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The effect of physico-chemical parameters and nutrients on the bioecology of *Chondracanthus acicularis* (Roth) Fredericq at three sites in the Moroccan Atlantic Ocean

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ABSTRACT

The red algae *Chondracanthus acicularis* (Roth) Frederiq is a carrageenophyte commercialized in Morocco. A biological study was done on three sites on the Moroccan Atlantic coast with different fishing intensities (SidiRahal, El Jadida, and SouiriaKdima) to evaluate the impact of physicochemical parameters on the algae distribution. Biometric studies (weight and size) were carried out on different thallus in addition to stereomicroscopic observations to identify fertile thallus. Additionally, measurements of physicochemical parameters and nutrients were conducted each month in situ and in the laboratory. This study's results showed that the highest biomass values were recorded in sampling sites characterized by optimal physicochemical and nutrient parameters, except for salinity. The highest biomass densities were recorded in April, February, and December at the Sidi Rahal site, El Jadida, and Souiria Kdima respectively. Minimum densities were recorded in December at the Sidi Rahal and El Jadida sites. While at SouiriaKdima, these minimum densities were recorded during October. The average thallus sizes were larger in Sidi Rahal relative to the other two sites. Minimum sizes were recorded during the winter period in the three studied sites. Conversely, maximum sizes were encountered in summer and early autumn. The thalli of *C. acicularis* were fertile during the winter season in the three studied sites showing a negative correlation with temperature and salinity. In conclusion, this study shows that indeed physicochemical parameters have a slight effect on the bioecological parameters. Furthermore, knowledge of the fertility period will allow establishing a management plan to conserve the species.

INTRODUCTION

In Morocco, carrageenan algae (Gigartinaceae) fishing activity has emerged over the past decade. In effect, Carrageenans are industrially important hydrocolloids that are found in various red seaweeds (Gigartinales, Rhodophyta).However, this activity remains limited to harvesting and raw material **(Mouradi *et al*., 2008).**The carrageenan industry has not experienced a development such as that of the agarophyte algae industry, which represents the leading industrial activity placing Morocco among the world's important producers**(El Mtili *et al*., 2013; Hanif *et al.*, 2014).**The main commercial species are *Chondracanthus acicularis* (*C. acicularis*) (Roth) Fredericq 1993, *Chondracanthus teedei* (*C. teedii*) (Mertens ex Roth) Kützing 1843 and *Gigartinapis tillata* (*G. pistillata*) (*S.G. G melin*) Stackhouse1809, due to the highest carrageenan yields (Pereira, 2013). These three species are characterized by different morphological aspects and can live in the same biotope; however, they are harvested or picked up without distinguishing them from one to another **(Guiry, 1984a).***Chondracanthus* species are characterized by having multiaxial erect thalli emerging from a discoid crustose holdfast, and growth by means of apical or marginal meristems **(Bulboa Contador *et al*., 2020).** The most important *C. acicularis* and *G. pistillata* world productions are carried out in the south of France, Spain, Portugal, and Morocco(Lembi and Waaland, 1989).It is expected that demand for carragenophytic red algae will continue to rise as they are distinguished by the quality of the carrageenan types they produce **(Guiry, 1984b)**. Gigartinaceae algae, despite their fairly significant potential, are faced with a real threat linked to the manner in which they are collected and the risks linked to overexploitation following high demand**(El Omari *et al*., 2007)**. In fact, collectors use a wide variety of cutting tools which often destroy its attachment system (rhizoids or other), an essential organ for algae regeneration. On the other hand, algae collection has grown strongly, and large quantities could have escaped the control system in certain regions. *C. acicularis* quantitative importance and the value of its products made it the focus of this work.

In order to ensure Gigartinaceae wild harvest sustainability, measures are implemented as part of the management plan put in place for red algae. Thus, scientific monitoring is carried out in support of measures to exploit these resources. Indeed, information on this species management is requiredand severely lacking in scientific literature. The aim of this work is to achieve an *in situ* monitoring and a bio-ecological study at three sites on the Moroccan Atlantic coast: Sidi Rahal (SR), El Jadida (EJ), and SouiriaKdima (SK). The goal is to identify the period of biological rest and highest biomass to establish a management plan for better conservation of the species.

MATERIALS AND METHODS

C. acicularis (Gigartinales, Gigartinacea) is a relatively uncommon, perennial, intertidal red alga. It grows in the lower intertidal and the shallow subtidal in sheltered or semi-exposed areas on the coast. It is often associated with silt or fine sand, where it forms conspicuous turfs. On the Atlantic coasts, the species has been reported from the British Isles to south Cameroon and from North Carolina south to Uruguay **(Dixon and Irvine, 1977).** Some difficulties have been experienced in distinguishing between certain forms of this species as *C. teedei* a species with a similar geographical distribution in the northeastern Atlantic. Intermediate forms in morphology have frequently been reported **(Gayral, 1958; Hoek and Donze, 1967; Dixon and Irvine, 1977;),** but a study has shown that the cultivated plants of these two Atlantic species do not hybridize **(Guiry, 1984a).** These difficulties of identification have been compounded by the virtual absence of *C. acicularis* reproductive materialreports, particularly in the North-East Atlantic.

1. Study area and sampling strategy

Samples of *C. acicularis* were collected monthly from January to December 2017, at extreme low tides, in a representative population of three studied carrageenophytes localized in Sidi Rahal (SR) (33°28.367 N; 7°59.647 W), El Jadida (EJ) (33°95.171 N; 8°32.366 W) and SouiriaKdima (SK) (32°03.047 N; 9°20.786 W) in the Northern Moroccan coast (Fig. 1).

All samples were collected randomly in different nine quadrats (25 x 25 cm) from the rocky shores of each sampling site. The samples were carefully removed **(de ABraga, 1990 and Chopin, 1997)** and placed in labeled plastic bags with seawater, and then transported to the laboratory to determine the biomass of the thalli, size, and reproductive structures. The identification and separation of *C. acicularis* from other species were accomplished utilizing a stereomicroscope after removing epizoites and epiphytes using freshwater.

For fresh biomass estimation, seaweeds were dried using tissue paper to remove excess water and moisture prior to weighing. For the determination of wet to dry weight ratios, samples were dried in a ventilated oven to monitor weight (60˚C, 48 h). Biomass was expressed as dry weight per square meter of the substrate. Collected seaweed was not sorted into sexual stages in this study.

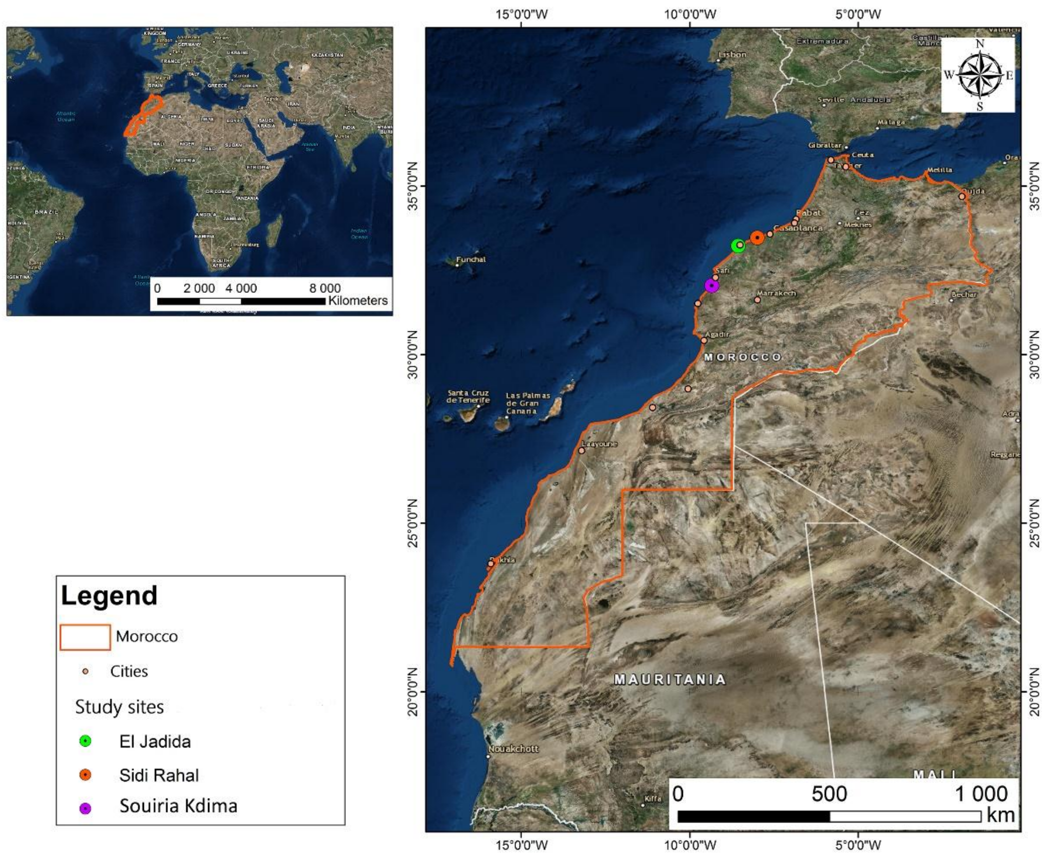

Fig. 1. Location of the three sampling sites of *Chondracanthus acicularis* along the Atlantic coast of Morocco.

2. Sampling and analytical methods

A sampling of physical parameters (temperature [°C], salinity [psu], pH, dissolved oxygen [ppm]) was carried out at 1 m depth by a multi CTD probe Hanna (HI9828 Multiparameter Water Quality Meter, USA). Seawater was collected, filtered using a 0.20 µm pore-sized filter, and stored in 50 ml polyethylene bottles. Nutrient analysis (nitrates (NO_3^-), nitrites (NO_2^-), phosphates (PO_4^{3-}), and silicates (SiO_4^{4-}) was achieved at the Physical Oceanography laboratory of INRH-Casablanca using an AA3 Axe Flow Type Auto-analyzer. The measuring unit used for all nutrients is [μmol l^{-1}].

3. Data analysis

To evaluate relative similarities among different sampling sites depending on the biological indicators (biomass, size, and fertility) and the physicochemical parameters, Principal Component Analysis (PCA) was applied as a graphic method via the package FactoMineR **(Lê *et al*., 2008)** implemented in R software, version 3.6.1 (R Core Team 2019). Moreover, to unveil the crude correlation structure within the bioecological parameters and among the physicochemical parameters, a Focused Principal Components Analysis (FPCA) was performed **(Falissard, 1996).** Thus, an FPCA of each bioecological parameter was carried out using the package ‘‘psy’’ implemented in the R statistical language. The FPCA gives a multidimensional graphical display that allows for the simultaneous visualization of both correlations between physicochemical parameters (predictors variables) and biological parameters as a particular variable of interest (outcome) and also correlations among physicochemical parameters themselves **(Falissard, 1999; Fricker, 2012).** The significance threshold used in the analysis was 5% (p = or < 0.05). Additionally, the season, which is a qualitative variable, was added as a supplementary variable.

RESULTS

1. Physicochemical data

In Sidi Rahal, salinity oscillated between 27.1 psu (December 2017) and 36.1 psu (November 2017) (Fig. 2a). The maximum dissolved oxygen value was 6.3 ppm in March, 6.21 ppm in December, and the minimum was 2.21 ppm in September (Fig. 2b) while the average water temperatures ranged from 15°C (January and March 2017) to 22°C (August 2017) (Fig. 2c). The maximum pH value was 8.9 (July 2017) and the minimum was 8 (June 2017). In El Jadida, the salinity oscillated between 31.5 psu (January 2017) and 38 psu (May/July 2017) (Fig. 2a). The maximum dissolved oxygen value was 6.21 ppm in December and the minimum was 2.21 ppm in September (Fig. 2b). Average water temperatures ranged from 15.6°C (January and February 2017) to 22°C (August 2017) (Fig. 2c). The maximum pH value was 8.3 (April 2017) and the minimum was 8.1 (May 2017). In Souiria Kdima, the salinity oscillated between 34.4 psu (January 2017) and 38.9 psu (July 2017) (Fig. 2a). The maximum dissolved oxygen value was 10.81 ppm in October and the minimum was 2.27 ppm in September (Fig. 2b). The average water temperature ranged from 15°C (January 2017) to 25.5°C (August 2017) (Fig. 2c). The maximum pH value was 8.3 (August 2017) and the minimum was 8.1 (July 2017).

2. Nutrients

Nutrient levels (NO_3^-, NO_2^-, PO_4^{3-}, and SiO_4^{4-}) varied with seasons as well as stations (Fig.s 2d–2g). In Sidi Rahal, nitrite (NO_2^-) levels ranged between 0.25 μmol l^{-1} (November) and 0.65 μmol l^{-1} (January) (Fig. 2f). Nitrates level (NO_3^-) ranged between 3.97 μmol l^{-1} (September) and 200 μmol l^{-1} (June) (Fig. 2g). Phosphates level (PO_4^{3-}) ranged between 0.42 μmol l^{-1} (August) and 4.74 μmol l^{-1} (January) (Fig. 2d). The level of (SiO_4^{4-}) ranged between 0.17 μmol l^{-1} (December) and 3.99 μmol l^{-1} (January) (Fig. 2e). In El Jadida, Nitrite (NO_2^-) levels ranged between 0.03 μmol l^{-1} (June and August) and 0.53 μmol l^{-1} (May) (Fig. 2f). Nitrates level (NO_3^-) ranged between 0.22 μmol l^{-1} (June) and 5.31 μmol l^{-1} (April) (Fig. 2g). Phosphates level (PO_4^{3-}) ranged between 0.13 μmol l^{-1} (April and August) and 0.34 μmol l^{-1} (October) (Fig. 2d). The level of (SiO_4^{4-}) ranged between 0.04 μmol l^{-1} (February) and 0.12 μmol l^{-1} (June) (Fig. 2e). In Souiria Kdima, Nitrite (NO_2^-) levels ranged between 0.04 μmol l^{-1} d (July) and 0.72 μmol l^{-1} (March)

(Fig. 2f). Nitrates level (NO_3^-) ranged between 0.12 μmol l^{-1} (July) and 4.32 μmol l^{-1} (August) (Fig. 2g). Phosphates level (PO_4^{3-}) ranged between 0.10 μmol l^{-1} (July) and 0.33 μmol l^{-1} (September) (Fig. 2d). The level of (SiO_4^{4-}) ranged between 0.05 μmol l^{-1} (December, May, June, July, and November) and 0.13 μmol l^{-1} (April) (Fig. 2e).

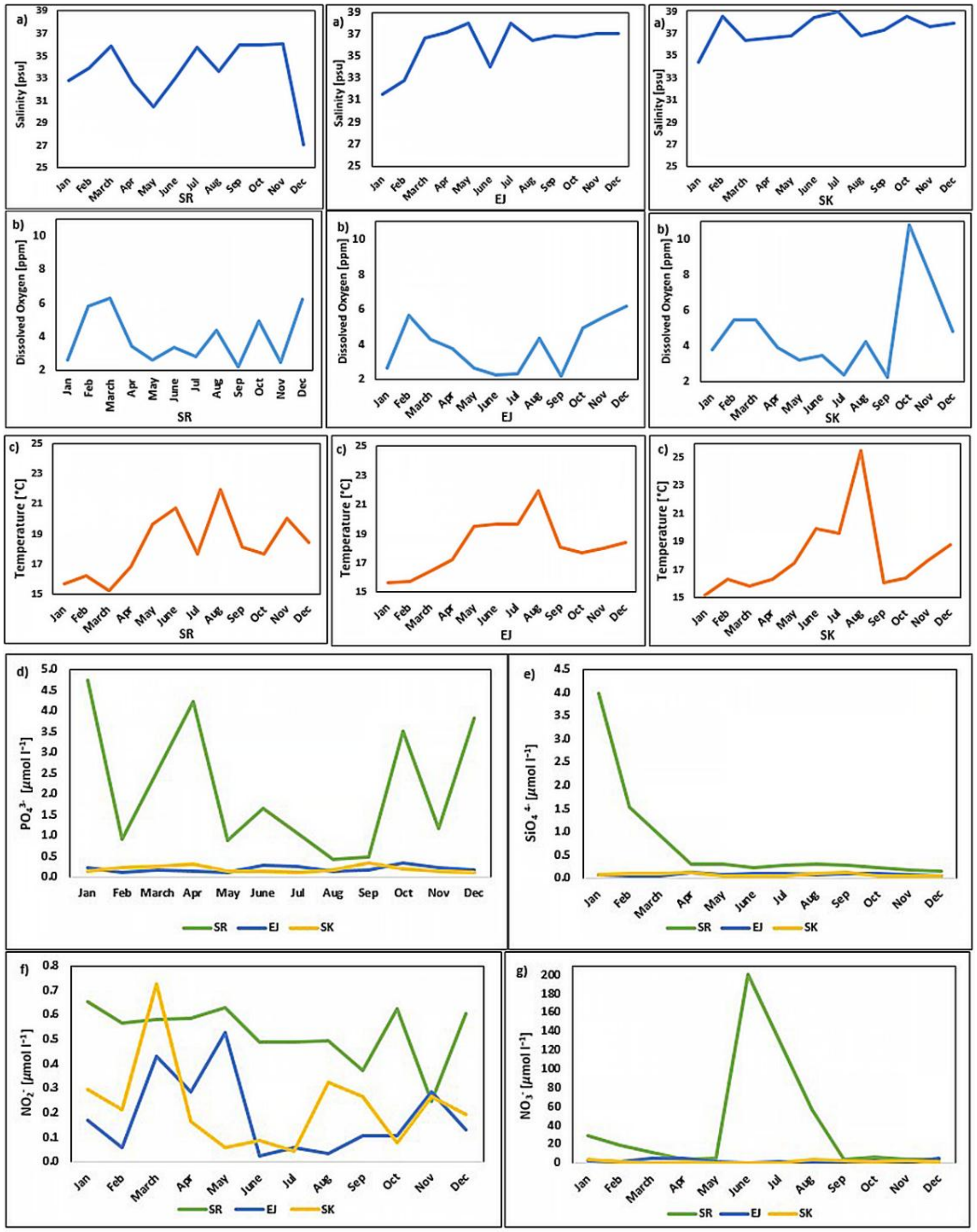

Fig. 2. Physicochemical parameters at the three sites Sidi Rahal (SR), El Jadida (EJ) and SouiriaKdima (SK) (Salinity / dissolved Oxygen /Water Temperature (a,b,c), Phosphates PO4 3- (d), Silicates SiO4 4- (e), Nitrites NO2 - (f) and Nitrates NO3 - (g).

3. Biomass and thallus's size

In Sidi Rahal, the average annual biomass of *C. acicularis* was 373.43g/m^2,with seasonal variation ranging from 165.25g/m^2,in the autumn to 493.71 g/m^2, in the spring (Fig. 3b). The maximum average thalli size was 3.83 cm (n = 30) (April 2017) and the minimum 0.83 (n =30) (December 2017) (Fig. 4a). In El Jadida, the average annual biomass of *C. acicularis* was 298.3g/m^2,with seasonal variation ranging from 215.1g/m^2,in the summer to 379.4g/m^2,in the spring (Fig. 3b).The maximum average thalli size was 3 ±0.2 cm (n = 30) (September 2017) and the minimum 1.48±0.2cm (n =30) (February 2017) (Fig. 4a). In Souiria Kdima the average annual biomass of *C. acicularis* was 121.5g/m^2, with seasonal variation ranging from 104.7 g/m^2, in the summer to140.8 g/m^2, in the spring (Fig. 3b). The maximum average thalli size was 3.17 ±0.9 cm (n = 30) (September 2017) and the minimum 0.61±0.2cm (n = 30) (February 2017) (Fig. 4a).

The average biomass and the size of the thalli generally show a seasonal trend. The biomass showed low values in autumn for Sidi Rahal and in summer for the two sites El Jadida and Souiria Kdima, while the highest values were recorded in early spring for all three sites. (Fig. 3b). When analyzing the size of the plants, Souiria Kdima and El Jadida share the same period of a maximum size of the biomass which is the autumn season and precisely the month of September while Sidi Rahal recorded its maximum size during the period of summer and spring with the maximum value being recorded during April. On the other hand, the three sites recorded a minimum value during the winter. (Fig.3a, 3b; Fig.4a, 4b).

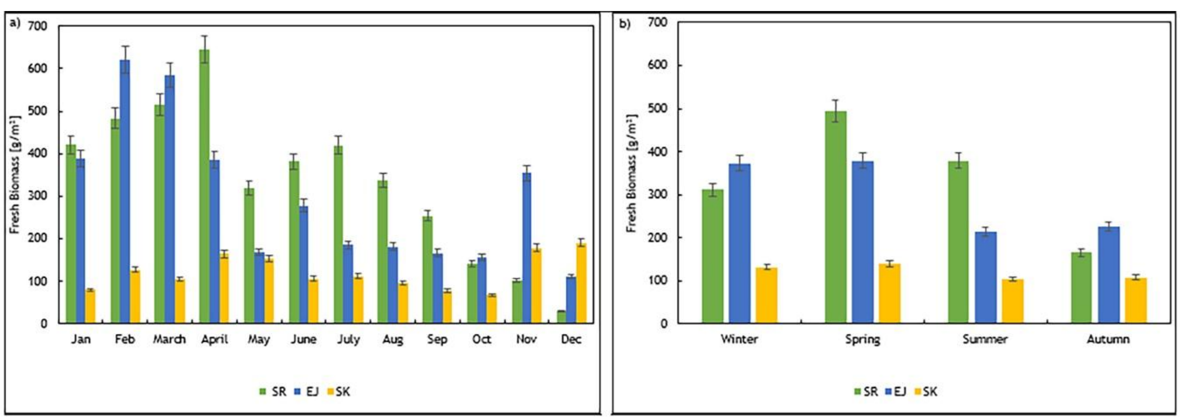

Fig. 3. Variation of the biomass of *Chondracanthus acicularis* at the three sites (SR, EJ, SK): (a) Monthly variation, (b) Seasonal variation.

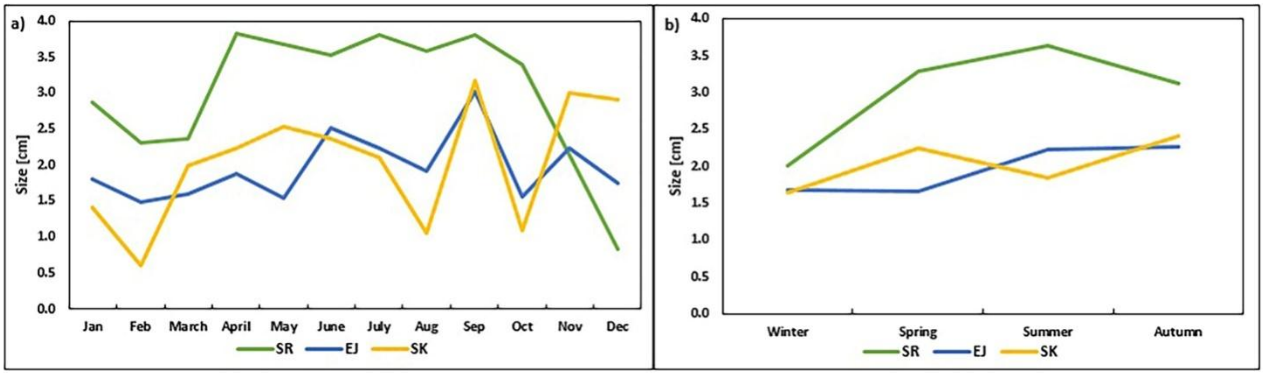

Fig. 4. Variation in size of *Chondracanthus acicularis* at the three sites (SR, EJ, SK): (a) Monthly variation; (b) Seasonal variation.

4. Fertility

In Sidi Rahal, reproductive thalli first appeared during winter then reached their maximum during spring, and then decreased and disappeared in the summer (Fig. 5b). In El Jadida, reproductive thalli first appeared during the beginning of winter; their proportion had increased during the season and remained stable until spring. During spring, a few reproductive thalli were still present, but they were absent through the summer months (Fig. 5b). Lastly, in Souiria Kdima, the proportion of reproductive thalli was high in winter reaching its maximum value in January, after that their proportion decreased in spring and disappeared during the summer and autumn seasons (Fig. 5).

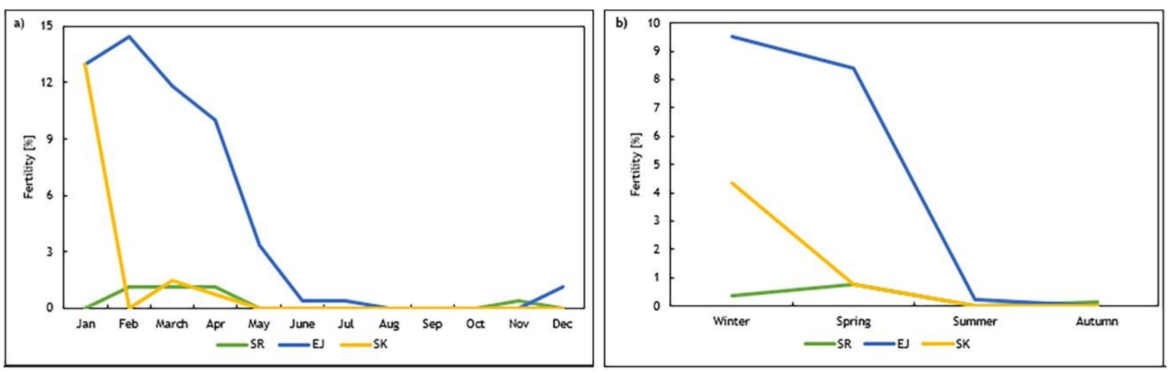

Fig. 5. Fertility temporal variation of *Chondracanthus acicularis* at the three sites (SR, EJ, SK): (a) Monthly variation, (b) Seasonal variation.

5. Wet to dry biomass conversion rate

The wet to dry weight ratios of *C. acicularis* ranged in Sidi Rahal from 26 to 37% with an average of 28.8% ± 1.61 (n = 30), in El Jadida from 26.1 to 32,3% with an average of 29.1% ± 1.94 (n = 30) and in Souiria Kdima from 26.4 to 34% with an average of 28.9% ± 1.62 (n = 30). The dry matter was maximal in January and March and minimal in October and December (Fig. 6).

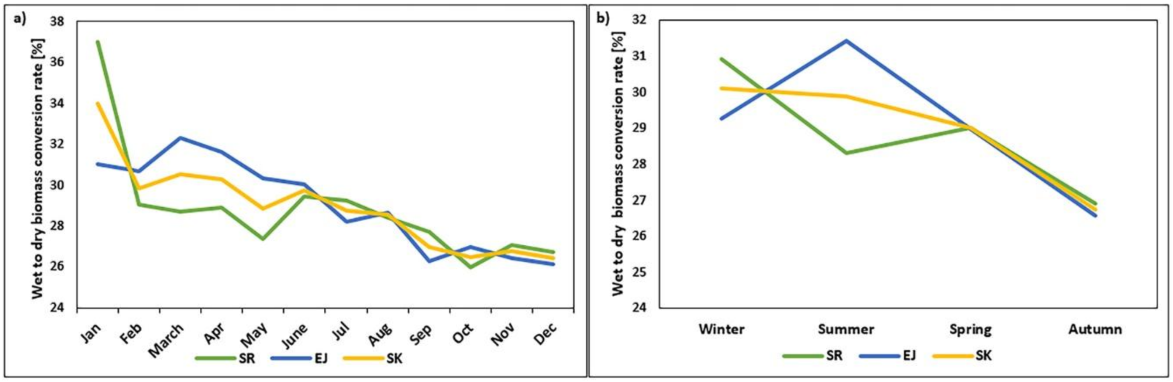

Fig. 6. Wet to dry biomass conversion rate of *Chondracanthus acicularis* at the three sites (SR, EJ, SK): (a) Monthly variation, (b) Seasonal variation.

6. Statistics

Correlations (Pearson's correlation) between each bioecological parameter (used as a particular variable of interest) and the other variables represented by FPCA, showed that biomass was significantly positively associated with fertility, $SiO_4^{4-}$and NO_2^-, whereas fertility was significantly negatively correlated to temperature and salinity, which explains its association to the season factor. Conversely, the length was not correlated to any of the other bioecological parameters, instead, it was significantly positively associated with temperature, pH, $NO_3^-$and SiO_4^{4-} (Fig. 7). The three diagrams of the FPCA also show that physicochemical variables form two independent and opposed clusters (the variables form two distinct groups of points that are roughly diametrically opposite): the salinity and the dissolved O_2 versus PO_4^{3-}, NO_2^-, NO_3^-, $SiO_4^{4-}$and pH. This means that the variables of the two clusters are negatively correlated but within each group, the variables are positively correlated (Fig. 7).

One-way ANOVA or Kruskal-Wallis test revealed a significant sampling site effect on the biomass and the size of *C. acicularis*. Thus, multiple comparisons test exhibited a difference in biomass existing between the average recorded at the Souiria Kdima site 121.5 g/m^{2}against the Sidi Rahal sites 373.43 g/m^{2}and El Jadida 298.3 g/m^{2}which were higher. Whereas, the thalli size in Sidi Rahal (3 cm on average) was higher than the samples collected in El Jadida and Souiria Kdima sites which recorded the same average size of 2 cm. The fertility averages, on the other hand, showed no significant differences between the three sites but only with a significance threshold of $\alpha = 6\%$. It was higher in El Jadida with 4.53% and very low in Souiria Kdima with 1.26% and in Sidi Rahal with 0.3%.

For the seasonal effect, both the biomass and the size do not show any significant differences throughout the year (2017). However, the average fertility was significantly higher in winter compared to summer and autumn. Hence, in Souiria Kdima, the reproductive thalli first appeared during the autumn and then reached their maximum during the winter until they begin decreasing in early spring up to complete disappearance in summer. In El Jadida, the reproductive thalli first appeared in December; their proportion had increased during January and remained stable up to March (Fig. 5). In April, few reproductive thalli were still present but they disappeared in summer. However, in Sidi Rahal, the proportion of reproductive thalli was consistently low throughout the whole year (2017) and comparable to the two other sites they were absent during the summer (Fig. 5).

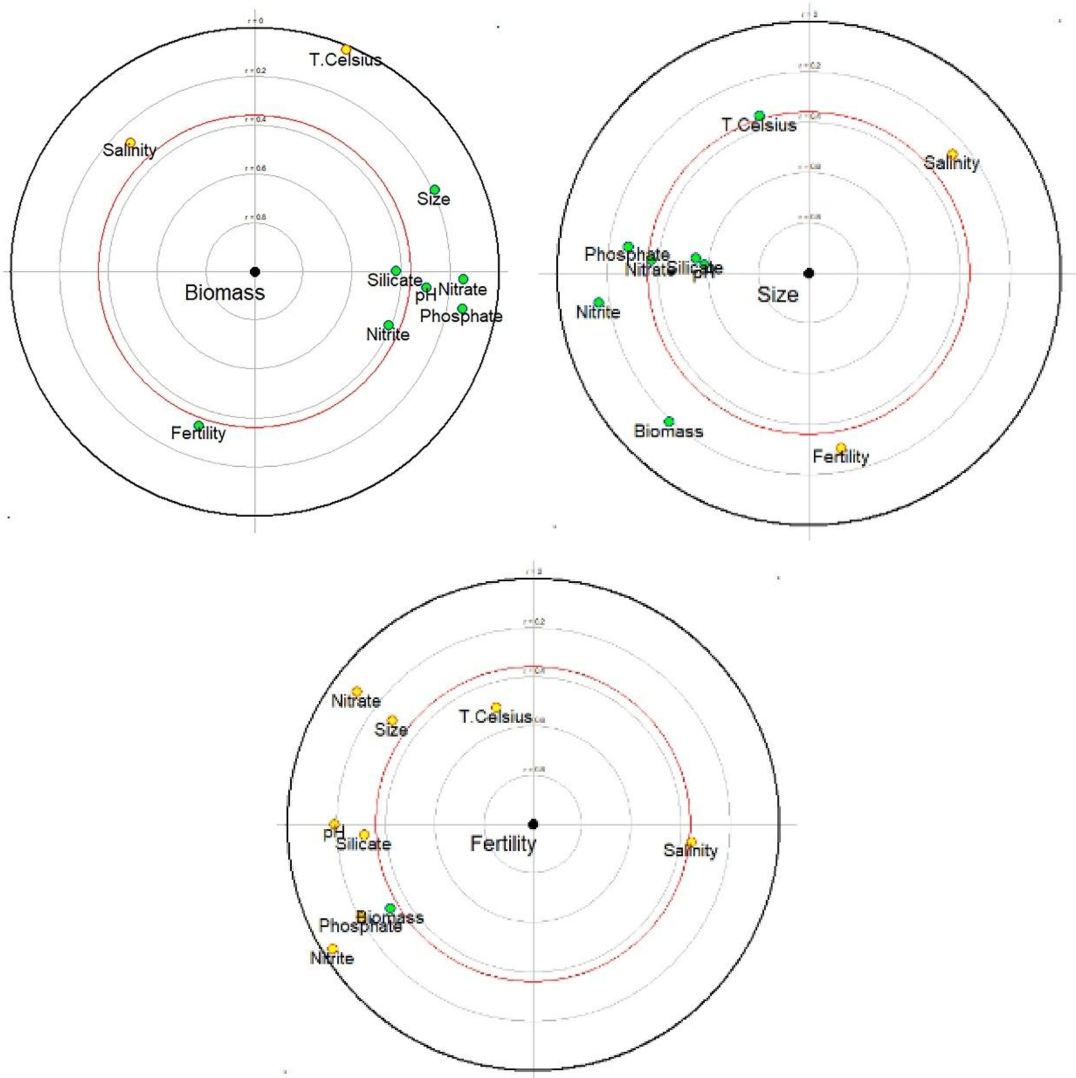

Fig. 7. Focused Principal Component Analysis (ACPF) on each of the biological parameters: Biomass in [g/m²], size in [cm], and fertility in [%]. The green points indicate the physicochemical parameters positively correlated with the variable of interest in the center. The yellow points indicate the physicochemical parameters correlated negatively with the variable in the center. The red circle indicates the significance threshold at 5% (the variables inside indicate significant correlations).

DISCUSSION

For the first time, the biology (biomass, size, and fertility) of *C. acicularis* was investigated in Morocco in relation to seasonality, physicochemical parameters, and nutrients. The results revealed that the mean biomass of *C. acicularis* was the highest in winter and spring (between January and April) at all stations (Fig. 3a). Similar results were reported for red macroalgae, such as *Chondracanthus teedei* (Mertens ex Roth), where they were abundant in spring **(Pereira and Mesquita, 2004).**

In addition, the highest values of biomass were recorded in sampling sites characterized by optimal physicochemical parameters and nutrients except for salinity (Fig. 2). It is well known that the conditions of the site directly influence the biomass of many seaweeds in terms of reduced levels of nutrients leading to lower levels of biomass**(Reith *et al.*, 2005; Elser *et al*., 2007;Pedersen *et al.*, 2010; Kerrison *et al.*, 2016)**. It was also revealed that for *Ecklonia radiata*, a minor increase in nutrient concentration in field-based mesocosms lead to a four times increase in biomass accumulation **(Falkenberg *et al.*, 2013).** Generally, red algae, such as *C. acicularis*, are characterized by an important protein content **(Dawes, 1998),** hence, variability of protein content automatically affects dry matter content. In this study, the highest values of dry matter content, which were registered between January and March, have coincided with relatively high biomass values, suggesting a link between biomass and protein content. Another study exhibited that protein levels were negatively correlated with water temperature and salinity**(Marinho-Soriano *et al*., 2006).** Meanwhile, the period between January and March was marked by decreased values of temperature and salinity, which indicates the influence on protein synthesis and, as a consequence, algae biomass.

The thallus sizes were relatively larger on average during summer in Sidi Rahal,and the minimum values were recorded in winter in the three stations. Our results were in concordance with those of Pereira and Mesquita (2004) and Pereira (2013), those authors revealed that the maximum size value in *Chondracanthus teedii* was also recorded during summer while the minimum value was recorded in winter**(Pereira and Mesquita, 2004 and Pereira, 2013).**

Fertility was higher in winter/spring and lower in summer in each of the three sites. On the Moroccan coast, tetrasporangial plants were detected in October **(Ardré, 1969)**, while cystocarpic plants have been detected from August to March **(Gayral, 1958).** Elsewhere, in the eastern Atlantic, Gayral (1966) reported reproductive cystocarpic plants of *C. acicularis* from November to February on the northern coast of France **(Gayral, 1966).** Seoane-Camba (1965) also found them in January and February on the northern coast of Spain, whereas tetrasporangial plants, were present between July and September in Portugal**(Seoane-Camba, 1965).** In this study, fertility varied throughout the year and was negatively correlated with temperature and salinity. This explains its association with the seasonal factor. Our results are in agreement with other fertility results in temperate regions tending to be seasonal, and consequently, seasonal fluctuations in the spore rain can be predicted **(Hoffmann, 1987).** In most cases, fertility is associated with periods of maximum growth so that the highest biomass usually overlaps with the highest fertility **(De Wreede, 1976; P. O., 1985;Trono and Tolentino, 1993; Hurtado and Ragaza, 1999).** Hurtado and Ragaza (1999)reported that the relationship between biomass and fertility can be determined from the life cycle of the plant and as the plant matures, there is an increase in thallus length, increase in the number and size of the leaves, vesicles, and appearance of the reproductive structures, all adding to the biomass of the plant **(Hurtado and Ragaza, 1999).** These observations are matching with our results that showed a positive and significant association between fertility and biomass where the peak of fertility was corresponding to the period of the highest biomass.

CONCLUSION

This study showed a positive and significant association between fertility and biomass where the peak of fertility was corresponding to the period of the highest biomass. Both fertility and biomass are negatively correlated with salinity. Furthermore, the physicochemical parameters and nutrients studied have a slight effect on biomass, size, and fertility. However, these environmental factors do not appear to be the only determinants because, in most cases, the correlation of these factors with the studied parameters remains unclear. Dry matter content may be affected by protein content. Studies have shown that protein content is negatively correlated with temperature and salinity, which is also the case for biomass. To further validate this hypothesis, which was already verified by other authors, studies on macronutrients' seasonal variability of *C. acicularis* are underway. To conclude, the distribution of *C. acicularis* also depends on the substrate's nature. Thus, the understanding of this alga's life cycle will be useful for decision-making on the management and conservation of this natural resource. The results of this study have established the first guidelines for the exploitation of *C. acicularis*. The collection of this carragenophytic red algae may be established during the summer and fall seasons for a period not exceeding three to four months. A biological rest however must be established at the beginning of winter to not disturb the biological cycle of this species.

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