Influence of sea cucumbers on chromophoric of dissolved organic matter in multitrophic aquaculture tanks

Seyed Mohammad Sadeghi-Nassaj [1] , **Teresa S. Catalá** [1] , **Pedro A. Álvarez** [2] , **Isabel Reche** [Corresp. 3]

[1] Ecología and Instituto del Agua, Universidad de Granada, Granada, Spain
[2] Av. de la Habana 10, iMare Natural S.L, Motril, Granada, Spain
[3] Ecología/ Facultad de Ciencias, Universidad de Granada, Granada, Spain

Corresponding Author: Isabel Reche
Email address: ireche@ugr.es

Background. The effluents of the mono-specific aquaculture contain high concentrations of dissolved nutrients and organic matter, which affect negatively water quality of the recipient aquatic ecosystems. A key feature of water quality is its transparency. Chromophoric dissolved organic matter (CDOM) determines most of the light transmission in the ultraviolet and blue bands in the aquatic ecosystems. A sustainable alternative to mono-specific aquaculture is the integrated multitrophic aquaculture that includes species trophically complementary named "extractive" species. Sea cucumbers are recognized as efficient extractive species, with a high potential to improve water quality, due to the consumption of particulate organic matter (POM). However, the effects of sea cucumbers on CDOM are still unknown. **Methods.** During one year, we biweekly monitored CDOM in two aquaculture tanks with different trophic structure. One of the tanks (-*holothurian* tank) only contained the primary species, *Anemonia sulcata*, whereas the other tank (+ *holothurian* tank) also contained individuals of *Holothuria tubulosa* and *H. forskali*. We routinely performed CDOM absorption spectra from 200 nm to 750 nm and determined quantitative (absorption coefficients at 325 nm) and qualitative (spectral slopes and molar absorption coefficients at 325 nm) optical parameters in the inlet waters, in the tanks, and in their corresponding effluents. **Results.** Absorption coefficients at 325 nm (a_{325}) and spectral slopes from 275 to 295 nm ($S_{275-295}$) were significantly lower in the effluents of the +*holothurian* tank (average: 0.33 and 16 μm^{-1}, respectively) than in the effluents of the −*holothurian* tank (average: 0.69 m^{-1} and 34 μm^{-1}, respectively), being the former similar to those found in the inlet waters (average: 0.32 m^{-1} and 22 μm^{-1}, respectively). This reduction in CDOM absorption appears to be mediated by the POM consumption by the holothurians. The reduction of POM concentration in the +*holothurian* tank may weaken the process of POM disaggregation into dissolved organic matter, which ultimately might have generated CDOM in the –*holothurian* tank. **Discussion.** Extractive species such as holothurians improve water transparency through POM consumption, likely because reduces POM disaggregation into CDOM. We suggest that CDOM monitoring in aquaculture facilities, using automatic probes or even remote sensing, could be a useful tool to trace the effectiveness of extractive species at large scales of time and space.

Influence of sea cucumbers on chromophoric dissolved organic matter in multitrophic aquaculture tanks

Seyed Mohammad Sadeghi-Nassaj[1], Teresa S. Catalá[1], Pedro A. Álvarez[2], and Isabel Reche[1]

[1]Departamento de Ecología and Instituto del Agua, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain

[2] iMare Natural S.L., Av. de la Habana 10, 18600 Motril, Granada, Spain

Corresponding author:

Isabel Reche

Email address: ireche@ugr.es

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Abstract:

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Methods. During one year, we biweekly monitored CDOM in two aquaculture tanks with different trophic structure. One of the tanks (–*holothurian* tank) only contained the primary species, *Anemonia sulcata*, whereas the other tank (+ *holothurian* tank) also contained individuals of *Holothuria tubulosa* and *H. forskali*. We routinely performed CDOM absorption spectra from 200 nm to 750 nm and determined quantitative (absorption coefficients at 325 nm) and qualitative (spectral slopes and molar absorption coefficients at 325 nm) optical parameters in the inlet waters, in the tanks, and in their corresponding effluents.

Results. Absorption coefficients at 325 nm (a_{325}) and spectral slopes from 275 to 295 nm ($S_{275-295}$) were significantly lower in the effluents of the +*holothurian* tank (average: 0.33 and 16 μm^{-1}, respectively) than in the effluents of the –*holothurian* tank (average: 0.69 m^{-1} and 34 μm^{-1}, respectively), being the former similar to those found in the inlet waters (average: 0.32 m^{-1} and 22 μm^{-1}, respectively). This reduction in CDOM absorption appears to be mediated by the POM consumption by the holothurians. The reduction of POM concentration in the +*holothurian* tank

may weaken the process of POM disaggregation into dissolved organic matter, which ultimately might have generated CDOM in the –*holothurian* tank.

Discussion. Extractive species such as holothurians improve water transparency through POM consumption, likely because reduces POM disaggregation into CDOM. We suggest that CDOM monitoring in aquaculture facilities, using automatic probes or even remote sensing, could be a useful tool to trace the effectiveness of extractive species at large scales of time and space.

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Introduction

The demographic growth of the human population has increased the global demand of fish and seafood (FAO, 2009). Since extractive fisheries are more and more reduced, the aquaculture is gaining relevance and, currently, accounts for more than 40% of human consumption of fish and seafood (Bostock *et al.*, 2010). Mono-specific aquaculture produces wastewater that usually contains high concentrations of organic matter as well as inorganic nutrients, antibiotics and uneaten food pellets (Read & Fernandes, 2003; Klinger & Naylor, 2012). At ecosystem level, the inputs of mineral nutrients associated with the aquaculture can produce problems of eutrophication (Ajin et al., 2016; Ruiz-Zarzuela et al., 2009). Moreover, the inputs of dissolved and particulate organic matter can reduce water transparency due to an increase in light backscattering and absorption (Ibarra et al. 2012; Del Bel Belluz et al. 2016). Therefore, a sustainable aquaculture is a global challenge for both scientists and food producers. The integrated multitrophic aquaculture (IMTA) is an alternative practice to alleviate the handicaps of the traditional, mono-specific aquaculture (Diana *et al.*, 2013). Unlike mono-specific aquaculture, IMTA uses trophically-complementary "extractive" species that consume excretion products, fecal and food wastes of the primary species (Chopin et al. 2012). Hence, it is desirable that the future expansion of aquaculture promotes this practice to reduce the inputs of organic matter in the environment, at the same time that aquaculture farmers obtain an economical value from the co-cultured species.

The chromophoric dissolved organic matter (CDOM) is the fraction of the dissolved organic matter (DOM) that absorbs light in the ultraviolet (UV) and, to a lesser extent, in the visible range of the spectrum. Therefore, CDOM is largely responsible for UV and blue light attenuation in marine ecosystems (Bricaud *et al.*, 1981; Nelson & Siegel, 2013). Since CDOM absorption overlaps one of the chlorophyll a absorption peaks, CDOM can diminish the potential for primary productivity. This fact affects the algorithms used in remote sensing to assess ocean color and to infer primary productivity (Carder et al. 1989; Siegel *et al.*, 2005; Ortega-Retuerta *et al.*, 2010). As remote sensing has been suggested as an excellent tool to monitoring offshore aquaculture at large scales (Populus *et al.*, 1995; Rajitha *et al.*, 2007; Saitoh *et al.*, 2011), the CDOM-chlorophyll a interference is important for the validation of those algorithms. However, the interaction between aquaculture and CDOM has been poorly explored (Ibarra *et al.* 2012; Nimptsch *et al.* 2015; Del Bel Belluz *et al.* 2016).

Sea cucumbers are extractive species with a high capacity to consume particulate organic matter (Nelson et al. 2012a,b; Yokoyama 2013, 2015). In addition, sea cucumbers are highly demanded food for human consumption in some countries (Purcell et al. 2013). Despite the effects of sea cucumbers on different components of the particulate organic matter has been extensively studied (Slater & Carton 2009; Slater *et al.* 2009; Nelson *et al.*, 2012a Zamora & Jeff 2011; Yokoyama 2013, 2015; Zhang *et al.*, 2014), their influence on the optical properties of the dissolved organic matter still remains unexplored (Zamora *et al.*, 2016).

In this study, we evaluate the effects of sea cucumbers (*Holothuria tubulosa* and *H. forskali*) on the optical properties of the dissolved organic matter in aquaculture tanks with *Anemonia sulcata* as primary species. *Anemonia sulcata* is a very palatable species, highly demanded for catering in Spain, with an additional great pharmacological interest. During one year, we compared the changes in DOM optical properties in a tank with holothurians and in another tank without them, exploring the main factors controlling these changes.

Material and Methods

Aquaculture tanks and sampling

We monitored during one year two aquaculture tanks located at the iMareNatural S.L. facilities (http://www.imarenatural.com) in Spain (36°44′38″ N, 3°35′59″ W). Each tank of 50,000 liters of capacity was connected directly with the coastal water by one inlet pipe and the effluent water was released by one outlet pipe. The seawater was pumped into the tanks at a continuous flow of 1,200 l h^{-1}. In one of the tanks, around 810 individuals of the primary specie, the sea anemone *Anemonia sulcata*, and 90 individuals of sea cucumbers *Holothuria tubulosa* and *H. forskali* were included (hereafter designated as + *holothurian* tank). In the other tank only ≈ 810 individuals of the primary specie were included (hereafter designated as – *holothurian* tank). Sea anemones were placed on floating structures (plastic boxes) within the tanks and were fed with fresh chopped fish, mainly *Scomber scombrus* (Chintiroglou and Koukouras 1992).

Chromophoric Dissolved Organic Matter (CDOM)

Absorption spectra of dissolved organic matter provide information on CDOM concentration and other qualitative properties. Absorption coefficients at specific wavelengths are used as proxies of CDOM concentration, and spectral slopes, spectral ratios and molar absorption coefficients, which are largely independent of the concentration, are surrogates of CDOM origin, molecular weight and chemical structure (Weishaar et al., 2003; Twardowski *et al.*, 2004; Helms et al., 2008; Nelson and Siegel, 2013).

Water samples from each tank were sampled biweekly from July 17th 2013 to August 20th 2014. Each sampling day, we took water samples from the inlet pipe, the center of the two tanks and from their corresponding effluents. To avoid that light can affect absorption measurements; we took the samples in pre-combusted (4h at 500 °C), acid-cleaned, amber glass bottles. They were kept in ice during transportation to the laboratory (about one hour from the tanks). Water samples were filtered through pre-combusted Whatman GF/F glass fiber filters of 0.7μm nominal pore size.

CDOM absorbance spectra were recorded at wavelengths from 200 nm to 750 nm at 1-nm interval using an UV/VIS Perkin Elmer spectrometer with a 10 cm-quartz cuvette. The spectrophotometer was connected to a computer with Lambda 25 software. The detection limit of the spectrophotometer (0.001Absorbance) corresponds to a CDOM absorption coefficient detection limit of 0.02 m^{-1}. Spectrum corrections due to residual scattering by fine size particle fractions, micro-air bubbles, or colloidal material present in the sample were performed by subtracting the average of the absorption between 600 and 700 nm (Green and Blough, 1994).

CDOM absorption coefficients, a_λ, were calculated using the next equation:

$$a_\lambda = 2.303\,\frac{Absorbance\,(\lambda) - Absorbance\,(600-700)}{l} \quad (1)$$

Where a_λ is the absorption coefficients in m^{-1} at each λ wavelength, *Absorbance (λ)* is the absorbance at wavelength λ, *Absorbance (600-700)* is the average absorbance from 600 to 700 nm, 2.303 is the factor that converts decadic to natural logarithms, l is the cuvette path length in m^{-1}.

Spectral slopes describe the shape decay of absorption coefficients vs. wavelengths. Slopes were calculated from the linear regression of log-transformed absorption coefficients in the wavelength bands 275-295 nm ($S_{275-295}$) and 350-400 nm ($S_{350-400}$) (Helms et al. 2008). The spectral slopes for both wavelength ranges were calculated as in equation 2.

$$a_\lambda = a_{\lambda ref}\, e^{-S(\lambda - \lambda_{ref})} \quad (2)$$

Where λ is the selected wavelength in nm, a_λ is the absorption coefficient at λ wavelength in m^{-1}, $a_{\lambda ref}$ is the absorption coefficient at a reference wavelength λ_{ref}, and S is the spectral slope. The spectral slope ratio (S_R) was calculated as the ratio of the spectral slope from 275 nm to 295 nm ($S_{275\text{-}295}$) to the spectral slope from 350 nm to 400 nm (Helms et al., 2008). In addition, we calculated the molar absorption coefficients at 325 nm (a^*_{325}) as the absorption coefficients at 325 nm normalized by the concentration of total organic carbon.

Ancillary data

Basic parameters as temperature (°C), pH, salinity (psu), total dissolved solids (TDS), and conductivity ($mScm^{-1}$) were measured in the tanks using a multi-parameter HANNA probe (HI9828 model). Total organic carbon (TOC) concentration was measured as non-purgeable organic carbon after a high-temperature catalytic oxidation using a Shimadzu TOC-V CSN. Samples, by triplicate, were acidified with hydrochloric acid and purged for 20 min to eliminate the remaining dissolved inorganic carbon. Three to five injections were analyzed for each sample. Standardization of the instrument was done with potassium hydrogen phthalate. Particulate organic matter (POM) was obtained filtering between 1.5 and 2.0 l of water through pre-weighed and pre-combusted (500°C for 4 h) Whatman GF/F glass fiber filters. The filters containing all the solids were dried at 60°C for >24 h and reweighed to determine the total mass (mineral + organic matter). Then, the organic fraction was burned by combusting the filters at 500°C for 6 h; finally, the filters were reweighed again to determine the mineral residue. POM was obtained after the subtraction of the mineral residue to the total mass. The concentration of chlorophyll-*a* was determined spectrophotometrically after pigment extraction with methanol (APHA 1992). Prokaryotic (bacteria and cyanobacteria) abundance was determined in triplicate using flow cytometry (Gasol and del Giorgio 2000) with a FACScalibur Becton Dickinson cytometer equipped with a laser emitting at 488 nm. Data were processed using Cell quest software.

Statistical analysis

To compare the temporal dynamics of CDOM optical parameters in the tank with holothurians vs. the tank without holothurians we performed paired t-test (for normally distributed variables) and Wilcoxon matched pairs test (for not-normally distributed variables) using the Statistica software (V8) and R 3.2.2. These statistical analyses ameliorate the problem of temporal pseudoreplication in this type of studies (Millar and Anderson 2004). Correlations between optical parameters of CDOM and potential controlling factors were performed using Statistica software (V8).

Results and discussion

During the study period, the pH in the inlet waters ranged from 7.71 to 8.31, the temperature from 13.58 ºC to 25.58 ºC, the salinity from 35.8 to 41.6 psu, the conductivity between 52.28 and 61.96 mS $cm^{-1}$and total dissolved solids from 18.26 to 30.84 ppt.

The quantity of CDOM was measured as absorption coefficient at 325 nm, since this wavelength is the most common in the literature (Nelson & Siegel 2013). In the inlet waters, the a_{325} values ranged from 0.06 to 0.83 m^{-1} (Table S1) and in the effluents of +*holothurian* and –*holothurian* tanks from 0.06 to 0.79 m^{-1} and from 0.37 to 1.27 m^{-1}, respectively (Tables S2 and S3). The absorption coefficients of the inlet waters (i.e. coastal waters of Western Mediterranean Sea) were similar to those ones found in other coastal waters (Catalá *et al.* 2013; Nima *et al.*, 2016) or in the open Mediterranean Sea (Bracchini *et al.*, 2010; Organelli *et al.* 2014). Systematically, throughout the whole study period, the effluents of the –*holothurian* tank showed higher a_{325} values (red triangles in Fig. 1a) than the effluents of the + *holothurian* tank (black squares in Fig. 1a).

The spectral slopes, the spectral slope ratios, and the molar extinction coefficients are qualitative parameters, which are independent of the CDOM concentration. The higher the spectral slope, the smaller the molecular weight of DOM is (Helms *et al.*, 2008). The slope in the band from 275 nm to 295 nm ($S_{275-295}$) is an optical parameter particularly sensitive to environmental changes as solar radiation or salinity (Helms *et al.* 2008; 2013; Catalá *et al.* 2013). In inlet waters, the values of $S_{275-295}$ ranged from 10 to 38 μm^{-1} (Table S1) and in the effluents of +*holothurian* and –*holothurian* tanks from 6 to 28 μm^{-1} and from 13 to 40 μm^{-1}, respectively (Tables S2 and S3). In the inlet waters, the values were similar to those reported for coastal and estuary waters, usually characterized with lower slopes (~ 15-25 μm^{-1}) than the values for the open ocean (~ 25-50 μm^{-1}) (Helms *et al.*, 2008; 2013 Catalá *et al.*, 2015). Like the a_{325} values, $S_{275-295}$ in the –*holothurian* effluent waters (red triangles in Fig.1b) showed consistently higher values than the inlet waters (white circles in Fig.1b) and the +*holothurian* effluents (black squares in Fig.1b). In the inlet waters, the spectral slope ratios (S_R) ranged from 0.6 to 2.6 (Table S1) and in the effluents of +*holothurian* and –*holothurian* tanks from 0.5 to 3.1 and from 0.4 to 3.9, respectively (Tables S2 and S3). The S_R values in –*holothurian* effluents (red triangles in Fig. 1c) showed consistently higher values than the inlet waters (white circles in Fig. 1c) and +*holothurian* effluents (black squares in Fig. 1c). In inlet waters, the molar absorption coefficients at 325 (a^*_{325}) ranged from 0.20 to 5.29 m^{-1}mg C l^{-1} (Table S1) and in the effluents of +*holothurian* and –*holothurian* tanks from 0.23 to 7.08 m^{-1} mg C $l^{-1}$and from 0.96 to 12.71 m^{-1}mg C l^{-1}, respectively (Tables S2 and S3). The a^*_{325} values in the inlet waters (white circles in

Fig. 1d) and in the effluents of +*holothurian* (black squares in Fig. 1d) were lower than the values in the effluents of –*holothurian* (red triangles in Fig. 1d).

To determine if the presence of holothurians, pooling all the data, modifies significantly DOM absorption properties we performed paired t-test or Wilcoxon matched pair tests (Table 1). In the Fig. 2 we pooled all the seasonal data in median values, 25-75 % percentiles and non-outliers values (Fig. 2). The a_{325} values in the –*holothurian* tank and effluents (Fig. 2a, red boxes) were significantly higher that the values in the +*holothurian* tank and effluents and the inlet waters (Table 1). A similar effect was found for the spectral slope (S_{275-29}) (Fig. 2b, red boxes), the spectral ratios (S_R) (Fig. 2c, red boxes) and the molar absorption coefficients (a^*_{325}) (Fig. 2d, red boxes). Indeed, we observed higher CDOM concentration, but with smaller molecular size (higher spectral slopes), in the tank without holothurians than in the tank with holothurians. On the other hand, the differences between the inlet waters and the +*holothurian* tank and effluent waters, although significant, were less relevant (Fig. 2, Table 1). Therefore, holothurians appear to reduce significantly CDOM concentration, particularly of compounds with comparatively higher molecular size.

These results suggest that the monoculture of *A. sulcata* increases CDOM in comparison with the inlet waters. The higher CDOM values in the –*holothurian* tank than in +*holothurian* tank can be related to: (1) a higher abundance of bacteria and their metabolic by-products or (2) a higher concentration of particulate organic matter (derived from uneaten food and microbial cells) with disaggregation in dissolved compounds. In both cases, an increment in CDOM concentration is expected. Several studies have shown that bacteria and phytoplankton can produce CDOM as metabolic by-products (Nelson *et al.*, 1998, 2004; Ortega-Retuerta *et al.* 2009; Romera-Castillo *et al.* 2010; Catalá *et al.* 2015; 2016). However, we did not find significant correlations between CDOM optical parameters and the concentration of chlorophyll-*a* or the abundance of bacteria (Fig. 3a). Therefore, phytoplankton and bacterial carbon processing appear to have minor importance in the study tanks. In contrast, we found significant and positive relationships between the concentration of POM and the absorption coefficients a_{325} in the inlet waters ($r^2 = 0.33$, $p = 0.004$; regression line $a_{325} = 0.21 + 0.141$ POM), in the *+holothurian* treatment ($r^2 = 0.41$, $p=0.002$; regression line $a_{325} = 0.20 + 0.079$ POM) and –*holothurian* treatment ($r^2=0.20$, $p=0.006$; regression line $a_{325} = 0.42 + 0.102$ POM) (Fig. 3b). Therefore, POM concentration in the tanks appears to be the main driver of CDOM dynamics. POM disaggregation into dissolved components is a common process in coastal waters (He *et al.* 2016), particularly under sunny conditions (Shank *et al.*, 2011; Pisani, *et al.* 2011). Holothurians consume several components of POM as phytoplankton cells, bacteria, uneaten food, animal feces, and transparent exopolymer particles (Hudson *et al.*, 2005; Slater *et al.*, 2009; Navarro *et al.*, 2013; Yokoyama, 2013; Wotton, 2011). Since holothurians reduce POM concentration in *+holothurian* tank by consumption (Sadeghi-Nassaj *et al.* in review), we think that the potential for POM disaggregation into DOM in this tank was significantly lower than in the –*holothurian* tank.

Overall, we found that the presence of holothurians, an extractive species, in aquaculture tanks reduces the concentration of CDOM and, consequently, improves water transparency in the tanks. Indeed, CDOM optical properties in the tank with holothurians were quite similar to the inlet waters both quantitatively (absorption coefficients) and qualitatively (spectral slopes and ratios). Therefore, the presence of extractive species in offshore aquaculture installations could effectively increase water transparency by reducing light absorption and scattering (Ibarra *et al.* 2012; Del Bel Belluz *et al.* 2016). Monitoring CDOM optical properties is an easy and inexpensive procedure; which is very sensitive to the changes caused by the extractive species in multitrophic aquaculture. Therefore, the use of CDOM probes, for long-term monitoring, or by remote sensing for large spatial scales, is a promising research area for the development of sustainable aquaculture.

All data for CDOM optical parameters and ancillary variables are in supplementary material (Table S1, S2, S3, S4, and S5).

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Figure captions

Figure 1 Seasonal dynamics of the optical parameters of chromophoric dissolved organic matter. Values of (a) absorption coefficients at 325 nm (a_{325}), (b) spectral slopes from 275 to 295 nm ($S_{275-295}$), (c) spectral slope ratios (S_R), and (d) molar absorption coefficients at 325 nm (a^*_{325}) in the inlet waters (white dots), in the effluent of the +*holothurian* tank (black squares) and in the effluent of the –*holothurian* tank (red triangles).

Figure 2 Median (line), the 25-75% percentile (box), and the non-outliers range (whisker) of the optical parameters of chromophoric dissolved organic matter. Values of (a) absorption coefficients at 325 nm (a_{325}), (b) spectral slopes from 275 to 295 nm ($S_{275-295}$), (c) spectral slope ratios (S_R), and (d) molar absorption coefficients at 325 nm (a^*_{325}) in the inlet water (white box), in the +*holothu*rian effluent and tank (black boxes) and in the –*holothurian* effluent and tank (red boxes).

Figure 3 Scatterplots of (a) absorption coefficients at 325 nm (a_{325}) *vs.* bacterial abundance and (b) absorption coefficients at 325 nm (a_{325}) *vs.* particulate organic matter. White dots are values for inlet waters, black squares are values for +*holothurian* (effluent and tank) waters red triangles are –*holothurian* (effluent and tank) waters. Correlation lines are shown when are significant for $p < 0.05$.

Figure 1

Seasonal dynamics of the optical parameters of chromophoric dissolved organic matter.

Figure 1 Seasonal dynamics of the optical parameters of chromophoric dissolved organic matter. Values of (a) absorption coefficients at 325 nm (a_{325}), (b) spectral slopes from 275 to 295 nm ($S_{275-295}$), (c) spectral slope ratios (SR), and (d) molar absorption coefficients at 325 nm (a^*_{325}) in the inlet waters (white dots), in the effluent of the +holothurian tank (black squares) and in the effluent of the –holothurian tank (red triangles).

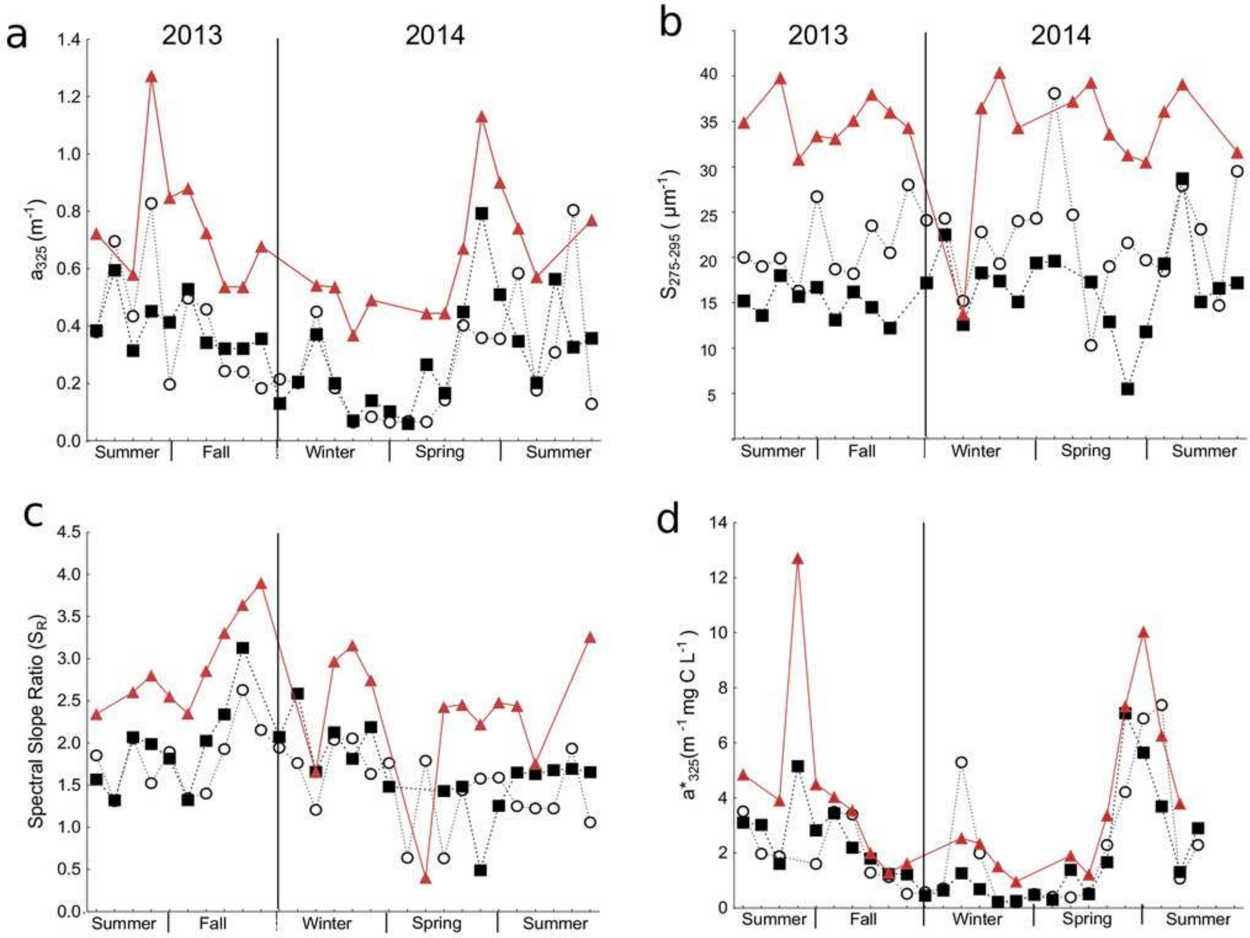
a
2013
2014
a_{325} (m^{-1})
Summer
Fall
Winter
Spring
Summer
b
2013
2014
$S_{275-295}$ (μm^{-1})
Summer
Fall
Winter
Spring
Summer
c
Spectral Slope Ratio (S_R)
Summer
Fall
Winter
Spring
Summer
d
a*$_{325}$(m^{-1} mg C L^{-1})
Summer
Fall
Winter
Spring
Summer

Figure 1

Figure 2

Median (line), the 25-75% percentile (box), and the non-outliers range (whisker) of the optical parameters of chromophoric dissolved organic matter.

Figure 2 Median (line), the 25-75% percentile (box), and the non-outliers range (whisker) of the optical parameters of chromophoric dissolved organic matter. Values of (a) absorption coefficients at 325 nm (a_{325}), (b) spectral slopes from 275 to 295 nm ($S_{275-295}$), (c) spectral slope ratios (S_R), and (d) molar absorption coefficients at 325 nm (a^*_{325}) in the inlet water (white box), in the +*holothu*rian effluent and tank (black boxes) and in the –*holothurian* effluent and tank (red boxes).

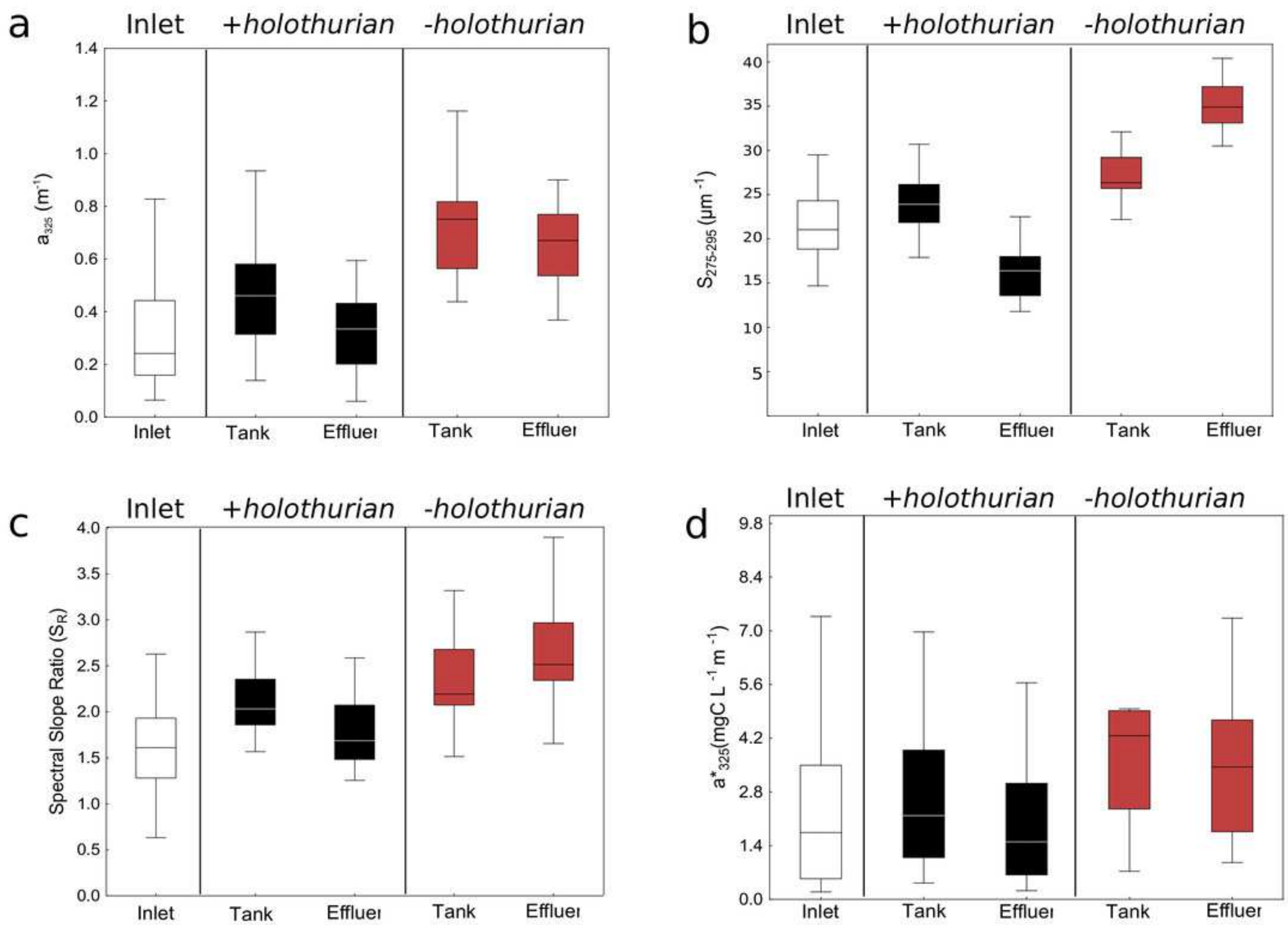
a
Inlet
+holothurian
-holothurian
a_{325} (m^{-1})
Inlet
Tank
Effluer
Tank
Effluer
b
$S_{275-295}$ (μm^{-1})
c
Spectral Slope Ratio (S_R)
d
a^*_{325}(mgC L^{-1} m^{-1})

Figure 2

Figure 3

Scatterplots of (a) absorption coefficients at 325 nm (a_{325}) *vs.* bacterial abundance and (b) absorption coefficients at 325 nm (a_{325}) *vs.* particulate organic matter.

Figure 3 Scatterplots of (a) absorption coefficients at 325 nm (a_{325}) *vs.* bacterial abundance and (b) absorption coefficients at 325 nm (a_{325}) *vs.* particulate organic matter. White dots are values for inlet waters, black squares are values for +*holothurian* waters (effluent and tank) red triangles are –*holothurian* waters (effluent and tank). Correlation lines are shown when are significant for $p < 0.05$.

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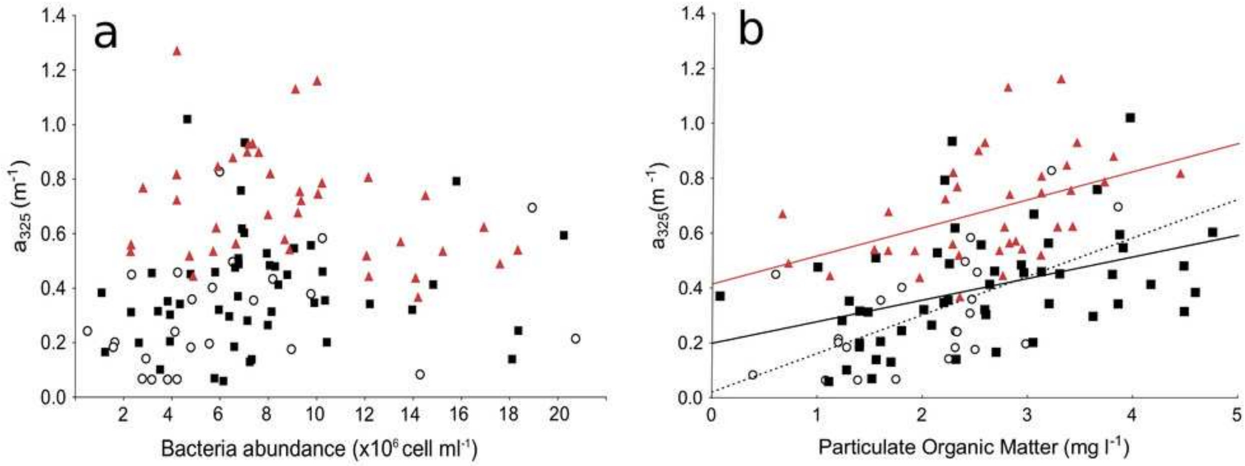
a
b
a_{325} (m^{-1})
Bacteria abundance (x10^6 cell ml^{-1})
Particulate Organic Matter (mg l^{-1})

Figure 3

Table 1 (on next page)

Results of paired t-test and Wilcoxon matched pairs test between the inlet waters and the tanks and their effluents for the CDOM optical properties

Table 1 Results of paired t-test (for normally distributed variables) and Wilcoxon matched pairs test (for not normally distributed variables) between the inlet waters and the tanks and their effluents for the CDOM optical properties considered in this study.

Table 1 Results of paired t-test (for normally distributed variables) and Wilcoxon matched pairs test (for not normally distributed variables) between the inlet waters and the tanks and their effluents for the CDOM optical properties considered in this study.
Bold means that there is a significant difference.

	Statistical Analysis	*t* or *z*	*p*- value	Statistical Analysis	*t* or *z*	*p*- value
Inlet vs. +*holothurian* tank				**Inlet vs. +*holothurian* effluent**		
a_{325}	Paired t-test	5.76	**0.0000**	Paired t-test	19.67	**0.0000**
$S_{275\text{-}295}$	Paired t-test	2.46	**0.0206**	Paired t-test	4.86	**0.0000**
S_R	Paired t-test	15.05	**0.0000**	Wilcoxon	2.35	**0.0188**
a^*_{325}	Paired t-test	3.34	**0.0026**	Paired t-test	6.77	**0.0000**
Inlet vs. -*holothurian* tank				**Inlet vs. –*holothurian* effluent**		
a_{325}	Paired t-test	25.36	**0.0000**	Paired t-test	24.91	**0.0000**
$S_{275\text{-}295}$	Wilcoxon	3.39	**0.0006**	Wilcoxon	3.98	**0.0000**
S_R	Paired t-test	6.07	**0.0000**	Paired t-test	6.18	**0.0000**
a^*_{325}	Paired t-test	4.68	**0.0001**	Paired t-test	3.74	**0.0013**
+*holothurian* vs. –*holothurian* tanks				**+*holothurian* vs. –*holothurian* effluents**		
a_{325}	Paired t-test	40.06	**0.0000**	Paired t-test	11.45	**0.0000**
$S_{275\text{-}295}$	Paired t-test	2.97	**0.0029**	Wilcoxon	3.82	**0.0001**
S_R	Paired t-test	15.54	**0.0000**	Wilcoxon test	3.78	**0.0001**
a^*_{325}	Paired t-test	4.90	**0.0001**	Paired t-test	6.07	**0.0000**

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