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Suspended cultivation of the Pacific oyster *Crassostrea gigas* in the Eastern Tropical Pacific

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Abstract The Pacific or Japanese oyster, *Crassostrea gigas*, was introduced into Ecuador in 1990; however, little is known about its cultivation in the Eastern Tropical Pacific. This study analyzes growth and the influence of environmental factors on two cohorts of *C. gigas* held in pearl nets suspended from a long line, in Ayangue Bay, Santa Elena Province, Ecuador. Juveniles of 4.8 ± 0.11 mm (cohort I) and 7.3 ± 0.11 mm (cohort II) shell length remained in suspended cultivation for 7 months (beginning August 2014) and 9 months (beginning March 2015), respectively. Intra-monthly, (during the first 2 months), monthly, and bi-monthly sampling of the dorsoventral length, dry biomass of the shell and soft tissues, and the fouling on the shell were determined. Temperature (in a continuous pattern), weekly salinity, and total seston and phytoplankton biomass (chlorophyll *a*) were determined. Results show that available food, indicated by chlorophyll *a*, and temperature allowed an adequate development of oysters in culture. Growth in shell size and soft tissues increased with higher temperatures. *Crassostrea gigas* could reach commercial size in less than 1 year of cultivation. Results of this study demonstrate the biological potential for Japanese oyster culture along the Ecuadorian coast.

Keywords Growth · Japanese oyster · Mollusks · Ostreidae · Pacific oyster · Tropical pacific

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Introduction

The Pacific or Japanese oyster, *Crassostrea gigas* (Thunberg 1793), is one of the most important mollusks in global aquaculture, with over 600,000 t produced in 2014 (FAO 2016). Adaptability of this species to factors influencing the physiology of marine invertebrates (temperature, salinity, etc.) has allowed it to thrive in different latitudes and systems (estuaries, lagoons, coastal areas, and offshore) of more than 70 countries (Miossec et al. 2009). On the American Pacific coast, it has been introduced into the USA, Canada, Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Peru, and Chile.

In Ecuador, a program has been initiated recently in several coastal communities to develop *C. gigas* culture using long lines, through initiatives of the Ministry of Agriculture, Livestock, Aquaculture, and Fisheries with the support of CENAIM-ESPOL (spat production). Results have been encouraging, which is the reason why the Japanese oyster is considered as one of the main species for the diversification of aquaculture in Ecuador. With the exception of Lombeida (1997) who reported results of studies of growth of Pacific oysters in reservoirs, drainage channels, and shrimp farming ponds, there have been few published reports on results of the culture of the Pacific oyster along the tropical eastern Pacific coast.

The present research is a contribution to knowledge about growth and the influence of environmental factors on *C. gigas* under suspended culture in equatorial waters, where there are important environmental changes due to the mixture of waters from several oceanographic phenomena, which could affect oyster physiology.

Materials and methods

The study was carried out in Ayangue Bay, Province of Santa Elena (1° 59′ 1.59″ S, 80° 45′ 35.15″ W), using an experimental long-line culture system. Two batches of seed produced at different times in the CENAIM-ESPOL hatchery were used. The first batch (cohort I), with an average dorsoventral shell length of 4.8 ± 0.11 mm, began on August 2014 and continued for 7 months; cultivation of the second batch (cohort II), with average of 7.3 ± 0.11 mm, began in March 2015 and was maintained for 9 months. Spat of each cohort was confined to several 35 × 35 cm pearl nets (initially 3 mm mesh size, increasing as the crop developed) at a density equivalent to 25–30% coverage of the pearl net base. The number of individuals per basket was reduced in each intra-monthly sampling, during the first 2 months, and later monthly or inter monthly, when five individuals were finally kept in each of three experimental pearl nets (three replicates). The remaining pearl nets were kept under the same conditions and served as replacement replicas for the experimental ones. The baskets were protected with a cover made of sardine fishing net (10 mm stretched mesh size), in order to avoid predation, mainly fish of the family Balistidae (Sonnenholzner et al. 2016).

Oysters sampled were cleaned of fouling with a spatula. The dorsoventral axis (length) and the dry biomass of the soft tissue, shell, and fouling were measured. For length, a digital caliper with precision 0.01 mm was used. To estimate biomasses, components were dehydrated by heating (60 °C) until reaching constant weight, determined with an analytical balance (0.001 g accuracy).

At the culture site, weekly samples of surface seawater (in triplicate) were taken at a depth 1–0.5 m with the aid of a 12-L plastic container, being careful not to alter the culture

environment. Subsequently, water subsamples were obtained for salinity determination using a 1‰ appreciation refractometer, as well as previously filtered water samples with a 153-μm pore size mesh to remove the macroplankton. These samples were transferred to dark plastic bottles (2 L) and transported to the laboratory, in order to estimate phytoplankton biomass by concentration of chlorophyll *a* and total seston. These analyses were performed by retaining the sample particles in Whatman GF/C filters using Millipore vacuum filtration equipment. For determination of chlorophyll *a*, the spectrophotometric method was used while seston content was determined using gravimetric techniques following Strickland and Parsons (1972). Temperature was continuously recorded every 30 min or 1 h using an electronic thermograph (Hobo, Onset, USA).

In order to evaluate the effect of environmental factors on oyster growth, an initial identification of those factors was made by constructing a Pearson correlation matrix and significance at $P < 0.1$ was determined using Fisher's r test. Only environmental variables significantly correlated with each growth parameter were used for the stepwise multiple regressions. The variability of the daily rate of growth parameters (length and mass of shell as well as tissue mass) were related to the variability of the mean of each environmental factor determined in each period through a stepwise multiple regression procedure, after Log $(X + 2)$ transformation of the data, following Hair et al. (1992). The input probability of the variables in the models was 0.05.

Results

Growth in shell length was generally continuous in both cohorts. Cohort I reached an average size of 67.6 ± 5.43 mm in 7 months of experimental culture. In cohort II, a growth recess occurred in June and September 2015, reaching 61.6 ± 3.03 and 77.8 ± 7.24 mm at 7 and 9 months of culture, respectively (Fig. 1a). Shell mass in both cohorts had slow growth during the first 4–5 months of culture, but then accelerated. Oysters of cohort I reached mean dry biomass values of 18.2 ± 1.71 g after 7 months, while those from cohort II reached 15.1 ± 1.99 g and 25.1 ± 1.64 g at 7 and 9 months of culture, respectively (Fig. 1b).

Dry biomass of soft tissues showed more outstanding changes than those of the shell (Fig. 1c). In cohort I, there were abrupt changes (increases and decreases in mass) at the beginning of the experiment during August–September 2014. Subsequently, the mass increased significantly until November 2014 when it decreased and remained low until the end of January; it increased again until the end of the experiment and reached a mass of 1.1 ± 0.24 g. Soft tissue masses in cohort II increased exponentially during the first 3 months (until the end of May 2015); then growth stopped until early August 2015 and increased again with high growth rates in August when it reached 0.9 ± 0.06 g. Later, the masses maintained values without great variation but declined during the last month to 0.8 ± 0.08 g.

In cohort I, fouling mass on the shell was evident in the fourth month of cultivation (December 2014) with an average value of 0.7 ± 0.12 g. There was no variation in the following month and then fouling mass increased and reached a weight of 3.7 ± 1.21 g at the end of the experiment. In cohort II, fouling mass was evident in the second month of culture (end of April 2015) with values of 0.4 ± 0.11 g, later remaining low in the next 3 months and then increasing until the end of September 2015 (2.1 ± 0.44 g) and then reduced and maintained in 2 g (Fig. 2a). The percentage of fouling mass associated to the mass of the shell followed a similar tendency of the accumulated fouling, except that in the first months the

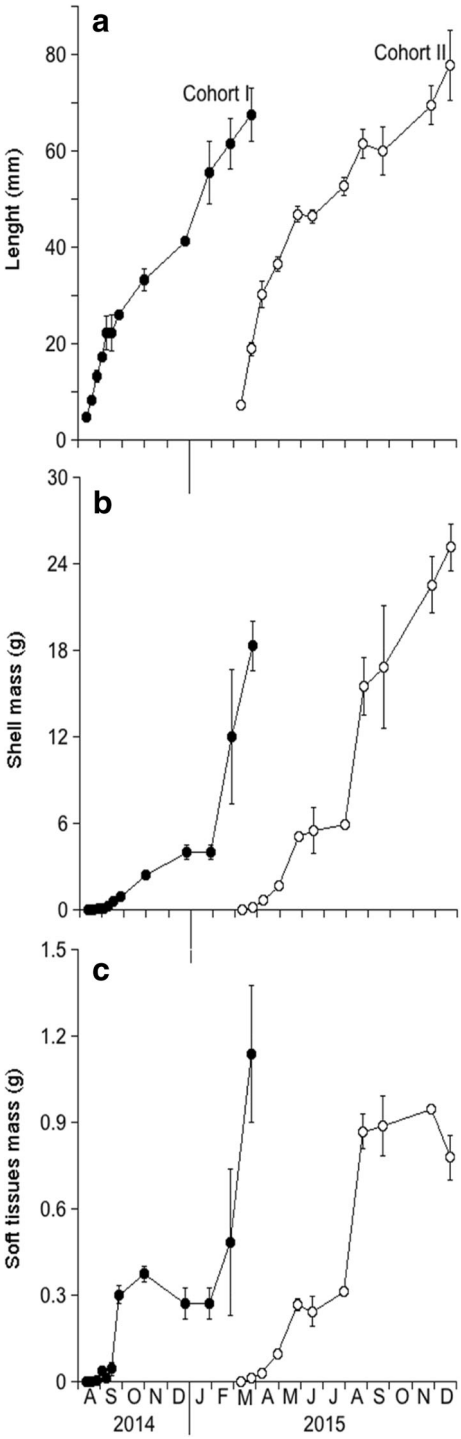

Fig. 1 Growth in length (dorsoventral axis; **a**), dry mass of shell (**b**), and soft tissues (**c**) of the Pacific oyster, *Crassostrea gigas*, under suspended culture conditions in Ayangue Bay, Santa Elena Province, Ecuador

values were elevated because the oyster shells did not show much growth. Values were in the range 12–22% and 3.5–27% for cohorts I and II, respectively (Fig. 2b).

The daily average seawater temperature during the experiment varied within a range of 23 to 29 °C, with minimum values (23.4–24) recorded in September 2014, early February 2015, mid-June 2015, and mid-August, while the maximum values (28–28.7 °C) were recorded during May–June 2015 (Fig. 3a). Values <26 °C were recorded from August to early December 2014 and from late July to late November 2015, and values >26 °C from early December 2014 to late July 2015. Intraday temperature variability was higher in mid-February than at the end of April 2015, with fluctuations of more than 6 °C (mid-March 2015, Fig. 3b). In September 2014, there was also an important and sustained intraday variability (1.5–4.7 °C), as well as from mid to late June 2015 (~ 4 ° C). In the rest of the year, the intraday variation was less than 1 °C indicating relatively constant temperatures during the day.

Total seston (Fig. 4a) showed great variability during the experimental period with values ranging from 2.5 to 14.5 mg/L. Chlorophyll *a* held values between 2 and 4 μg/L mostly (Fig. 4b). However, peaks of chlorophyll *a* were observed during August–September 2014 (5.1 μg/L), January 2015 (7.3 μg/L), and particularly between mid-October and mid-

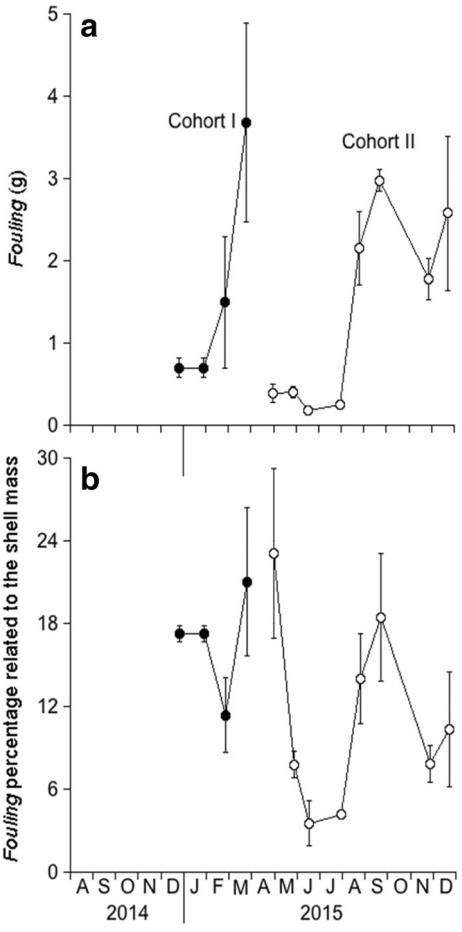

Fig. 2 Total mass of fouling (**a**) and percentage related to shell mass (**b**) of the Pacific oyster, *Crassostrea gigas*, under suspended culture conditions

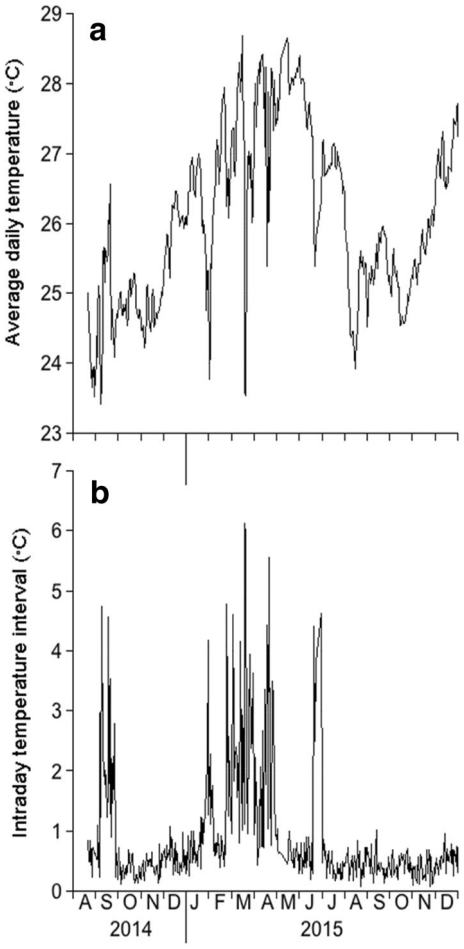

Fig. 3 Daily average (**a**) and intraday (**b**) temperature variability at the culture site of the Pacific oyster *Crassostrea gigas* under suspended cultivation at Ayangue Bay, Santa Elena Province, Ecuador

November 2015 (7–7.8 μg/L). On the other hand, salinity did not show a defined pattern but remained within a range of 32 (early July 2015) to 36 (end of November 2015) (Fig. 4c).

Multivariate analyses (combining all the variability generated by cohorts I and II) yielded little explanation of the observed shell growth variance, since only 16% was explained by the intraday temperature variability (Table 1). However, 43 and 47% of growth variance (shell length and mass) was explained by temperature and chlorophyll *a* variability, respectively. The variability of growth in each of the components in cohort II was explained (34–37%) by intraday temperature variability.

Discussion

Shell growth, both in length and mass, of the two *C. gigas* cohorts under suspended culture conditions in Ayangue, Province of Santa Elena, Ecuador, followed a linear trend in the first months with a decrease in slope in the later months, a characteristic of bivalve growth (Urban 2002). Likewise, growth rates of 0.28 and 0.25 mm/day in length of the cohorts I and II,

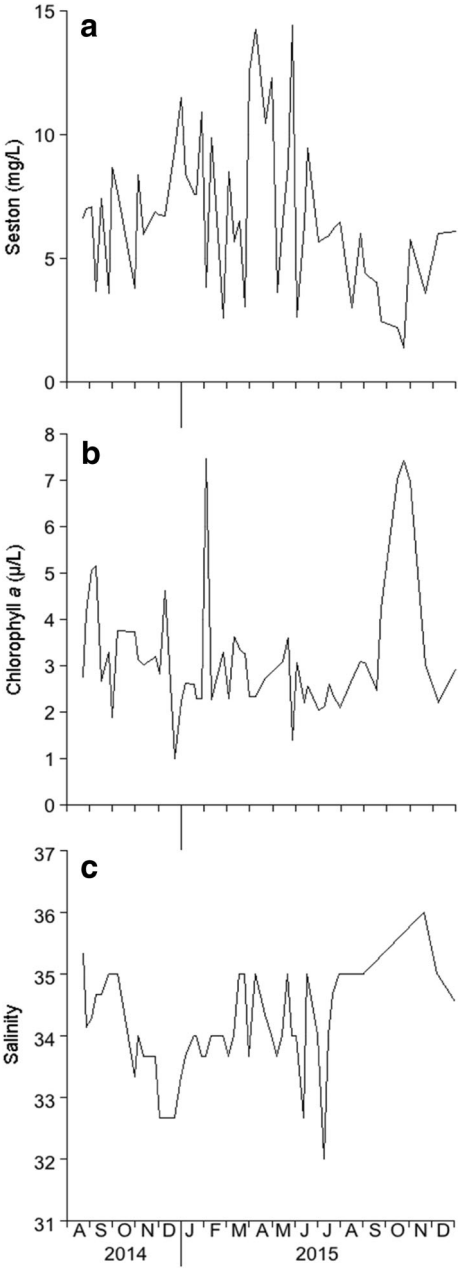

Fig. 4 Total seston (**a**), phytoplankton biomass estimated by chlorophyll *a* (**b**), and salinity (**c**) at the culture site of the Pacific oyster *Crassostrea gigas* at Ayangue Bay, Santa Elena Province, Ecuador

respectively, are concurrent with the growth rates observed in the subtropical coast of the Mexican Pacific, which are the highest recorded for the species (Chávez-Villalba 2014). With these growth rates, organisms of 90–110 mm in length could be produced in the first year of culture.

The study site was located in a transition zone of the American Pacific, characterized by a remarkable spatial and temporal variability of oceanographic conditions (Allauca 1990). This

Table 1 Models of *Crassostrea gigas* growth in length, mass of shell, and soft tissues under suspended culture conditions in Ayangue Bay, Santa Elena Province, Ecuador, according to the determined environmental variables and a multiple stepwise regression analysis. IDTemp = intraday temperature variability, Chlo *a* = chlorophyll *a*. Data analyzed and expressed in Log ($X + 2$). The input of independent variables to the model was established in $P < 0.05$. NS = non-significant. Fouling content was not included as an independent variable in the models because there were not enough data about this parameter

Growth	Models	R^2
All cohorts		
Shell length		NS
Shell mass	−0.27 + 0.12 IDTemp	0.16
Tissues mass		NS
Cohort I		
Shell length	0.70–2.77 Temperature	0.47
Shell mass	0.20 + 0.16 Chlo *a*	0.43
Tissues mass		NS
Cohort II		
Shell length	0.28 + 0.28 IDTemp	0.35
Shell mass	0.22 + 0.22 IDTemp	0.37
Tissues mass	0.24 + 0.17 IDTemp	0.34

is due to the fact that on the coast of Ecuador and particularly in the peninsular region where two of the largest marine ecosystems converge: The Central American Pacific Coastal and The Humboldt systems, establishing a remarkable transition zone characterized by a significant spatial and temporal variability of the physical-oceanographic conditions.

Oscillation of salinity in the culture site was only 2.5 units, and this variation does not seem to generate remarkable effects on the physiological processes of bivalve mollusks (Bernard 1983, Griffiths & Griffiths 1987, Lodeiros & Himmelman 2000). As for temperature, the values showed a general tendency in agreement with the summer cycle of the southern hemisphere. Some authors point out that the most important environmental factor in *C. gigas* cultivation is temperature (Castillo-Durán et al. 2010, Chávez-Villalba 2014). In the current study, temperature variability was, within its daily average or its intraday variance, the factor that explained most the growth variability in the models tested.

In cohort I (which showed the highest growth rate), the explanation of the variance of growth in the models was the highest (> 40%), being the daily average temperature (inversely proportional) and the phytoplankton biomass estimated by chlorophyll *a*, the factors included in the prediction models, indicating the importance of these factors in the modulation of oyster growth. A general observation was that temperatures > 26 °C promoted greater growth in both cohorts. This behavior contrasts with that observed in subtropical areas (North Pacific coast of Mexico), where summer temperature increases inhibit growth of both shell and soft tissue in *C. gigas* (Chávez-Villalba et al. 2010). Although *C. gigas* is a temperate and cold water organism, it has an euryhaline and eurythermic character (Miossec et al. 2009), which allows it to survive and adapt to temperature ranges from − 2 to 35 °C (Héral & Deslous-Paoli 1990); however, events of high temperatures, particularly in summer, can negatively influence growth and survival, associating stress and susceptibility to diseases (Kantzow et al. 2016).

Until now, there were no records of the introduction *C. gigas* into Ecuadorian waters since the species was brought in for aquaculture activities in the early 1990s (Alvarez et al. 2008). Hence, it is probable that the Pacific oyster has adapted, by selection, to local high temperatures and their variability, since the intraday variation can reach up to 6 °C, but this does not

seem to have negative effects on growth. This variable was also included in the growth models and explained it in a positive way.

According to Ren & Ross (2001), the organic particulate material of seston contributes little to growth of *C. gigas*. The current study supports this hypothesis, since there was no correlation between seston abundance with changes in growth. As for phytoplankton, studies that model the growth of *C. gigas* using chlorophyll *a* as the main component of the food indicate that it is the main source of growth support for the species (Ren & Schiel 2008). Mean values of chlorophyll *a* reported by the previous authors (0.8–1.6 μg/L) are lower than the range found in the study site, where values above 2 μg/L were generally recorded. This indicates that the food available was not a limiting factor (Saxby 2002). On the contrary, oysters had enough food to promote their growth throughout the year. In addition to including chlorophyll *a* concentration in the growth models, towards the end of the experiment, a higher growth of cohort II was observed concurrently with the higher concentrations of chlorophyll *a* (7–7.8 μg/L).

Although environmental factors do not appear to have a negative effect on oyster growth, it was found that soft tissues did not follow a typical exponential growth trend of bivalves since both cohorts showed a phase of growth reduction. This indicates that other external stressor factors, such as predation, may be influencing oyster growth locally, or it could be related to physiological processes such as reproduction (not studied in the experiment) where the energy demands are high (Lodeiros and Himmelman 2000). Gametogenesis is a process that requires a lot of energy, and in the case of *C. gigas*, this high requirement of energy is manifested when juveniles begin their sexual maturation (Royer et al. 2008). Research on reproduction of *C. gigas* related to growth and environmental variability on the Ecuadorian coast is necessary to verify the above hypotheses.

Reproduction studies are also necessary when considering aquaculture management of an introduced species such as the Japanese oyster. This is important for management of mature organisms in hatchery and spat production, as well as for determining how the reproductive processes of the species occur when it is cultivated. In the Mexican coast, *C. gigas* matures and partially spawns under culture conditions, but, apparently, high temperatures do not allow development and/or the fixation of larvae (Chávez-Villalba 2014). There is no evidence of recruitment of the species for natural banks in tropical areas of the American Pacific coast, and this would apply to the Ecuadorian coast as well. This may suggest the feasibility of expanding *C. gigas* culture in the tropical and subtropical American Pacific with a view of low environmental impact, particularly in the benthic communities. However, constant monitoring of oysters during cultivation is necessary since it has been observed that recruitment of the species occurs increasingly at higher temperatures (Crooks et al. 2015), probably as a process of species adaptation to environmental conditions of each place where it was introduced.

Fouling is an important factor in the growth of bivalve mollusks in culture (Lacoste & Gaertner-Mazouni 2015) and particularly in *C. gigas* (Chávez-Villalba 2014). Fouling on the shells in both cohorts initially increased and then decreased, which seems to indicate a biocontrol action by other community organisms within the pearl nets. This coincides with results of the use of sea urchins (organisms sporadically found inside the culture baskets) as fouling biocontrol in *C. gigas* culture along the Ecuadorian coast (Sonnenlhozner et al. 2017). Although a fouling effect on growth was not found, observations of abundant fouling on both the mesh of the baskets (unregistered) and on the shell (up to 27% related to shell weight), it may be important in the cultivation of the species. This can cause problems in filtration and other physiological processes in bivalves of horizontal disposition (Lodeiros 2002), as is the

case of *C. gigas* under cultivation conditions. In addition, the fouling in the baskets makes it necessary to intensify maintenance and cleaning, which can have effects on production costs. In fact, this was the reason for abandoning the cultivation of *C. gigas* by members of Cooperativa Isla de la Plata in Puerto Cayo, Manabí Province, Ecuador, after persistent heavy fouling by barnacles (J. Alio, pers. com.).

Results of this study show that both available food and temperature at the study site were favorable for Pacific oyster development under cultivation conditions. In particular, temperature had a significant effect on growth of *C. gigas*; high values (>26 °C) increased growth rates in both cohorts at different times of the year. Although tissue mass did not exhibit the typical exponential growth behavior, probably due to reproductive events, in general, growth rates were high and allowed prediction that the species can reach commercial sizes (80–90 mm) in less than 1 year of culture. Although the survival study was not analyzed in the present study, it is estimated that the accumulated survival in both cohorts was 60–65%, which is considered adequate for *C. gigas* culture (Chávez-Villalba 2014). This study shows that there is a potential to intensify cultivation of the species along the Ecuadorian coast. At the same time, studies are recommended on reproduction and those that allow to minimize the environmental stress and to optimize the culture techniques.

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