Ulva blooms in the southwestern Gulf of California: Reproduction and biomass

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ABSTRACT

Ulvacean blooms are generally characterized by one or more *Ulva* species, some of which are common to blooms across a broad geographic range. In tropical environments the identified stimuli that induce reproductive development of *Ulva* are restricted to temperature, salinity, dehydration and fragmentation. Culture studies have been prolific in describing the reproduction of *Ulva* species, but its reproductive changes in natural environment have not been described yet. Hence, seasonal changes were described in reproductive stages and their relationship with biomass and environmental factors of *Ulva* species at four macroalgal blooms in a subtropical bay. Eight *Ulva* species were found: *U. acanthophora*, *U. clathrata*, *U. flexuosa*, *U. intestinalis*, *U. lactuca*, *U. lobata*, *U. nematoidea* and *U. rigida*. Reproductive stage and biomass varied according to site and season. Five species showed four reproductive stages (vegetative, thallus with fully differentiated zooids in formation and empty cells after zooids release); for the remaining species only vegetative thalli were found. *Ulva rigida* showed the highest biomass values, followed by *U. acanthophora*, following a seasonal pattern.

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1. Introduction

Ulvacean blooms are generally characterized by one or more species of *Ulva*, some of which are common to blooms across a broad geographic range. Their success depends on their rapid growth rate and opportunistic life history (Merceron et al., 2007). In temperate and cold waters, reproduction, proliferation and liberation of *Ulva* zoospores, gametes, and asexual zooids are driven by a wide range of stimuli (e.g. lunar periodicity, dehydration, fragmentation, salinity, and temperature changes); in contrast, the identified stimuli that induce reproductive development of *Ulva* in tropical environments are restricted to temperature, salinity, dehydration, and fragmentation (Carl et al., 2014).

It is also known that changes in biomass could be attributed to seasonal changes, mainly in temperature, light intensity, water movement, tides and nutrients (Whitehouse and Lapointe, 2015). When these variables are appropriate, the release of propagules takes place; then when settlement occurs, they give rise to macroalgal blooms or "green tides". For most species of green macroalgae, the micro-propagule stage and macroscopic vegetative fragments contribute to their opportunistic life history (Worm and Lotze, 2006; Fang et al., 2012).

In the Gulf of California, seasonal cycles of seaweed biomass have been reported with the maximum biomass in spring and its subsequent disappearance in late summer for most of the species; these changes have been related to temperature peaks and changes in nutrient concentrations in the water column (Pacheco-Ruíz et al., 2002; Casas-Valdez et al., 2016). Changes in *Ulva* biomass have been reported in Bahía de La Paz with biomass values that ranged from 351 to 537 g m^{-2} (Águila-Ramírez et al., 2005); these peaks of *Ulva* biomass in the bay have been attributed to favorable temperature and nutrient enrichment from anthropogenic activities (Piñón-Gimate et al., 2017).

Culture studies have been prolific in describing the reproduction of *Ulva* species; however, reproductive changes for these species have not been described yet in their natural environment; thus, the objective of this study was to describe seasonal changes in the reproductive stages of *Ulva* species and their relationship with

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biomass and environmental factors at four macroalgal blooms in a subtropical bay.

2. Material and methods

2.1. Study area

Bahía de La Paz is a semi-protected water body located in the western littoral of the Gulf of California. The climate is arid dry (BWh): annual evaporation (215 mm) exceeds annual precipitation (180 mm); the maximum rainfall in the bay occurs between July and October and it is associated with southeast winds, tropical storms and hurricanes; dominant winds come from the northwest from November to March (García, 1981). Four sites were chosen due to the presence of *Ulva* blooms: El Portugués, San Juan de la Costa, Casa del Marino, and El Tecolote (Fig. 1). The study period was from February 2013 to October 2015.

2.2. Reproductive stages

To determine the changes in the reproductive stages of *Ulva*, 10 thalli of each morphotype (larger than 1.0 cm) were collected from eight permanent PVC pipe quadrats (0.50 × 0.50 m) previously placed in each bloom area at random. Collected thalli were separated by species, properly rinsed and transported in plastic bags to the laboratory where they were fixated in 4% formaldehyde for further microscopic analyses.

In order to obtain reproductive stages of each thallus, three segments were observed: the apex, center, and thallus base. Sections were cut from the thallus for laminar morphologies, which consisted of 25 × 25 mm and a 25 mm long portion for tubular morphologies; a semi-permanent slide preparation was made for each segment per specimen. Each semi-permanent preparation was observed through the microscope where superficial cells were observed at 40 × (Nikon Eclipse E100).

The reproductive stages were classified according to Pettett (2009) as follows: initially chloroplasts were visible to the interior of the cell giving a totally greenish coloration (stage a); reproduction started when chloroplasts were displaced to one side of the cell, and early zooid formation began in the interior of the cell (stage b); later zooids filled the interior of the cell (stage c); and finally, zooids were released and the cells became empty producing a clear coloration (stage d). Based on this classification we obtained the photographs for reproductive stages for each species we found.

The reproductive stages were obtained by recording the number of cells at each stage in each section, which were divided by the total number of cells counted and then multiplied by 100 to obtain the stage percentage. The reproductive stage with the highest percentage was assigned as the reproductive stage at that time and site for that *Ulva* species (Carl et al., 2014).

2.3. Seasonal biomass

To obtain macroalgal biomass data for each bloom, three transects were laid out perpendicular to the shore line according to the length of each bloom mat with one transect at each end and the third one in the middle of the bloom. Five equidistant points were defined for each transect with end points at the shoreline and at the far edge of the bloom. At each point, macroalgae were collected by free diving from four, non-overlapping, randomly placed quadrants (0.25 m^{-2}) (Alveal and Romo 1995) every three months during the study period. Samples were washed in the field and each morphotype was separated by hand; each separate morphotype was weighed in a digital scale (±0.1, wet weight) to determine biomass, and a subsample was fixed with 4% formaldehyde-seawater for taxonomic identification for the region with keys from Abbot and Hollenberg (1992) and Norris (2010). *Ulva* species were identified based on external and internal morphological characters (i.e. thallus shape with or without branches, thallus size, margin shape, arrangement, cell size and shape). The nearest species name was assigned for each morphotype collected. Even though molecular analyses are recommended for definite species identification of this genus, they were not available at this time for this study.

2.4. Environmental variables

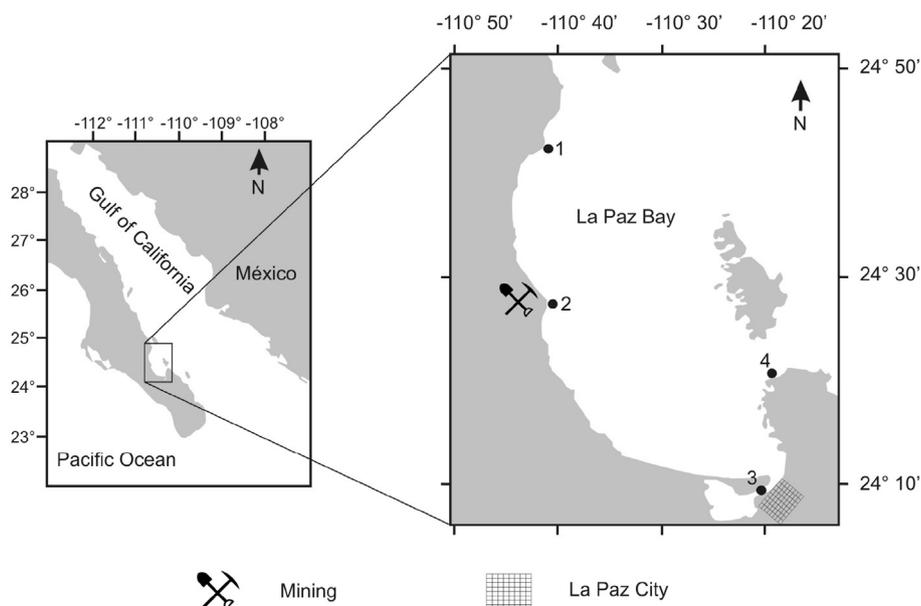In each site, data of sea surface temperature, turbidity and

Fig. 1. Study sites for *Ulva* species. 1. El Portugués, 2. San Juan de la Costa, 3. Casa del Marino, 4. El Tecolote.
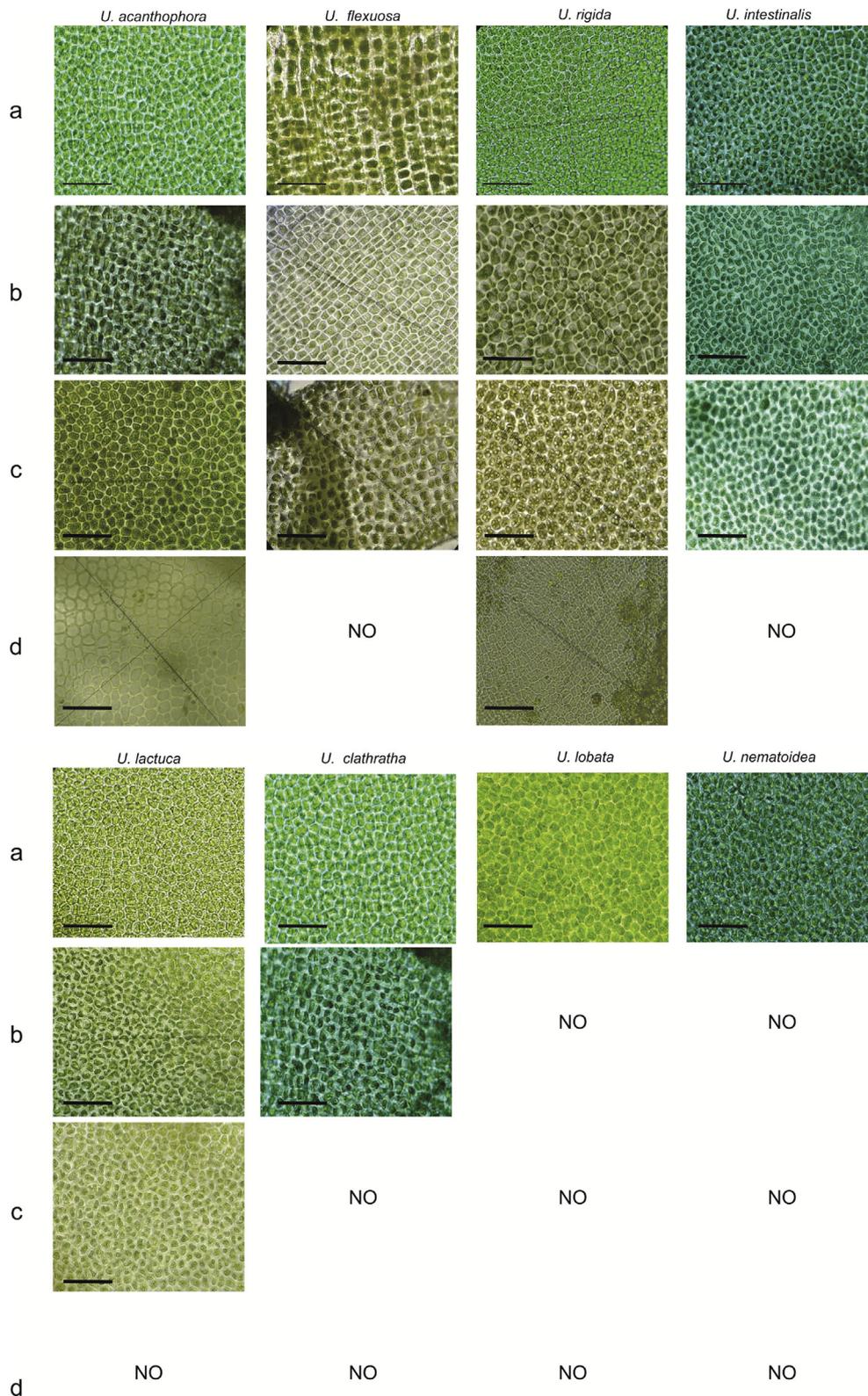
Fig. 2. Reproductive stages: vegetative stages (a); thallus with zooids in formation (b); with formed zooids (c); and empty cells after zooids release (d); for the species *Ulva acanthophora, U. flexuosa, U. rigida, U. intestinalis, U. lactuca, U. clathrata, U. lobata* and *U. nematoidea*. NO = not observed.

nutrients (DIN, TN, PO_4 and TP) of the water column were taken as described in Chávez-Sánchez et al. (2017), with which seasonal mean values for winter, spring, summer and autumn were obtained.

2.5. Data analysis

To test for significant annual, seasonal, and spatial differences in *Ulva* biomass, as well as in temperature and nutrient concentration
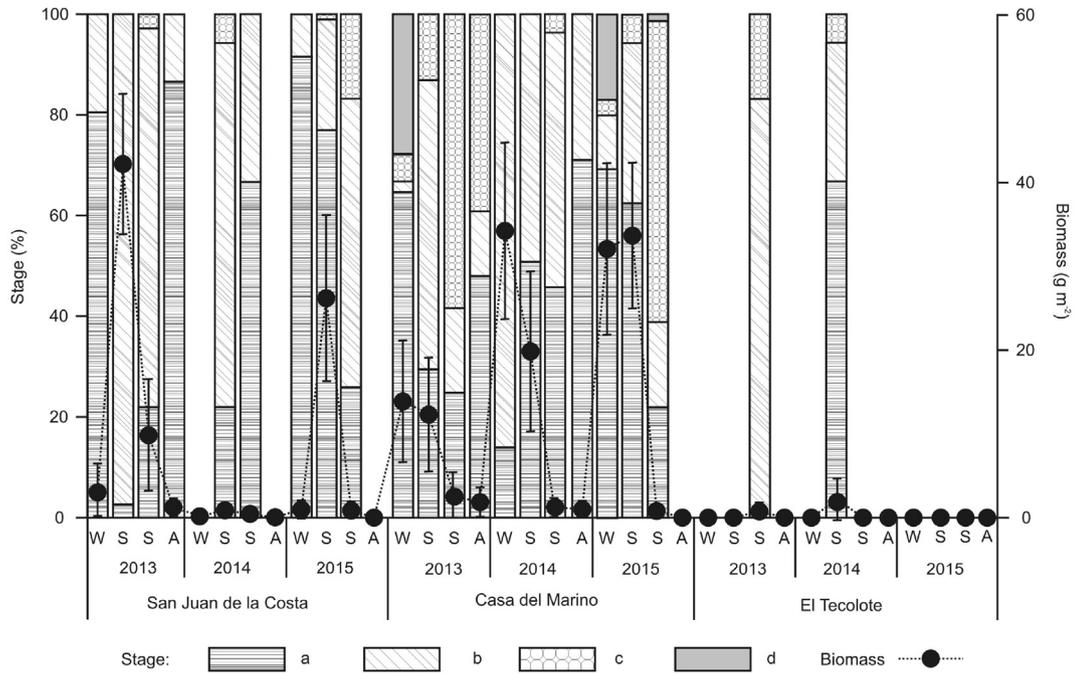
Fig. 3. Reproductive stage percentages and biomass of *Ulva acanthophora*. *a*: vegetative stage; *b*: reproduction starts; *c*: zooids are filling the interior of the cell; and *d*: zooids are released and the cells become empty.

in the water column, normality (Kolmogorov-Smirnov) and Levine homogeneity tests were performed, followed by a multifactorial ANOVA ($p < 0.05$) and *post-hoc* comparisons using Tukey's tests (Zar, 2010). Regression analyses were done for biomass and environmental factors by site but only significant ($p < 0.05$) correlations

were observed. Reproductive stages were analyzed with a Multidimensional Scaling ordination analysis (MDS) in order to check similarities between samples depending on the dominant reproductive stage at each season and site (Stress < 0.1 significant). The analyses were performed with the software STATISTICA 7.1
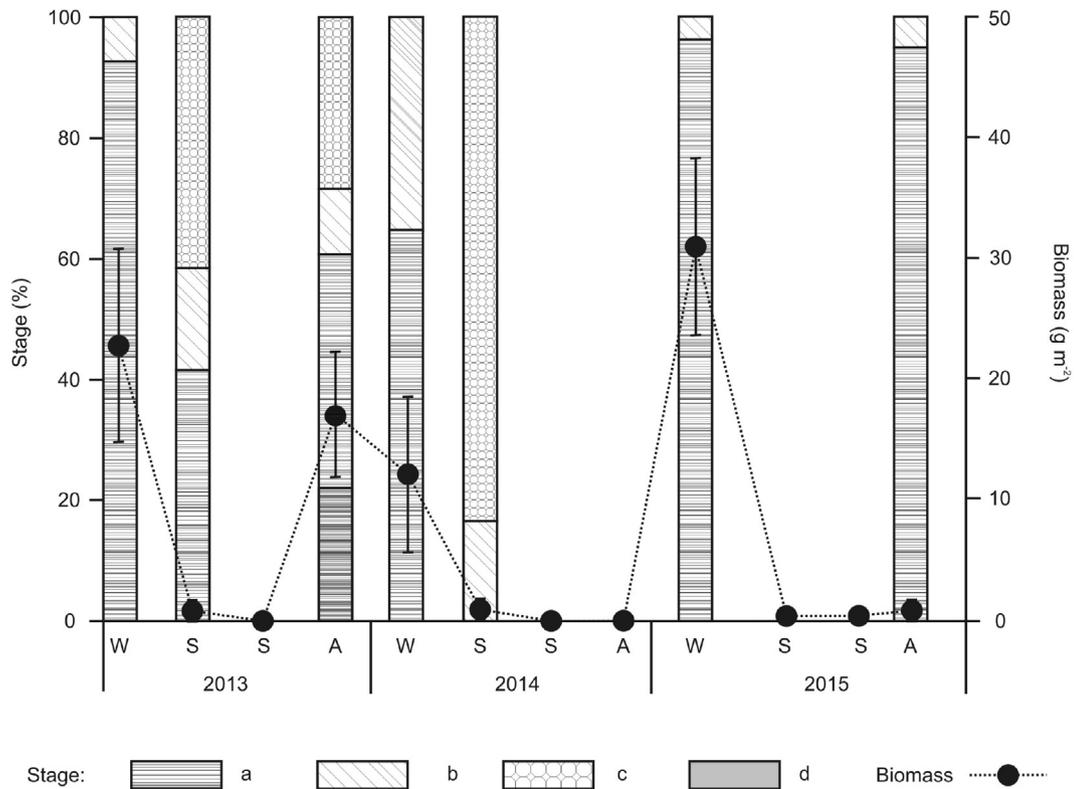

Fig. 4. Reproductive stage percentages and biomass of *Ulva flexuosa*. *a*: vegetative stage; *b*: reproduction starts and *c*: zooids are filling the interior of the cell.
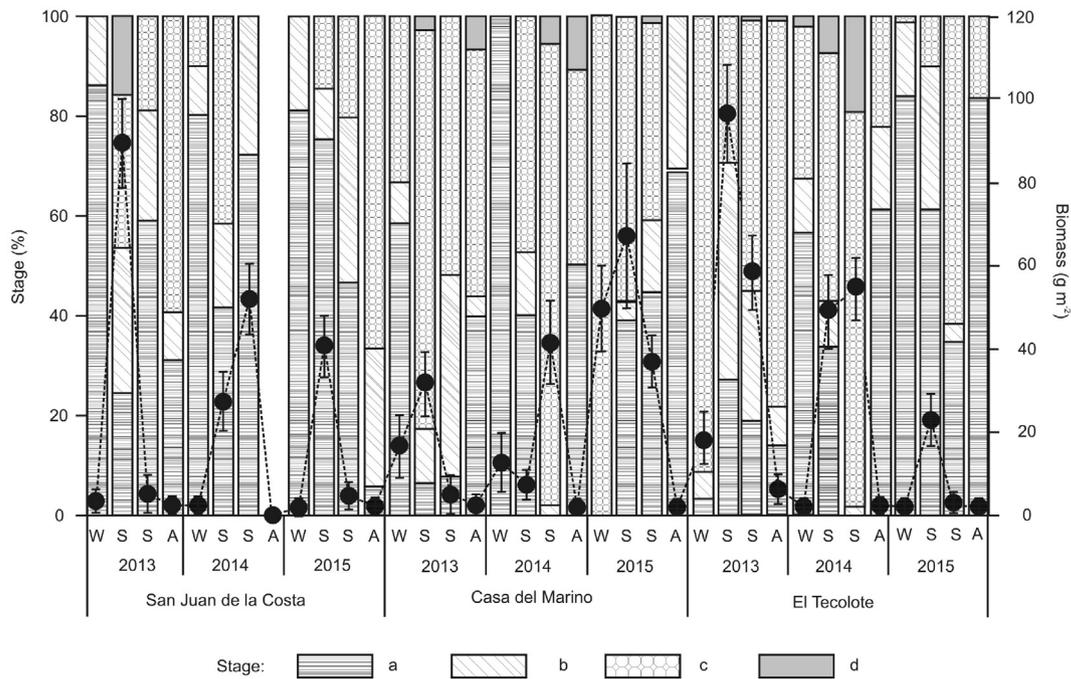
Fig. 5. Reproductive stage percentages and biomass of *Ulva rigida*. *a*: vegetative stage, *b*: reproduction starts; *c*: zooids are filling the interior of the cell; and *d*: zooids are released and the cells become empty.

(StatSoft, 2013) and PRIMER ver. 6.0 (Clarke and Warwick, 2001).

3. Results

Eight *Ulva* species were found: *U. acanthophora*, *U. clathrata*, *U. flexuosa*, *U. intestinalis*, *U. lactuca*, *U. lobata*, *U. nematoidea* and *U. rigida*, whose presence, reproductive stage and biomass varied according to site and season. For the species *U. acanthophora, U. flexuosa, U. rigida, U. intestinalis, U. lactuca* and *U. clathrata*, vegetative stages (a), thallus with zooids in formation (b) with formed zooids (c) and empty cells after zooid release (d) were observed (Fig. 2). For *U. lobata* and *U. nematoidea*, only vegetative thallus were found when present (Fig. 2). Differences could be observed in the coloration of the slides depending on *Ulva* species.

3.1. Reproductive stages and biomass

3.1.1. Ulva acanthophora

For *U. acanthophora* the four reproductive stages were found, of which the most common was stage *a* (47.2%). It was found in every sampling site and season although this stage was only present at El Tecolote in the summer of 2014. The predominant stages at Casa del Marino and San Juan de la Costa were *a* and *b*; at San Juan de la Costa, the three-year stage *c* was present only in summer (Fig. 3). The MDS analysis showed similarities between San Juan de la Costa and Casa del Marino with a predominance of the *b* stage during spring and summer; during spring and winter, they showed similarities above 60% with a predominance of the reproductive stage *a*. At Casa del Marino summer and autumn were similar above 60% with *c* and *a* reproductive stages (Fig. 6a). Its highest biomass was found at San Juan de la Costa ($p < 0.05$) for spring 2013 (42.3 ± 8.4 g m^{-2}). A different pattern of the three years was observed at Casa del Marino with the highest biomass values in winter (27 ± 9.3 g m^{-2}) and spring (22.3 ± 8.3 g m^{-2}). At El Tecolote its biomass values (0.2 ± 0.1 g m^{-2}) were very low ($p < 0.05$) during the three years (Fig. 3).

3.1.2. Ulva flexuosa

U. flexuosa was present only at El Portugués where three stages were observed (*a*, *b* and *c*). During the three years the vegetative stage was predominant in winter; stage *c* showed a higher percentage (83.3%) in spring 2014. The highest biomass for *U. flexuosa* was found during winter 2015 ($p < 0.05$; 31.3 ± 7.7 g m^{-2}) (Fig. 4).

3.1.3. Ulva rigida

For *U. rigida* the four reproductive stages were observed and the most common was *c*. At San Juan de la Costa the presence of each stage was variable; however, it could be observed that stage *a* was dominant during the winter of the three years, and then in autumn stage *c* was dominant in 2013 and 2015. At Casa del Marino stage *c* was predominant until the autumn of 2014, while in winter only was stage *a* present; stage *c* was again predominant from summer to winter 2015. For El Tecolote stage *c* was dominant; however, stage *a* showed the highest percentage above all in 2015 (Fig. 5). The MDS analysis showed similarities above 80% between El Tecolote and Casa del Marino during winter and summer and some samples of spring and autumn with a predominance of stage *c* while samples from San Juan de la Costa, Casa del Marino and El Tecolote showed a similarity above 60% during winter, spring and summer with a predominance of stage *a* (Fig. 6b). This species showed its highest biomass at San Juan de la Costa and El Tecolote during spring 2013 (89.6 ± 10.3 g m^{-2}, 96.6 ± 11.4 g m^{-2}, respectively); this pattern was not repeated since biomass values were almost half ($p < 0.05$) at the same sites during spring (27.6 ± 7.1 g m^{-2}, 40.8 ± 7.8 g m^{-2}) and summer 2014 (52.2 ± 8.8 g m^{-2}, 55.5 ± 9.7 g m^{-2}). *Ulva rigida* showed an important decrease in its biomass at El Tecolote (42.6 ± 7.2 g m^{-2}, 5.3 ± 2.8 g m^{-2}) and San Juan de la Costa (24.3 ± 4.4 g m^{-2}, 10.1 ± 2.8 g m^{-2}) while at Casa del Marino its biomass increased (13.8 ± 5.6 g m^{-2}, 37.1 ± 7.5 g m^{-2}) (Fig. 5) from 2013 to 2015.

The species *U. intestinalis, U. clathrata, U. lactuca, U. lobata* and *U. nematoidea* were occasionally present; the dominant reproductive stages were *a* and *b*, and some thalli presented stage *c*
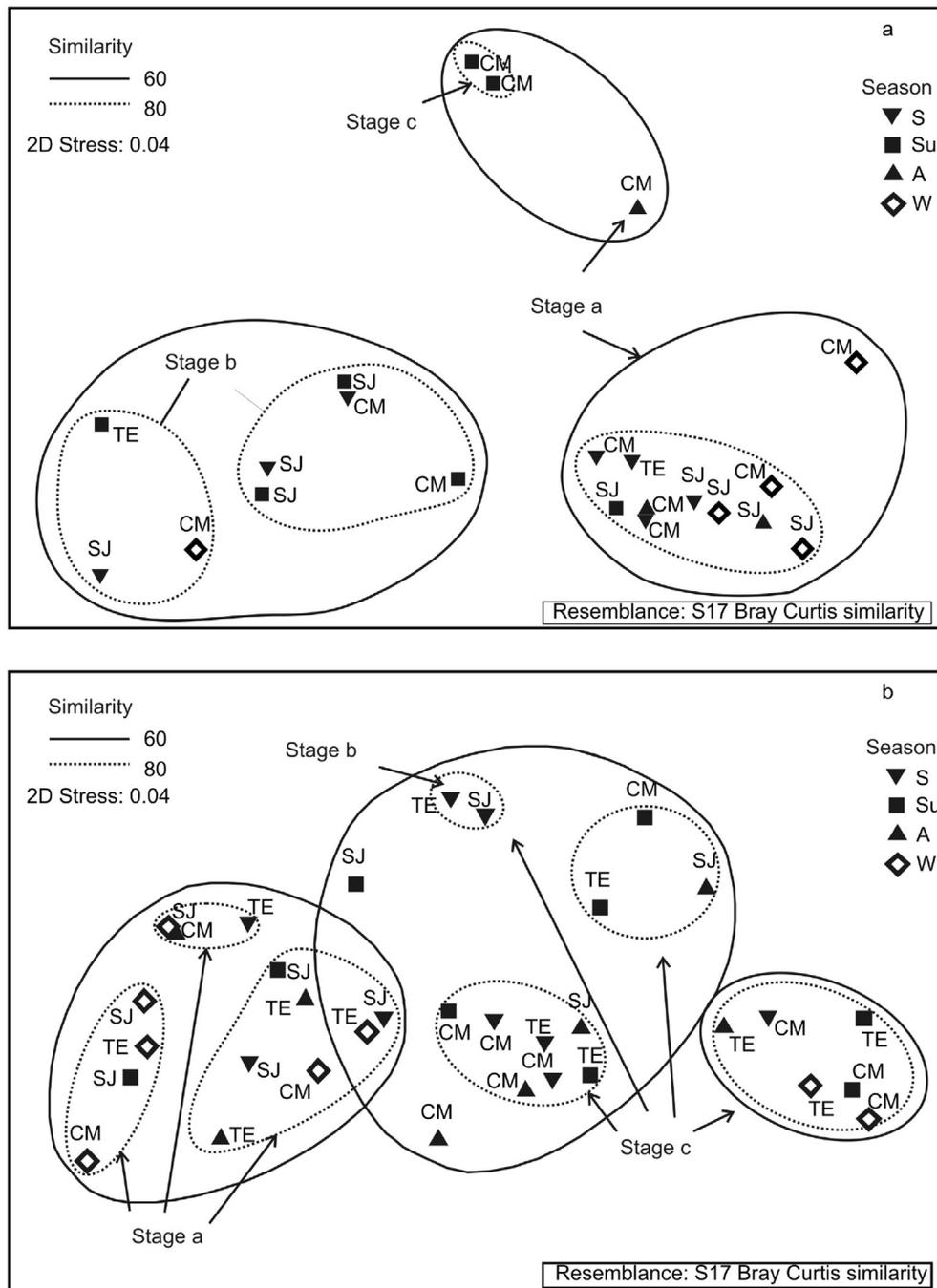
Fig. 6. Multidimensional scaling (MDS) analysis for ordering similarities between samples depending on the mean seasonal percentage of the reproductive stage for *U. acanthophora* (a) and *U. rigida* (b). SJC: San Juan de la Costa, CM: Casa del Marino, TE: Tecolote, W: Winter, S: Spring, Su: Summer, A: Autumn.

sporadically; these species had little biomass (<7 g m^{-2}).

3.2. Hydrographic parameters and nutrients

Temperature showed a similar seasonal pattern in the four sites during the three years with an increase from spring to late summer and early autumn, decreasing to early winter (Table 1). Mean temperature during summer and autumn 2014 (29.06 ± 1.5, 28.2 ± 1.4 °C; respectively) and 2015 (29.2 ± 1.5, 29.4 ± 0.9 °C, respectively) was higher than 2013 (26.5 ± 1.5, 26.7 ± 1.1 °C, $p < 0.05$). Salinity showed little variation between the seasons of each year and sites (Table 1); higher salinities (36.5 ± 0.4 psμ) could

be observed mostly at Casa del Marino depending on the season of the year ($p < 0.05$). Nutrients showed a broad variation, but no seasonal pattern could be observed. Dissolved inorganic nitrogen (DIN), TN and TP mean seasonal values were higher at San Juan de la Costa and lower at El Tecolote ($p < 0.05$) in general (Table 2).

3.3. Linear regression

Stage ***d*** of *U. acanthophora* was observed inversely related to biomass (Fig. 7a), temperature (Fig. 7b) and ammonia (Fig. 7c) and positively correlated with total phosphorus (Fig. 7d) at Casa del Marino. For the same species at San Juan de la Costa, reproductive

Table 1
Mean seasonal values (±Standard Error) of surface seawater temperature (SST) and salinity at El Portugués (POR), San Juan de la Costa (SJC), Casa del Marino (CM) and El Tecolote (TEC).

	Site	2013				2014				2015			
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
SST (°C)	POR	22.8 ± 0.1[a]	23.1 ± 0.3	26.4 ± 0.1	26.7 ± 0.1	23 ± 0.1	25.9 ± 0.2	29.1 ± 0.3[b]	28.4 ± 0.4[b]	22 ± 0.1[a]	24.7 ± 0.4	29.3 ± 0.2[b]	28.6 ± 0.9[b]
	SJC	23.8 ± 0.7	23.7 ± 0.9	27.5 ± 0.2	26.2 ± 0.1	25.1 ± 0.3	26.9 ± 0.5	29.5 ± 1.2[b]	28.7 ± 0.3[b]	25.7 ± 0.2	27.1 ± 0.9	30.4 ± 0.1[b]	29.9 ± 0.2[b]
	CM	24.5 ± 0.3	24.1 ± 0.8	26.4 ± 1.1	25.9 ± 0.2	23.5 ± 0.3	27.6 ± 1.3	28.6 ± 0.8[b]	28.3 ± 0.7[b]	23.4 ± 0.6	24.7 ± 0.9	29.4 ± 0.2[b]	29.3 ± 0.1[b]
	TEC	22.5 ± 0.1[a]	23.2 ± 0.4	24.7 ± 0.4	26.1 ± 0.4	24.1 ± 0.1	26.8 ± 0.2	27.9 ± 0.6	29.0 ± 0.1	21.2 ± 0.6[a]	24.0 ± 0.4	28.8 ± 0.7[b]	29.7 ± 0.1[b]
Salinity	POR	35.5 ± 0.2	34.7 ± 0.8	34.5 ± 0.4	35 ± 0.5	35.6 ± 0.3	35.5 ± 0.1	34.7 ± 0.4	34.4 ± 0.3	34.9 ± 0.4	34.0 ± 1.0	34.6 ± 0.3	35 ± 0.2
	SJC	35 ± 1.6	35.5 ± 0.5	35.4 ± 0.6	35 ± 0.3	35.9 ± 0.3	34.8 ± 0.2	35.3 ± 0.7	34.7 ± 0.4	35.5 ± 0.2	35.3 ± 0.2	35.1 ± 0.1	34.7 ± 0.1
	CM	36.5 ± 0.4[b]	35.6 ± 0.1	36 ± 0.2[b]	34.4 ± 0.1	35.6 ± 0.1	36.4 ± 0.2[b]	35.8 ± 0.3	34.9 ± 0.3	35.3 ± 0.1	35 ± 0.3	35.6 ± 0.1	35.3 ± 0.1
	TEC	35.8 ± 0.3	35.7 ± 0.4	35.5 ± 0.1	33.6 ± 2.3[a]	35.7 ± 0.2	35.9 ± 0.2	35.4 ± 0.1	35.0 ± 0.1	35.4 ± 0.1	34.9 ± 0.2	35.4 ± 0.2	34.8 ± 0.2

Note: different super index letter denotes significant differences between sites and season.

stage *c* was inversely correlated with biomass (Fig. 7e). Reproductive stage *c* of *U. flexuosa* showed an inverse correlation with salinity (Fig. 7f).

4. Discussion

Reproductive stages and biomass values of *Ulva* species at this subtropical bay were variable depending on site, season and *Ulva* species. Only three out of eight *Ulva* species showed the four reproductive stages and high biomass values. Seasonal patterns in macroalgal biomass had been previously observed for other coastal lagoons of the Gulf of California (Piñón-Gimate et al., 2008) and also in La Paz Bay depending on the species (Águila-Ramírez et al., 2005). It was found that within a season, *Ulva* biomass seemed to respond to temperature increases from above 23 °C; depending on species biomass, peaks could be reached from 24 to 27 °C; these changes in temperature could be observed from late winter until early summer; afterwards, temperatures were too high and *Ulva* tended to disappear. Nutrient supply at the four sites was not a limiting factor since nutrient concentrations (see Table 2) could be considered high for this bay (Piñón-Gimate et al., 2017). Fong et al. (1996) indicated that when N supply was sufficient, the other factors (salinity and temperature) could also play important roles in seasonal succession of green macroalgae. As observed in other studies, temperature alone would not solely drive biomass growth since most studies have shown that nutrient supply could overlap

seasonal growth of macroalgae (Liu et al., 2013). For example, the warmer temperatures recorded in the Yellow Sea could have favored nutrient uptake rates and hence favored faster growth. Therefore, dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen (DON) seemed to determine seasonal pattern of biomass increases of the *Ulva prolifera* bloom in the Yellow Sea (Shi et al., 2015). In our study it was observed that temperature and other factors for late spring and early summer were not limiting for the increase in biomass of *U. rigida*, a species of wide distribution. However, for *U. acanthophora* temperature was an important factor as it has been reported with a temperate affinity (Table 3) (Casas-Valdez et al., 2000).

Temperature is an important factor that triggers zoospore release, and *Ulva* has been known to be extremely tolerant to temperature changes; for example, *U. pertusa* maximal sporulation percentages occurred from 15 to 20 °C (Han and Choi, 2005), and the optimum for *U. mutabilis* sporulation was from 21 to 22 °C (Nordby, 1977). According to our data, the temperature range at which changes in reproductive stages were produced in field populations for each of the species of *Ulva* were different (Table 3).

Salinity can also influence spore release by affecting turgor pressure and diameter of the pore of sporangia (Han and Choi, 2005). Several species of *Ulva* have shown an optimum liberation rate of zoospores at salinities of 35 psµ. For example, Han et al. (2008) found a maximal spore release for *U. pertusa* from 25 to 35 psµ. Thallus growth was higher at salinity levels above 20 psµ,

Table 2
Mean seasonal values (±Standard Error) of dissolved inorganic nitrogen (DIN), total nitrogen (TN), orthophosphates (PO$_4$) and total phosphorus (TP) at El Portugués (POR), San Juan de la Costa (SJC), Casa del Marino (CM) and El Tecolote (TEC).

	Site	2013				2014				2015			
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
DIN (µmol l^{-1})	POR	1.8 ± 1.1[a]	5.1 ± 2.1	3.1 ± 1.3	1.9 ± 1.0[a]	1.6 ± 0.8	2.1 ± 0.4	2.5 ± 1.6	3.1 ± 1.4	1.2 ± 0.7[a]	2.9 ± 1.5	1.9 ± 1.3[a]	2.4 ± 0.9
	SJC	18.3 ± 2.2	16.4 ± 0.8	40.1 ± 5.0[b]	2.5 ± 0.9	18.3 ± 2.9	19.2 ± 3.1	9.5 ± 2.1	61.0 ± 7.5[b]	10.1 ± 1.4	12.8 ± 4.3	2.8 ± 1.2	89.7 ± 6.7[b]
	CM	1.5 ± 0.7[a]	7.4 ± 2.4	4.7 ± 1.9	11.0 ± 3.1	3.8 ± 1.1	7.0 ± 1.9	4.1 ± 2.2	1.4 ± 0.6	12.7 ± 2.7	9.9 ± 3.5	3.2 ± 1.6	1.2 ± 0.5[a]
	TEC	4.9 ± 2.1	4.5 ± 1.5	5.5 ± 1.4	4.7 ± 1.9	2.0 ± 1.2[a]	1.7 ± 0.9	2.3 ± 1.0	1.6 ± 0.9	2.4 ± 1.7	0.9 ± 0.6	2.6 ± 2.0	1.2 ± 0.6[a]
TN (µmol l^{-1})	POR	6.5 ± 0.9[a]	12.2 ± 2.5	17.0 ± 3.4	15.3 ± 3.1	6.7 ± 1.6[a]	6.0 ± 1.0[a]	10.0 ± 2.2	33.1 ± 5.4	10.0 ± 2.6	9.1 ± 1.8	9.8 ± 1.6	20.0 ± 2.8
	SJC	30.4 ± 3.6[b]	20.5 ± 3.0	49.0 ± 5.8[b]	14.0 ± 1.2	9.7 ± 2.8	4.8 ± 2.4	28.7 ± 3.5	98.8 ± 8.5[b]	18.9 ± 2.3	29.5 ± 4.3	15.6 ± 3.2	134.4 ± 7.8[b]
	CM	27.1 ± 4	35.5 ± 3.1	21.8 ± 3.7	25.2 ± 3.8	53.8 ± 5.3	15.5 ± 0.6	26.9 ± 4.0	48.5 ± 7.7[b]	17.0 ± 2.9	39.8 ± 7.0[b]	19.7 ± 3.5	35.5 ± 3.6
	TEC	19.7 ± 4.5	15.7 ± 2	12.8 ± 1.8	21.7 ± 4.3	26.1 ± 3.1	31.5 ± 2.0	15.1 ± 2.4	28.6 ± 4.8	8.8 ± 1.5	16.0 ± 3.3	9.5 ± 1.9	19.3 ± 3.3
PO$_4$ (µmol l^{-1})	PORT	0.5 ± 0.5	1.0 ± 0.8	1.1 ± 0.8	1.2 ± 0.9	0.9 ± 0.8	1.5 ± 0.8	0.7 ± 0.5	0.8 ± 1.0	0.8 ± 0.4	0.9 ± 0.3	0.4 ± 0.5	1.3 ± 1.0
	SJC	0.3 ± 0.6[a]	1.3 ± 0.5	1.8 ± 1.2	0.5 ± 0.3	1.4 ± 0.7	5.3 ± 2.1[b]	0.9 ± 0.9	1.7 ± 1.2	2.8 ± 1.2[b]	2.7 ± 1.9[b]	0.2 ± 0.4	2.4 ± 1.1[b]
	CM	0.9 ± 0.5	0.7 ± 0.6	0.6 ± 0.5	0.5 ± 0.6	0.8 ± 0.5	2.1 ± 1.0[b]	1.1 ± 0.9	0.7 ± 0.4	1.5 ± 0.5	0.5 ± 0.6	0.4 ± 0.3	0.8 ± 0.3
	TEC	0.5 ± 0.8	1.1 ± 0.8	1.1 ± 0.7	0.6 ± 0.5	1.0 ± 0.7	1.2 ± 0.8	0.4 ± 0.4	0.5 ± 0.5	0.6 ± 0.4	0.4 ± 0.4	0.3 ± 0.4[a]	0.5 ± 0.5
TP (µmol l^{-1})	PORT	0.8 ± 0.1[a]	1 ± 0.5	1.0 ± 0.5	0.7 ± 0.2[a]	0.9 ± 0.5[a]	0.8 ± 0.5[a]	1.3 ± 0.8	0.7 ± 0.3[a]	3.1 ± 0.9	1.9 ± 0.6	1.3 ± 0.8	0.7 ± 0.2[a]
	SJC	1.0 ± 0.4	2 ± 1	2.6 ± 1.4	0.9 ± 0.3	1.7 ± 1.1	1.5 ± 0.9	1.7 ± 1.0	9.8 ± 2.6[b]	3.8 ± 1.1	3.4 ± 1.8	1.7 ± 0.4	12.3 ± 2.6[b]
	CM	1.6 ± 0.7	1.6 ± 0.7	1.2 ± 0.7	0.9 ± 0.5	2.7 ± 0.8	1.3 ± 0.4	1.4 ± 0.8	1.2 ± 0.7	2.8 ± 0.9	1.6 ± 0.9	1.4 ± 0.7	1.4 ± 0.8
	TEC	1 ± 0.5	1.1 ± 0.4	1.0 ± 0.4	1.1 ± 0.5	1.4 ± 0.8	1.1 ± 0.5	1.8 ± 1.3	1.1 ± 0.8	2.2 ± 0.9	1.8 ± 0.9	0.9 ± 0.4[a]	1.2 ± 0.9

Note: different super index letter denotes significant differences between sites and season.
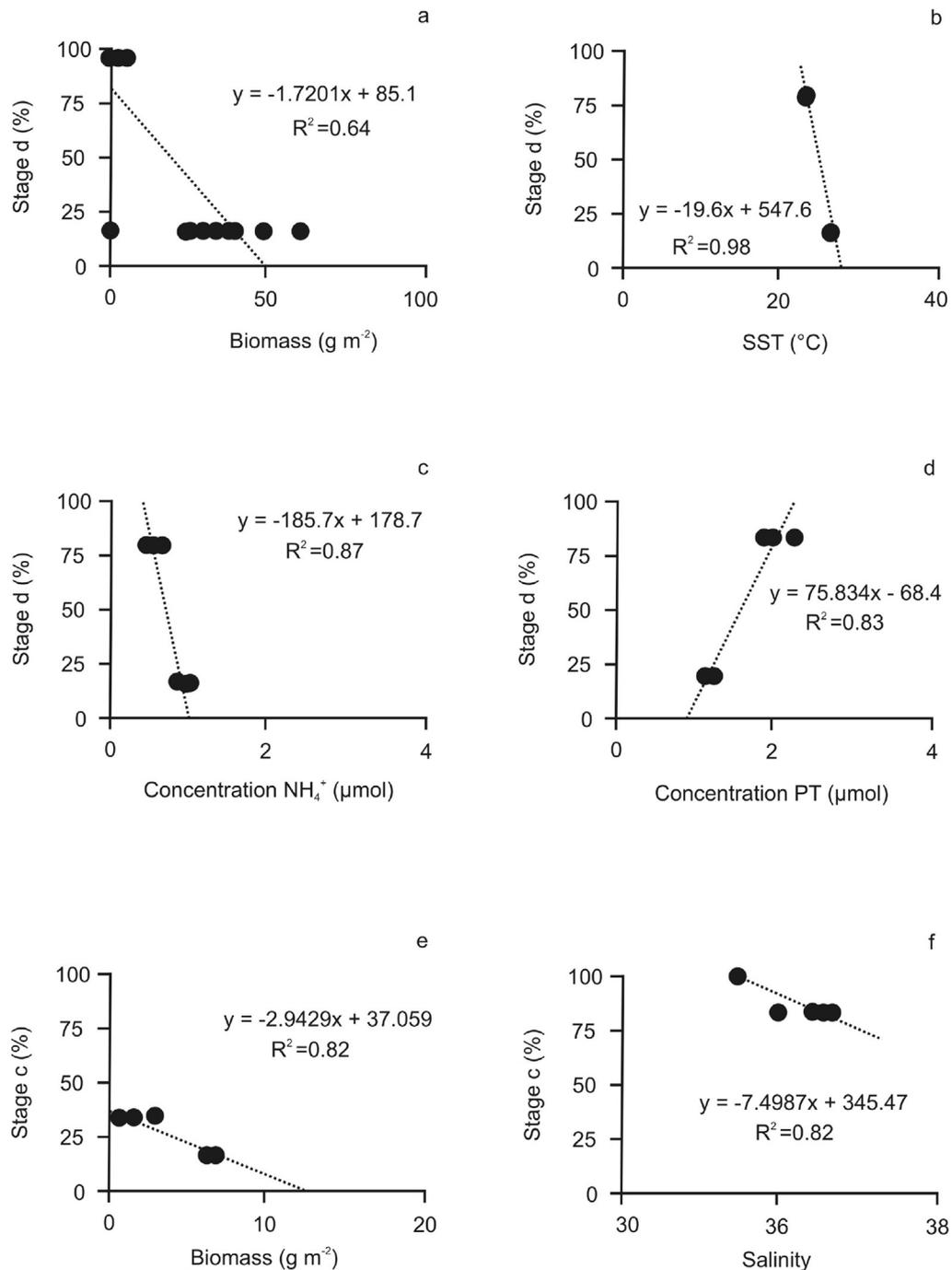
Fig. 7. Correlation between the percentage of reproductive stage *d* of the species *Ulva acanthophora* and its seasonal biomass (a); superficial seawater temperature (b); ammonium (c); and total phosphorus (d) at Casa del Marino. Correlation between reproductive stage *c* of the species *U. acanthophora* and its seasonal biomass (e) at San Juan de la Costa; and reproductive stage *c* of the species *U. flexuosa* and salinity (f) at El Portugués.

and the highest values were obtained especially at 35 psµ (Sousa et al., 2007). Other species have also shown a tendency for optimal spore release rates at ocean salinities (35 psµ), our data showed a similar response since salinity values where reproductive stages were found ranged from 34.7 to 35.6 psµ (Table 3). In La Paz Bay salinity fluctuated from 32 to 35.6 psµ without significant variation along the year; therefore, *Ulva* species are in optimal conditions for production of reproductive stages.

Zoospore release is influenced by nutrients; for example, Imchen (2012) showed that nitrate concentration of 15 µmol l^{-1}

and phosphate of 4 µmol l^{-1} had a significant effect on the biomass of released zoospores. Probably, formation of zoospores starts when phosphorus concentrations increases, and the release of zoospores occurred later when nitrogen concentrations reached their highest peak. This idea is supported by our data since a higher percentage of reproductive stage *d* (zoospores already released) was present for *U. acanthophora* at higher concentrations of total phosphorus while nitrogen and particularly ammonia were inversely correlated with the same stage for this species.

Photoperiod has been reported to have a significant effect on

Table 3
Ranges of the hydrographic variables that are considered optimum for the presence of any given reproductive stages and/or the highest biomass values for each of the most abundant species of *Ulva* at El Portugués (PORT), San Juan de La Costa (SJC), Casa del Marino (CM), El Tecolote (TEC).

Species	Site	SST (°C)	Salinity	Turbidity (NTU)	NID (μmol l^{-1})	NT (μmol l^{-1})	PO$_4$ (μmol l^{-1})	PT (μmol l^{-1})	Reproductive stage	Biomass (g m^{-2})
U. acanthophora	SJC CM	21.7–25	35.2–35.9	13.4–26.4	0.7–16.9	15.1–22.2	0.3–3.1	1.1–1.9	a, b, c, d	34.6–42.3
U. flexuosa	PORT	20.2–22.3	35.2–36.0	0–49.6	1.4–1.6	5.9–19.9	0.1–2.0	0.7–3.1	a, b, c	22.9–31.3
U. rigida	SJC CM TEC	22.5–26.3	35.7–36.9	0–41.4	1.2–11.4	8.7–31.4	0.1–1.5	0.7–1.8	a, b, c, d	89.6–96.6

formation and release of swarmers (Corradi et al., 2006; Carl et al., 2014); extended light periods may result in low discharge, particularly in temperate environments where day length plays a key role in the reproduction of seaweeds (Carl et al., 2014). It was not possible to measure the photoperiod in the field; however, it was expected that spring and earlier summer days would favor the presence of zooid release stages due to longer days (Chávez-Sánchez et al., 2017).

Biomass showed different seasonal patterns depending on the species and site. Biomass ranged from 22.9 to 96.6 g m^{-2} associated to nutrient concentrations of 0.7–11.4 μmol l^{-1} DIN, 5.9–31.4 μmol l^{-1} TN and 0.7–3.1 μmol l^{-1} TP, these values were within the range observed by other authors. *Ulva* biomass values (63.3 g m^{-2}) were associated to DIN, high total nitrogen and phosphorous concentrations (2.0 ± 0.1, 48.9 ± 0.1 and 12.2 ± 0.2 μmol l^{-1}, respectively) in a previous study at different sites in La Paz Bay (Chávez-Sánchez, 2012). Moreover, in coastal lagoons of Sinaloa, *Ulva* values oscillated from 10 to 110 g m^{-2} and were associated to nutrient concentrations of 4–40 μmol l^{-1} DIN, 20–100 μmol l^{-1} TN and from 1 to 5 μmol l^{-1} TP. These results suggested that nutrients are not limiting factors for the development of *Ulva*, and therefore changes in biomass depend on the biogeographic affinities of the species and substratum availability at each site as we observed. A rapid increase in nutrient concentrations could result in higher biomass values such as the green tides observed in other parts of the world, for example, the *Ulva* bloom in Quingdao, China in 2008 where nutrient concentrations (DIN concentrations found ranging from 0.65 to 11.4 μmol l^{-1}) triggered the development of the bloom (Liu et al., 2013).

The reproductive stages showed no correlation neither with biomass (only reproductive stage *d* and biomass of *U. acanthophora* that showed an inverse correlation) nor season or site. However, in laboratory experiments it has been shown that conditions inducing early zoosporangium development produced lower biomass; therefore, the optimal conditions to obtain zoospores may be different from those that maximize biomass (Ruangchuay et al., 2012). Kim et al. (2011) and Mantri et al. (2011) mentioned that somatic cells of *Ulva* spp. could grow under a wide range of temperatures and salinity. For reproduction, no information could be found for the region of the Gulf of California. Several studies have demonstrated that some environmental parameters trigger zoospore formation; however, the reported studies (Sousa et al., 2007; Mantri et al., 2011; Carl et al., 2014), were done under laboratory conditions, hence the importance of the present study since the observations were made directly from field samples.

In experimental studies, Carl et al. (2014) showed that their *Ulva* sp. 3 had a high capacity for rapid and intense growth under tropical conditions since preexisting vegetative cells of *Ulva* can transform directly into reproductive cells forming zoospores and gametes by meiosis and mitotic division, respectively. It could be the case for La Paz bay, where some conditions trigger the release of zoospores and others allow the increase of biomass instead.

Descriptions of reproductive stages of *Ulva* in the field have been scarcely described. Some reproductive stages could be seen as an opportunistic strategy of *Ulva* to rapidly repopulate extended areas

in places where nutrient enrichment allows their rapid growth. Vegetative or reproductive stages showed a seasonal pattern depending on site and species where certain values of temperature combined with salinity, light (photoperiod) and nutrients were favorable either for vegetative growth (which will be reflected in biomass increase) or the appearance of certain reproductive stages.

Field studies, such as this, are necessary since the presence of certain reproductive stages could indicate favorable conditions for the development of excessive macroalgal biomass.

Rapid and intense growth and reproduction in subtropical conditions of other geographical areas has lead to the formation of *Ulva* blooms or "green tides" that have an ecological and economic impact on extensive areas (Liu et al., 2013). Therefore, the monitoring of temperature and nutrient loadings in our study area could be used to predict their effect on the production of macroalgal blooms.

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