

Research Article

## The mangrove oyster *Saccostrea palmula* is an option for artisanal aquaculture development in the Mexican Central Pacific

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**ABSTRACT.** The mangrove oyster *Saccostrea palmula* is a relatively small but emerging species in aquaculture, and it is necessary to resume and increase studies on its cultivation. Therefore, we carried out a study that includes three main components for developing *S. palmula* culture in the Mexican Central Pacific: spat collection, pre-growth phase (eight weeks), and grow-out phase (six months). We tested natural (shell strings) and artificial (coupelles) collectors placed in contrasting environmental conditions: marine (La Boquita) and estuarine (Cuyutlan). The spat was pre-grown in suspended Australian trays at the two collection sites and under in-land pond conditions. Then, it was pooled under an estuarine environment and in a single lot for the final culture phase in Juluapan lagoon (all sites in Colima, Mexico). Spat collection was higher in coupelles ( $858.3 \pm 175.6$  spat coupelle<sup>-1</sup>) than in shell strings. The number of spat collected (13,062 spat), final pre-growth size ( $26.5 \pm 0.6$  mm shell height), and pre-growth survival (94.7%) were higher in La Boquita (marine environment). The final culture phase in Juluapan (estuarine conditions) was successful, recording an average height of  $54.2 \pm 4.9$  mm and average total weight of  $30.8 \pm 6.9$  g after six months of culture, after spat collection and pre-growth periods. We found the largest oyster, *S. palmula*, ever recorded (shell height 83.3 mm and total weight 59.4 g), and we confirmed that *S. palmula* has potential for artisanal aquaculture and socioeconomic regional development in the Mexican Central Pacific.

**Keywords:** *Saccostrea palmula*; oyster spat; pre-growth; grow-out; marine-estuarine aquaculture

### INTRODUCTION

The mangrove (=palmate) oyster *Saccostrea palmula* (P.P. Carpenter, 1857) is distributed from San Ignacio Lagoon (26.8°N) on the Pacific coast of Baja California Peninsula and from El Soldado Lagoon (27.6°N) in Sonora (Gulf of California) to Bayovar, Piura, Peru (5.9°S), the Cocos Island in Costa Rica and the

Galápagos Islands in Ecuador (Coan & Valentich-Scott 2012, Lodeiros et al. 2020). This oyster normally lives strongly attached to the roots of the red mangrove *Rhizophora mangle* and on rocks in the adjacent intertidal zones, but it can also be found at depths of 7 m (Félix-Pico et al. 2011, 2015). The coastal communities use the species as food, where it is exploited in an artisanal way, and some people take

advantage of the shells to make handicrafts (Holguín-Quiñones & González-Pedraza 1994). Nevertheless, there are no evaluations of the socioeconomic characteristics of the fishery or market demands (Asere et al. 2019), which are necessary to sustain this and other forms of exploitation (aquaculture).

*S. palmula* is a species with relatively few studies. Some contributions have been made to reproduction (Cabrera-Peña et al. 2001, Romo-Piñera 2005, Romo-Piñera et al. 2015, Alvarado 2018), using this oyster as a monitor for metals and metalloids (mercury, cadmium, copper, selenium) (Páez-Osuna & Osuna-Martínez 2015, Sepúlveda et al. 2021), or studying its role as a host of pathogens like *Perkinsus marinus* (Cáceres-Martínez et al. 2012). Growth-related studies indicate that the palmate oyster is a relatively small species. For example, Cruz & Jiménez (1994) reported a maximum shell length of 45 mm in Costa Rica, while in the estuary Morales of this country, Cabrera-Peña et al. (2001) found an average size of 39 mm shell length but a maximum size of 67 mm and a weight of 25 g. In addition, in Bahía de La Paz (Mexico), Félix-Pico et al. (2015) found a range size from 14 to 60 mm, indicating that the species can grow up to 50 mm annually. So far, the maximum size reported for the species is 75 mm in shell height (Keen 1971). Species growth data have been fitted using the von Bertalanffy growth model, obtaining the equation  $TL = 68.2 (1 - e^{-0.1577t})$  (Cabrera-Peña et al. 2001).

This oyster is seen as a species that can be produced through aquaculture. For instance, Molina-Camacho (1989) performed experimental cultivation using Nestier® plastic trays suspended from a longline system in Magdalena Bay (Mexico). The results were not very promising since the seeded juveniles showed marginal growth during cultivation. At the same time, some experiments were made to collect natural spat of the species by testing different substrates (car tires, fiberglass-resin sheets, polyethylene sheets, and scallop shells) also in Magdalena Bay and La Paz Bay (Mexico) (Chávez-Villalba & Cáceres-Martínez 1991, 1994). Similar results were obtained in both locations, with car tires being the most efficient collectors and recruitment throughout the year, with peaks from March to June. Although Cáceres-Martínez et al. (2012) reported sampling of palmate oysters for pathogen studies from various farming sites in Sinaloa (Mexico), to our knowledge, there is no published evidence on these oyster farms, and there are no reports on other collecting and farming studies of the species.

Due to the above, it is necessary to resume studies on the cultivation of *S. palmula* to determine the

species' potential to be exploited through aquaculture activities (Chávez-Villalba et al. 2021). Under this context, we performed a study considering the following objectives: 1) test two types of collectors (coupelles and shell strings) to obtain wild spat of the species, 2) cultivate the spat collected considering a pre-growth phase under contrasting conditions (marine, estuarine and in-land pond environments), and 3) perform the final growth-out cultivation phase in estuarine conditions.

## MATERIALS AND METHODS

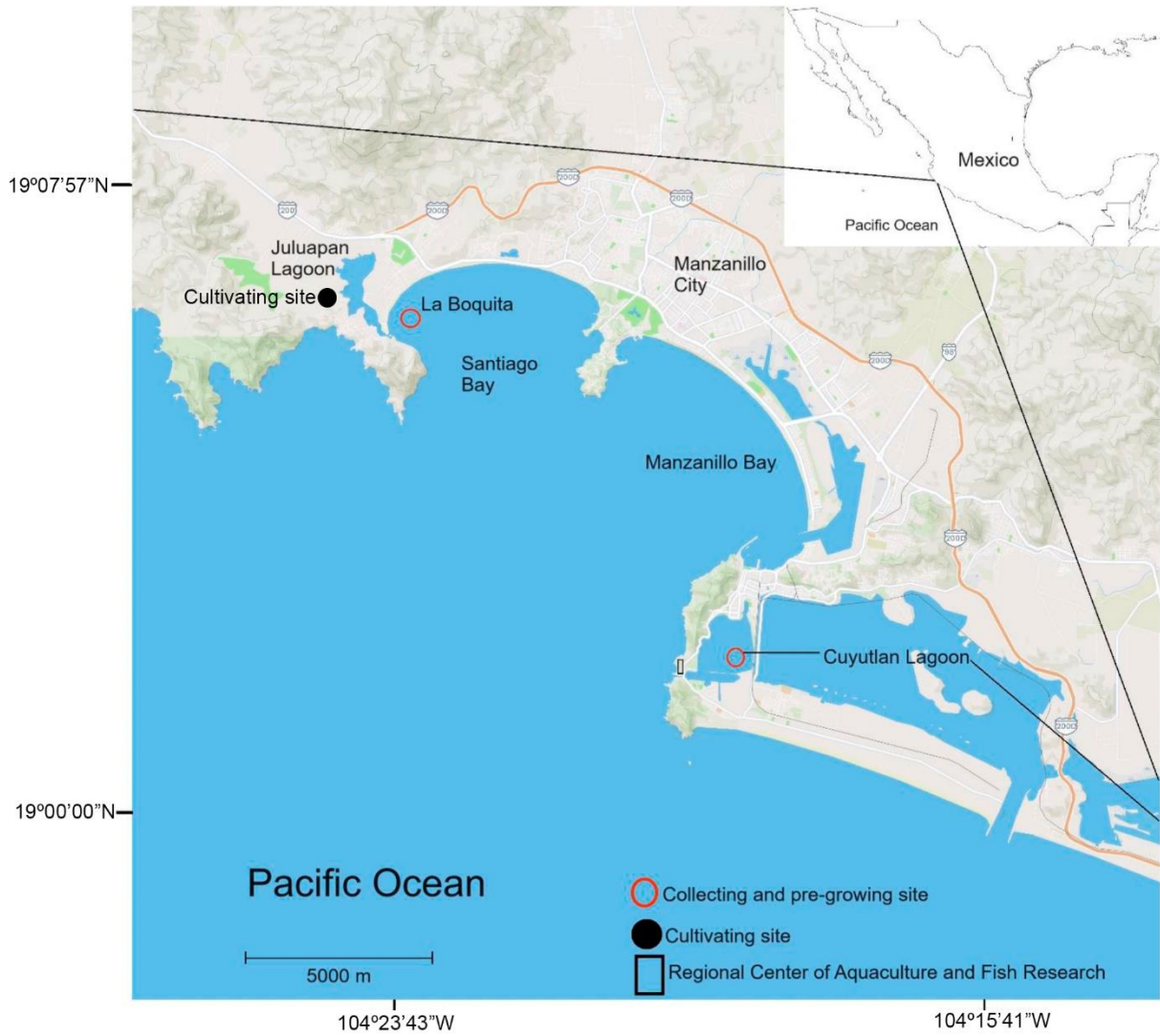
### Study area

Collecting and cultivating experiments were performed in three sites of the Mexican Central Pacific, specifically on the coasts of Colima (Mexico): La Boquita beach within Bahía Santiago (Santiago Bay) (19°02'06.79"N, 104°20'05.15"W), represented the marine condition site characterized by being an area 20 m deep, and Cuyutlan lagoon (18°56'-19°03'N, 104°00'-104°19'W) and Juluapan lagoon (19°07'36"-19°06'18"N, 104°24'-104°2'51"W) are typical bodies of water with low relief coastal plains representing estuarine conditions (Fig. 1): these lagoon sites will be referred to only as Cuyutlan and Juluapan throughout the document. The oysters were also maintained for pre-growth in inland ponds with the supply of cultured microalgae within the aquaculture facilities of the Centro Regional de Investigación Acuícola y Pesquera - Manzanillo (CRIAP) (Regional Centre of Aquaculture and Fish Research).

We include a graphic methodological reference (Fig. 2) to illustrate the experimental design of our study.

### Spat collection

Wild spats of *S. palmula* were collected using two types of collectors, shell strings and coupelles (Fig. 3). A shell string collector consisted of 20 oyster shells placed outside up and 10 cm apart on ropes 2 m long. The coupelle collector consisted of 48 discs (calcium carbonate-alloyed polyethylene) -16 cm in diameter- which were placed one on top of the other in a row on a PVC tube (¾") containing a rope with a weight of 0.5 kg at one end and a safety snap at the other. A total of 30 collectors of each type were built, placing 15 units each at the La Boquita site and 15 units each at the Cuyutlan site. The collectors in La Boquita remained inside the ocean for 50 days, from July 21 to September 9, 2020, while the collectors in Cuyutlan remained inside the lagoon for 43 days, from July 14 to August



**Figure 1.** Spat collection and cultivation sites for the palmate oyster *Saccostrea palmula* in Colima (Mexico).

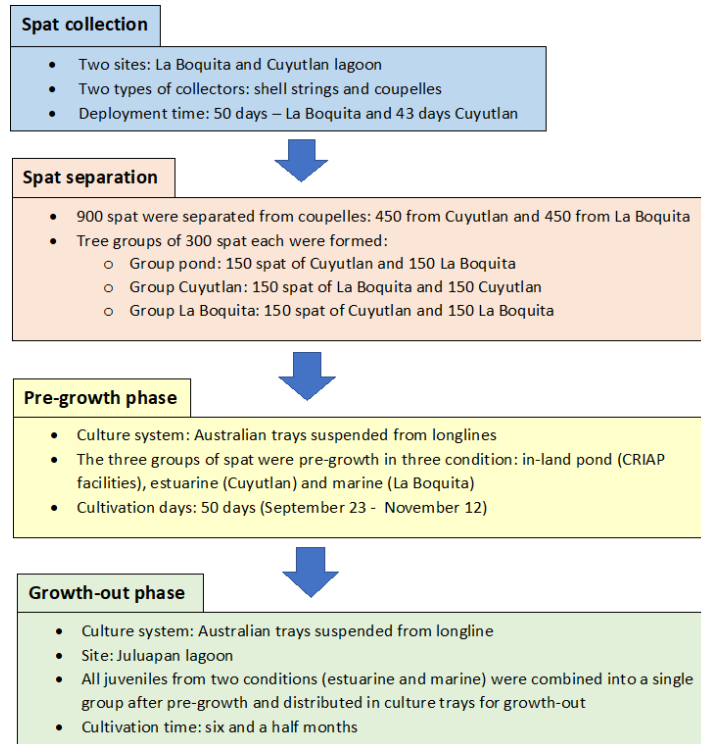
26, 2020. The spat/collector area was used to compare the results.

At the end of the collection periods, the collectors were taken to the CRIAP, where they were placed temporarily in 10,000 L ponds with circulating seawater and aeration; seawater was replaced daily. The spat fixed in the 30-shell string of both sites was counted to compare their number with the number of spat captured in the coupelles. The discs of each coupelles were inspected to separate the spat fixed on both sides, which were placed inside 1 mm mesh plastic bags (0.6 m length and 0.3 m width) and maintained inside the ponds. Of the total spat collected from the coupelles in the two collection sites (Fig. 2), 900 juveniles (spat) were combined (450 spat from Cuyutlan and 450 spat from La Boquita) and indivi-

dually marked with labels made of acetate sheets with printed numerical code that was cut and then pasted with cyanoacrylate glue to the left valve of oysters. The shell height (SH), length (SL), and width (SW) of the shell were measured for each detached spat with an electronic caliper, and the individual body weight (BW) was obtained using an electronic scale.

### Pre-growth phase

As a cultivation method, we used Australian oyster trays suspended from a longline system for the pre-growth phase. The tagged spat was placed directly inside the cultivation containers, considering a density of 50 oysters per tray, two trays per unit, and three units per site (replicates). The pre-growth phase was performed under three different environmental condi-



**Figure 2.** Graphic flowchart indicating the experimental design (phases and sequence of experiments) carried out for the artisanal cultivation of the palmate oyster *Saccostrea palmula*.

tions and combined an equal number of spats collected only of coupelles and two sites (three groups of 300 spat; 150 from La Boquita and 150 from Cuyutlan) (Fig. 2). Therefore, three cultivating units were placed in La Boquita having marine conditions, other three units were located in Cuyutlan having estuarine conditions, and the last three units were placed inside 100 L in-land ponds in the aquaculture laboratory of CRIAP, which represented the controlled conditions. The pre-growth phase was from September 22-24 until November 12, 2020 (~50 days). Sampling was conducted weekly to recover all the juveniles from the cultivating units. Juvenile oysters were cleaned and then measured and weighed individually; SH (dorsal-ventral axis), SL (anterior-posterior axis), and SW (right-left axis) were registered with an electronic caliper while the total fresh BW was recorded with an electronic balance.

The juvenile oysters maintained at CRIAP were fed three times a day with a 1:1 combination of *Chaetoceros gracilis* and *Isochrysis galbana* at a concentration of 125 cells  $\mu\text{L}^{-1} \text{d}^{-1}$ , respectively (Helm et al. 2006). For in-land ponds, the seawater in the holding ponds was changed 100% each morning, washing the oyster units and then filling the pond with

clean seawater. The rest of the time, the seawater in the ponds was connected to a recirculation system with biological filters, UV radiation, and plastic hoses that provided continuous aeration.

At the end of the pre-growing phase, all the juvenile oysters from the ponds, Cuyutlan, and La Boquita sites were moved to the CRIAP Manzanillo unit and placed in a 35,000 L capacity pond (100% water change daily). The oysters remained for 18 days with no supplemented food, only receiving raw seawater from the adjacent sea. The surviving oysters from the pre-growth experiment (~800), plus other 13,000 juveniles obtained during spat collection in Cuyutlan and La Boquita that remained fed on raw seawater at CRIAP in a 35,000 L pond, were combined and transferred to Juluapan, Santiago Bay (Colima), on December for final growth-out.

**Growth-out phase**

For this last phase, the combined group containing oysters from La Boquita and Cuyutlan, as well as those collected but kept in the CRIAP, were placed in the Australian trays not exceeding 25% of the surface of each unit (avoiding exceeding the carrying capacity of the container). A sample of 150 oysters was measured

and weighed at the beginning of the experiment, and from then, biometrics were performed every 15 days. Cleaning or interchange of trays and maintenance (take-off of predators) of the culture units was carried out monthly. The growth-out period lasted approximately six and a half months, from December 1, 2020, to June 19, 2021.

### Obtaining environmental variables at each stage

During the pre-growth phase, temperature ( $^{\circ}\text{C}$ ), salinity, and oxygen concentration ( $\text{mg L}^{-1}$ ) were measured in every sampling at 1.5 m deep in each location with a multiparameter probe (YSI-556). In the final phase, no measurements were made *in situ*, but monthly values of nocturnal sea surface temperature (NSST) and chlorophyll-*a* concentration (Chl-*a*) at 4 km scale processed by NASA Goddard Space Flight Center, Ocean Ecology Laboratory, Ocean Biology Processing Group (NASA 2014)- were also obtained, using a one-year temporality (2020). With the polygon previously obtained from the regionalization of the study area (Manzanillo), information was obtained from all the pixels (pxs) that coincided in the area using the SeaDAS<sup>®</sup> program. In the case of the CRIAP laboratory, the variables in the pond were recorded every three days with the probe.

### Data analysis

Kolmogorov-Smirnov normality and Bartlett homoscedasticity tests ( $\alpha = 0.05$ ) were applied to the data. If the assumptions mentioned above were not met, the corresponding non-parametric or free distribution tests were applied (Zar 2010). A one-way ANOVA was applied to determine significant differences in oyster size and weight between locations during the pre-growth phase (marine, estuarine-lagoon, and pond). Tukey's multiple comparison method determined significant differences between treatment means per experiment ( $\alpha = 0.05$ ). Finally, a Pearson analysis was performed to correlate the increase in size with environmental conditions (temperature, salinity, and oxygen) and biological productivity (Chl-*a*). The statistical package, SigmaStat v.3.1 (2004), was used.

The association between SL and BW growth, with water environmental (temperature, salinity, dissolved oxygen) and biological (Chl-*a* concentration) variables in laboratory condition (pond), the estuarine-lagoon (Cuyutlan), and (La Boquita) marine condition was explored with a Principal components analysis (PCA). The statistical package PC-ORD v.4.0 was used.

## RESULTS

### Spat collection

Spat settlement was observed in both collectors, with a greater amount on the inner face of the shells and discs and with depth. The two-way ANOVA test showed significant effects of both site and type of collector ( $P < 0.05$ ). A total of 12,874 spat were found in the coupelles and 188 in the shells from La Boquita (13,062 spat), while in Cuyutlan, 2,017 spat were collected from the coupelles and 66 from the shells. Therefore, the total spat obtained from both sites was 15,145. The average number of spat settlements was  $858.3 \pm 175.6$  per coupelle and  $13.4 \pm 4.8$  per shell string for La Boquita, and  $138.5 \pm 46.2$  per coupelle and  $4.4 \pm 1.5$  for shell string for Cuyutlan (Table 1).

The average SH of oysters was similar in both La Boquita ( $15.8 \pm 3.2$  mm) and Cuyutlan ( $16.2 \pm 3.4$  mm), showing a size range of 1 to 28 and 1 to 30 mm, respectively (Fig. 3). In terms of SL, the average was also similar between locations ( $15.4 \pm 3.5$  mm La Boquita and  $15.6 \pm 3.8$  mm Cuyutlan) showing a size range of 2-29 and 2-27 mm in that order. The average SW was also comparable ( $2.4 \pm 0.7$  mm La Boquita and  $2.6 \pm 0.8$  mm Cuyutlan) and presented an equal size range (1-5 mm). Finally, the average BW of the spat was  $0.49 \pm 0.2$  g for La Boquita and  $0.56 \pm 0.3$  g for Cuyutlan, while the weight range was 0.09-1.8 and 0.1-2.2 g respectively (Fig. 4).

On the other hand, the relationship between SH and BW of the spat in both sites was negative allometric, whose equation was  $\text{BW} = 0.1527 \text{SH}^{2.2508}$  ( $R^2 = 0.683$ ) for La Boquita and  $\text{BW} = 0.1529 \text{SH}^{2.2923}$  ( $R^2 = 0.677$ ) for Cuyutlan. The relationship of SH and SL of the shell in both sites was linear, whose equation was:

$\text{SL} = 0.6511 \text{SH} + 0.6363$  ( $R^2 = 0.360$ ) for La Boquita and  $\text{SL} = 0.6168 \text{SH} + 0.6432$  ( $R^2 = 0.310$ ) for Cuyutlan.

### Pre-growth phase (eight weeks)

The pre-growth survival was generally high, with 83% for Cuyutlan, 88.7% for ponds, and 94.7% for La Boquita. The juveniles at La Boquita showed better performance, recording greater increases in SH ( $F = 162$ ,  $P < 0.5$ ), SL ( $F = 72.62$ ,  $P < 0.05$ ), SW ( $F = 225.1$ ,  $P < 0.05$ ), and BW ( $F = 178.7$ ,  $P < 0.05$ ), than at Cuyutlan and ponds (Fig. 5).

The two-way analysis of variance with a randomized design indicated significant differences ( $P < 0.05$ ) in the daily growth rate (DGR) during pre-growth, as well as in the interaction of factors. Oyster

**Table 1.** Number of spat collected from the palmate oyster *Saccostrea palmula* by site and type of collector. SD: standard deviation.

Site collector	La Boquita		Cuyutlan	
	Coupelle	Shell string	Coupelle	Shell string
1	1112	13	157	3
2	985	14	92	5
3	925	20	175	6
4	1022	8	95	6
5	682	24	142	3
6	769	14	148	4
7	598	7	57	7
8	1054	9	164	5
9	867	15	178	2
10	720	13	63	3
11	961	8	172	4
12	547	15	65	5
13	750	17	158	3
14	834	11	170	4
15	1048	-	181	6
Average ± SD	858.3 ± 175.6	13.4 ± 4.8	134.5 ± 46.2	4.4 ± 1.5
Spat collected	12,874	188	2,017	66



**Figure 3.** a) Shell string and b) coupelle collectors for the wild spat collection of the palmate oyster *Saccostrea palmula* in the Mexican Central Pacific.

spat collected at Cuyutlan and grown in the ponds, Cuyutlan and La Boquita, showed higher DGR (Tukey's test  $P < 0.05$ ), respectively; and the spat collected at La Boquita and grown at the same site, Cuyutlan and ponds showed lower DGR (Tukey's test  $P < 0.05$ ) (Fig. 6).

During the pre-growth phase, PCA revealed correlations between oyster growth in SH and BW, with temperature, salinity, dissolved oxygen, and Chl-*a* concentration, mainly for the La Boquita site and partially in Cuyutlan. The first component (PC1) explains 80.6% of the total variation, and PC2 explains

only 19.4% of the total variation. The first axis correlated positively with temperature, dissolved oxygen, and Chl-*a* and negatively with salinity. The second axis correlated negatively with all variables, particularly salinity (-0.92). The total cumulative variation of the two components (PC1 + PC2) explains 100% (Fig. 7).

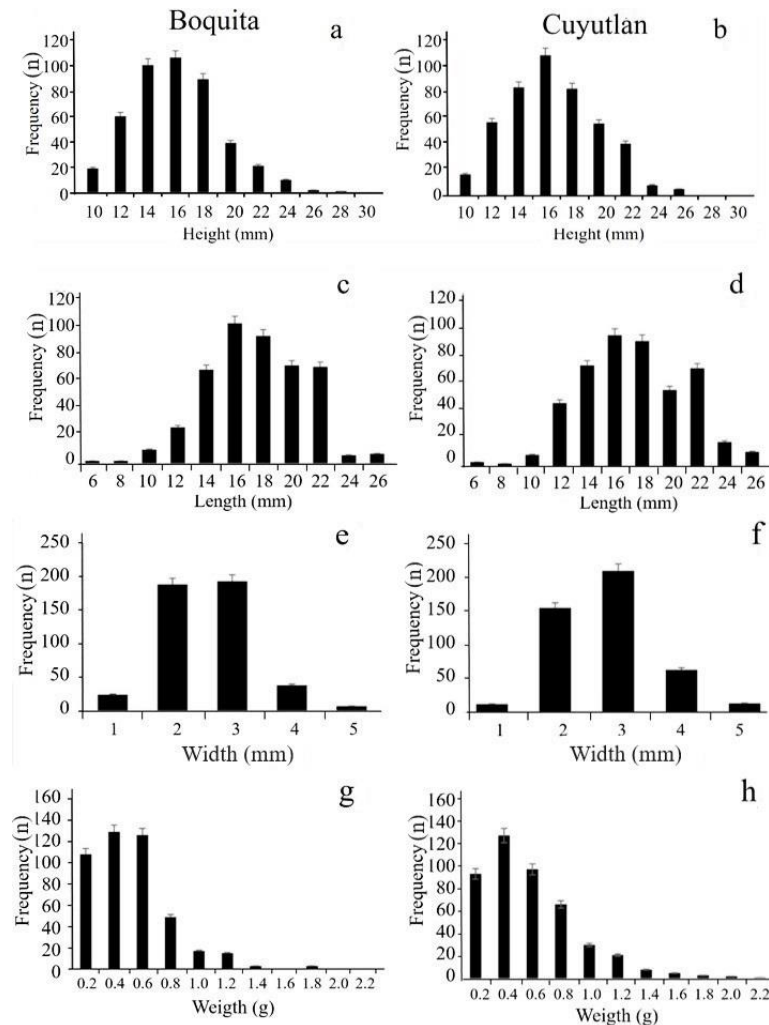
**Growth-out phase**

At the end of seven months (November 2020 to June 2021) of the growth-out phase for juveniles in suspended units in Juluapan (Colima), oysters recorded an average SH of  $54.2 \pm 4.9$  mm and  $30.8 \pm 6.9$  g average BW, with maximums of 83.3 mm in SH and 59.4 g in BW, respectively. The average DGR was  $0.177$  mm d<sup>-1</sup> in SH and  $0.169$  g d<sup>-1</sup> in BW, with maximum DGR values from December 2020 to February and from April to June for SH and BW (Fig. 8), reaching the adult size in less than seven months of culture. The final oyster survival was 97.3%.

**Environmental variables**

**Pre-growth phase**

Surface water temperatures at La Boquita and Cuyutlan showed a similar pattern during the pre-growth phase, decreasing from an average of 30°C in September to 29°C in November; the average water temperature in the pond was  $28.6 \pm 0.8$ °C during the eight weeks. Salinity also followed a similar pattern at both sites (sea, lagoon), remaining above 34, except September



**Figure 4.** Frequency distribution of shell height, length, and width, as well as the body weight of the spat of the palmate oyster *Saccostrea palmula* collected from the coupelles in a,c,e,g) La Boquita and in b,d,f,h) Cuyutlan.

29-30 and October 01, when it reached values of 26, caused by tropical storms in Manzanillo, Colima, during those dates (2020). Dissolved oxygen concentration recorded mean values above ( $6 \pm 1.11 \text{ mg L}^{-1}$ ), with a minimum value of  $4.4 \text{ mg L}^{-1}$  and a maximum of  $8.5 \text{ mg L}^{-1}$ . The variation in Chl-*a* concentration was similar at Cuyutlan ( $0.10 \text{ mg m}^{-3}$ ) and La Boquita ( $0.14 \text{ mg m}^{-3}$ ), with increases at both sites during October ( $1.15 \text{ mg m}^{-3}$ ), decreasing towards November ( $0.28 \text{ mg m}^{-3}$ ) (Fig. 9).

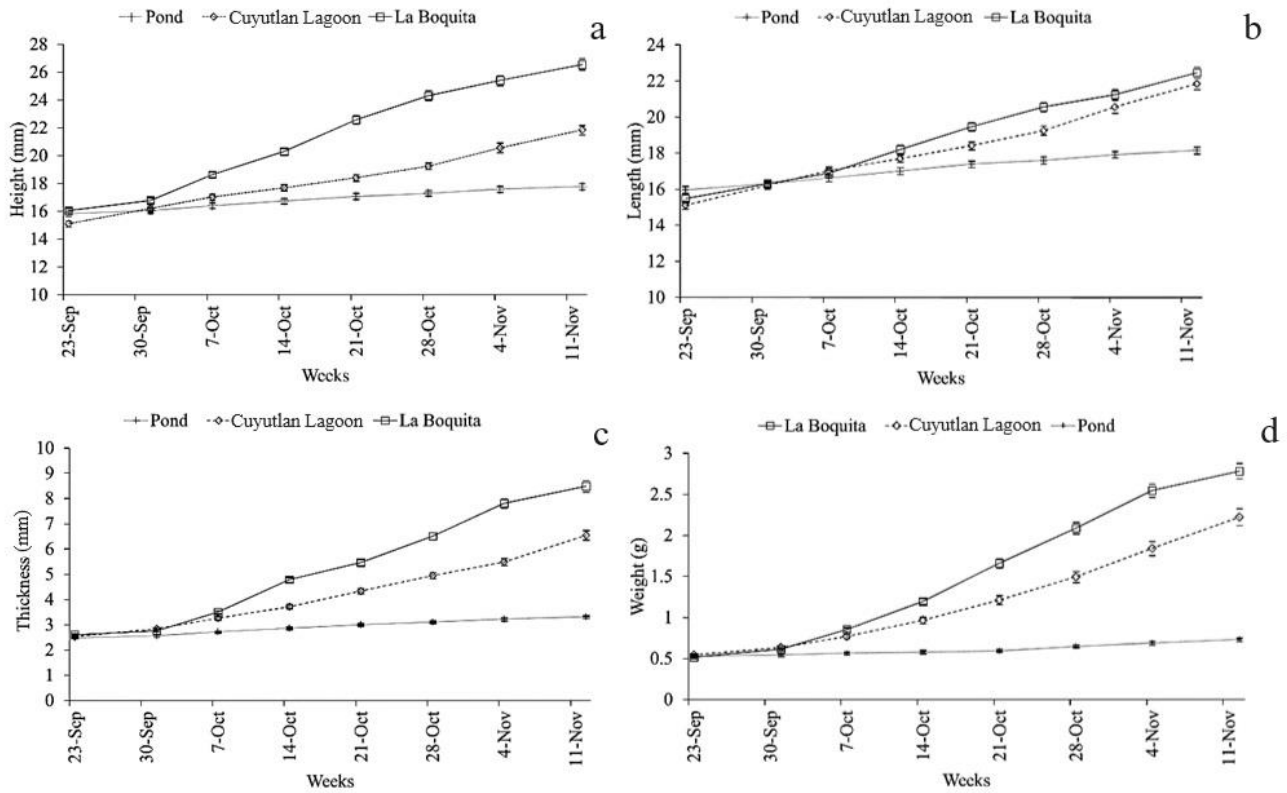
### Final growth phase

The temperature in Juluapan from November 2020 to June 2021 ranged from  $25.5$  to  $28.8^\circ\text{C}$ , with lower temperatures from February ( $25.4^\circ\text{C}$ ) to April ( $26.9^\circ\text{C}$ ). Chl-*a* concentration in the same period ranged from  $4.1$  to  $0.2 \text{ mg m}^{-3}$ , with highest concentrations in March ( $4.1 \text{ mg m}^{-3}$ ) and April ( $3.3 \text{ mg m}^{-3}$ ) (Fig. 10).

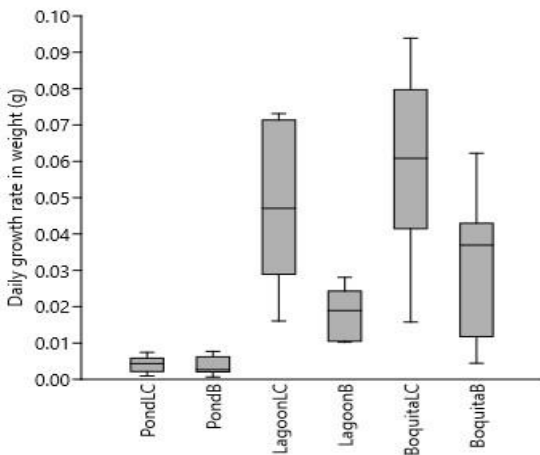
## DISCUSSION

### Spat collection

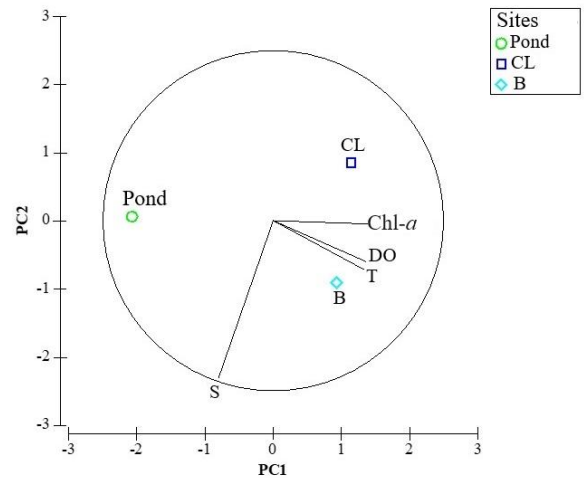
The two substrates for collecting oyster spat promoted its growth in all experimental sites. Although there are proven substrates for spat collection of several species of bivalve mollusks (Dunn et al. 2014, La Peyre et al. 2014, Hasan et al. 2021), little is known about the collection of the mangrove oyster *S. palmula*. For example, there are only two studies made by Chávez-Villalba & Cáceres-Martínez (1991, 1994) reporting the spat collection of this oyster in natural and artificial substrates suspended in the shallow sublittoral of La Paz Bay and in an estuary of Magdalena Bay, Baja California Sur (Mexico). Our results coincide in the preference for spat settlement on artificial substrate and in not finding any relationship between the number of



**Figure 5.** Mean value (± standard error) of a) shell height, b) length, c) spat thickness and d) body weight of the palmate oyster *Saccostrea palmula* during the pre-growth phase from September 23 to November 12, 2020 in ponds, Cuyutlan and La Boquita.

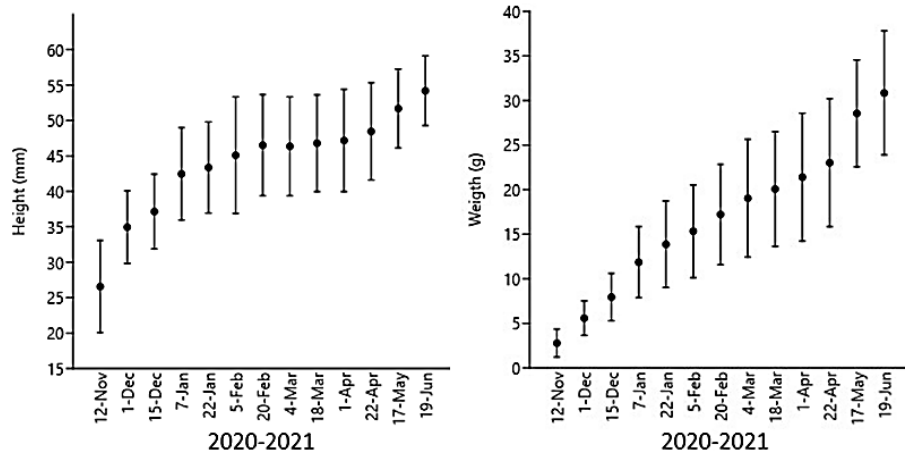


**Figure 6.** Daily growth rates of the palmate oyster *Saccostrea palmula* in body weight (spat) kept in ponds (PondLC, PondB), in Cuyutlan (LagoonLC, LagoonB), and La Boquita (BoquitaLC, BoquitaB). B: La Boquita and LC: Cuyutlan.

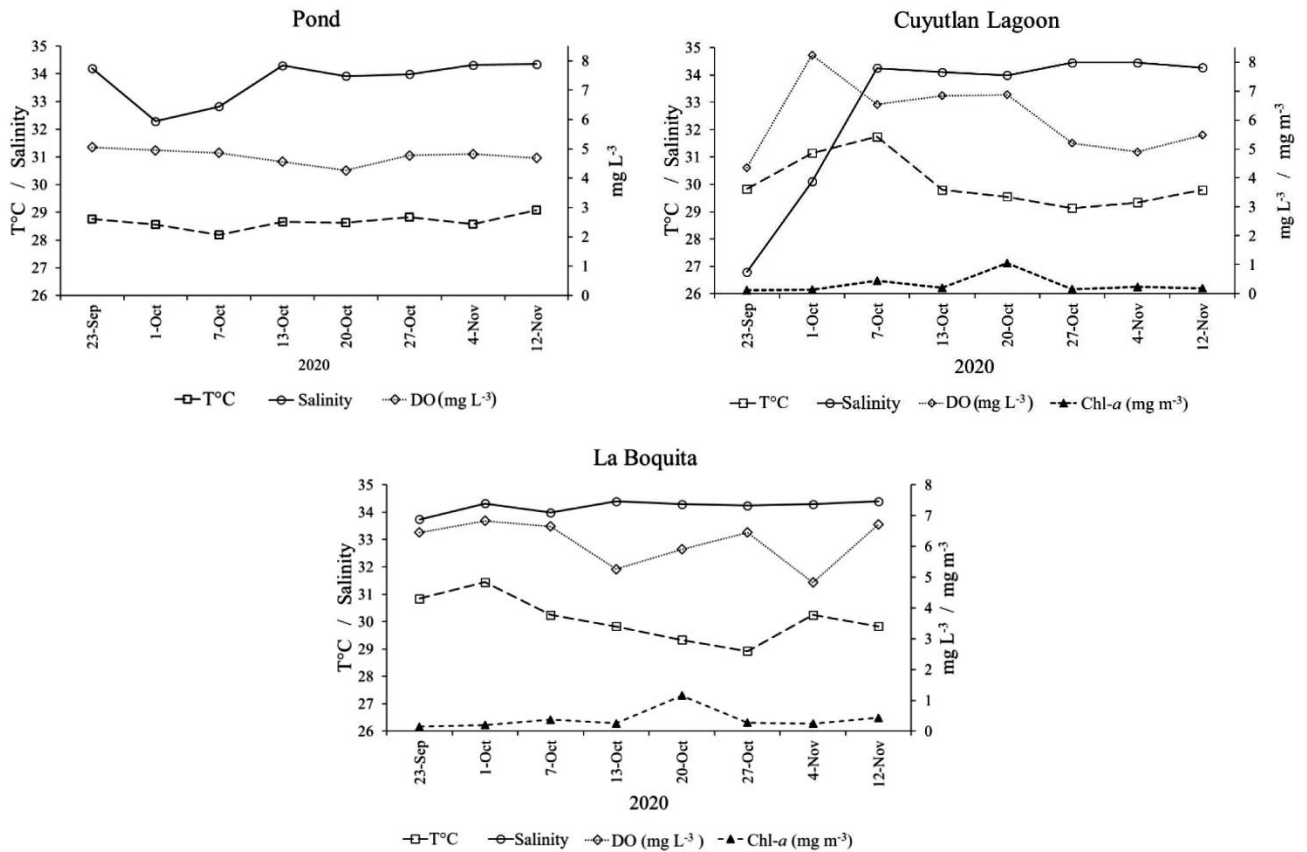


**Figure 7.** Bray-Curtis ordination of spat growth of the palmate oyster *Saccostrea palmula* in ponds, in La Boquita (B), and Cuyutlan (CL), during the eight weeks of pre-growth, considering the association of the variable temperature (T), salinity (S), dissolved oxygen (DO) and chlorophyll-a (Chl-a) concentration.





**Figure 8.** Growth curves in shell height and body weight during the growth-out phase of the palmate oyster *Saccostrea palmula* in Juluapan (Colima, Mexico).

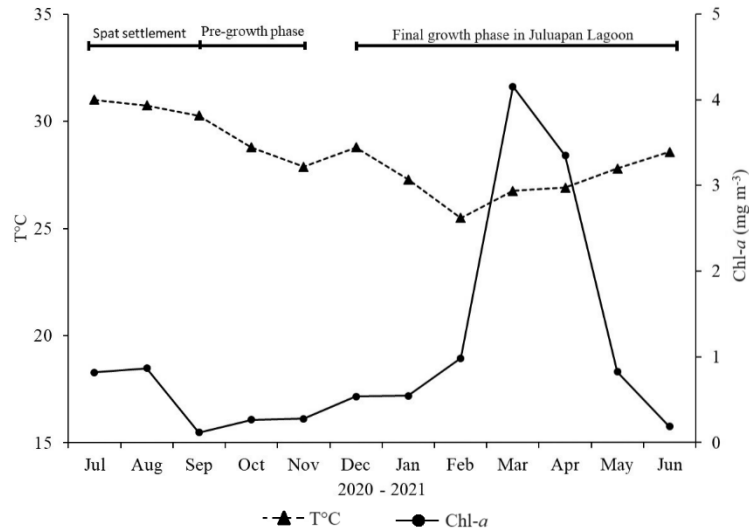


**Figure 9.** Mean values of environmental variables during pre-growth of the palmate oyster *Saccostrea palmula* at different culture sites: La Boquita, Cuyutlan, and ponds. T°C: temperature, DO: dissolved oxygen, Chl-a: chlorophyll-a.

spat collected and temperature (°C), salinity, and dissolved oxygen concentration (mg L<sup>-1</sup>).

Around the second week of monitoring shell and couple strings (July 28, 2020), there was evidence of

spat collected in both devices' middle and deep sections, which could indicate that both substrates already showed maturation on their surface with the presence of biofilm (a film of bacteria and cyanobacteria that develop and prepare the solid substrate for the



**Figure 10.** Values of temperature (T°C) and chlorophyll-a concentration (Chl-a (mg m<sup>-3</sup>)) during the pre-growth (La Boquita, Cuyutlan, and ponds) and final growth-out phase of the palmate oyster *Saccostrea palmula* at Juluapan Lagoon. T°C: Dotted line and triangles is the T°C and the solid line and black circles is the Chl-a.

settlement of marine invertebrates); a necessary condition for the settlement of aquatic invertebrate larvae, in addition to other physical conditions such as gravity, light, surface orientation, color, among others (Michener & Kenny 1991, Taylor et al. 1998). Poirier et al. (2019) exposed experimental substrates for 72 h with unfiltered seawater and developed biofilms, settling *Crassostrea virginica* larvae after two weeks under pond conditions. Similarly, Tamburri et al. (2008) began to record the settlement of *C. ariakensis* in the substrates offered in the first 15 days in an experiment that recreated a mesocosm and a wide variety of natural and artificial substrates. This time is more or less consistent with the time it took for the oyster larvae to settle on the shell strings and coupelles, as these devices were placed on July 14, 2020, in Cuyutlan, and one week later in the case of La Boquita (July 21, 2020). Nevertheless, some authors report a shorter settlement time for larvae of bivalve species under pond conditions, such as Su et al. (2007), who recorded settlement of *Pinctanda martensii* spat on the experimental substrates from the fourth day.

Several authors indicate a preference for spat settlement of various bivalve species on natural solid substrates, such as shell surfaces or mangrove roots, and in the case of artificial substrates, such as plastic and other manufactured materials (Devakie & Ali 2002, Tamburri et al. 2008, Almeida-Funo et al. 2019, Hasan et al. 2021). The settlement process is linked to the maturation conditions of the substrate surface, whether natural or artificial (Crisp 1974), and of the oyster spat

in particular (Tamburri et al. 1996). In our study, the collectors spent a different time in La Boquita (51 days) than in Cuyutlan Lagoon (43 days), but both times provided a good margin for the oyster spat to settle. The collectors in La Boquita stayed longer due to the passage of the tropical storm (Hurricane Hernan) through the study area. On the other hand, Poirier et al. (2019) suggest that oyster spat will preferentially settle on the bottom on a natural substrate (shell) than on an artificial substrate suspended in the water column. However, the best collection results were obtained with the coupelles, composed of a PVC plastic disc mixed with calcium carbonate from the shells. According to Tamburri et al. (2008), these materials, together with the roughness of the surface of the coupelles (concentric lines) on both sides, make them attractive for larval settlement compared to natural substrates. For example, our results indicated an acceptable settlement density of 247.7 spat m<sup>-2</sup> on the coupelles. This system is also practical for recovering the devices suspended in the water column since it allows handling from the surface for cleaning and spat registration. All this was verified during our operations in Cuyutlan and the shallow sublittoral of La Boquita.

Another highlight is that the spat was mainly attached to the bottom of the shells and the coupelles. The spat settles on the shell's concave part and inside the coupelles' discs. This pattern revealed a clear negative phototropism to the effect of surface area illumination during the day and moonlit nights or urban areas. Negative phototropism is documented in *P.*

*martensii* as it mostly attaches to dark substrate and lower illumination (Su et al. 2007). Similar work is reported by Saucedo et al. (2005), who reported a higher spat set of *Pinctada mazatlanica* on dark substrate (red/black), indicating that settlement of these bivalves prefers dark substrates and areas with lower illumination.

Our results showed differences in abundance and structure of sizes and weight in the spat depending on the collection site. For example, the highest collection was recorded in La Boquita. Still, the spat collected in Cuyutlan presented a distribution of sizes and weight in class intervals to the right with greater frequency, that is, of greater size and weight even with the difference in submerged time, and this could be linked to different environmental conditions and circulation of currents between sites; in Cuyutlan the spat found more suitable conditions as greater food supply and lower dynamics. Hasan et al. (2021) obtained similar results, with a higher density of oyster spat fixed on the artificial substrate (tire rubber) under higher salinity and Chl-*a* concentration conditions. The effect of the environment (site) and the substrate (natural and artificial) on the settlement of the larva plays a relevant role in the collection of the native spat of *S. palmula* in the region, which, with proper management, can be incorporated as a mitigation alternative to fishing natural oyster banks by local fishermen. Likewise, the efficiency of natural substrates other than shells in collecting spat of the species in other natural areas of the region should be evaluated.

### Pre-growth

The variation in temperature, salinity, dissolved oxygen, and Chl-*a* in the experimental sites during the first 50 days of spat growth between September and November 2020 was in an optimal range for the adequate development of the mangrove oyster. For example, the temperature oscillation range was between 27.2-31.7°C, while the salinity range was between 26-34, implying no environmental stress. In Cuyutlan, Sosa-Avalos et al. (2013) report a thermal oscillation from 26.8 in May to 31.9 in October, where salinity decreases significantly in the rainy months (August, September, and October) to values close to 26 during tropical storms and hurricanes. During the eight-week experimentation period, the dissolved oxygen concentration was within the optimal range, with a minimum of 4.4 mg L<sup>-1</sup> and an average of more than 6 mg L<sup>-1</sup>. However, Sosa-Avalos et al. (2013) recorded lower concentrations for July (2.7 mg L<sup>-1</sup>), October (2.3 mg L<sup>-1</sup>), and December (3.7 mg L<sup>-1</sup>), values that may

limit the optimal development of the oyster and other species in this coastal lagoon. Juárez-Pedroza (2013) indicated that since the expansion of the Tepalcates channel in 2005 in Cuyutlan, like the even older Ventanas channel, the hydrodynamics of this lagoon have changed because it presents a greater exchange of seawater with the lagoon system, reflected in the levels of tides, water mirror, speed and direction of the currents, as well as environmental variables and therefore positive changes in the distribution of the mangrove community and of the entire estuarine-lagoon ecosystem.

In the case of Chl-*a* concentration, various authors report the months of highest concentration during winter (December, January, February) and spring (March, April, May) and the lowest concentration, from June to November, both in Santiago Bay, where La Boquita site is located, as in Cuyutlan (Sosa-Avalos et al. 2006, 2013). This condition of lower biological production in the study area could have affected the development of this oyster (growth) during the eight weeks of pre-growth. Nevertheless, filter-feeding organisms, such as those of the Ostreidae family, in addition to the phytoplankton load as the main food, filter suspended particles (bacteria, microzooplankton, detritus, dissolved organic matter) contributing a relevant percentage of the available food when the blooms of phytoplankton are lower (Gosling 2003). In the study carried out by Castillo-Durán et al. (2010) with *Crassostrea corteziensis* and *C. gigas* in the coastal lagoon Las Guasimas (Sonora), the concentration of Chl-*a* was not a limiting factor for the growth of juvenile organisms, but the high concentrations of seston were.

Spat oyster growth under pond conditions was significantly lower in size and weight, probably due to various or multifactorial causes, mainly food. For instance, stress is caused by confinement in the pond, where the spat is placed inside a mosquito net bag. An oyster container could affect growth by hindering the free flow of water and limiting the food offered through filtration. Although the suggested ration (250 cells μL<sup>-1</sup> d<sup>-1</sup>) offered in the pond was similar to that used by some authors (Helm et al. 2006, Argüello-Guevara et al. 2013), likely, the oyster juveniles did not fully filter the concentration of microalgae in the pond, due to the reduced movement of water inside the pond, concerning the dynamics of the water column of the natural environment of La Boquita and Cuyutlan. Although growth in the pond was marginal, survival was higher (>88%) than spat growth in Cuyutlan (83%). The temperature and dissolved oxygen were lower in the

pond during the eight weeks of pre-growth concerning Cuyutlan and La Boquita sites, which could influence juvenile growth. For example, the temperature was constant, but from two to three degrees less than in the other two sites, in addition to a lower dissolved oxygen concentration of up to  $4 \text{ mg L}^{-1}$  less. All this could stress the oysters and send the energy from the food only to maintenance functions, but not to the increase in size and weight. Something similar occurred in the growth evaluation of *C. gigas* and *C. corteziensis* during summer and winter in Las Guasimas Lagoon (Sonora), where both species showed lower growth with lower temperatures and dissolved oxygen concentration (Castillo-Durán et al. 2010).

Unlike the results in pond conditions, *S. palmula* juveniles in marine and estuarine conditions developed well, and it was shown that the pre-growth of the species could be carried out in either of these two environments, which is an advantage for groups of producers who want to develop cultivation activities since they have more than one alternative from where to choose.

### Growth-out

In this study, the spat was collected and pre-grown at three different sites (marine, estuarine, and ponds), and the initial idea was to continue farming in both environments until the oysters reached adult size. However, due to the COVID-19 pandemic, it was decided to combine all the juveniles in a single group, move them to Juluapan, and follow them up to adult size. Despite all this, the results obtained in the final phase are very interesting due to the good growth observed. For example, the only available reference on this species' culture was in estuarine conditions (Magdalena Bay, Mexico), which showed poor growth results since juveniles only increased 5 mm in average size in six months of suspended culture (Molina-Camacho 1989). In contrast, the results here showed that the juveniles increased 30 mm in SH in seven months of culture, showing constant rises in size and weight throughout the entire experiment. The results are similar or even better when compared to other small oysters, such as *C. rhizophorae* in suspended culture; Wedler (1980) recorded 70 mm shell oysters after eight months, while Villarroel et al. (2004) obtained only a 15 mm increase in SH of 30 mm juveniles after six months. According to Félix-Pico (2015), *S. palmula* can grow up to 50 mm in natural conditions during one year, so growth in culture could be even higher.

As mentioned in the introduction, *S. palmula* is a relatively small species with a maximum SH of 45 mm

in Costa Rica (Cruz & Jiménez 1994), 67 mm SH, and 25 g BW also in Costa Rica (Cabrera-Peña et al. 2001), 60 mm SH in Mexico (Félix-Pico et al. 2015), or 75 mm SH according to Keen (1971). Nevertheless, in our study, we found an individual with an SH of 83.3 mm and a BW of 59.3 g, which could represent the largest *S. palmula* oyster ever found. Good growth results and these organisms with large sizes can only be obtained under optimal environmental conditions, which indicates that the Juluapan site is an ideal site to develop the species' culture.

### CONCLUSIONS

To our knowledge, there is no published evidence on collecting and farming studies of the species *S. palmula*. Still, our experiments showed satisfactory results in the collection phase and the species' pre-growth and final growth. According to our findings, the best collection results were obtained with the artificial substrate (coupelles) in both marine and estuarine conditions. Although some differences were detected between the spat collected at both sites, the two environments are conducive to obtaining spat. Likewise, the pre-growth was adequate in both sites. The follow-up during the final phase showed encouraging results, producing oysters of a size and weight above what was expected. The final culture phase in Juluapan (estuarine conditions) was successful, recording an average SH of  $54.2 \pm 4.9$  mm and an average BW of  $30.8 \pm 6.9$  g. A large organism was obtained with a size and weight never recorded before (SH = 83.3 mm and BW = 59.4 g). All of the above leads us to highly recommend *S. palmula* as a potential alternative to develop artisanal aquaculture activities in the Mexican Central Pacific. We are also considering market studies and trying to generate protocols for producing spat in a hatchery.

### Data availability

Data are available on request from authors.

### Credit author contribution

M. Durand-Acosta: conceptualization, validation, methodology, formal analysis, writing-original draft; J. Chávez-Villalba: conceptualization, analysis performed, review and editing; S.M. Abad-Rosales: conceptualization, analysis performed, review and editing; S.F. Cisneros-Gaytán: funding acquisition, project administration, supervision; E. López-Uriarte: conceptualization, funding acquisition, project administration, supervision, analysis performed, review and editing.

### Conflict of interest

The authors declare that they have no conflicts of interest.

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