W.L. and Chantey M.H. (eds), *Cult. Mar. Invertebr. Anim.* Plenum Publ. New York 29-60. https://doi.org/10.1007/978-1-4615-8714-9_3
- Hamilton M., Kube P., Elliott N., et al. 2009. Development of a breeding strategy for hybrid abalone. *Proc. Assoc. Adv. Anim. Breed. Genet.* 18: 350-353.
- Hernández J., Uriarte I., Viana M.T., et al. 2009. Growth performance of weaning red abalone (*Haliotis rufescens*) fed with *Macrocystis pyrifera* plantlets and *Porphyra columbina* compared with a formulated diet. *Aquac. Res.* 40: 1694-1702. <https://doi.org/10.1111/j.1365-2109.2009.02267.x>
- Hopkins K.D. 1992. Reporting fish growth: A review of the basics. *J. World Aquac. Soc.* 23: 173-179. <https://doi.org/10.1111/j.1749-7345.1992.tb00766.x>
- Johnston D., Moltshaniwskyj N., Wells J. 2005. Development of the radula and digestive system of juvenile blacklip abalone (*Haliotis rubra*): Potential factors responsible for variable weaning success on artificial diets. *Aquaculture* 250: 341-355. <https://doi.org/10.1016/j.aquaculture.2005.03.012>
- Kawamura T., Saito T., Takami H., Yamashita Y. 1995. Dietary value of benthic diatoms for the growth of post-larval abalone *Haliotis discus hannai*. *J. Exp. Mar. Bio. Ecol.* 194: 189-199. [https://doi.org/10.1016/0022-0981\(95\)00099-2](https://doi.org/10.1016/0022-0981(95)00099-2)
- Lafarga-De la Cruz F., Gallardo-Escárate C. 2011. Intraspecific and interspecific hybrids in *Haliotis*: Natural and experimental evidence and its impact on abalone aquaculture. *Rev. Aquac.* 3: 74-99. <https://doi.org/10.1111/j.1753-5131.2011.01045.x>
- Li J., Wang M., Fang J., et al. 2018. A comparison of offspring growth and survival among a wild and a selected strain of the Pacific abalone (*Haliotis discus hannai*) and their hybrids. 495: 721-725. <https://doi.org/10.1016/j.aquaculture.2018.06.071>
- Liang S., Luo X., You W., Luo L., Ke C. 2014. The role of hybridization in improving the immune response and thermal tolerance of abalone. *Fish Shellfish Immunol.* 39: 69-77. <https://doi.org/10.1016/j.fsi.2014.04.014>
- Martínez-Ponce D.R., Searcy-Bernal R. 1998. Grazing rates of red abalone (*Haliotis rufescens*) postlarvae feeding on the benthic diatom *Navicula incerta*. *J. Shellfish Res.* 17: 627-630.
- Morales-Bojórquez E., Muciño-Díaz M.O., Vélez-Barajas J.A. 2008. Analysis of the decline of the abalone fishery (*Haliotis fulgens* and *H. corrugata*) along the westcentral coast of the Baja California peninsula, Mexico. *J. Shellfish Res.* 27: 865-870. [https://doi.org/10.2983/0730-8000\(2008\)27\[865:AOTDO-T\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2008)27[865:AOTDO-T]2.0.CO;2)

- Morse D.E., Duncan H., Hooker N., Morse A. 1977. Hydrogen peroxide induces spawning in mollusks, with activation of prostaglandin endoperoxide synthetase. *Science* 196: 298-300.
<https://doi.org/10.1126/science.403609>
- Muñoz P., Ambler R., Bulboa C. 2012. Settlement, Survival, and post-larval growth of red abalone, *Haliotis rufescens*, on polycarbonate plates treated with germlings of *Ulva* sp. *J. World Aquac. Soc.* 43: 890-895.
<https://doi.org/10.1111/j.1749-7345.2012.00615.x>
- Ortiz J., Uquiche E., Robert P., et al. 2009. Functional and nutritional value of the Chilean seaweeds *Codium fragile*, *Gracilaria chilensis* and *Macrocystis pyrifera*. *Eur. J. Lipid Sci. Technol.* 111: 320-327.
<https://doi.org/10.1002/ejlt.200800140>
- Parker F., Davidson M., Freeman K., et al. 2007. Investigation of optimal temperature and light conditions for three benthic diatoms and their suitability to commercial scale nursery culture of abalone (*Haliotis laevis*). *J. Shellfish Res.* 26: 751-761.
[https://doi.org/10.2983/0730-8000\(2007\)26\[751:IOOTAL\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2007)26[751:IOOTAL]2.0.CO;2)
- Revilla-Lovano S., Sandoval-Gil J.M., Zertuche-Gonzalez J.A., et al. 2021. Physiological responses and productivity of the seaweed *Ulva ohnoi* (Chlorophyta) under changing cultivation conditions in pilot large land-based ponds. *Algal Research*, 56: 102316.
<https://doi.org/10.1016/j.algal.2021.102316>
- Searcy-Bernal R., Pérez-Sánchez E., Anguiano-Beltrán C., Flores-Aguilar R. 2007. Metamorphosis and postlarval growth of abalone *Haliotis rufescens* in a Mexican commercial hatchery. *J. Shellfish Res.* 26: 783-787.
[https://doi.org/10.2983/0730-8000\(2007\)26\[783:MAPGOA\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2007)26[783:MAPGOA]2.0.CO;2)
- Searcy-Bernal R., Ramade-Villanueva M.R., Altamira B. 2010. Current Status of abalone fisheries and culture in Mexico. *J. Shellfish Res.* 29: 573-576.
<https://doi.org/10.2983/035.029.0304>
- Simental-Trinidad J.A., Sánchez-Saavedra M.P., Correa-Reyes J.G. 2001. Biochemical composition of benthic marine diatoms using as culture medium a common agricultural fertilizer. *J. Shellfish Res.* 20: 611-617.
- Simental J.A., Sanchez-Saavedra M.D., Flores-Acevedo N. 2004. Growth and survival of juvenile red abalone (*Haliotis rufescens*) fed with macroalgae enriched with a benthic diatom film. *J. Shellfish Res.* 23: 995-999.
- Siqueiros-Beltrones D.A., Domenico V. 2000. Grazing selectivity of red abalone *Haliotis rufescens* postlarvae on benthic diatom films under culture conditions. *J. World Aquac. Soc.* 31: 239-246.
<https://doi.org/10.1111/j.1749-7345.2000.tb00359.x>
- Strain L.W.S., Borowitzka M.A., Daume S. 2006. Growth and survival of juvenile greenlip abalone (*Haliotis laevis*) feeding on germlings of the macroalgae *Ulva* sp. *J. Shellfish Res.* 25: 239-247.
[https://doi.org/10.2983/0730-8000\(2006\)25\[239:GASOJ-G\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2006)25[239:GASOJ-G]2.0.CO;2)
- Takami H., Kawamura T., Yamashita Y. et al. 2002. Effects of delayed metamorphosis on larval competence, and postlarval survival and growth of abalone *Haliotis discus hannai*. *Aquaculture* 213: 311-322.
[https://doi.org/10.1016/S0044-8486\(02\)00338-1](https://doi.org/10.1016/S0044-8486(02)00338-1)
- Valenzuela-Miranda D., Del Río-Portilla M.A., Gallardo-Escárate C. 2015. Characterization of the growth-related transcriptome in California red abalone (*Haliotis rufescens*) through RNA-Seq analysis. *Mar. Genomics* 24: 199-202.
<https://doi.org/10.1016/j.margen.2015.05.009>
- Viana M.T., López L.M., Salas A. 1993. Diet development for juvenile abalone *Haliotis fulgens* Evaluation of two artificial diets and macroalgae. *Aquaculture* 117: 149-156.
[https://doi.org/10.1016/0044-8486\(93\)90131-H](https://doi.org/10.1016/0044-8486(93)90131-H)
- Viana M.T., D'Abramo L.R., Gonzalez M.A., et al. 2007. Energy and nutrient utilization of juvenile green abalone (*Haliotis fulgens*) during starvation. *Aquaculture* 264: 323-329.
<https://doi.org/10.1016/j.aquaculture.2007.01.004>
- Zertuche-González J.A., Sandoval-Gil J.M., Rangel-Mendoza L.K., et al. 2021. Seasonal and interannual production of sea lettuce (*Ulva* sp.) in outdoor cultures based on commercial size ponds. *J. World Aquac. Soc.* 52: 1047-1058.
<https://doi.org/10.1111/jwas.12773>

SUPPLEMENTARY MATERIAL

Table S1. – Results from the slope analysis of the relative growth rate performed with the *emtrends* function from the *emmeans* R package.

\$emtrends

Line=RR:

Diet	Week.trend	SE	df	lower.CL	upper.CL
M	6.06	0.444	7814	5.18	6.93
MN	9.27	0.445	7814	8.4	10.14
MU	8.03	0.446	7814	7.16	8.91
MUN	8.70	0.453	7814	7.82	9.59
U	8.54	0.444	7814	7.67	9.41
UN	6.43	0.446	7814	5.56	7.31

Line=RG:

Diet	Week.trend	SE	df	lower.CL	upper.CL
M	4.42	0.458	7814	3.52	5.32
MN	8.56	0.447	7814	7.68	9.43
MU	7.09	0.447	7814	6.21	7.96
MUN	8.79	0.449	7814	7.91	9.67
U	6.92	0.446	7814	6.04	7.79
UN	8.38	0.447	7814	7.51	9.26

Confidence level used: 0.95

\$contrasts

Line=RR:

	Contrast estimate	SE	df	t.ratio	p.value
M-MN	-3.21	0.629	7814	-5.11	<.0001
M-MU	-1.98	0.629	7814	-3.14	0.0210
M-MUN	-2.65	0.634	7814	-4.17	0.0004
M-U	-2.48	0.629	7814	-3.95	0.0011
M-UN	-0.37	0.630	7814	-0.59	0.9914
MN-MU	1.24	0.629	7814	1.96	0.3647
MN-MUN	0.56	0.634	7814	0.89	0.9493
MN-U	0.73	0.629	7814	1.16	0.8557
MN-UN	2.84	0.630	7814	4.50	0.0001
MU-MUN	-0.67	0.635	7814	-1.06	0.8986
MU-U	-0.51	0.629	7814	-0.80	0.9670
MU-UN	1.60	0.631	7814	2.54	0.1131
MUN-U	0.17	0.634	7814	0.26	0.9998
MUN-UN	2.27	0.636	7814	3.57	0.0048
U-UN	2.11	0.630	7814	3.35	0.0107

Line=RG:

	Contrast estimate	SE	df	T ratio	P value
M-MN	-4.14	0.640	7814	-6.47	<.0001
M-MU	-2.67	0.640	7814	-4.17	0.0004
M-MUN	-4.37	0.642	7814	-6.82	<.0001
M-U	-2.50	0.639	7814	-3.91	0.0013
M-UN	-3.96	0.640	7814	-6.19	<.0001
MN-MU	1.47	0.632	7814	2.33	0.1840
MN-MUN	-0.24	0.634	7814	-0.37	0.9991
MN-U	1.64	0.631	7814	2.60	0.0974
MN-UN	0.18	0.632	7814	0.28	0.9998
MU-MUN	-1.71	0.634	7814	-2.69	0.0772
MU-U	0.17	0.631	7814	0.27	0.9998
MU-UN	-1.30	0.632	7814	-2.05	0.3153
MUN-U	1.88	0.633	7814	2.97	0.0359
MUN-UN	0.41	0.634	7814	0.65	0.9874
U-UN	-1.47	0.631	7814	-2.32	0.1852

P value adjustment: Tukey method for comparing a family of six estimates.

Table S2. – Results from all the pairwise comparisons for survival analysis performed with the *pairwise_survdiff* function from the *survminer* R package.

	diet=M, cross=RG	diet=M, cross=RR	diet=MN, cross=RG	diet=MN, cross=RR	diet=U, cross=RG	diet=U, cross=RR	diet=MU, cross=RG	diet=MU, cross=RR	diet=UN, cross=RG	diet=UN, cross=RR	diet=MUN, cross=RG
diet=M, cross=RR	0.702	---	---	---	---	---	---	---	---	---	---
diet=MN, cross=RG	0.001	0.007	---	---	---	---	---	---	---	---	---
diet=MN, cross=RR	0.007	0.029	0.653	---	---	---	---	---	---	---	---
diet=U, cross=RG	0.000	0.006	0.961	0.628	---	---	---	---	---	---	---
diet=U, cross=RR	0.360	0.232	0.000	0.000	0.000	---	---	---	---	---	---
diet=MU, cross=RG	0.326	0.583	0.038	0.127	0.029	0.052	---	---	---	---	---
diet=MU, cross=RR	0.450	0.666	0.023	0.083	0.017	0.087	0.848	---	---	---	---
diet=UN, cross=RG	0.007	0.026	0.659	0.973	0.629	0.000	0.121	0.077	---	---	---
diet=UN, cross=RR	0.113	0.247	0.161	0.384	0.127	0.012	0.615	0.489	0.362	---	---
diet=MUN, cross=RG	0.827	0.933	0.006	0.023	0.004	0.279	0.502	0.623	0.020	0.208	---
diet=MUN, cross=RR	0.000	0.001	0.653	0.362	0.666	0.000	0.011	0.006	0.368	0.052	0.001