Science of the Total Environment 618 (2018) 718–735

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Assessment of ecosystem health disturbance in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as sentinel species

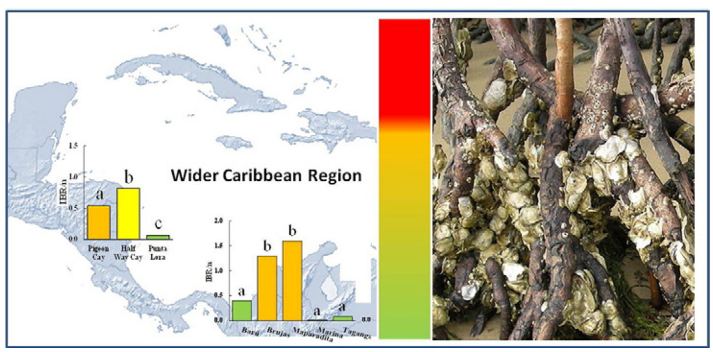
J. Aguirre-Rubí [a,b,c], A. Luna-Acosta [d,e], M. Ortiz-Zarragoitia [a,b], B. Zaldibar [a,b], U. Izagirre [a,b], M.J. Ahrens [d], L. Villamil [d,f], I. Marigómez [a,b,*]

[a] CBET Res. Grp., Dept. Zoology & Animal Cell Biology, Univ. Basque Country (UPV/EHU), Leioa, Basque Country, Spain
[b] Research Centre for Experimental Marine Biology and Biotechnology (Plentzia Marine Station; PiE-UPV/EHU), Univ. Basque Country, Plentzia, Basque Country, Spain
[c] National Autonomous Univ. Nicaragua-León (UNAN-León), León, Nicaragua
[d] Dept. Biological and Environmental Sciences, Univ. Jorge Tadeo Lozano (UJTL), Bogotá, Colombia
[e] Dept. Ecology and Territory, Pontificia Univ. Javeriana, Bogotá, Colombia
[f] Biosciencies Doctoral Program, Faculty of Engineering, Univ. de La Sabana, Colombia

HIGHLIGHTS

- Health status of wild oysters, determined in Caribbean mangrove conditions
- A toolbox of non-sophisticated biological effects endpoints was applied.
- Signs of ecosystem health disturbance, related to contaminants and sewage
- Approach suitable for assessing health disturbance in mangrove-lined coastal ecosystems
- Mangrove cupped oysters, proper sentinels for tropical coastal biomonitoring

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 May 2017
Received in revised form 28 July 2017
Accepted 10 August 2017
Available online 19 October 2017

Editor: D. Barcelo

Keywords:
Mangrove cupped oyster
Biomarkers
Condition
Reproduction
Histopathology
Ecosystem health
Biomonitoring

ABSTRACT

This investigation was aimed at contributing to develop a suitable multi-biomarker approach for pollution monitoring in mangrove-lined Caribbean coastal systems using as sentinel species, the mangrove cupped oyster, *Crassostrea rhizophorae*. A pilot field study was carried out in 8 localities (3 in Nicaragua; 5 in Colombia), characterized by different environmental conditions and subjected to different levels and types of pollution. Samples were collected in the rainy and dry seasons of 2012–2013. The biological effects at different levels of biological complexity (Stress-on-Stress response, reproduction, condition index, tissue-level biomarkers and histopathology) were determined as indicators of health disturbance, integrated as IBR/n index, and compared with tissue burdens of contaminants in order to achieve an integrative biomonitoring approach. Though modulated by natural variables and confounding factors, different indicators of oyster health, alone and in combination, were related to the presence of different profiles and levels of contaminants present at low-to-moderate levels. Different mixtures of persistent (As, Cd, PAHs) and emerging chemical pollutants (musk fragrances), in combination with different levels of organic and particulate matter resulting from seasonal oceanographic variability and sewage discharges, and environmental factors (salinity, temperature) elicited a different degree of disturbance in ecosystem health condition, as reflected in sentinel *C. rhizophorae*. As a result, IBR/n was correlated with pollution

* Corresponding author.
E-mail address: ionan.marigomez@ehu.eus (I. Marigómez).

https://doi.org/10.1016/j.scitotenv.2017.08.098

indices, even though the levels of biological indicators of health disturbance and pollutants were low-to-moderate, and seasonality and the incidence of confounding factors were remarkable. Our study supports the use of simple methodological approaches to diagnose anomalies in the health status of oysters from different localities and to identify potential causing agents and reflect disturbances in ecosystem health. Consequently, the easy methodological approach used herein is useful for the assessment of health disturbance in a variety of mangrove-lined Caribbean coastal systems using mangrove cupped oysters as sentinel species.

1. Introduction

Mangrove ecosystems are threatened by a combination of natural disasters, tourism, aquaculture, deforestation and chemical pollution (Bayen, 2012; Lewis et al., 2011). In mangroves of the Wider Caribbean Region (WCR, UNEP Regional Seas Programme), pollution monitoring programmes have been carried out since the 1970′s to determine the concentrations of pesticides and other persistent organic pollutants (POPs), PAHs and metals in seawater, sediments, seafood and in biomonitor species (Fernandez et al., 2007; Sericano et al., 1995). Particularly, mangrove cupped oysters (*Crassostrea rhizophorae*) have been proposed as biomonitors for pollution monitoring in mangrove ecosystems (Aguirre-Rubí et al., 2017; Silva et al., 2006).

Marine pollution monitoring cannot be based solely on chemical data because these do not provide any indication of the magnitude of deleterious effects exerted to biota and ecosystems by combinations of pollutants in multiple stress scenarios (Allan et al., 2006; Cajaraville et al., 2000). Consequently, it is recommended to include both chemical and biological effects endpoints in pollution monitoring programs (ICES, 2011, 2012). Thus, biomarkers are "early warning signals" commonly used to assess the biological effects exerted by mixtures of chemicals on sentinel species in complex environmental conditions (Cajaraville et al., 1993; Marigómez et al., 2013); which have recently become an integral component of environmental monitoring programmes in several countries (Schettino et al., 2012).

"Biological effects" monitoring based on the biomarker approach is not currently being carried out in the Caribbean coastal zone. Although information about the biological effects exerted by chemical pollutants on mangrove oysters is gaining attention (Alves et al., 2002; Maranho et al., 2012; Rebelo et al., 2003; Zanette et al., 2008), the monitoring capacities and facilities in many WCR countries can be far away from those required to conduct sophisticated and most-advanced biomarker-based monitoring using oysters as sentinels. However, as very recently stated by Blaise et al. (2016), effortless and low cost biomarkers can provide basic knowledge on animal health and water quality and are technically achievable everywhere. Accordingly, and aware of the technical and logistic limitations (e.g., dealing with limited accessibility and difficulties for secure sample transportation and quality in situ processing), a toolbox of biological effects endpoints was selected to assess the potential of the mangrove cupped oyster, *C. rhizophorae*, as sentinel for pollution monitoring in Caribbean mangroves and coastal zones. This toolbox included undemanding measurements of responses at population and individual level, and histopathological analyses; these latter allow scoring responses at systemic and tissue levels on the basis of low-cost, solid and straightforward technology.

The Stress-on-Stress (SoS) response has been recommended by ICES (2012) for monitoring programmes as an indicator of mussel health status (Hellou and Law, 2003). The successful application of SoS response as biomarker for environmental monitoring in mussels led to its subsequent wider application to other bivalve species, especially in subarctic and temperate regions (Blaise et al., 2016). SoS response is a cost-effective test, in which the capacity of bivalves to survive on air is scored as a measure of resilience (Smaal et al., 1991; Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995). It has been applied in the field to detect effects of urban discharges to estuarine and coastal waters using both native (Hellou and Law, 2003) and transplanted mussels (Moles and Hale, 2003), as well as for assessing oil spill impact (Thomas et al., 1999) and in laboratory experiments (Eertman et al., 1995; Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995). It is not as sensitive as some core biomarkers of general stress (e.g. lysosomal membrane stability) but is more sensitive than others and its methodology is simple, rapid and low-cost (Viarengo et al., 1995). Flesh Condition Index (FCI) reflects the physiological status of bivalves and it has been reported that it is reduced on exposure to chemical pollutants (Mubiana et al., 2006). SoS response and FCI are considered low-cost biomarkers that are easy to be applied with little monetary or logistic investment (Blaise et al., 2016).

Gamete development and gonad histopathology were included in the toolbox of biological effects endpoints because the gametogenic cycle is a backbone reference to understand health condition and the biological effects of pollutants, and because changes in normal gametogenic cycle and reproduction disturbances (e.g. intersex) are well-known biological effects of chemical pollutants (Ortiz-Zarragoitia and Cajaraville, 2010; Ortiz-Zarragoitia et al., 2011). Finally, digestive gland histopathology was also incorporated into the toolbox, because a wide range of contaminants, including metals, pesticides and PAHs, is known to provoke histopathological alterations in this tissue (Au, 2004; Bignell et al., 2012; Garmendia et al., 2011; Kim and Powell, 2007; Kim et al., 2008).

For the purpose of contributing to develop a suitable multi-biomarker approach for pollution monitoring in Caribbean mangroves and coastal zones using as sentinel species the mangrove cupped oyster, *C. rhizophorae*, a pilot field study was carried out in 2012–2013 in 8 localities (3 in Nicaragua and 5 in Colombia) characterized by different levels and types of pollution. Samples were collected in the rainy and dry seasons of 2012–2013. Results describing the tissue levels of pollutants were published in a preceding paper (Aguirre-Rubí et al., 2017), in which the suitability of mangrove cupped oysters as biomonitors for Caribbean mangroves and coastal zones was confirmed. In the present investigation, the biological effects at different levels of biological complexity (stress on stress response, reproduction, condition index, tissue-level biomarkers and histopathological condition) were determined, integrated as IBR/n index (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006; Marigómez et al., 2013), and compared with tissue burden of chemical pollutants (Aguirre-Rubí et al., 2017) in order to achieve an integrative biomonitoring approach.

2. Material and methods

2.1. Sampling sites and sample collection

Different representative scenarios of mangrove ecosystems from the Caribbean were selected. In Nicaragua, subtidal (<1 m depth) oyster reefs were sampled in two localities (Fig. 1a): Bluefields (sampling sites: Punta Lora and Half Way Cay) and Pearl Lagoon (sampling site: Pigeon Cay). Punta Lora was considered as a prospective reference site (remote from urban settlements) whilst Half Way Cay and Pigeon Cay were selected as potentially polluted areas influenced by aquatic transport and urban discharges (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013). In Colombia, intertidal prop roots of red mangrove trees (*Rhizophorae mangle*) were sampled in Cartagena Bay and Barbacoas Bay (Fig. 1b) and in Taganga Bay (Fig. 1c), and intertidal rocky habitat

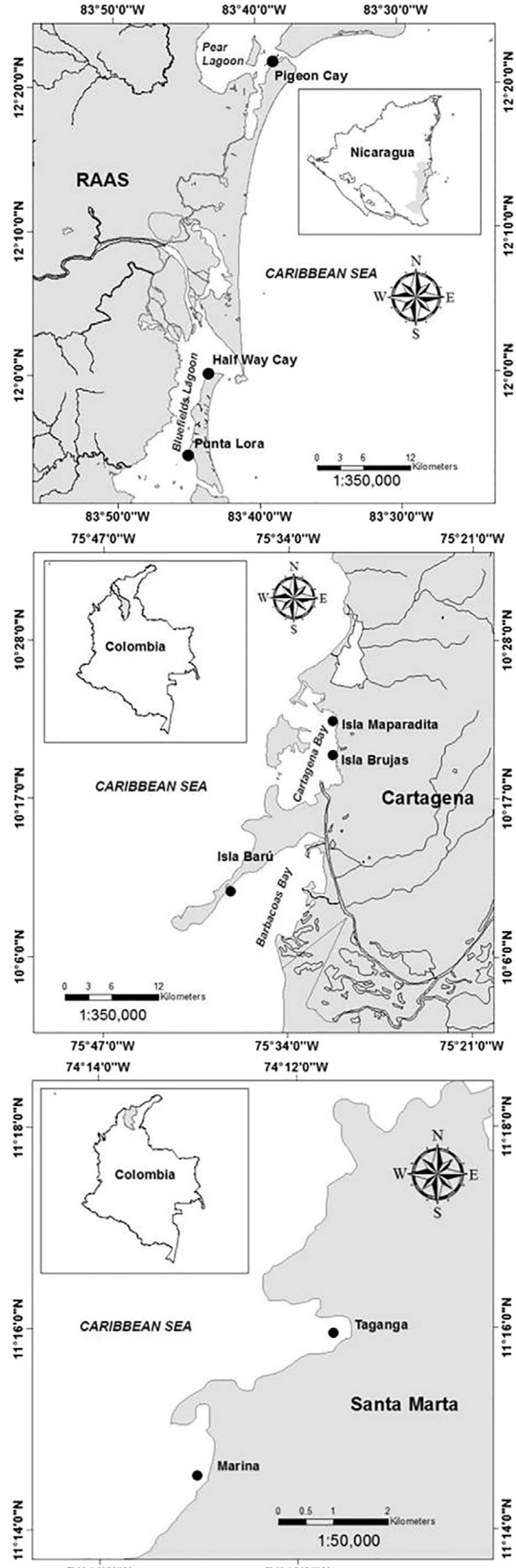


Fig. 1. Caribbean coastal maps from Nicaragua and Colombia, showing the localities where oysters *Crassostrea rhizophorae* were collected in 2012–2013. Nicaraguan coast: Pigeon Cay (12°21′42.50″N-83°38′32.11″W); Half Way Cay (11°59′58.73″N-83°43′28.24″W) and Punta Lora (11°54′21.02″N-83°44′58.68″W). Colombian coast: Isla Barú (10°10′56.02″N-75°38′21.74″W); Isla Brujas (10°19′59.07″N-75°30′47.24″W) and Isla Maparadita (10°22′21.09″N-75°30′48.65″W) in Cartagena Bay and Marina Santa Marta (11°14′31.59″N-74°13′05.24″W) and Taganga (11°16′07.68″N-74°11′37.37″W) in Santa Marta Bay.

in Santa Marta Bay (Fig. 1c). In Cartagena Bay, a mangrove-lined islet 200 m north of Isla Maparadita (0.5 km offshore the Terminal of Cartagena Port) and Isla Brujas were selected as seemingly polluted sites, as shown in previous studies (Vivas-Aguas et al., 2010, 2014). Isla Brujas is an islet adjacent to the industrial zone of Mamonal (oil refineries, petrochemicals, and asphalt, cement and smelting plants) that also receives the direct impact of the Dique Channel. This channel was dug five centuries ago to connect the Magdalena River with the Cartagena Bay for navigation and constitutes a major source of sediments and chemical pollution to Cartagena Bay (Vivas-Aguas et al., 2014). In addition, Isla Barú in Barbacoas Bay was selected as a putative reference site; however, it is also subjected to discharges from the a Magdalena River to which it communicates by smaller channels via the Dique Channel since the 1950′s (Gómez-Giraldo et al., 2009). In Santa Marta Bay, the Marina of Santa Marta was selected as a sampling site subject to strong anthropogenic influence (García et al., 2012). The nearby Taganga Bay was chosen a priori as a reference site. Sampling was carried out over one year (2012–2013) in the rainy season (October 2012) and in the dry season (March 2013). Environmental conditions and anthropogenic impacts and contamination were different between Nicaraguan and Colombian sampling sites, as well as among localities within each study area (ESM 1). In parallel, the levels of a variety of pollutants in each locality were determined (Aguirre-Rubí et al., 2017). Briefly, low tissue concentrations of metals (e.g., Ag, As, Pb, Cd) and PAHs, moderate-to-high tissue concentrations of Hg, HCHs, DDTs, detectable levels of chlorpyrifos, PCBs and BDE85 (in Pigeon Cay) and negligible levels of musks were recorded in Nicaraguan oysters. The profile of POPs was different in the oysters from the Colombian localities where (a) the tissue concentration of HCHs, chlorpyrifos, PCBs and PBDEs were in the range of non-detected to low; (b) noticeable tissue concentrations of musk fragrances were recorded in the dry season; and (c) the levels of Ag, As, Pb, Cd, and PAHs in several localities ranged from moderate to extremely high.

Up to 85 mangrove cupped oysters (*Crassostrea rhizophorae*) were simultaneously collected per sampling site, of which 25 were used for the chemical assessment (Aguirre-Rubí et al., 2017) and the remaining 60 for biological effects analyses presented herein. Upon collection, oysters were placed in 15 L plastic boxes (2 individuals/L) in seawater at ambient temperature and transported to the laboratory (for 3–6 h) before processing. Due to logistic problems, sampling could not be conducted in Pigeon Cay during the dry season.

2.2. Condition and histopathology

2.2.1. Stress on stress (SoS) test

The time until 50% mortality (LT_{50}) was calculated by means of the "survival-in-air" SoS test (Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995). For this purpose, 30 oysters from each site and sampling period were placed over wet paper on plastic trays at constant room temperature and 100% humidity. Survival was assessed daily and oysters were considered to be dead when their valves gaped and failed to close when they were physically stimulated, or simply presented a bad smell.

2.2.2. Flesh condition index

Shell length (L) was determined as the length in mm of the largest valve (the long axis measured to the nearest 0.1 mm with Vernier callipers; Dame, 1972). Flesh condition index (FCI) was intended to be determined as FDW/SDW, where FDW is the flesh dry weight (soft parts dried at 105 °C and weighed to the nearest 0.1 mg; Lobel and Wright, 1982) and SDW is the shell dry weight (dried at room temperature and measured in g; Crosby and Gale, 1990). However, this method was only applied in Colombian oysters because SDW could not be properly determined in Nicaraguan oysters as their shells were often broken. Instead, alternative estimates of SDW and L were obtained on the basis of shell cavity volume (SCV) values upon applying model functions (Crosby and Gale, 1990) adjusted to local population traits; as derived from direct SDW and L measurements carried out only in a particular set of selected (unbroken) oysters collected in the three Nicaraguan localities in October 2013. SCV was determined according to Rebelo et al. (2005). Briefly, moulds of the shell internal cavity were made using plasticine and introduced into a water-filled glass column to verify water displacement. Displaced water dripped through a tap in the column and was collected in a Petri dish over a balance; SCV (expressed in mL) was calculated via the mass of the displaced water; thus, FCI = FDW/SCV = 100 × FDW (g)/SCV (mL) (Scott and Lawrence, 1982). This alternative method was decided after the 2012 sampling campaign and therefore it could not be applied to Colombian oysters collected in 2012. Thus, it was decided to keep on using FDW/SDW in Colombia and FDW/SCV in Nicaragua for inter-seasonal comparisons at regional scale and to calculate FDW/L as an oyster FCI suitable for inter-regional comparisons. Moreover, using L as the reference to construct condition indices in oysters (e.g. FDW/L or FDW/L^3; Hellou et al., 2003) overcomes uncertainties associated to unpredictable variations in SDW and difficulties resulting from inaccurate SCV determinations (Blaise et al., 2016).

2.2.3. Histology and histopathology

Upon dissection of their soft body ($N = 20$ oysters/sample), central cross-sectioned slices (3–5 mm thick, including digestive gland, gonad, mantle and additional tissues) were fixed in Davidson's fixative for 48 h and paraffin embedded. Microtome sections (5 μm) were obtained using an automated rotary Leica RM 2255 microtome (Leica Microsystems, Nussloch, Germany), stained with haematoxylin-eosin and examined under the light microscope (Olympus BX61; Tokyo, Japan) for gamete development determination and histopathological diagnosis (Kim et al., 2006).

2.2.4. Gamete development and reproductive disturbance

Gamete developmental stages were examined microscopically. Slides of 20 oysters per sample were examined individually under the light microscope using 10× and 20× objective lenses. The sex of each animal was recorded and the total number of female and male oysters was calculated for each sampling time and locality, and with these data, the sex ratio was calculated. Further on, the sex ratio index (SRI) was calculated as the G value of females-to-males ratio (F:M) upon the gross assumption of theoretical F:M ratios (after Vélez, 1982) of 2.3:1 in Nicaragua rainy season, 1:1 in Nicaragua dry season and Colombia rainy season, and 1:1.3 in Colombia dry season. Intersex index (IXI) was calculated as the prevalence of intersex (and hermaphroditic) individuals at each locality and season. The prevalence of gamete developmental stages was determined upon scoring the maturity of the follicles and gametes according to Kim et al. (2006); and also a gonad index (GI) value was assigned (modified after Ortiz-Zarragoitia and Cajaraville, 2010): 1 = undifferentiated (GI = 0); 2 = early development (GI = 1); 3 = mid development (GI = 2); 4 = late development (GI = 4); 5 = full development (GI = 5); 6 = early spawning (GI = 3); 7 = advanced spawning (GI = 2) and 8 = post-spawning (GI = 1). Gamete mass index (GMI) was calculated as the log ratio of the sum of the prevalence of developmental stages 2–5 (gonad tissue growth) to the sum of the prevalence of stages 6–8 (gonad tissue loss). Undifferentiated index (UDI) was estimated as the prevalence of stage 1. Oocyte atresia prevalence was recorded in female oysters and its intensity was scored (0–4) individually according to the scale for abnormal gonad development proposed by Kim et al. (2006) for mussels. Oocyte atresia index (OAI) was calculated by multiplying prevalence × intensity. Reproductive Anomalies Index (RAI) was calculated as weighted average of the deviation from the theoretical maximum anomalies of the specific indices of reproductive disturbance, according to the following formula:

$$\mathrm{RAI} = \frac{1}{5} \times \left(\frac{\mathrm{CF} \times \mathrm{SRI}}{\mathrm{SRI}_{50}} + \frac{\mathrm{CF} \times \mathrm{IXI}}{\mathrm{IXI}_{50}} + \frac{\mathrm{CF} \times \mathrm{GMI}}{\mathrm{GMI}_{max}} + \frac{\mathrm{CF} \times \mathrm{UDI}}{\mathrm{UDI}_{max}} + \frac{\mathrm{CF} \times \mathrm{OAI}}{\mathrm{OAI}_{50}} \right)$$

where CF = ×100/1.6 (thus RAI changes between 0 and 100); SRI_{50} = 27.7 for a 50% deviation from the theoretical sex ratio; IXI_{50} = 50, when a 50% prevalence of intersex individuals occurs; (c) where GMI_{max} = 2, when 100% of the gametes are mature in absence of any sign of spawning; (d) UDI_{max} = 100, when 100% of the individuals do not present differentiated gametes; and OAI_{50} = 2, the median value of the scale for atresia scoring after Kim et al. (2006).

2.2.5. Digestive gland histopathology

Slides of 20 oysters per sample were examined individually under the light microscope using 10×, 20× or 40× objective lenses. Parasites and histopathological alterations were scored using either quantitative or presence/absence scales (Kim et al., 2006). Intracellular ciliates, unidentified intracellular protists, *Nematopsis* sp., metazoans (trematodes, cestodes), *Rickettsia*/*Chlamydia*-like organisms (R/CLO) and granulocytomas were recorded quantitatively following procedures previously described (Kim et al., 2006; Kim and Powell, 2007; Garmendia et al., 2011). Quantitative scores were made by keeping a running count of the incidences as the slide was scanned to avoid re-examination of each slide multiple times for each category. Haemocytic infiltration (without distinction between focal and diffuse), brown cell aggregates and disseminated neoplasia were scored as present/absent for each individual oyster. Prevalence and intensity of histopathological lesions were determined in Nicaragua, and prevalence in Colombia, according to Kim and Powell (2007) and Garmendia et al. (2011), as follows:

$$\mathrm{Prevalence} = \frac{\mathrm{N.affected\ individuals} \times 100}{\mathrm{N.examined\ individuals}}$$

$$\mathrm{Intensity} = \frac{\sum_{i=1}^{n} \left(\begin{array}{c}\mathrm{N.occurrences\ of\ parasite} \\ \mathrm{or\ pathology\ per\ individual}\end{array}\right)}{\mathrm{N.affected\ individuals}}$$

The inflammatory response index (IRI) was calculated as the sum of the prevalence values of oedema, granulocytomas and disseminated neoplasia. The parasitic infestation index (PII) was calculated as the sum of the prevalence values calculated individually for each of the 5 groups of parasites aforementioned. The digestive gland atrophy index (DGAI) was estimated according to Kim and Powell (2007).

2.2.6. Integrated biomarker response (IBR)

IBR index was based on the integration of five biological responses from cellular to community levels (DGAI, IRI, FDW/L, RAI and $1/LT_{50}$) according to Beliaeff and Burgeot (2002), Broeg and Lehtonen (2006), and Marigómez et al. (2013). Calculations were based on a multivariate graphic method, according to the following procedure: (1) calculation of the mean and standard deviation for each sample; (2) standardization of data for each sample: $x_i' = (x_i - x)/s$; where, x_i' = standardised value of the biomarker; x_i = mean value of a biomarker from each sample; x = general mean value of x_i calculated from all compared samples (data set); s = standard deviation of x_i calculated from all samples; (3) addition of the standardised value obtained for each sample to the absolute standardised value of the minimum value in the data set: $y_i = x_i' + |x_{min}'|$; (4) calculation of the radar plot triangular areas as $A_i = (0.59 \times (y_i \times y_{i+1})/2$, where "$y_i$" and "$y_{i+1}$" are the standardised values of each biomarker and its next one in the radar plot, respectively, and 0.59 is sin α (α: radial angle for a pentagonal radar plot; α = 2π/5);

and (5) calculation of the IBR index which is the summing-up of all the radar plot triangular areas ($IBR = \sum A_i$) (Beliaeff and Burgeot, 2002). Since the IBR value is directly dependent on the number of biomarkers in the data set, the obtained IBR value was divided by the number of biomarkers used to calculate IBR/n (Broeg and Lehtonen, 2006).

2.3. Statistical analyses

Statistical analyses were carried out with the aid of SPSS version 22 statistical package (IBM SPSS, Armonk, NY, USA). Normal distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test) of data were determined before proceeding with subsequent analyses. Due to the high variability between individuals, even after normal distribution transformation of the data, non-parametric analyses were carried out. Significant differences in the prevalence of inflammatory responses and parasitic lesions and in health condition indices were analysed by the Z score test. Survival values of SoS test were analysed with the non-parametric Kaplan-Meier analysis, followed by the Tarone-Ware post hoc test for comparisons between localities for each season and between seasons for each locality. Differences in SCV, L, SDW, FCI (FDW/SCV and FDW/SDW), DGAI and GI were determined by applying a Kruskal–Wallis test, followed by Dunn's post hoc test. Sex ratio bias was studied using the G test of association, comparing total number of female and male mussels and normalizing for theoretical gender bias. In all the tests used herein, significant differences were established at $p < 0.05$.

3. Results

3.1. Nicaragua

Shell L, FDW, SCV and SDW were smaller ($p < 0.05$) in Pigeon Cay than in Half Way Cay and Punta Lora in the rainy season; likewise, all these biometric parameters presented lower values ($p < 0.05$) in the dry season than in the rainy season in the latter two localities (Table 1). Moreover, FDW was less in Half Way Cay than in Punta Lora at both seasons. A similar pattern was observed regarding condition indices. FSW/SCV, SDW/L and FDW/L values were lower in Pigeon Cay than in Half Way Cay and Punta Lora during the rainy season, lower in the dry season than in the rainy season in the latter two localities and smaller in Half Way Cay than in Punta Lora in the rainy season (Fig. 2a–c).

Sex ratio values for each sampling location at each sampling time are shown in Table 1. Statistically significant bias in the sex ratio towards females (2.82:1; G value = 8.65, $p < 0.05$) was detected for the whole Nicaraguan population of mangrove cupped oysters in comparison with the theoretical sex ratio of 1.65:1 (proxy based on the average between 2.3:1 (rainy season) and 1.1 (dry season) sex ratios). This bias towards females was also found in the rainy season (3.75:1; G value = 4.95, $p = 0.03$) but not in the dry season (1.21:1; G value = 5.08, $p = 0.18$), when a 22.5% of the oysters presented undifferentiated gonads. Indeed, the three studied localities showed a significant bias towards females in the rainy season (Table 1). No intersex was recorded. Different gamete development stages were observed depending on the season and the locality (Figs. 3 and 4). In the rainy season, gamete development was delayed in Pigeon Cay in comparison with the other two localities, and most advanced in Punta Lora (Fig. 3a). In the dry season, gamete development was more advanced in Half Way Cay than in Punta Lora (Fig. 3a). Accordingly, significant differences ($p < 0.05$) were observed in GI between seasons in Half Way Cay and between Half Way Cay and Punta Lora in both seasons (Table 1). In contrast, no differences in GMI and UDI were recorded (Table 1). The levels of oocyte atresia (Fig. 4c) were similar in both seasons in all three localities except in Half Way Cay in the dry season, when no oocyte atresia was recorded (Table 1). As a whole, RAI reflects only a low-to-moderate (<10) reproductive disturbance in Pigeon Cay and Half Way Cay, mainly featured by deviations in the expected SRI and GMI values (Fig. 2d).

Histopathological examination revealed the presence of a variety of parasites such as intracellular ciliates, undetermined intracellular protists, *Nematopsis* sp., trematodes and *Rickettsia*/*Chlamydia*-like organisms (R/CLO) (Fig. 5). Some parasites had a local impact such as metazoans (Half Cay in dry season) and R/CLO (Punta Lora in dry season) (Fig. 5g and i). Other parasites such as the unidentified intracellular protists were found sporadically (Fig. 5e). Ostensibly, parasite prevalence was low in Punta Lora in the rainy season and PI was higher in Half Way Cay than in Punta Lora all over the year (Table 1). Nevertheless, the intensity of parasitic infestations was generally low. It is worth noting that intracellular ciliates (Fig. 5f) and *Nematopsis* sp. (Fig. 5h) presented the highest prevalence values throughout the entire study period; whilst highest intensities were seemingly recorded for unidentified intracellular protists (Table 1) in the rainy season. PII values were higher during the rainy season in Pigeon Cay and Half Way Cay than in Punta Lora, where PII values increased in the dry season (Fig. 2e).

Unlike in the dry season, the histological integrity of the digestive gland tissue was seemingly altered in all the studied localities in the rainy season (Fig. 5a and b). A large part of the digestive gland tissue was occupied by disorganized interstitial connective tissue (ICT) with haemocytic infiltration (Fig. 5b and d) and the caliper of digestive ducts was particularly undersized (Fig. 5b and f). Inflammatory responses such as massive haemocytic infiltrations of ICT (oedema), brown cell aggregates, granulocytomas and disseminated neoplasia were observed (Fig. 5b–d). In contrast, prevalence of inflammatory responses was apparently higher in the dry season than in the rainy season and more so in Half Way Cay than in Punta Lora; however, differences after applying the Z score test could only be established at $p < 0.1$ (Table 1). Prevalence of brown cell aggregates was always high whereas only one granulocytoma was found in one oyster from Pigeon Cay in the rainy season (Fig. 5d). Two isolated cases of oysters with disseminated neoplasia were recorded in the rainy season (Table 1; Fig. 5c). Unlike for the case of PII, IRI was higher in the dry season than in the rainy season (Fig. 2f); moreover, its values were always higher in Pigeon Cay and Half Way Cay than in Punta Lora.

Digestive alveoli presenting severe atrophy (with a wide lumen and thin epithelium) and separated by ample areas of ICT (Fig. 5b, d and f) were observed all over the year, but their incidence was particularly high in the rainy season. In addition, digestive cell vacuolisation (Fig. 5e) was recorded in a few specimens (<10%) from Half Way Cay during both seasons. Thus, in the rainy season DGAI was similar in the three localities and always higher than in the dry season, especially in Punta Lora ($p < 0.05$; Fig. 2g). In contrast, in the dry season DGAI differed between localities, with higher values in Half Way Cay than in Punta Lora ($p < 0.05$; Table 2).

LT_{50}, as determined by the SoS assay, was significantly different between seasons for Half Way Cay and Punta Lora (Tarone-Ware test, $p < 0.05$) but not between localities for a given season (Fig. 2h). The highest LT_{50} values were observed in the rainy season, whilst 2–3 fold lower values were recorded in the dry season (Fig. 2h).

Five indices of biological response (DGAI, IRI, FDW/L, RAI, LT_{50}) were represented in radar plots (Fig. 7). The depicted profiles revealed that in the rainy season low levels of biological complexity (DGAI and IRI) and reproduction (RAI) were responsive in Half Way Cay and Punta Lora whilst all the indices except LT_{50} (SoS) responded in Pigeon Cay. Conversely, quite different profiles were observed in the dry season, with responses at more complex levels of biological organisation and especially in SoS. Thus, significant differences were found between localities in the IBR/n index in the rainy season after applying the Z score test, with IBR/n values being lowest in Punta Lora, intermediate in Half Way Cay and highest in Pigeon Cay (Fig. 7).

Table 1
Condition and histopathology of *Crassostrea rhizophorae* from shallow subtidal oyster reefs of Nicaraguan mangrove lagoons. Shell cavity volume, condition index and atrophy their values are mean ± standard error ($n = 20$). Sex Ratio Index = SRI's G value. The superscript letters (a and b) indicate significant differences between groups ($p < 0.05$). Asterisks (*) indicate seasonal difference for a locality ($p < 0.05$); ♯, significantly different from the theoretical gender bias (Vélez, 1982) according to the G test ($p < 0.05$). [1], estimated based on model functions.

	Rainy season			Dry season	
	Pigeon Cay	Half Way Cay	Punta Lora	Half Cay	Punta Lora
Shell length (L; mm)[1]	59.7 ± 2.1^{a}	79.9 ± 2.6^{b}	84.8 ± 2.4^{b}	$70.4 \pm 4.0^{*}$	$72.4 \pm 2.6^{*}$
Flesh dry-wt (FDW; mg)	118.4 ± 16.3^{a}	296.1 ± 22.6^{b}	370.3 ± 26.1^{b}	$113.3 \pm 16.3^{a*}$	$149.5 \pm 13.9^{b*}$
Shell cavity volume (SCV; mL)	4.9 ± 2.5^{a}	9.6 ± 4.4^{b}	10.9 ± 4.3^{b}	$7.3 \pm 4.4^{*}$	$7.4 \pm 2.8^{*}$
Shell dry-wt (SDW; g)[1]	11.2 ± 1.1^{a}	24.0 ± 1.8^{b}	27.2 ± 1.7^{b}	$18.4 \pm 2.5^{a*}$	$18.6 \pm 1.6^{a*}$
Sex ratio (Sex ratio index)	$5.2:1^{♯}$ (3.91)	$6.4:1^{♯}$ (5.74)	$2.1:1^{♯}$ (0.09)	$1:1.4^{*}$ (0.01)	$1.7:1^{*}$ (2.16)
Intersex index	0	0	0	0	0
Gonad index	3.5 ± 0.2^{b}	3.8 ± 0.2^{b}	3.3 ± 0.1^{a}	$2.1 \pm 0.4^{a*}$	3.7 ± 0.3^{b}
Gametogenic mass index	0.77	0.70	0.10	0.10	0.34
Undifferentiated index	0.05	0.08	0.00	0.00	0.00
Oocyte atresia prevalence	0.387	0.406	0.222	0.000	0.333
Oocyte atresia intensity	0.806 ± 1.327	0.594 ± 0.979	0.407 ± 0.971	0.000^{a*}	0.417 ± 0.669^{b}
Oocyte atresia index	0.312	0.241	0.090	0.000	0.139
Prevalence (oedema) %	18.5	35	2.5	89	44
Prevalence (BCA) %	95	100	97.5	89	100
Prevalence (granulocytomas) %	5	0	0	0	0
Preval. (dissem. neoplasia) %	5	5	0	0	0
Prevalence (ciliates) %	*29*	*23.5*	*0*	*17*	*33*
Preval. (Undert. Intra. Prot.) %	*2.5*	*10*	*0*	*11*	*17*
Prevalence (Total Intr. Prot.) %	*31.5*	*33.5*	*0*	*28*	*50*
Prev. (*Nematopsis* sp.)%[(1)]	97.5	76	25.5	89	39
Prevalence (Metazoan) %	0	0	0	11	0
Prevalence (R/CLO) %	0	0	0	0	10

[1] Intensity (individuals per section) = 62.5 (PC-RS); 12.5 (HC-RS); 9 (HC-DS); 2.5 (PL-RS); 2 (PL-DS).

3.2. Colombia

Shell L was highest at Marina Santa Marta and lowest at Isla Brujas in both seasons, without any seasonal variability (Table 2). The highest FDW values were recorded in Isla Maparadita and Marina Santa Marta in the rainy season, whilst values were much lower in all the localities except Marina Santa Marta in the dry season (Table 2). The highest SDW values were found in Marina Santa Marta in both seasons, while SDW was significantly lower in the dry season than in the rainy season in Isla Brujas, Isla Maparadita and Taganga ($p < 0.05$, Table 2). The highest FDW/SDW was recorded in Isla Maparadita followed by Taganga and Isla Barú, with lower values in the dry season than in the rainy season in Isla Maparadita and Isla Barú ($p < 0.05$; Fig. 6a). The highest SDW/L was recorded in Marina Santa Marta in both seasons, with lower values in the dry season than in the rainy season in Isla Brujas, Isla Maparadita and Taganga, and higher values in the dry season than in the rainy season in Isla Barú ($p < 0.05$, Fig. 6b). The highest FDW/L values were recorded in Isla Maparadita and Marina Santa Marta in the rainy season and in Isla Barú and in Marina Santa Marta in the dry season ($p < 0.05$, Fig. 6b).

Sex ratio values for each sampling location at each sampling time are shown in Table 2. No statistically significant bias in the sex ratio of the whole studied population (1.14:1; G value = 2.55, $p = 0.11$) was detected in comparison with the theoretical sex ratio of 1:1.14 resulting from the average between 1:1 (rainy season) and 1:1.33 (dry season) sex ratios. Accordingly, no bias was found in any season (rainy season: 1.16:1; G value = 0.80, $p = 0.37$; dry season: 1:1.06; G value = 0.925, $p = 0.34$). Among the studied localities, only Taganga showed a significant bias to female condition in both seasons (Table 2). Intersex cases were sporadically found: one female from Marina Santa Marta in the rainy season, and one female from Isla Barú and one from Marina Santa Marta and one male from Isla Brujas in the dry season (Table 2). All these cases corresponded to hermaphrodites as they showed separate male and female gonad follicles (Fig. 4d–f).

Most oysters were in advanced gamete development stages (e.g., spawning and spawned; Fig. 3b). In the rainy season, Marina Santa Marta and Taganga showed the most advanced gamete development; however, the opposite was found in the dry season. Significant differences in GI were found between seasons in Isla Brujas and Taganga and between localities for both seasons (Fig. 3b). Likewise, differences in GMI and UDI were recorded between localities but no between seasons (Table 2). Atretic oocytes were only observed in one oyster from Marina Santa Marta in the rainy season. As a whole, RAI reflects only a low-to-moderate (<10) reproductive disturbance, mainly featured by deviations in the expected SRI, GMI and UDI values (Fig. 6d).

A variety of parasites such as intracellular ciliates, *Nematopsis* sp., metazoan and R/CLO were found although, in general terms, both prevalence and intensity were low throughout the study (Table 2; Figs. 5 and 6e). The highest PII values were found in Isla Brujas, where in addition PII was significantly higher in the dry season than in the rainy season (most likely due to the moderate-to-high prevalence of *Nematopsis* sp.); likewise, PII tended to be higher in the dry season than in the rainy season in all the localities (Fig. 6e).

Overall, no major alteration in the histological integrity of the digestive gland tissue was recorded. However, in the dry season the prevalence of oedema in digestive gland tissue in all the localities was high, especially in Isla Barú and Marina Santa Marta, and the prevalence of brown cell aggregates in Marina Santa Marta was moderately high (Table 2). Granulocytomas and disseminated neoplasia were not recorded. Prevalence of inflammatory responses was apparently higher in the dry season than in the rainy season (Table 2). IRI values were higher in Isla Brujas than in the other localities in the dry season, when they were seemingly higher in all the localities; although seasonal differences were not significant in Isla Maparadita and Isla Brujas because IRI values were also conspicuous in the rainy season (Fig. 6f). Medium values of DGAI (score ~ 1–2) were recorded in all the localities all along the study. Nevertheless, DGAI values were significantly higher in the dry season than in the rainy season in Isla Brujas and, vice versa, higher in the rainy season than in the dry season in Taganga (Fig. 6g). Additionally, DGAI was significantly different among localities in the dry season, with highest values in Isla Brujas followed by Isla Maparadita (Fig. 6g).

LT_{50} values for the SoS assay were significantly different between seasons, especially in Isla Barú, Isla Brujas and Isla Maparadita ($p < 0.05$, Fig. 6h); the lowest LT_{50} values being recorded in these localities in the rainy season and less markedly in the dry season, even though LT_{50} values recorded in Isla Brujas continued to be the lowest (Fig. 6h).

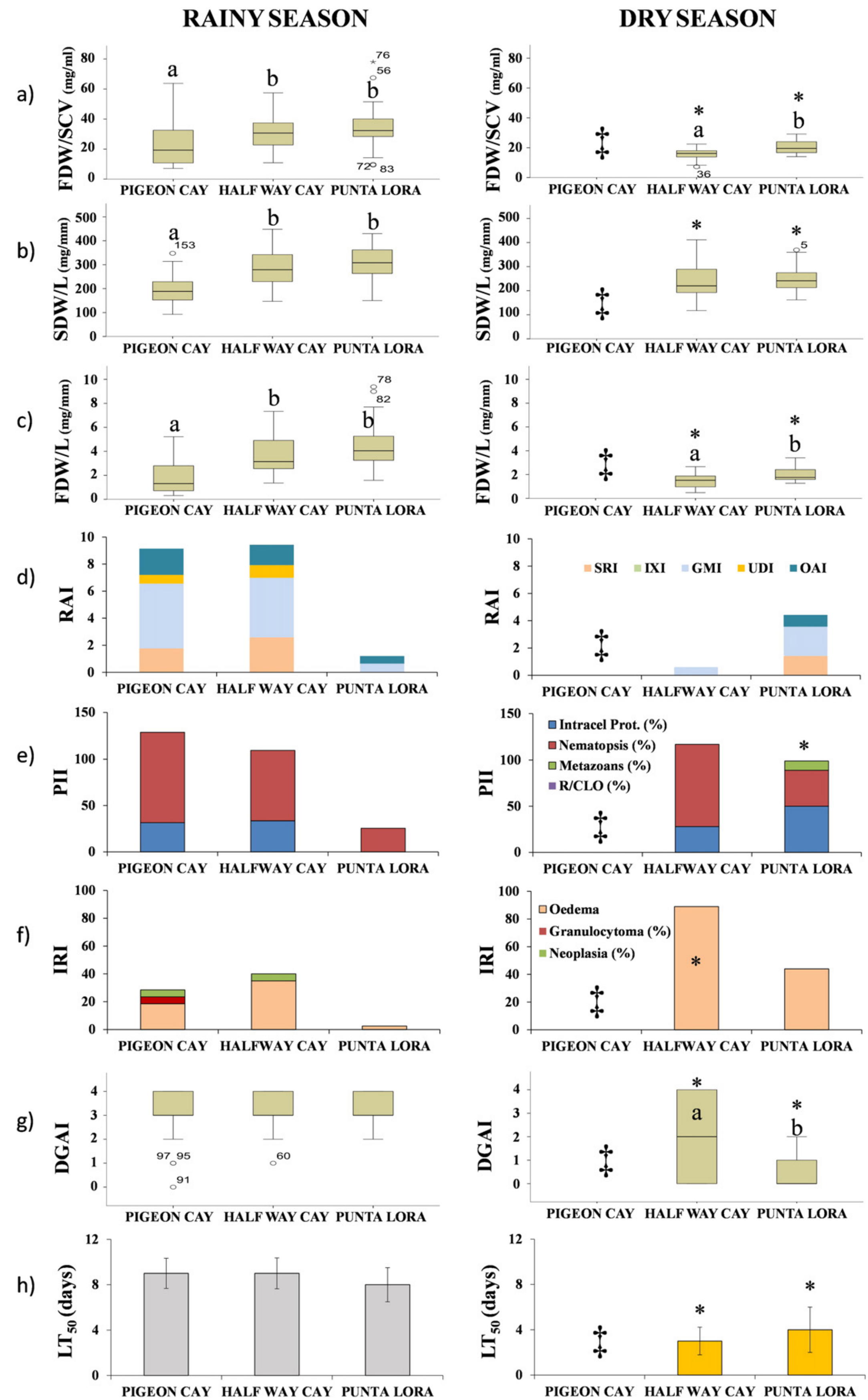


Fig. 2. Biomarkers of anomalies in oyster health indicative of disturbance in ecosystem health for the rainy and the dry seasons in Nicaraguan mangrove lagoons (Pigeon Cay, Half Way Cay and Punta Lora). Different letters denote statistically significant differences between localities and asterisk (*) indicate seasonal differences for a locality ($p < 0.05$). SDW/L, shell growth index; FDW/SCV and FDW/L, condition indices; RAI, reproductive anomalies index; IRI, inflammatory response index; DGAI, digestive gland atrophy index; LT_{50}, median survival time after SOS test. ‡, no data.

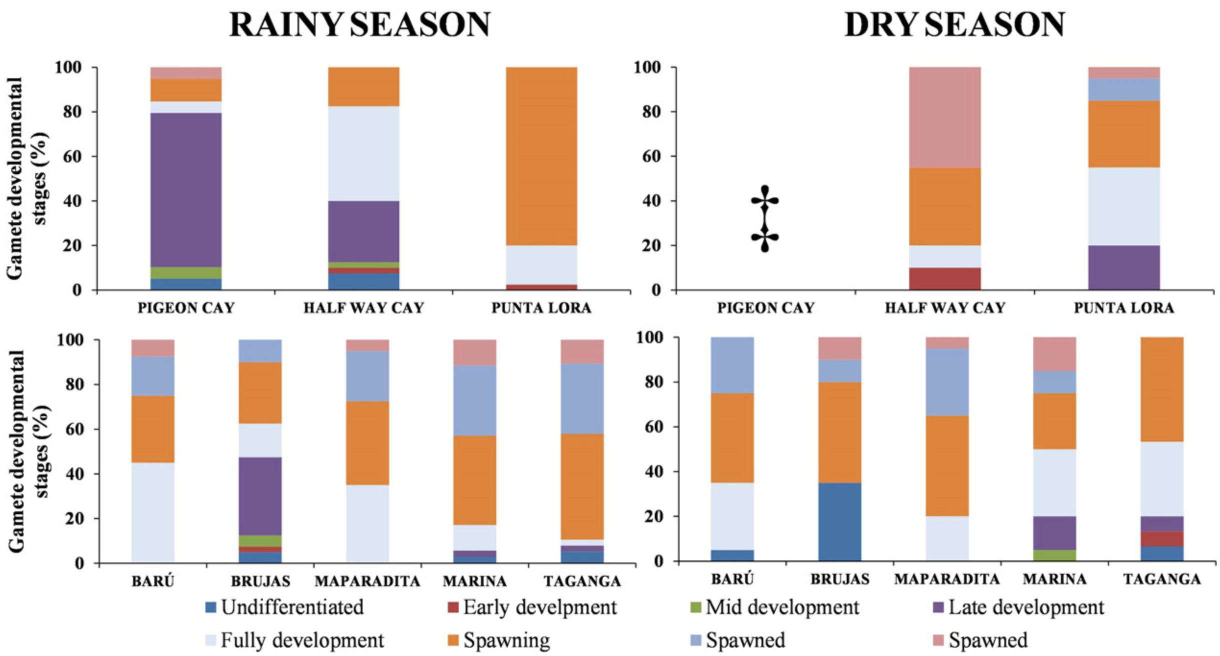


Fig. 3. Gamete development stages of oyster *Crassostrea rhizophorae* collected from Nicaragua (a) and Colombia (b) at the rainy and the dry seasons. ‡, no data.

The profiles depicted by the 5 indices of biological response varied with season (radar plots in Fig. 7b), except in Marina and Taranga where FDW/L and RAI were equally responsive at both seasons. In the rainy season, LT_{50} was particularly sensitive in Isla Barú, Isla Brujas and Isla Maparadita, whilst in the dry season responses at mid-to-high level of biological complexity (FDW/L and RAI) were elicited in Isla Barú and at low-to-mid level (DGAI, IRI and FDW/L) in Isla Maparadita and, especially, in Isla Brujas (Fig. 7b). As a result, IBR/n index showed significant differences in both seasons (Fig. 7b): Isla Brujas and Isla Maparadita showed higher IBR/n values than Isla Barú, Marina Santa Marta and Taganga in the rainy season, Isla Brujas being the locality with the highest IBR/n values in the dry season.

4. Discussion

The habitats and environmental conditions of Nicaragua and Colombia were different, so it comes as no surprise that the anthropogenic impact and the profile and levels of pollutants were different as well (Aguirre-Rubí et al., 2017; Dumailo, 2003; Mancera-Pineda et al., 2013; Vivas-Aguas et al., 2014). In Nicaragua, shallow subtidal oyster reefs were investigated, whereas in Colombia intertidal prop roots of mangrove trees and intertidal rocky habitats were sampled. Moreover, SST and salinity were higher in Colombia than in Nicaragua, with very high SST (>33 °C) in the former and extreme low salinities (<2 PSU) in the rainy season in the latter (ESM 1). Another key variable to keep in mind for inter-regional comparisons was that oysters from Nicaragua (shell $L_{max} = 7.3 \pm 1.7$ cm) and Colombia (Shell $L_{max} = 3.7 \pm 0.9$ cm) corresponded to different size classes, which were the most abundant ones at each corresponding sampling site.

4.1. Nicaragua

Oyster shell length varied with season, with the largest animals being collected in Half Way Cay and Punta Lora in the rainy season. Accordingly, FDW, L and SCV were higher in Half Way Cay and Punta Lora than in Pigeon Cay in the rainy season, which suggests that growth was reduced in Pigeon Cay oysters.

Generally, stress is associated with a decrease in flesh condition, whereas a higher abundance of food and hormonally active substances will increase flesh condition (Smaal and van Stralen, 1990). Different indices are available to determine flesh condition in oysters (Crosby and Gale, 1990). In Nicaragua, FDW/SCV and FDW/L were clearly lower in the dry season than in the rainy season, and lower in Pigeon Cay than in the other two localities. Seasonal changes in FCI have been previously reported in mangrove oysters (Nascimento and Pereira, 1980; Rebelo et al., 2005). However, the seasonal pattern may vary depending on locally relevant differences in nutritional and reproductive status, as well as on environmental constrains (e.g., the presence of pollutants). Thus, unlike in the present study, in *C. gigas* and *C. corteziensis* from the Gulf of California lagoons, FDW/SCV was higher in the dry season than in the rainy season (Osuna-Martínez et al., 2010). Likewise, high FDW/SCV and FDW/L values were reported in *C. rhizophorae* both in the dry season, associated to high levels of nutrients in the water (Rebelo et al., 2005), but also in the rainy season, concomitantly with full gonad maturation (Nascimento and Pereira, 1980). For the present study, high FDW/L values would have been expected for the dry season, together with advanced gametogenic stages; this seems to apply when the rainy and the dry seasons are compared in Half Way Cay, and to a lesser extent in Punta Lora, where a higher incidence of spawning stages in the dry season than in the rainy season coincides with lower FDW/L values. However, the lowest FDW/L was recorded in Pigeon Cay, where gamete development was seemingly arrested in the rainy season (late gamete development stage was dominant resulting in high GI values) and the highest in Punta Lora in the rainy season, where spawning stages were dominant. Therefore, reproduction does not seem to be the main factor influencing FDW/L in the rainy season.

Conversely, the low FDW/SCV and FDW/L values found in Pigeon Cay in comparison with Half Way Cay and Punta Lora might be attributed to the presence of pollutants (e.g., PAHs, PCBs and PBDEs), as reflected in high pollution indices (PLI: Pollution Load Index, Tomlinson et al., 1980; CPI: Chemical Pollution Index, Bellas et al., 2011) s reported by Aguirre-Rubí et al. (2017). In this regard, FDW/SCV was proposed as an inexpensive, quick, representative and responsive tool for monitoring pollution (Scott and Lawrence, 1982), commonly used to estimate growth differences among oysters living in different environmental conditions (Austin et al., 1993). Likewise,

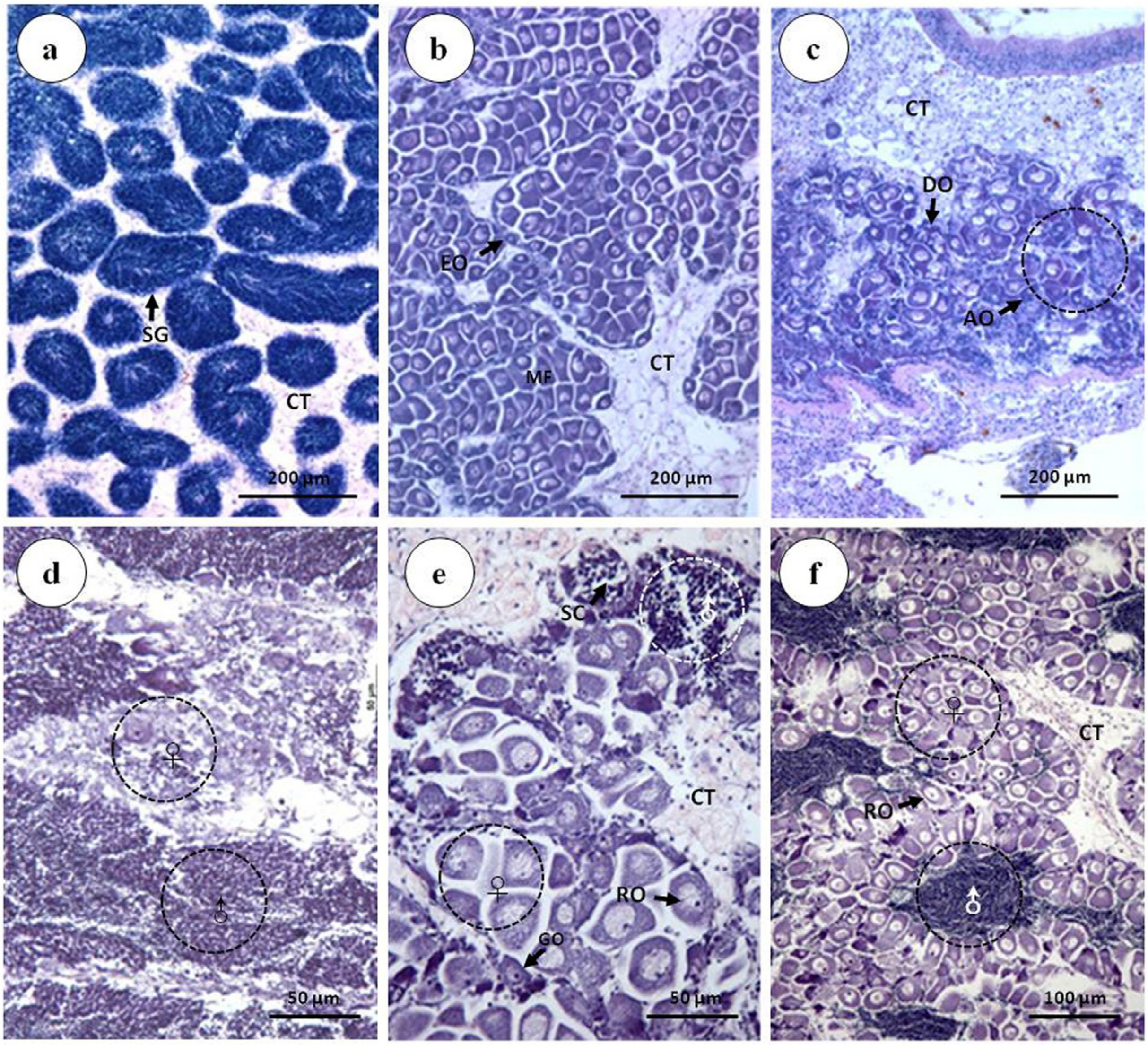


Fig. 4. Light micrographs of gonad sections and intersex individuals of *Crassostrea rhizophorae*. a) normal gonad of a male; b) normal gonad of a female; c) female from Half Way Cay with atretic oocytes; d) male from Isla Brujas with intersex; e) female from Marina with intersex; f) female from Isla Barú with intersex. AO, atretic oocytes; EO, early oocyte; GO, growing oocyte; DO, degenerated ovum; RO, ripe ovum; SG, spermatogonia; SC, spermatocytes; CT, connective tissue; MF, mature follicles; ♀, female germ cells; ♂, male germ cells.

FDW/SCV provides indication of the nutritional status of oysters and of whether they are subject to stress conditions (Crosby and Gale, 1990). For example, in *C. virginica*, exposure to metals such as Cu and Cr is directly related with a decrease in FDW/SCV (Aguilar et al., 2012). Likewise, FDW/SCV was found to be lower in *C. rhizophorae* from Brazilian estuaries with higher levels of PAHs and PCBs (Valdez Domingos et al., 2007).

Reproduction anomalies tended to be more prevalent in the rainy season than in the dry season and more marked in Pigeon Cay and Half Way Cay than in Punta Lora where, conversely, anomalies were most evident in the dry season. It is worth noting that the so-called reproductive anomalies do not necessarily mean deleterious or pathological effects but simply deviations from the mean values, which might be indicative of potential disturbances in oysters reproduction. The main anomalies recorded in this study included alterations in sex ratio, gamete development delay/arrest and oocyte atresia.

Crassostrea oysters are protandrous hermaphrodites and sex ratio values can vary depending on the species, age, size and local conditions (Park et al., 2012). Sex appears to be determined by a single gene with a dominant male allele M and a recessive protandrous allele F, such that FF animals are protandrous and MF animals are permanent males (Powell et al., 2011). In *C. rhizophorae*, females predominate and can reach up to 90% of the population although their prevalence may vary with size and season (Vélez, 1982). In raft cultivated *C. rhizophorae* (>20 mm shell length), females were predominant (male/female ratio = 0.2–0.6) during the rainy season and especially in the largest size classes (Gordon, 1988). Accordingly, sex ratio was skewed towards females in Nicaragua; however, feminisation went beyond the expected values (according to criteria by Vélez, 1982) in the rainy season, especially in Pigeon Cay and Half Way Cay but also in Punta Lora. Sex ratio in Punta Lora oysters was comparable to that reported for *C. rhizophorae* in the Laguna Grande de Obispo, in Venezuela (F:M, 2.42:1; Montes-M et al., 2007), and in Guaratuba Bay (F:M, 1.6:1) and in two localities at Camanu Bay (F:M, 1.9:1 y 2.1:1) in Brazil (Christo and Absher, 2006; Lenz and Boehs, 2011). A predominance of females was also observed in *Crassostrea brasiliana* from Brazil (Castilho-Westphal et al., 2015). Sex ratio values in Pigeon Cay and Half Way Cay, however, were much higher. This apparent anomaly could be due to the large size of the oysters (L_{max} > 80 mm), or caused by exposure to endocrine disrupting compounds (e.g. pesticides) in the rainy season. Sex ratio skew towards females has been reported in bivalve populations from sites polluted with tributyltin, metals and PAHs (Gagne et al., 2003; Gauthier-Clerc

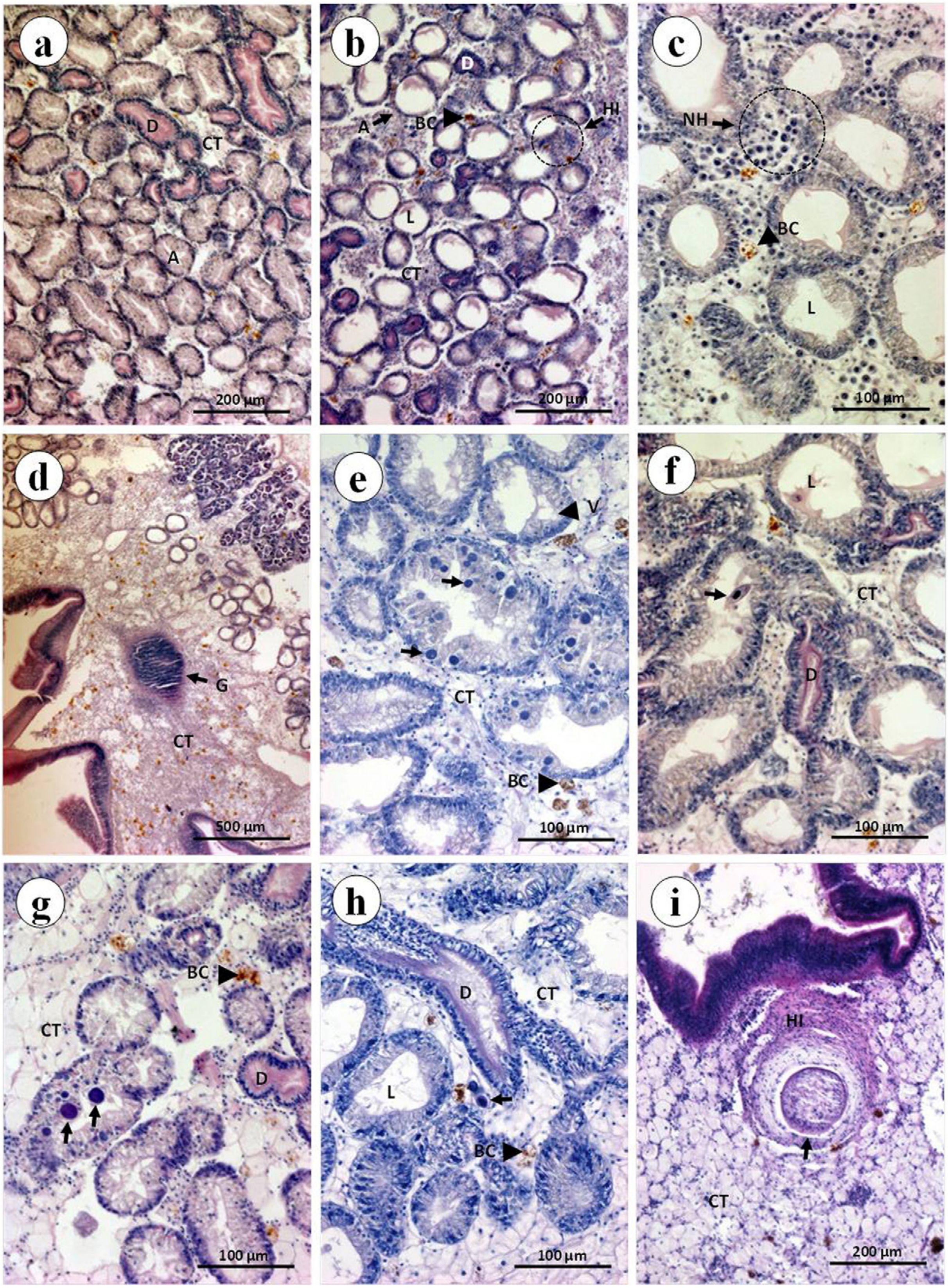


Fig. 5. Paraffin sections of the digestive gland of *Crassotrea rhizophorae* stained with haematoxylin-eosin: a) normal digestive gland b) digestive gland with diffuse infiltration haemocyte and atrophied alveoli; c) disseminated neoplasia; d) granulocytoma and diffuse infiltration haemocytic; e) unidentified protists; f) ciliate; g) R/CLO; h) *Nematopsis* sp.; i) metazoan. Abbreviations: A, alveoli; D, duct; CT, connective tissue; HI, haemocytic infiltration; L, lumen; NH, neoplastic haemocytes. Arrows, tissue alterations; arrowheads indicate the presence of brown cell (BC) aggregates and vacuolization (V).

Table 2
Condition and histopathology of *Crassostrea rhizophorae* from intertidal roots/docks of Colombian mangrove swamps. Shell cavity volume, condition index and atrophy their values are mean ± standard error ($n = 20$). Sex Ratio Index = SRI's G value. The superscript letters (a, b and c) indicate significant differences between pairs of localities ($p < 0.05$). Asterisk (*) indicate seasonal difference for a locality ($p < 0.05$); ♯, significantly different from the theoretical gender bias (Vélez, 1982) according to the G test ($p < 0.05$).

	Rainy season					Dry season				
	Isla Barú	Isla Brujas	Isla Maparadita	Marina Santa Marta	Taganga	Isla Barú	Isla Brujas	Isla Ma-paradita	Marina Santa Marta	Taganga
Shell length (L; mm)	39.3 ± 1.7^{bc}	33.3 ± 1.1^{a}	34.1 ± 0.8^{ab}	41.5 ± 1.9^{c}	33.7 ± 1.3^{a}	35.6 ± 1.6^{a}	34.1 ± 1.9^{a}	35.7 ± 0.9^{a}	41.1 ± 1.6^{b}	39.3 ± 3.1^{ab}
Flesh dry-wt (FDW; mg)	103.1 ± 7.6^{a}	95.3 ± 8.3^{a}	231.1 ± 26.1^{b}	153.7 ± 9.6^{b}	70.7 ± 6.0^{a}	91.7 ± 8.1^{b}	$56.7 \pm 8.9^{a*}$	$62.7 \pm 6.6^{a*}$	155.4 ± 17.3^{c}	53.5 ± 6.3^{a}
Shell dry-wt (SDW; g)	3.2 ± 1.7^{a}	4.3 ± 2.5^{b}	3.7 ± 2.2^{ab}	6.5 ± 2.9^{c}	2.7 ± 1.2^{a}	4.1 ± 2.0^{c}	$2.6 \pm 1.2^{b*}$	$1.7 \pm 0.6^{ab*}$	6.3 ± 2.8^{d}	$1.4 \pm 0.7^{a*}$
Sex ratio (sex ratio index)	1.2:1 (0.40)	1:1.1 (0.11)	1.2:1 (0.4)	1.4:1 (0.76)	2:1♯ (4.08)	1.3:1 (1.17)	1:2.3[(1)] (0.80)*	1.5:1 (2.37)	1:1.4 (0.00)	2.5:1[(1)] (4.67)
Intersex index	0	0	0	2.5	0	5	5	0	5	0
Gonad index	3.6 ± 0.2^{b}	3.3 ± 0.2^{b}	3.4 ± 0.2^{b}	2.6 ± 0.2^{a}	2.4 ± 0.2^{a}	3.2 ± 0.3^{b}	$1.6 \pm 0.3^{a*}$	3.0 ± 0.3^{b}	3.3 ± 0.3^{b}	$3.4 \pm 0.4^{b*}$
Gamet. mass index	$0.26^{(a)}$	$0.40^{(b)}$	$0.19^{(a)}$	$0.07^{(a)}$	$0.02^{(a)}$	$0.16^{(a)}$	$0.00^{(b)}$	$0.10^{(a)}$	$0.30^{(a)}$	$0.30^{(a)}$
Undifferent. index	0.00	0.05	0.0	0.03	0.05	0.05^{a}	0.35^{b}	0.00^{a}	0.00^{a}	0.07^{a}
Oocyte atresia prevalence	0^{a}	0^{a}	0^{a}	0.053^{b}	0^{a}	0	0	0	0	0
Oocyte Atresia intensity	0^{a}	0^{a}	0^{a}	0.053 ± 0.101^{b}	0^{a}	0	0	0	0	0
Oocyte atresia index	0^{a}	0^{a}	0^{a}	0.003^{b}	0^{a}	0	0	0	0	0
Prevalence (oedema) %	$2.5^{(a)}$	$15^{(a)}$	$30^{(b)}$	$0^{(a)}$	$0^{(a)}$	$85^{(b)}$	$55^{(a)}$	$45^{(a)}$	$70^{(a)}$	$40^{(a)}$
Prevalence (BCA) %	0^{a}	0^{a}	2.5^{a}	22.5^{b}	2.5^{a}	10^{a}	5^{a}	0^{a}	45^{b}	5^{a}
Prevalence (granulocytomas) %	0	0	0	0	0	0	0	0	0	0
Prevalence (dissemin. neoplasia) %	0	0	0	0	0	0	0	0	0	0
Prevalence (ciliates) %	$2.5^{(a)}$	$7.5^{(b)}$	$2.5^{(a)}$	$0^{(a)}$	$0^{(a)}$	$10^{(a)}$	$15^{(b)}$	$0^{(a)}$	$5^{(a)}$	$5^{(a)}$
Prevalence (Undert. Intra. Prot.) %	0	0	0	0	0	0	0	0	0	0
Prevalence (Total Intr. Prot.) %	*2.5* $^{(a)}$	*7.5* $^{(b)}$	*2.5* $^{(a)}$	*0* $^{(a)}$	*0* $^{(a)}$	*10* $^{(a)}$	*15* $^{(b)}$	*0* $^{(a)}$	*5* $^{(a)}$	*5* $^{(a)}$
Prevalence (*Nematopsis* sp.) %	0	2.5	2.5	0	0	0^{a}	30^{b}	0^{a}	0^{a}	0^{a}
Prevalence (Metazoan) %	0	2.5	0	0	2.5	0^{a}	0^{a}	10^{b}	0^{a}	0^{a}
Prevalence (R/CLO) %	0^{a}	5^{b}	0^{a}	0^{a}	0^{a}	10^{b}	0^{a}	10^{b}	0^{a}	5^{a}

[1] >30% of oysters with undifferentiated gonad.

et al., 2002). Likewise, feminizing effects were reported in bivalves exposed to sewage effluents and to urban and agricultural runoff waters (Blaise et al., 2003; Gagne et al., 2001; Quinn et al., 2004; Ortiz-Zarragoitia and Cajaraville, 2010). In contrast, sex ratio skew towards males was recorded in Half Way Cay in the dry season. A similar masculinisation was reported in summer for *C. rhizophorae* by Lenz and Boehs (2011).

Mangrove cupped oysters reproduce continuously throughout the year with peaks of seasonal spawning associated with temperature and salinity; however, spawning and spatfall are continuous in lagoons with temperatures higher than 25 °C (Vélez, 1982), like Bluefields and Pearl Lagoon. As seen in diverse species of *Crassostrea* from different regions of the world, GI is correlated with seawater temperature, salinity and quantity and quality of the food supply (Christo and Absher, 2006). At least 2–3 spawning peaks per reproductive cycle have been reported in *C. rhizophorae* from different regions, but the main one seems to be in the rainy season (Villarroel et al., 2004; Lenz and Boehs, 2011). However, our results suggest that wild mangrove oysters from Nicaraguan Caribbean follow a continuous reproductive cycle, albeit peaks of more intense spawning could not be disregarded. Similarly, *C. virginica* from Mecoacan was reported to spawn all along the year (George-Zamora et al., 2003). More detailed studies on wild mangrove oyster populations would be required in this respect. Nevertheless, gamete development was seemingly delayed in Pigeon Cay in comparison with Punta Lora in the rainy season, with Half Way Cay in between. In the dry season, however, gamete development was delayed in Punta Lora in comparison with Half Way Cay. In bivalves, exposure to chemicals such as oils, PAHs, DDTs, and alkylphenols causes alterations in gamete development and enhances oocyte atresia (Binelli et al., 2001; Lowe, 1988; Ortiz-Zarragoitia et al., 2011). Delayed gametogenesis was reported in bivalve populations from sites polluted with tributyltin, metals and PAHs (Gagne et al., 2003; Gauthier-Clerc et al., 2002). The occurrence of gamete development arrest in Pigeon Cay and Half Way Cay in the rainy season and Punta Lora in the dry season may, therefore, be due to pollutant specific effects. Interestingly, the highest tissue levels of PAHs were recorded in oysters from Pigeon Cay, followed by oysters from Half Way Cay; as well as the highest levels of PBDEs (Aguirre-Rubí et al., 2017). Conversely, organochlorine pesticides (OCPs) do not seem to be related to effects on gamete development, as the highest tissue levels were recorded in oysters from Punta Lora in the rainy season (Aguirre-Rubí et al., 2017) in absence of apparent effects on gametogenesis.

Oocyte atresia is a normal process in late spawning and post spawning stages, as described in e.g. *C. brasiliana* (Castilho-Westphal et al., 2015). In the present study, atresia was recorded at higher occurrence in Pigeon Cay and Half Way Cay than in Punta Lora in the rainy season and its intensity values were particularly high in the two former localities resulting in significantly higher OAI values. Enhanced oocyte atresia could be a sign of gonad resorption and spawning abortion aimed at re-directing energy resources from reproduction to counteract stressors (e.g. pollutants). Exposure to chemical pollutants is known to enhance oocyte atresia (Binelli et al., 2001; Lowe, 1988; Ortiz-Zarragoitia et al., 2011). In agreement, the levels of oocyte atresia can be related to the PLI and CPI values recorded in the studied localities (Aguirre-Rubí et al., 2017).

RAI integrated the above discussed reproductive anomalies and showed that these were most relevant in Pigeon Cay and Half Way Cay in the rainy season and to a lesser extent in Punta Lora in the dry season. Though these reproductive anomalies might have resulted from natural causes (e.g. old large sized oysters tend to be female with atretic oocytes) the observed inter-site differences suggested that additional stressors might be acting (e.g., pollutants, nutrient availability, hypoxia or extreme salinity).

Although the intensity of parasitic infestations was generally low, it was higher in Half Way Cay than in Punta Lora in both sampling occasions; parasite prevalence being especially low in the rainy season in

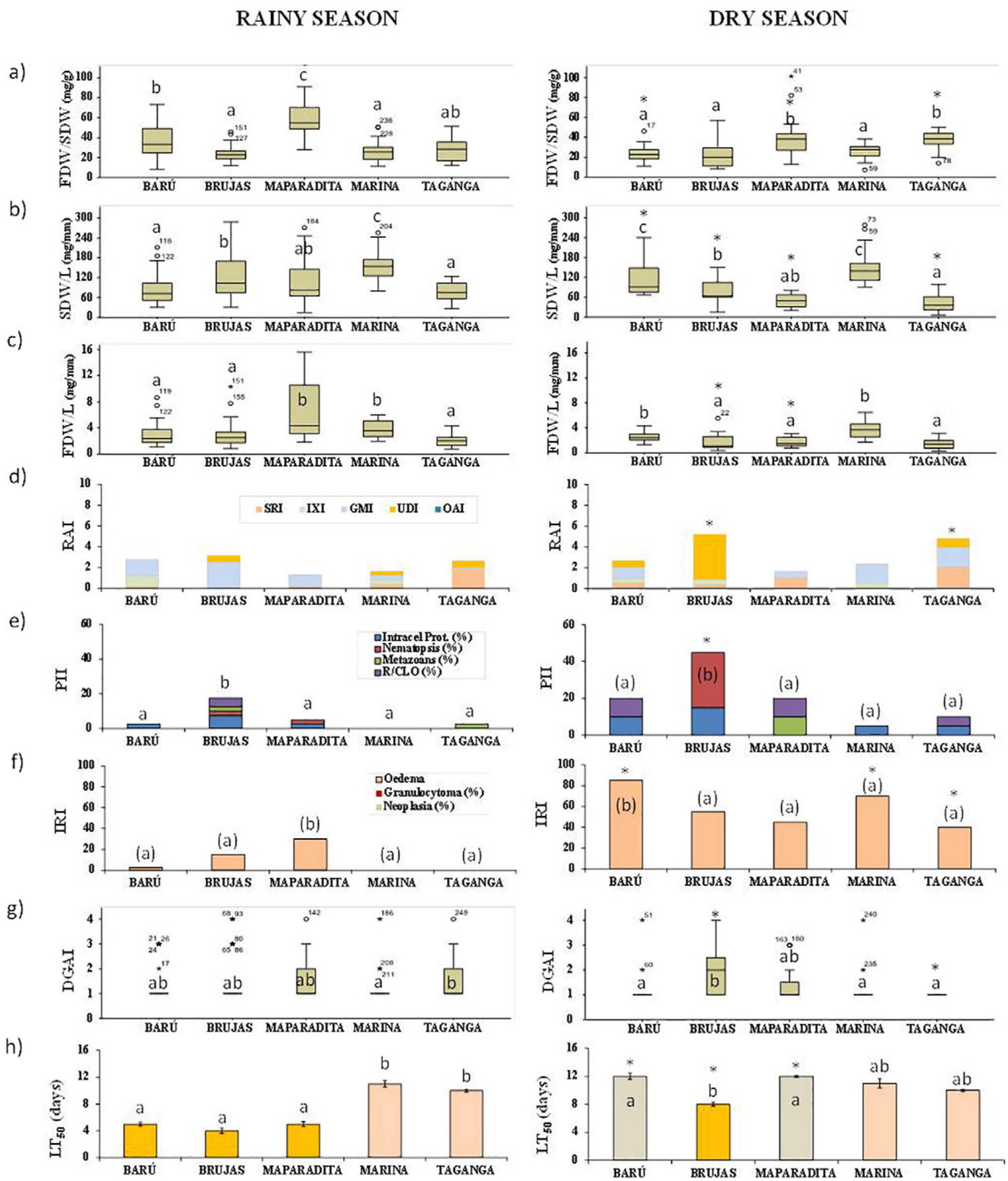


Fig. 6. Biomarkers of anomalies in oyster health indicative of disturbance in ecosystem health for the rainy and the dry seasons in Colombian mangrove swamps (Isla Barú, Isla Brujas, Isla Maparadita, Marina Santa Marta and Taganga). Different letters denote statistically significant differences between localities and asterisk (*) indicate seasonal differences for a locality ($p < 0.05$; $p < 0.1$, when shown between brackets). SDW/L, shell growth index; FDW/SDW and FDW/L, condition indices; RAI, reproductive anomalies index; IRI, inflammatory response index; DGAI, digestive gland atrophy index; LT_{50}, median survival time after SOS test. ‡, no data.

Punta Lora. Most frequently recorded parasites were *Nematopsis* sp. and ciliates. *Nematopsis* sp. seems to be a common parasite in Caribbean *C. rhizophorae* (Brandão et al., 2013; Nascimento et al., 1986; Sabry et al., 2007, 2011, 2013), mussels (Boehs et al., 2010; Ceuta and Boehs, 2012; Garmendia et al., 2011) and other oyster species (Afsar et al., 2014; Aguirre-Macedo et al., 2007). No seasonal trend was observed in the prevalence of *Nematopsis* sp.. Similarly, *Nematopsis* was recorded throughout the year in *Mytella guyanensis* (Boehs et al., 2010; Ceuta and Boehs, 2012) but a marked seasonality was reported in *C. rhizophorae* (Brandão et al., 2013; Nascimento et al., 1986; Sabry et al., 2007). Prevalence recorded in Pigeon Cay, and Half Way Cay can be considered high (Brandão et al., 2013). At low infection intensities, this protist only causes inflammatory responses although at high intensities it can be harmful (Bower et al., 1994).

As a result, PII was high in Half Way Cay in both seasons and in Pigeon Cay, and moderate in Punta Lora in the dry season. The differences do not seem to be related to size (or age) because oysters from Pigeon Cay are the smallest but present the highest PII value; whilst usually large and aged individuals exhibit more severe degree of parasitisation. Likewise, FCI does not seem to be relevant because oysters with similar

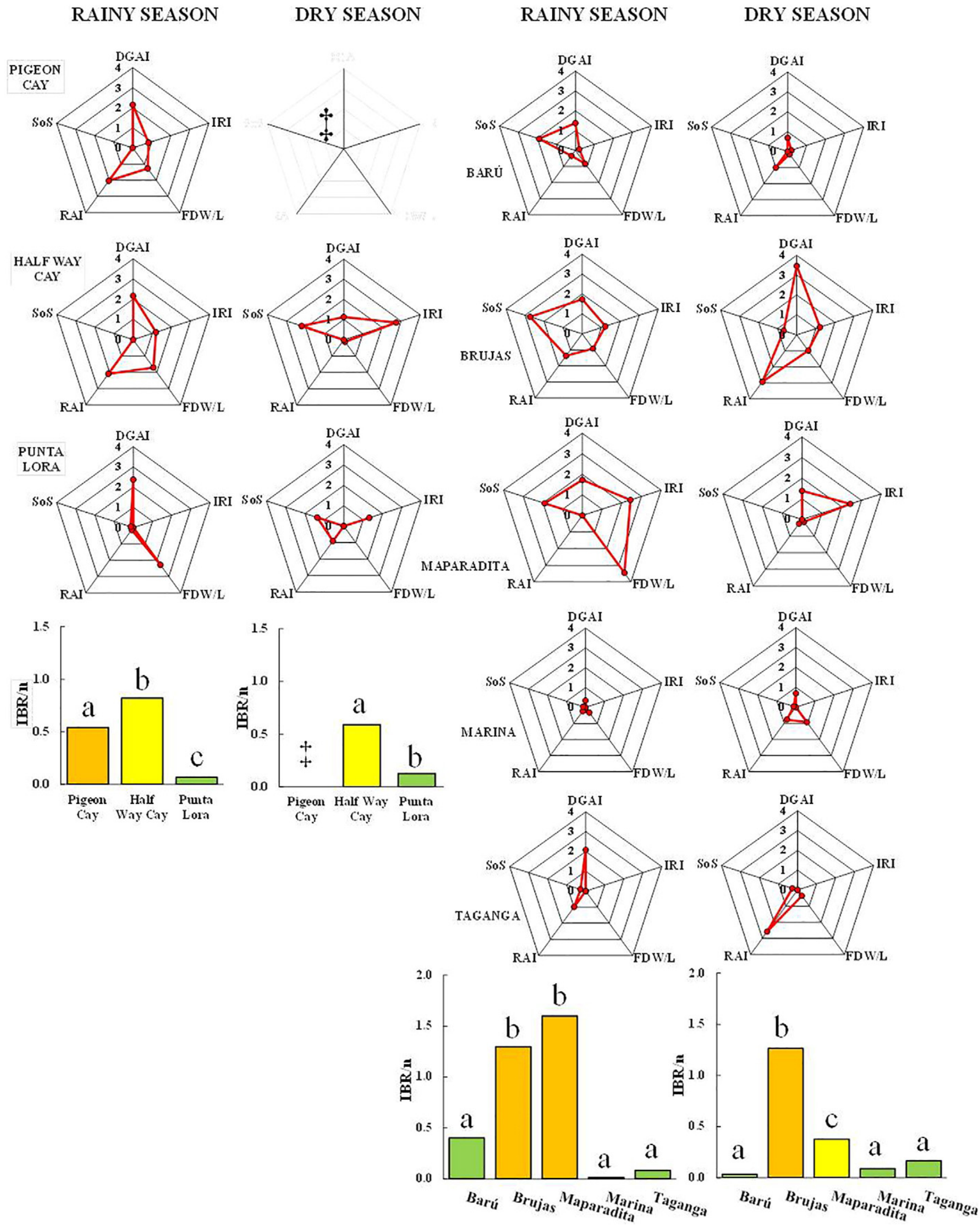


Fig. 7. Radar plots for biomarkers and the corresponding IBR/n index for the rainy and the dry seasons in Nicaraguan mangrove lagoons (left) and intertidal roots/docks of Colombian mangrove swamps (right). Different letters denote statistically significant differences between pairs of means according to the *Z*-score test ($p < 0.05$). DGAI, digestive gland atrophy index; IRI, inflammation index; FDW/L, condition index; RAI, reproductive anomalies index; LT_{50}, median survival time after SOS test. Nicaraguan sampling localities: PC, Pigeon Cay; HC, Half Cay; PL, Punta Lora. Colombian sampling localities: B, Isla Barú; E, Isla Brujas; C, Isla Maparadita; M, Marina Santa Marta; T, Taganga. ‡, no data.

FDW/L values show different parasite load. It is conceivable that environmental stressors or pollution exposure provokes immunodeficiency or that high turbidity and levels of suspended particulate or organic matter in the water column facilitates parasitic infestation. In presence of high levels of suspended organic matter, growth of bacteria and algae and then of protozoans, their predators, is enhanced (La Rosa et al., 2001). Thus, the abundance of ciliates has been proposed as an indicator of the host health and the level of eutrophication (Palm and Dobberstein, 1999). The presence of ciliates depends on environmental factors such as temperature, salinity and suspended matter (e.g., turbidity) and has been suggested to be indicative of organic pollution resulting from e.g., domestic sewage (Brandão et al., 2013; Chu et al., 2002; Marcogliese and Cone, 1997). Ciliates are believed to be comensals that only may result harmful at high intensities and not always, as reported in *C. rhizophorae* in Brazil (Boehs et al., 2010; Brandão et al., 2013; Nascimento et al., 1986)

Disorganized ICT with haemocyte infiltration and shrinkage of digestive diverticula, digestive alveoli atrophy and digestive cell vacuolisation were observed in all three studied Nicaraguan localities, mainly in the rainy season. Comparable histopathological alterations were interpreted as a stress response to pollution enhancement in the rainy season (Valdez Domingos et al., 2007). Granulocytomas and disseminated neoplasia were only found, at low prevalence, in Pigeon Cay and Half Way Cay. These inflammatory lesions have been associated to the presence of pollutants but also to pathogens (Auffret, 1988; De Vico and Carella, 2012; Garmendia et al., 2011; Kim et al., 2008). Oedema and brown cell aggregates were observed during both sampling occasions. Whilst the prevalence of brown cell aggregates was close to 100% in all the cases, the prevalence of oedema was higher in the dry season than in the rainy season and higher in Half Way Cay than in Punta Lora and Pigeon Cay, with highest values in Half Way Cay in the dry season. However, intensity values of brown cell aggregates were low except in Pigeon Cay that exceeded the scores in other localities by a factor of 5 to 30 times. Elevated levels of haemocytic infiltration and oedema are general responses to chemical pollutants in molluscs (Au, 2004). These inflammatory responses were shown to be related to the tissue concentration of Cd and hydrocarbons in *C. virginica* from Mexican coastal ecosystem (Gold-Bouchot et al., 1995), and were suggested as indicators of pollution-induced stress for native oysters in coastal lagoons (Gold-Bouchot et al., 1995).

IRI runs in parallel with PII suggesting that most inflammatory responses were associated to the presence of parasites and pathogens, in agreement with Carella et al. (2015). Nevertheless, other aetiologies cannot be dismissed because the prevalence of the two former and the intensity of oedemas were higher in Pigeon Cay and Half Way Cay in the rain season, where oysters presented the highest pollutant tissue levels (Aguirre-Rubí et al., 2017). Moreover, DGAI revealed that digestive gland atrophy indicative of general stress (Apeti et al., 2014; Couch, 1985; Garmendia et al., 2011; Kim and Powell, 2007) seemed to be higher in the rainy season than in the dry one, with some signals of moderate stress in Half Way Cay in the dry season. Pollution by chemicals in mixture is a potential factor activating latent infection in oysters (Chu et al., 2002).

Stress-on-Stress responses showed a very different profile in comparison with other general stress biomarkers such as DGAI. LT_{50} was in the range of 3–9 d, with the shortest values being recorded in the dry season. This seems to indicate that oysters were more resistant to additional stress in the rainy season than in the dry season, which would not be expected from a stressed organism. Certainly, a better individual condition could explain these inconsistent results, which correspond to the cases of Half Way Cay and Punta Lora in the rainy season (high FDW/L and high LT_{50}). However, it does not apply for the case of Pigeon Cay (low FDW/L and high LT_{50}). On the other hand, the LT_{50} recorded in Nicaragua are not actually high in comparison with e.g. Colombia oysters and therefore the resilience of oysters could be considered affected at regional scale and especially in the dry season. According to our records there is no previous data on SoS response in *C. rhizophorae* so that LT_{50} values can be only interpreted relatively. Thus, LT_{50} in Nicaragua (~8 d) does not reach the values of 12 d recorded in some localities in Colombia in this study, with a more marked decrease than those reported in other species to discriminate polluted and reference sites (Blaise et al., 2016). In contrast, the low LT_{50} values recorded in Nicaragua in comparison with Colombian oysters could also be explained because Nicaraguan oysters were larger, as concluded for other species (Thomas et al., 1999). However, Pigeon Cay oysters while being smaller than Half Way Cay and Punta Lorain oysters during the rainy season did not display higher LT_{50} values than oysters from the two latter localities, which suggests some additional effect other than size on SoS in Pigeon Cay. The low LT_{50} values recorded in the dry season can be explained by different, not necessarily opposing, causes known to lead to reduced survival-in air in bivalves (Hellou and Law, 2003; Smaal et al., 1991; Thomas et al., 1999; Viarengo et al., 1995), such as: poor individual condition (low CI and FDW/L), reproductive stress during spawning, unfavourable environmental conditions (hypoxia and high turbidity and salinity) and seasonal sources of pollutants (i.e., pesticides, etc.).

The present approach thus suggests that oysters are not equally healthy in the different localities and seasons investigated herein, which is evidenced by the integrated biomarker response (IBR) index. This provides an integrated view on the health status of sentinel organisms even though seasonal variability could act as a confounding factor, as previously suggested for bivalves from temperate regions subject to pollution (Leiniö and Lehtonen, 2005; Marigómez et al., 2013). Integrating these anomalies in the oysters' health status into the IBR/n index provided a general indication of ecosystem health disturbance (Marigómez et al., 2013). The lowest IBR was that recorded in Half Way Cay followed by Pigeon Cay, both with comparable health profiles, whilst ecosystem health was less disturbed in Punta Lora, where the signal provided by DGAI in the rainy season might be indicative of a recent distressing episode, unlike the situation in other localities that seems to be more pervasive. In summary, complex multi-factorial sources of health disturbance of oysters seem to co-occur over a regional background of low-to-moderate pollution (e.g. by pesticides and domestic sewage; e.g. as shown in ESM 1) in Nicaraguan Caribbean coast and therefore correlation between IBR/n and pollution indices such as PLI and CPI was not significant. Though individual biological responses at each locality and season were generally consistent, the lack of correlation may be due to the strong influence of the physiological status (modulated by multi-factorial drivers and seasonal variation under natural field conditions) of the oysters on these measurements.

4.2. Colombia

Shell length, in the range of 33–42 mm, was quite variable among the five localities, with oysters from Marina Santa Marta being the largest. Moreover, SDW values were exceptionally high in Marina Santa Marta in both seasons, as were SDW/L ratios in Marina Santa Marta (both seasons), Isla Brujas (rainy season) and, to a lesser extent, in Isla Barú (dry season). Abnormal shell growth, including e.g. hypertrophy and chambering, has been associated with exposure to endocrine disruptors such as TBTs, which are characteristic of marinas and dockyards (Chagot et al., 1990); which could be the case for Marina Santa Marta.

The highest FDW/SDW was recorded in Isla Maparadita, with lower values in the dry season than in the rainy season. Although it is commonly used in the case of mussels as an indicator of individual condition (Lobel and Wright, 1982; Smaal and van Stralen, 1990), FDW/SDW is known to have some limited value as an index of nutritive status or recent stress in oysters; both flesh and shell growth are disparately variable throughout the year in tropical oysters and unpredictable variations in SDW can also result from natural mineral incrustations and stochastically occurring shell break-offs (Blaise et al., 2016; Crosby and Gale, 1990). In contrast, FDW/L overcomes uncertainties associated to unpredictable variations in SDW (Blaise et al., 2016; Hellou et al., 2003). Herein, higher FDW/L values were recorded in all the localities in the rainy season than in the dry season. Oscillations in condition index have been related to periods of post-spawning occurring towards the end of each rainy season (Gordon, 1988). However, this does not seem to be the case here, as spawning in the Caribbean is continuous. Furthermore, following this argument, one would expect finding an inverse relationship between FDW/L values and degree of gonad development, but no significant relationship was evident. Likewise, the high FDW/L values recorded in the rainy season could also be explained by seasonal increases in suspended organic matter. Interestingly, seasonal increase in FCI in oysters is positively correlated with microbial load but FCI decreases with too high microbial tissue loads due to sewage pollution, which causes lower assimilation efficiency (Jana et al., 2014). In the present study, the faecal bacteria load is higher in the rainy season than in the dry season, which coincides with high FWD/L values. Finally, seasonal fluctuations in FCI could be explained partially by changes in salinity and number of days with extreme temperature ranges, which may result in decreased feeding activity or quality diet abundance (Austin et al., 1993). Water salinity at Isla Maparadita during the rainy season dropped more strongly than in any other locality studied. As a whole, in Santa Marta Bay, upwelling in the dry season leads to increased salinity, decreased temperature and turbidity and enrichment of inorganic nutrients due to the contribution of deep marine waters. In the rainy season, outwelling occurs, mediated by tropical rainfall and relevant river loads of sediment, organic matter and domestic sewage to the Santa Marta Bay; this results in decreased salinity and dissolved oxygen, and increased temperature, turbidity and phosphorus and chlorophyll concentrations (Mancera-Pineda et al., 2013). The Gaira and Manzanares rivers contribute with freshwater and sediments to Santa Marta Bay during the rainy season, together with inorganic nutrients, domestic sewage and litter. Furthermore, mineral coal transport and loading activity in the nearby port might contribute to enhance sedimentation and sediment re-suspension thus leading to episodically elevated levels of suspended particulate matter. Therefore, FDW/L would be governed by the nutritional status of by the influence of stressors or specific contamination sources (e.g. domestic sewage) rather than by seasonality in gamete development and spawning.

According to the SRI, evidence of sex ratio skew towards females could be envisaged in Taganga oysters in both seasons. This locality is influenced by discharges from the nearby marine outfall of Santa Marta and could therefore be recipient of potentially feminizing chemical pollutants. The potential presence of endocrine disrupting chemicals is a matter of concern; these chemicals include oestrogens, anti-oestrogens, anti-androgens, substances that reduce other steroid hormone levels, toxicants that affect the central nervous system, and toxicants that affect hormonal status, which typically occur in mixtures in domestic sewage effluents and industrial wastes (Depledge and Billinghurst, 1999). Moreover, a certain arrest in gametogenesis was observed in Isla Brujas in the dry season, with a high prevalence of oysters with undifferentiated follicles. In the rainy season, Santa Marta Marina and Taganga showed the most advanced gamete development, whereas the opposite was found in the dry season. Oocyte atresia was virtually non-existent and therefore RAI was mainly influenced by altered sex ratios and arrest/delay of the gametogenic cycle, with the highest scores recorded in Isla Brujas and Taganga, followed by Isla Barú. The observed low-to-moderate reproduction anomalies, therefore, seem to be related to pollutant exposure rather than to nutritional or environmental conditions.

The prevalence of parasites (such as *Nematopsis* sp., ciliates, intracellular protists, R/CLOs and metazoans) was low in all the samples; though somewhat higher in the dry season than in the rainy season. These parasites are common in *C. rhizophorae* and related bivalves and their seasonal variations and their relation to environmental conditions have been previously reported. (Aguirre-Macedo et al., 2007; Boehs et al., 2010; Brandão et al., 2013; Ceuta and Boehs, 2012; Marcogliese and Cone, 1997; Nascimento et al., 1986; Sabry et al., 2007, 2011, 2013). R/CLOs have been previously recorded in the digestive gland cells of *C. rhizophorae* (Brandão et al., 2013; Sabry et al., 2013) with prevalence usually below 30% and absence of pathological effects. In *C. rhizophorae* from Maraú River in Brazil, low prevalence (3.3%) of metazoan and R/CLO was also reported in the rainy season in comparison with the dry season (10–13.3%; Brandão et al., 2013). Likewise, low prevalence (1.76%) of the metazoan *Tylocephalum* was found in *C. rhizophorae* in the dry season, associated with high water temperature (Sabry et al., 2007), as well as for R/CLO in the dry season in *Mytella guyanesis* (Ceuta and Boehs, 2012). Isla Brujas, especially in the dry season, exhibited moderate prevalence of *Nematopsis* sp. and ciliates. As a result, PII was higher in Isla Brujas (both seasons) than in the other localities, followed by Isla Barú and Isla Maparadita in the dry season. Pollution by chemicals in mixture is a potential factor activating latent infection in oysters (Chu et al., 2002). Isla Brujas receives the input from the Dique Channel, which is a main source of sediments and chemical pollution in the Cartagena Bay (Vivas-Aguas et al., 2010).

Regarding inflammatory responses, only oedema and brown cell accumulation were recorded, and these were particularly high in the dry season. During this season there might be some common causing agent, but most especially in Isla Barú and Marina, be this a particular pollutant, sewage contamination or environmental conditions. Inflammatory responses in bivalves are generally considered to be indicative of stress, due to parasitosis (Cochennec-Laureau et al., 2003; Garmendia et al., 2011) or xenobiotic compounds (Gold-Bouchot et al., 1995; Sheir and Handy, 2010). Elevated levels of haemocytic infiltration and oedema are general responses to chemical pollutants in molluscs (Au, 2004). T. Accordingly, IRI exhibited the same trends as PII in the rainy season but not at all in the dry season. DGAI, which represents a fast and enduring biological response to environmental stressors (Garmendia et al., 2011; Marigómez et al., 2013), only exhibited a low-to-moderate increase in Isla Brujas in the dry season and therefore it did not provide any clear evidence of health impairment.

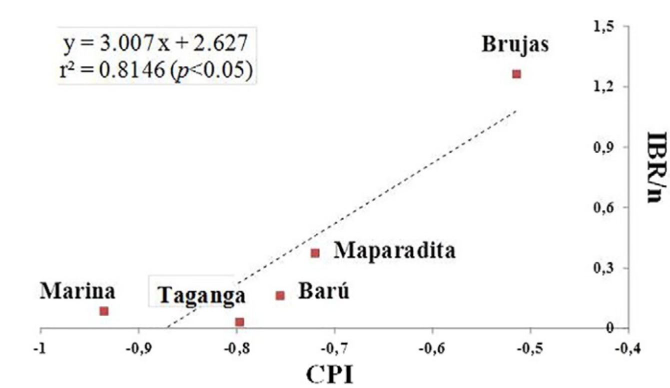
LT_{50} was in the range of 4–12 d for the Colombian sampling locations. Oysters from Isla Brujas in the dry season and, most markedly, those from Isla Barú, Isla Brujas and Isla Maparadita in the rainy season exhibited very low LT_{50} values. Unlike in Nicaraguan mangrove cupped oysters, for which the lowest LT_{50} values were recorded in the dry season, Colombian oysters seem to be more resistant to additional stress in the dry season than in the rainy season, as it would be expected from a stressed organism. Moreover, LT_{50} values were generally higher than in Nicaragua, which might be related to the smaller size of the Colombian oysters or to the poorer health condition of the Nicaraguan oysters at regional scale, as discussed above. Overall, the SoS response profile in the dry season resembled the one recorded for DGAI but, conversely, these biological responses were apparently not at all related in the rainy season, nor related to other observed health and growth parameters, neither with gamete development. Likewise, LT_{50} values could not be related to the pollutant tissue burdens reported in the oysters (Aguirre-Rubí et al., 2017). In the case of intertidal mussels from temperate regions, LT_{50} values around 3–6 d have been reported on exposure to Cu, Cd, PCB congeners, Aroclor 1254, and individual PAHs, in comparison with >10 d recorded in control and reference mussels (Eertman et al., 1995; Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995). Thus, as a general rule, LT_{50} values >10 d are indicative of healthy condition whereas values <6 d are indicative of stressed mussels (Martínez-Gómez et al., 2017; Viarengo et al., 1995). These critical values, however, can vary depending on species and the nutritional, reproductive and physiological status of the organisms and on environmental factors (Babarro and de Zwaan, 2008; Hellou and Law, 2003; Koukouzika and Dimitriadis, 2005; Thomas et al., 1999). Likewise, they can be different for different species, e.g. even if they are sympatric *Mytilus* spp. (Hellou and Law, 2003), and therefore the present results obtained for mangrove cupped oysters must be interpreted cautiously.

Though modulated by natural variables and confounding factors, different indicators of health disturbance, alone and in combination, were related to the presence of different profiles and levels of chemical pollutants present at low-to-moderate levels. Thus, IBR/n discriminated Isla Brujas and Isla Maparadita from the other localities in both seasons as well as Isla Brujas from Isla Maparadita in the dry season. Considering altogether the present results and the pollutant tissue levels reported for these mangrove cupped oysters (Aguirre-Rubí et al., 2017), it can be concluded that different mixtures of a variety of persistent (e.g., As, Cd, PAHs) and emerging chemical pollutants (e.g. musk fragrances) in combination with different levels of organic and particulate matter resulting from upwelling and sewage discharges, and environmental factors (salinity, temperature) elicit a different degree of disturbance in oyster health condition in mangrove-lined Caribbean coastal ecosystems, as reflected in sentinel *C. rhizophorae*. As a result, IBR/n was correlated with pollution indices such as PLI and CPI (Fig. 8), even though the levels of biological anomalies used as indicators of health disturbance and the levels of pollutants were in general terms low-to-moderate and modulated by biological variables and environmental factors that varied seasonally.

4.3. Assessment of ecosystem health disturbance

Integrative approaches for marine and coastal pollution monitoring involve the use of methodological standards in both sampling, analysis of samples and interpretation of results (ICES, 2011). Assessment of ecosystem health disturbance for the monitoring of pollution and its biological consequences in tropical and subtropical coastal regions is constrained by (a) limitations for systematic routine sampling (e.g. accessibility to sampling sites is expensive and complicated, and may be hampered by natural phenomena such as hurricanes, cyclones and earthquakes); (b) the limited knowledge on the biology and ecology of local/regional target species; (c) the lack of adequate sample transportation to the laboratory (this may take long time under non-optimal conditions for sample quality preservation); and (d) technological hurdles (e.g. for cryo-processing and cryo-sample transportation) that impede the application of core endpoints elsewhere applied in coastal biomonitoring. Moreover, quantifying a suite of biomarkers often requires purchasing of dedicated equipment, special wares, and expensive reagents not always easy to afford by developing countries (Blaise et al., 2016).

As previously established by Blaise et al. (2016) for mussels of subarctic and temperate regions, our study supports the use of simple, low-cost methodological approaches to diagnose anomalies in the health status of mangrove cupped oysters from different localities and to identify potential causing agents. These anomalies may reflect disturbances in ecosystem health that can be associated to chemical pollutants, eutrophication (e.g. from domestic sewage) as well as to naturally or human-driven anomalies and extremes in environmental factors such as salinity, dissolved oxygen, suspended matter, etc. Consequently, the low-cost methodological approach used herein is useful for the assessment of health status and disturbance in a variety Caribbean coastal ecosystems using mangrove cupped oysters as sentinel species. Likewise, this approach is useful to identify potential sources of health disturbance, be these chemical pollutants or other stressors, which can further on be identified following specific and more sophisticated and cost-intensive analytical procedures.



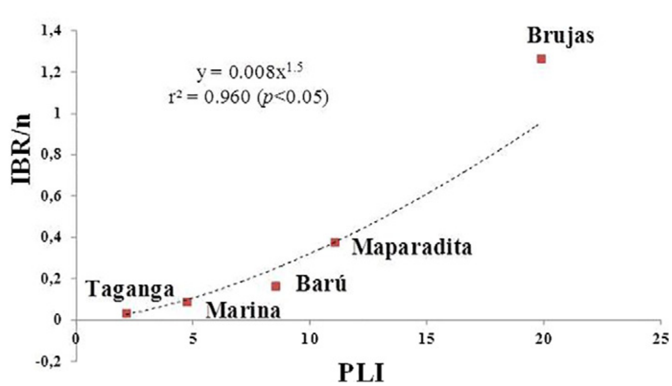


Fig. 8. Regression models and correlation between IBR/n and CPI (a) and PLI (b) in Colombian mangrove swamps in the dry season. CPI and PLI data obtained by Aguirre-Rubí et al. (2017). CPI, Chemical Pollution Index (Bellas et al., 2011); PLI, Pollution Load Index (Tomlinson et al., 1980).

Acknowledgements

Authors are indebted to Drs. L Garmendia and N Etxebarria from PiE-UPV/EHU and to colleagues from the Department of Biology (Environ Educ Prog, UNAN-León) for their invaluable assistance for sampling. This work was funded by Spanish Agency for International Development Cooperation (CARIBIOPOL-AECID 11-CAP2-1595) and supported by the Spanish Ministry of Economy and Finance (BMW-CTM2012-40203-C02-01), by The University of the Basque Country UPV/EHU (UFI 11/37) and by the Basque Government through Consolidated Research Groups fellowship (IT810-B). We also thank the scientific staff of the department of biology from Bluefields Indian and Caribbean University (BICU) for their support in the logistic and help with sampling, and the Research Centre on Health, Work and Environment (CISTA, UNAN-León) for their collaboration in the pre-treatment of samples for chemical analysis. We are also very grateful to A. Moncaleano, S. Casseres, P. Rojas and S. Lopera in Colombia for their collaboration in SoS and histological analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.08.098.

References

Afsar, N., Siddiqui, G., Roberts, D., 2014. Parasite inspection in five commercially important oyster species (Mollusca: Bivalvia) of Pakistan. Aust. J. Basic Appl. Sci. 10, 220–225.

Aguilar, C.A., Montalvo, C., Rodríguez, L., Cerón, J.G., Cerón, R.M., 2012. American oyster (*Crassostrea virginica*) and sediments as a coastal zone pollution monitor by heavy metals. Int. J. Environ. Sci. Technol. 9, 579–586.

Aguirre-Macedo, M.L., Simá-Álvarez, R.A., Román-Magaña, M.K., Güemez-Ricalde, J.I., 2007. Parasite survey of the eastern oyster *Crassostrea virginica* in coastal lagoons of the southern Gulf of Mexico. J. Aquat. Anim. Health 19, 270–279.

Aguirre-Rubí, J.R., Luna-Acosta, A., Etxebarria, N., Soto, M., Espinoza, F., Ahrens, M.J., Marigómez, I., 2017. Assessment of chemical pollution in mangrove-lined caribbean coastal systems using the oyster *Crassostrea rhizophorae* as biomonitor species. Envir Sci Pollut Res. https://doi.org/10.1007/s11356-017-9159-2.

Allan, I., Vrana, B., Greenwood, R., Mills, G., Roig, B., Gonzalez, C., 2006. A "toolbox" for biological and chemical monitoring requirements for the European Union's Water Framework Directive. Talanta 69, 302–322.

Alves, S.R.C., Severino, P.C., Ibbotsom, D.P., Silva, A.Z.X., Lopes, F.R.A.S., Saenz, L.A., Bainy, A.C.D., 2002. Effects of furadan in the brown mussel *Perna perna* and in the mangrove oyster *Crassostrea rhizophorae*. Mar. Environ. Res. 54, 241–245.
Apeti, D.A., Kim, Y., Lauenstein, G., Tull, J., Warner, R., 2014. Occurrence of Parasites and Diseases in Oysters and Mussels of the U.S. Coastal Waters. National Status and Trends, the Mussel Watch Monitoring Program. NOAA Technical Memorandum NOSS/NCCOS 182. Silver Spring, MD p. 51.
Au, D.W.T., 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Mar. Pollut. Bull. 48, 817–834.
Auffret, M., 1988. Histopathological changes related to chemical contamination in *Mytilus edulis* from field and experimental conditions. Mar. Ecol. Prog. Ser. 46, 101–107.
Austin, H., Haven, D.S., Moustafa, M.S., 1993. The relationship between trends in a condition index of American oysters *Crassostrea virginica*, and environmental parameters in three Virginia estuaries. Estuaries 16, 362–374.
Babarro, J.M.F., de Zwaan, A., 2008. Anaerobic survival potential of four bivalves from different habitats. A comparative survey. Comp. Biochem. Physiol. 151A, 108–113.
Bayen, S., 2012. Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: a review. Environ. Int. 48, 84–101.
Beliaeff, B., Burgeot, T., 2002. Integrated biomarker response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem. 21, 1316–1322.
Bellas, J., Nieto, O., Beiras, R., 2011. Integrative assessment of coastal pollution: development and evaluation of sediment quality criteria from chemical contamination and ecotoxicological data. Cont. Shelf Res. 31, 448–456.
Bignell, J., Cajaraville, M.P., Marigómez, I., 2012. Background document: histopathology of mussels (*Mytilus* spp.) for health assessment in biological effects monitoring. In: Davies, I.M., Vethaak, A.D. (Eds.), Integrated Monitoring of Chemicals and Their Effects. ICES Coop Res Rep N. 315, Copenhagen, Denmark, pp. 111–120.
Binelli, A., Bacchetta, R., Vailati, G., Galassi, S., Privini, A., 2001. DDT contamination in Lake Maggiore (N. Italy) and effects on zebra mussel spawning. Chemosphere 45, 409–415.
Blaise, C., Gagné, F., Burgeot, T., 2016. Three simple biomarkers useful in conducting water quality assessments with bivalve mollusks. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-016-6908-6.
Blaise, C., Gagné, F., Salazar, M., Salazar, S., Trottier, S., Hansen, P.-D., 2003. Experimentally-induced feminisation of freshwater mussels after long-term exposure to a municipal effluent. Fresenius Environ. Bull. 12, 865–870.
Boehs, G., Villalba, A., Ceuta, L.O., Luz, J.R., 2010. Parasites of three commercially exploited bivalve mollusc species of the estuarine region of the Cachoeira river (Ilhéus, Bahia, Brazil). J. Invertebr. Pathol. 103, 43–47.
Bower, S.M., McGladdery, S.E., Price, I.M., 1994. Synopsis of infectious diseases and parasites of commercially exploited shellfish. Annu. Rev. Fish Dis. 4, 1–199.
Brandão, R.P., Boehs, G., Da Silva, P.M., 2013. Health assessment of the oyster *Crassostrea rhizophorae* on the southern coast of Bahia, northeastern Brazil. Rev. Bras. Parasitol. Vet. 22, 84–91.
Broeg, K., Lehtonen, K.K., 2006. Indices for the assessment of environmental pollution of the Baltic Sea coasts: integrated assessment of a multi-biomarker approach. Mar. Pollut. Bull. 53, 508–522.
Cajaraville, M.P., Bebianno, M.J., Blasco, J., Porte, C., Sarasquete, C., Viarengo, A., 2000. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Sci. Total Environ. 247, 295–311.
Cajaraville, M.P., Marigómez, I., Angulo, E., 1993. Correlation between cellular and organismic responses to oil-induced environmental stress in mussels. Sci. Total Environ. 134 (Suppl), 1353–1371.
Carella, F., Feist, S., Bignell, J.P., De Vico, G., 2015. Comparative pathology in bivalves: aetiological agents and disease processes. J. Invertebr. Pathol. 131, 107–120.
Castilho-Westphal, G.G., Magnani, F.P., Ostrensky, A., 2015. Gonad morphology and reproductive cycle of the mangrove oyster *Crassostrea brasiliana* (Lamarck, 1819) in the baía de Guaratuba, Paraná, Brazil. Acta Zool. 96, 99–107.
Ceuta, L.O., Boehs, G., 2012. Parasites of the mangrove mussel *Mytella guyanensis* (Bivalvia: Mytilidae) in Camamu Bay, Bahia, Brazil. Braz. J. Biol. 72, 421–427.
Chagot, D., Alzieu, C., Sanjuan, J., Grizel, H., 1990. Sublethal and histopathological effects of trace levels of tributyltin fluoride on adult oysters *Crassostrea gigas*. Aquat. Living Resour. 3, 121–130.
Christo, S.W., Absher, T.M., 2006. Reproductive period of *Crassostrea rhizophorae* (Guilding, 1828) and *Crassostrea brasiliana* (Lamark, 1819) (Bivalvia: Ostreidae) in Guaratuba Bay, Paraná, Brazil. J. Coast. Res. 1215–1218.
Chu, F.L.E., Volety, A.K., Hale, R.C., Huang, Y., 2002. Cellular responses and disease expression in oysters (*Crassostrea virginica*) exposed to suspended field contaminated sediments. Mar. Environ. Res. 53, 17–35.
Cochennec-Laureau, N., Auffret, M., Renault, T., Langlade, A., 2003. Changes in circulating and tissue-infiltrating hemocyte parameters of European flat oysters, *Ostrea edulis*, naturally infected with *Bonamia ostreae*. J. Invertebr. Pathol. 83, 23–30.
Couch, J.A., 1985. Prospective study of infectious and non-infectious diseases in oysters and fishes in three Gulf of Mexico estuaries. Dis. Aquat. Org. 1, 59–82.
Crosby, M., Gale, L., 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. J. Shellfish Res. 9, 233–237.
Dame, R.F., 1972. Comparison of various allometric relationships in intertidal and subtidal American oysters. Fish. Bull. 70, 1121–1126.
De Vico, G., Carella, F., 2012. Morphological features of the inflammatory response in molluscs. Res. Vet. Sci. 93, 1109–1115.
Depledge, M.H., Billinghurst, Z., 1999. Ecological significance of endocrine disruption in marine invertebrates. Mar. Pollut. Bull. 39, 32–38.
Dumailo, S., 2003. Evaluación de la problemática ambiental por medio del estudio de algunos aspectos de sedimentación y contaminación en la laguna de Bluefields, RAAS, Nicaragua. MSc Thesis. CIRA-UNAN, p. 242.
Ebanks-Mongalo, B.F., Suárez-Sánchez, J., Siu-Estrada, E., Montoya-Arguello, J.J., Mairena-Valdivia, D.A., Flores-Pacheco, A., Van der Wal, J.C., Valencia-Quintana, P., 2013. Concentración de Plaguicidas en Agua, Sedimentos y Ostiones (*Crassostrea rizophorae*) de la Laguna de Bluefields, RAAS, Nicaragua. WANI, pp. 49–54.
Eertman, R.H.M., Groenink, C.L.F.M.G., Sandee, B., Hummel, H., Smaal, A.C., 1995. Response of the blue mussel *Mytilus edulis* L. following exposure to PAHs or contaminated sediment. Mar. Environ. Res. 39, 169–173.
Fernandez, A., Singh, A., Jaffé, R., 2007. A literature review on trace metals and organic compounds of anthropogenic origin in the Wider Caribbean Region. Mar. Pollut. Bull. 54, 1681–1691.
Gagne, F., Blaise, C., Lachance, B., Sunahara, G.I., Sabik, H., 2001. Evidence of coprostanol estrogenicity to the freshwater mussel *Elliptio complanata*. Environ. Pollut. 115, 97–106.
Gagne, F., Blaise, C., Pellerin, J., Pelletier, E., Douville, M., Gauthier-Clerc, S., Viglino, L., 2003. Sex alteration in soft-shell clams (*Mya arenaria*) in an intertidal zone of the Saint Lawrence River (Quebec, Canada). Comp. Biochem. Physiol. 134C, 189–198.
García, F., Palacio, C., Garcia, U., 2012. Water quality at Santa Marta coastal area (Colombia). Dyna 79, 85–94.
Garmendia, L., Soto, M., Vicario, U., Kim, Y., Cajaraville, M., Marigómez, I., 2011. Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: tissue-level biomarkers and histopathology. J. Environ. Monit. 13, 915–932.
Gauthier-Clerc, S., Pellerin, J., Blaise, C., Gagne, F., 2002. Delayed gametogenesis of *Mya arenaria* in the Saguenay fjord (Canada): a consequence of endocrine disruptors? Comp. Biochem. Physiol. 131C, 457–467.
GEF-REPCar, 2011. Agricultural Pesticide Residues in Caribbean Coastal Waters. Colombia, Costa Rica and Nicaragua 2008–2011. Reducing Pesticides Run-off to the Caribbean Sea (GEF-REPCar) Rpt UNEP. p. 63.
George-Zamora, A., Sevilla-Hernández, L.M., Aldana-Aranda, D., 2003. Ciclo gonádico del ostión americano *Crassostrea virginica* (Lamellibranchia: Ostreidae) en Mecoacán, Tabasco, México Arturo. Rev. Biol. Trop. 51, 109–117.
Gold-Bouchot, G., Simá, R., Zapata-Pérez, O., Güemez, J., 1995. Histopathological effects of petroleum hydrocarbons and heavy metals on the American oyster, *Crassostrea virginica*, from Tabasco, Mexico. Mar. Pollut. Bull. 31, 439–445.
Gómez-Giraldo, A., Osorio, A., Toro, F.M., Osorio, J.D., Alvarez, O.A., Arrieta, A., 2009. Circulation patterns in the "Barbacoas" bay and its influence on sediment transport to the "Rosario" islands. Avances. Rec. Hidraul. 20, 21–40.
Gordon, C.M., 1988. Sex ratio condition and glycogen content of raft cultivated mangrove oysters *Crassostrea rhizophorae*. J. Shellfish Res. 72, 395–400.
Hellou, J., Law, R.J., 2003. Stress on stress response of wild mussels, *Mytilus edulis* and *Mytilus trossulus*, as an indicator of ecosystem health. Environ. Pollut. 126, 407–416.
Hellou, J., Yeats, P., Steller, S., Gagne, F., 2003. Chemical contaminants and biological indicators of mussel health during gametogenesis. Environ. Toxicol. Chem. 22, 2080–2087.
ICES, 2011. Report of the Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC), 14-18 March 2011. Copenhagen, Denmark. International Council for the Exploration of the Sea CM 2011/ACOM:30. p. 265.
ICES, 2012. Report of the Working Group on Biological Effects of Contaminants (WGBEC). International Council for the Exploration of the Sea CM 2012/SSGHIE: 04. p. 131.
Jana, H., Mondal, K.C., Maity, C., Das, A., Halder, S.K., Mitra, A., Pati, B.R., 2014. Effect of microbial load on the condition index of the edible oyster, *Saccostrea cucullata*, in the Sundarbans, India. Internat. J. Aquat. Biol. 2, 238–245.
Kim, Y., Ashton-Alcox, K.A., Powell, E.N., 2006. Histological Techniques for Marine Bivalve Molluscs: Update. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 27.
Kim, Y., Powell, E.N., 2007. Distribution of parasites and pathologies in sentinel bivalves: NOAA status and trends "Mussel Watch" program. J. Shellfish Res. 26, 1115–1151.
Kim, Y., Powell, E.N., Wade, T.L., Presley, B.J., 2008. Relationship of parasites and pathologies to contaminant body burden in sentinel bivalves: NOAA status and trends "mussel watch"program. Mar. Environ. Res. 65, 101–127.
Koukouzika, N., Dimitriadis, V.K., 2005. Multiple biomarker comparison in *Mytilus galloprovincialis* from the Greece coast: "lysosomal membrane stability, neutral red retention, micronucleus frequency and stress on stress". Ecotoxicology 14, 449–463.
La Rosa, T., Mirto, S., Marino, A., Alonzo, V., Maugeri, T.L., Mazzola, A., 2001. Heterotrophic bacteria community and pollution indicators of mussel-farm impact in the Gulf of Gaeta (Tyrrhenian Sea). Mar. Environ. Res. 52 (4), 301–321.
Leiniö, S., Lehtonen, K.K., 2005. Seasonal variability in biomarkers in the bivalves *Mytilus edulis* and *Macoma balthica* from the northern Baltic Sea. Comp. Biochem. Physiol. 140C, 408–421.
Lenz, T., Boehs, G., 2011. (Bivalvia: Ostreidae) en la Bahía de Camamu, Bahia, Brasil. Rev. Biol. Trop. 59, 137–149.
Lewis, M., Pryor, R., Wilking, L., 2011. Fate and effect of anthropogenic chemicals in mangrove ecosystems: a review. Environ. Pollut. 159, 2328–2346.
Lobel, P.B., Wright, D.A., 1982. Relationship between body zinc concentration and allometric growth measurements in the mussel *Mytilus edulis*. Mar. Biol. 66, 145–150.
Lowe, D.M., 1988. Alterations in cellular structure of *Mytilus edulis* resulting from exposure to environmental contaminants under field and experimental conditions. Mar. Ecol. Prog. Ser. 46, 91–100.
Mancera-Pineda, J.E., Pinto, G., Vilardy, S., 2013. Seasonal patterns of water masses distribution in Santa Marta bay, Colombian Caribbean: relative importance of upwelling and outwelling. Bol. Invest. Mar. Cost. 42 (329), 360.
Maranho, L.A., Pereira, C.D.S., Choueri, R.B., Cesar, A., Gusso-Choueri, P.K., Torres, R.J., Abessa, D.M.D.S., Morais, R.D., Mozeto, A.A., DelValls, T.A., Martín-Díaz, M.L., 2012. The application of biochemical responses to assess environmental quality of tropical estuaries: field surveys. J. Environ. Monit. 14, 2608.
Marcogliese, D.J., Cone, D.K., 1997. Parasite communities as indicators of ecosystem stress. Parassitologia 39, 227–232.

Marigómez, I., Garmendia, L., Soto, M., Orbea, A., Izagirre, U., Cajaraville, M.P., 2013. Marine ecosystem health status assessment through integrative biomarker indices: a comparative study after the Prestige oil spill "Mussel Watch". Ecotoxicology 22, 486–505.

Martínez-Gómez, C., Robinson, C.D., Burgeot, T., Gubbins, M., Halldórson, H.P., Albentosa, M., Bignell, J.P., Hylland, K., Vethaak, D., 2017. Biomarkers of general stress in mussels as common indicators for marine biomonitoring programmes in Europe: the ICON experience. Mar. Environ. Res. 124, 70–80.

Moles, A., Hale, N., 2003. Use of physiological responses in *Mytilus trossulus* as integrative bioindicators of sewage pollution. Mar. Pollut. Bull. 46, 954–958.

Montes-M, A., Prieto-Arcas, A., Ruiz, L.J., 2007. Abundancia, biomasa y proporción sexual en una población natural de la ostra (*Crassostrea rhizophorae*) en la Laguna grande de Obispo, Estado Sucre, Venezuela. Bol. Ctro. Invest. Biol. 41, 485–501.

Mubiana, V.K., Vercauteren, K., Blust, R., 2006. The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in *Mytilus edulis*. Environ. Pollut. 144, 272–279.

Nascimento, I.A., Pereira, S.A., 1980. Changes in the condition index for mangrove oysters (*Crassostrea rhizophorae*) from Todos os Santos Bay, Salvador, Brazil. Aquaculture 20, 9–15.

Nascimento, I.A., Smith, D.H., Kern, F., Pereira, S.A., 1986. Pathological findings in *Crassostrea rhizophorae* from Todos os Santos Bay, Bahia, Brazil. J. Invertebr. Pathol. 47, 340–349.

Ortiz-Zarragoitia, M., Cajaraville, M.P., 2010. Intersex and oocyte atresia in a mussel population from the Biosphere's Reserve of Urdaibai (Bay of Biscay). Ecotoxicol. Environ. Saf. 73, 693–701.

Ortiz-Zarragoitia, M., Garmendia, L., Barbero, M.C., Serrano, T., Marigómez, I., Cajaraville, M.P., 2011. Effects of the fuel oil spilled by the Prestige tanker on reproduction parameters of wild mussel populations. J. Environ. Monit. 13, 84–94.

Osuna-Martínez, C.C., Páez-Osuna, F., Alonso-Rodríguez, R., 2010. Mercury in cultured oysters (*Crassostrea gigas* Thunberg, 1793 and *C. corteziensis* Hertlein, 1951) from four coastal lagoons of the SE Gulf of California, Mexico. Bull. Environ. Contam. Toxicol. 85, 339–343.

Palm, H.W., Dobberstein, R.C., 1999. Occurence of trichodinid ciliates (Peritricha: Urceolariidae) in the Kiel Fjord, Baltic Sea, and its possible use as a biological indicator. Parasitol. Res. 85, 726–732.

Park, J.J., Kim, H., Kang, S.W., An, C.M., Lee, S.H., Gye, M.C., Lee, J.S., 2012. Sex ratio and sex reversal in two-year-old class of oyster, *Crassostrea gigas* (Bivalvia: Ostreidae). Dev. Reprod. 16, 385–388.

Powell, E.N., Klinck, J.M., Hofmann, E.E., 2011. Generation time and the stability of sex-determining alleles in oyster populations as deduced using a gene-based population dynamics model. J. Theor. Biol. 271, 27–43.

Quinn, B., Gagne, F., Costello, M., McKenzie, C., Wilson, J., Mothersill, C., 2004. The endocrine disrupting effect of municipal effluent on the zebra mussel (*Dreissena polymorpha*). Aquat. Toxicol. 66, 279–292.

Rebelo, M.F., Amaral, M.C.R., Pfeiffer, W.C., 2005. Oyster condition index in *Crassostrea rhizophorae* (Guilding 1828) from a heavy-metal polluted coastal lagoon. J Biol]–>Braz. J. Biol. 65, 345–351.

Rebelo, M.F., Pfeiffer, W.C., da Silva, H., Moraes, M.O., 2003. Cloning and detection of metallothionein mRNA by RT-PCR in mangrove oysters (*Crassostrea rhizophorae*). Aquat. Toxicol. 64, 359–362.

Sabry, R.C., Da Silva, P.M., Gesteira, T.C.V., Pontinha, V.A., Magalhães, A.R.M., 2011. Pathological study of oysters *Crassostrea gigas* from culture and *C. rhizophorae* from natural stock of Santa Catarina Island, SC, Brazil. Aquaculture 320, 43–50.

Sabry, R.C., Gesteira, T.C.V., Boehs, G., 2007. First record of parasitism in the mangrove oyster *Crassostrea rhizophorae* (Bivalvia: Ostreidae) at Jaguaribe River estuary - Ceará, Brazil. Braz. J. Biol. 67 (755), 758.

Sabry, R.C., Gesteira, T.C.V., Magalhães, A.R.M., Barracco, M.A., Guertler, C., Ferreira, L.P., Vianna, R.T., da Silva, P.M., 2013. Parasitological survey of mangrove oyster, *Crassostrea rhizophorae*, in the Pacoti river estuary, Ceará State, Brazil. J. Invertebr. Pathol. 112, 24–32.

Schettino, T., Caricato, R., Calisi, A., Giordano, M.E., Lionetto, M.G., 2012. Biomarker approach in marine monitoring and assessment: new insights and perspectives. Open Environ. Sci. 6, 20–27.

Scott, G.I., Lawrence, D.R., 1982. The American oyster as a coastal zone pollution monitor: a pilot study. Estuaries 5, 40–46.

Sericano, J.L., Wade, T.L., Jackson, T.J., Brooks, J.M., Tripp, B.W., Farrington, J.W., Mee, L.D., Readmann, J.W., Villeneuve, J.P., Goldberg, E.D., 1995. Trace organic contamination in the Americas: an overview of the US National Status & Trends and the International "Mussel Watch" programmes. Mar. Pollut. Bull. 31, 214–225.

Sheir, S.K., Handy, R.D., 2010. Tissue injury and cellular immune responses to cadmium chloride exposure in the common mussel *Mytilus edulis*: modulation by lipopolysaccharide. Arch. Environ. Contam. Toxicol. 59, 602–613.

Silva, C.A.R., Smith, B.D., Rainbow, P.S., 2006. Comparative biomonitors of coastal trace metal contamination in tropical South America (N. Brazil). Mar. Environ. Res. 61, 439–455.

Smaal, A.C., van Stralen, M.R., 1990. Average annual growth and condition of mussels as a function of food source. Hydrobiologia 195, 179–188.

Smaal, A.C., Wagenvoort, A., Hemelraad, J., Akkerman, I., 1991. Response to stress of mussels (*Mytilus edulis*) exposed in dutch tidal waters. Comp. Biochem. Physiol. 100C, 197–200.

Thomas, R.E., Harris, P.M., Rice, S.D., 1999. Survival in air of *Mytilus trossulus* following long-term exposure to spilled Exxon Valdez crude oil in Prince William sound. Comp. Biochem. Physiol. 122C, 147–152.

Tomlinson, D.L., Wilson, J.G., Harris, C.R., Jeffrey, D.W., 1980. Problems in assessment of heavy metals in estuaries and the formation of Pollution Index. Helgoländer Meeresun. 33, 566–575.

Valdez Domingos, F.X., Azevedo, M., Silva, M.D., Randi, M.A.F., Freire, C.A., Silva de Assis, H.C., Oliveira Ribeiro, C.A., 2007. Multibiomarker assessment of three Brazilian estuaries using oysters as bioindicators. Environ. Res. 105, 350–363.

Veldhuizen-Tsoerkan, M.B., Holwerda, D.A., Zandee, D.I., 1991. Anoxic survival time and metabolic parameters as stress indices in sea mussels exposed to cadmium or polychlorinated biphenyls. Arch. Environ. Contam. Toxicol. 20, 259–265.

Vélez, A., 1982. Hermafroditismo en la ostra de mangle *Crassostrea rhizophorae*. Bol. Inst. Oceanogr. Univ. Oriente. 21, 129–132.

Viarengo, A., Canesi, L., Pertica, M., Mancinelli, G., Accomando, R., Smaal, A.C., Orunesu, M., 1995. Stress on stress response: a simple monitoring tool in the assessment of general stress syndrome in mussels. Mar. Environ. Res. 39, 245–248.

Villarroel, E., Buitrago, E., Lodeiros, C., 2004. Identification of environmental factors affecting growth and survival of the tropical oyster *Crassostrea rhizophorae* in suspended culture in the Golfo de Cariaco, Venezuela. Rev. Cient. Ciencias Vet. 14, 28–35.

Vivas-Aguas, L., Sánchez, J., Betancourt, J., Quintero, M., Moreno, Y., Santana, C., Cuadrado, I., Ibarra, K., Rios, M., Obando, P., Sánchez, D., 2014. Diagnóstico y Evaluación de la Calidad Ambiental Marina en el Caribe y Pacifico Colombiano. REDCAM Inf Téc 2013. INVEMAR. Santa Marta. p. 314.

Vivas-Aguas, L., Tosic, M., Sánchez, J., Narváez, S., Cadavid, B., Bautista, P., Betancourt, J., Parra, J., Echeverri, L., Espinosa, L., 2010. Diagnóstico y Evaluación de la Calidad Ambiental Marina en el Caribe y Pacifico Colombiano. REDCAM Inf Téc 2010. INVEMAR. Santa Marta. p. 208.

Zanette, J., Flores Nunes, F., Dias Medeiros, I., Siebert, M.N., Mattos, J.J., Lüchmann, K.H., Rodrigues de Melo, C.M., Dias Bainy, A.C., 2008. Comparison of the antioxidant defense system in *Crassostrea rhizophorae* and *Crassostrea gigas* exposed to domestic sewage discharges. Mar. Environ. Res. 66, 196–198.