Lat. Am. J. Aquat. Res., 41(2): 296-304, 2013
"Proceedings of the 3rd Brazilian Congress of Marine Biology"
A.C. Marques, L.V.C. Lotufo, P.C. Paiva, P.T.C. Chaves & S.N. Leitão (Guest Editors)
DOI: 10.3856/vol41-issue2-fulltext-8

Research Article

Effects of the secondary metabolites from *Canistrocarpus cervicornis* (Dictyotales, Phaeophyceae) on fertilization and early development of the sea urchin *Lytechinus variegatus*

Fredy A. Ortiz-Ramírez[1], Magui Aparecida-Vallim[1]
Diana Negrão-Cavalcanti[1] & Valéria Laneuville-Teixeira[1]
[1]Programa de Pós-Graduação em Biologia Marinha, Departamento de Biologia Marinha
Instituto de Biologia, Universidade Federal Fluminense
CP 100.644, Niterói, RJ, 24001-970, Brazil

ABSTRACT. Marine organisms are rich sources of natural products that, among other activities, help to maintain the species equilibrium. Samples of the marine brown alga *Canistrocarpus cervicornis* (Kützing) De Paula & De Clerck were collected in Búzios, Rio de Janeiro, Brazil, in June 2006. The extract was obtained in CH_2Cl_2 and subjected to fractionation by chromatographic methods in order to isolate and purify the compound (4R, 7R, 14S)-4α, 7α-diacethoxy-14-hydroxydolastane-1(15), 8-dien. Then, the effects of the extract and the dolastane diterpene on zygotes and gametes of the sea urchin *Lytechinus variegatus* were evaluated. The exposure of male and female gametes to the *C. cervicornis* extract promoted, respectively, a reduction of 10-30% in fertilization and a 20 to 70% decrease in the number of eggs. Furthermore, the exposure of zygotes to the extract inhibited their development up to 86.7 ± 1.6% (at a concentration of 250 μg mL^{-1}), as well as generating abnormalities in 39-50% of zygotes. The results of the dolastane diterpene showed no evidence of inhibition in the zygotes' development, thought it was proved to induce anomalies. At higher concentrations (25 and 50 μg mL^{-1}), it was possible to observe cell lyses.

Keywords: natural products, diterpenes, brown seaweed, dolastanes, *Lytechinus variegatus*, Brazil.

Efectos de los metabolitos secundarios de *Canistrocarpus cervicornis* (Dictyotales, Phaeophyceae) sobre la fertilización y desarrollo embrionario temprano del erizo del mar *Lytechinus variegatus*

RESUMEN. Los organismos marinos son fuentes ricas en productos naturales que, entre otras actividades, ayudan a mantener el equilibrio entre las especies. Se colectaron muestras de *Canistrocarpus cervicornis* (Kützing) De Paula & De Clerck en Armação de Buzios, Estado de Rio de Janeiro, Brasil, durante junio de 2006 y se sometieron a extracción en CH_2Cl_2 hasta obtener un extracto bruto. Mediante técnicas tradicionales de fitoquímica se aisló y purificó el producto natural mayoritario dolastano (4R, 7R, 14S)-4α, 7α-diacetoxi-14-hidroxidolasta-1(15),8-dieno. Posteriormente, se evaluaron los efectos en diferentes concentraciones del extracto bruto en gametos y del extracto bruto y dolastano en cigotos del erizo de mar *Lytechinus variegatus*. Los resultados sugieren que cuando los gametos son expuestos al extracto bruto se promueve una disminución en las tasas de fecundación, 10 a 30% (espermatozoides) y de 20 a 70% (óvulos). Además, cuando los cigotos fueron expuestos al extracto bruto se inhibió el desarrollo en un 86,7% ± 1,6 (250 μg mL^{-1}) y se generaron cigotos anormales que variaron entre 39 y 50%. Los resultados de los experimentos con el dolastano no mostraron evidencias de inhibición del desarrollo embrionario. Sin embargo, se constató la inducción de anomalías y en las mayores concentraciones (25 y 50 μg mL^{-1}) se registró el evento de lisis en los cigotos.

Palabras clave: productos naturales, dolastanos, algas pardas, diterpenos, *Lytechinus variegatus*, Brasil.

Corresponding author: Fredy A. Ortiz-Ramírez (faortizr@gmail.com)

INTRODUCTION

In the course of evolution, marine organisms have developed different strategies to ensure their survival. As a consequence, many of these species are rich sources of natural products that can help keep the balance among species (Harper *et al.*, 2001). Such substances have also biotechnological potential as antibiotic, antiviral, cytotoxic and antitumor (Blunt *et al.*, 2010).

The brown algae (Phaeophyceae, Ochrophyte) are very abundant in the tropical region, especially the Fucales and the Dictyotales. More than 1,140 secondary metabolites were reported for them. Among these, there are rich sources of terpenes, especially diterpenes, belonging to the order Dictyotales (Maschek & Baker, 2008), and around 300 diterpene shave been isolated from at least 35 species of the family Dictyotaceae collected worldwide (Vallim *et al.*, 2005). These algae are chemically characterized by the production of dolastane and secodolastane diterpenes which are obtained from the cyclization the precursor geranilgeraniol between positions 1 and 11 (Teixeira *et al.*, 1986a, 1986b; Teixeira & Kelecom, 1988; De Paula, 2001; Vallim *et al.*, 2005; De Paula *et al.*, 2007).

Canistrocarpus cervicornis is known for exhibiting a chemical profile composed by dolastane and the secodolastane diterpenes (Teixeira *et al.*, 1986a, 1986b; Teixeira & Kelecom, 1988; Oliveira *et al.*, 2008). The studies revealed that some of these secondary metabolites inhibit the sodium potassium ATPase (Garcia *et al.*, 2009), as well as present antiviral (Vallim *et al.*, 2010), antifouling (Bianco *et al.*, 2009), and anti-herbivore (Pereira *et al.*, 2002; Bianco *et al.*, 2010) activities.

Interdisciplinary studies have shown that the algae are not passive participants in the biological interactions between producers and consumers. Given the development of new methods of chemical analysis and adequate experimental drawings, it is widely accepted that algae have chemical defense mechanisms against their consumers and also against threats caused by their intra-specific relations, which makes them important agents in the structure of marine communities (Potin *et al.*, 2002). Thus, the capacity of chemical defense of some algae (*Coilodesme californica* (Ruprecht) Kjellman, *Dictyota flabellata* (F.S. Collins) Setchell & N.L. Gardner, *Laurencia obtusa* (Hudson) J.V. Lamouroux, etc.) against herbivores has been shown (Steinberg, 1985; Hay & Fenical, 1988; Pereira *et al.*, 2003).

In fact, the ecological relations mediated by natural products from macro algae have been widely studied. Therefore, numerous types of diterpenes were pointed out as chemical defenses capable to inhibit the herbivory (Pereira *et al.*, 2004; Vallim *et al.*, 2007; Pereira & Da Gama, 2008; Bianco *et al.*, 2010). Also, diterpene alcohols found in algae from the genus *Dictyota* reduce the growth of herbivorous fishes (Hay *et al.*, 1988), as well as decreasing the survival, growth or reproduction of many species of amphipod (Cruz-Rivera & Hay, 2003). However, the consequences of their effects on the mechanisms of fecundation and embryonic development to population control of herbivores have been little studied.

In this context, *C. cervicornis* has also shown strategies of chemical defense against the gastropod *Astraea latispina* (Pereira *et al.*, 2002) and the sea urchin *Lytechinus variegatus* (Bianco *et al.*, 2010). However, ecological studies relating the chemical components of *C. cervicornis* and its effects on gametes and early life stages of *L. variegates* are not yet known.

The aim of the present work was, thus, to evaluate the effect of the crude extract in dichloromethane (CH_2Cl_2) from *C. cervicornis* and its major diterpene (4R, 7R, 14S)-4α, 7α-diacethoxy-14-hydroxydolastane-1(15), 8-dien on the gametes and early life stages *in vitro* of the sea urchin *L. variegatus*.

MATERIALS AND METHODS

Algal material

Canistrocarpus cervicornis was collected in June 2006 at Praia do Forno, Armação de Búzios, Rio de Janeiro State (22°45'42"S, 41°52'27"W) by snorkeling. Intact samples of alga were cleaned from epiphytic organisms and washed with sea water. The specimens were deposited at the Herbarium of the Universidade do Rio de Janeiro (HRJ10754).

Extraction and isolation

The air-dried algae were extracted five times with 100% CH_2Cl_2 at room temperature (25°C) for 24 h. The solvent was evaporated under reduced pressure, yielding a brownish residue (14 g). Crude extract (5 g) was subjected to silica gel chromatography (4 x 40 cm) and eluted with a gradient of n-hexane to methanol in order to obtain 228 fractions. Fractions F_{97} to F_{99} (771 mg) were combined and subjected to silica gel column chromatography and eluted with n-hexane and ethyl acetate (4:6), affording the pure compound (4R, 7R, 14S)-4α, 7α-diacethoxy-14-hydroxydolastane-1(15), 8-dien (Fig. 1). The diterpene was identified by comparison of physical and spectroscopic data (^{13}C and ^{1}H NMR data) with reported values (Sun *et al.*, 1981).

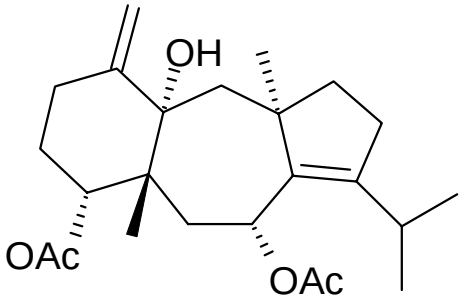

Figure 1. Structure of the major dolastane diterpene of *Canistrocarpus cervicornis*.

All solvents were HPLC grade. Analytical thin-layer chromatography (TLC) separations were carried out on Merck silica gel 60 F-254 (0.2 mm) percolated aluminum plates. Once developed, plates were visualized by spraying 2% ceric sulphate in sulfuric acid, followed by gentle heating. Silica gel 60 (Merck, 70-230 and 230-400 mesh) was used for column chromatography. NMR spectra were recorded in CDCl3 (100% Aldrich) on a Varian Unity Plus 300 spectrometer using TMS as internal standard.

Collection of gametes and storage

Lytechinus variegatus were collected in Itaipú Beach, Niterói, Rio de Janeiro, Brasil (23°00'34"S, 44°26'10"W) by snorkeling. Eggs and sperm had been obtained by previous spawning induction with injections of 1-3 mL KCl 0.5M. Eggs stocks (16,000 eggs mL^{-1}) were prepared in artificial seawater Sea Red® (ASW) and the salinity was adjusted to 32 psu adding ultra-pure water (Milli-Q®). Sperm were obtained by direct extraction with a pipette Pasteur and stock solutions (1:9 ASW) were preserved at refrigeration in Eppendorf® (2-5ºC).

Crude extract and dolastane solutions

Stock solution of the crude extract (500 µg mL^{-1} in DMSO) were diluted at 10 mL ASW and it was utilized for preparing sequential dilutions of 250, 125, 62.5 and 31.25 µg mL. Stock solution of the dolastane (100 µg mL^{-1} in DMSO) was diluted at 10 mL ASW and it was utilized for preparing sequential dilutions of 50, 25, 12.5 and 6.25 µg mL^{-1}.

Lytechinus variegatus embryonic development

One ml of stock solution of motile sperm were added to the egg suspension and carefully stirred for 30s to allow fertilization. Afterwards, fertilization was confirmed by observation of the fertilization envelope in at least 80% of the eggs after 5 min. 1 mL of fertilized eggs were deposited in plates of 3 mL in triplicate for both treatment and control. Then, we added 1 mL of the solutions of crude extract and dolastane isolated from *C. cervicornis* to treatment, whereas in control we used 1 mL of ASW. Eggs were incubated at a final volume of 2 mL a 25 ± 2°C. After two hours, we added 2 drops of 10% formaldehyde. This time was sufficient for zygotes reach to cleavage IV. Finally, the unfertilized eggs, fertilized not divided, eggs cleaved in I, II, III, IV stages and abnormal zygotes (A) were counted from aliquots of 1 mL of each sample in camera Sedgwick-Rafter, on 10 random points. This method was adapted from tests used in cytotoxicity, ecotoxicity, and preliminary studies of pharmacological activity (Lera *et al.*, 2006; Semenova *et al.*, 2006; Kiselyov *et al.*, 2010; Magalhães *et al.*, 2010).

Eggs pre-fertilization tests

1 mL of eggs and 1 mL of each dilution of the extract of *C. cervicornis* were deposited in plaques of 3 mL capacity. After 10 min, a solution with 20 µL of sperm was added and maintained at mild agitation for 30 sec. Each experiment was performed in triplicate and 1 mL of ASW was added to control. The incubation period, the fixation and counting of zygotes followed the same procedure previously described.

Sperm pre-fertilization tests

Twenty µL of sperm solution and 1 mL of each dilution of the extract of *C. cervicornis* were deposited in plaques of 3 mL capacity. After 10 min, a solution with 1 mL of eggs was added and maintained at mild agitation for 30 sec following the same procedure previously described.

Statistical analysis

The results of the experiments with crude extract and dolastane were analyzed by nonparametric Kruskal-Wallis. In the pre-fertilization tests, multiple comparisons were performed using the statistical analysis Infostat 2009 (Di Rienzo *et al.*, 2009), and then analyzed by Wilcoxon (Mann-Whitney U) when significant differences were recorded. All statistical tests were conducted according to Zar (1996), adopting the significance level $\alpha < 0.05$.

RESULTS

Lytechinus variegatus embryonic development

The embryonic development experiments started after that the elevation of the fertilization membrane was confirmed in at least 80% of the eggs (Fig. 2b). The unfertilized eggs, the ones fertilized without cleavage, the zygotes in cleavages I-IV and the anomalous (A)

were evaluated in each experiment and typical examples of these categories can be found in Fig. 2.

The results demonstrated that, at all concentrations, the number of fertilized eggs not cleaved increased in proportion to the concentration of the crude extract. Besides, most of the few eggs that reached cleavage generated anomalous, which resulted in impracticability of the development up to cleavage IV. Indeed, cleavages III and IV presented significant differences ($P < 0.0001$) when compared to control, since we observed merely between zero and 1% of individuals on cleavage III and, consequently, no eggs on cleavage IV. The effect of the addition of the crude extract at the smallest concentration caused an increase in the percentage of anomalous, whereas the greatest concentrations caused a very strong inhibition of cleavages, even though it was not accompanied by a rise in the percentage of anomalous. The results of the tests with the dolastane revealed that there were no significant differences ($P > 0.05$) between treatment and control, except for the variables III, IV and A. The differences found in cleavages III and IV correspond to the greatest concentrations (25 y 50 µg mL^{-1}), which the value of proportions was overestimated due to the lyses of zygotes. Therefore, the proportion of 9.85 ± 8.8% was registered in cleavage IV for the concentration of 25 µg mL^{-1} (dolastane), while a 0.8 ± 1.4% rate was registered for the concentration of 50 µg mL^{-1} (dolastane).

The results of anomalous zygotes were established differently for the crude extract and dolastane. The response caused by the crude extract was inversely proportional to the concentration; in other words, the smallest concentration caused the highest anomaly rates. On the other hand, the response caused by different dilutions of dolastane did not have significant difference in values of anomalous zygotes ($P > 0.05$). However, at all dilutions tested, the presence of anomalous was found with significant difference compared to the control. This indicates that this secondary metabolite can be responsible for the anomalies observed in the experiments with crude

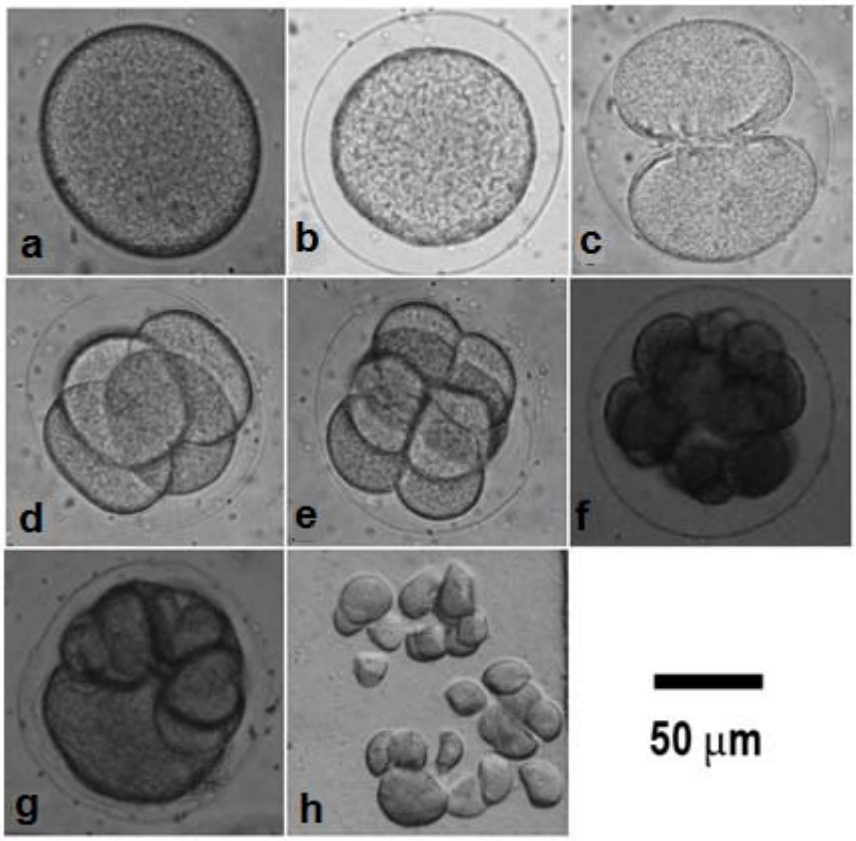

Figure 2. Early stages of the embryonic development of *Lytechinus variegatus* and events registered during experiments. a) Unfertilized egg, b) egg fertilized without cleavage, c) first cleavage, two blastomeres, d) second cleavage, four blastomeres, e) third cleavage, eight blastomeres, f) fourth cleavage, 16 blastomeres, g) abnormal zygotes, and h) lyses.

extract, especially in the smallest concentrations of the extract (3.125-12.5 µg mL^{-1}) (Table 1).

Pre-fertilization tests

In our experiments, the male gametes showed less resistance to the compounds of *C. cervicornis* than the female at the smallest concentration (15.62 µg mL^{-1}). The increase of the concentration of the crude extract in the treatment of gametes before fecundation promoted a rise in the number of unfertilized eggs. Indeed, the sperm were susceptible to the exposure to crude extract, since the percentage of unfertilized eggs was superior to 70% in all concentrations tested. No significant difference was noticed among these concentrations ($P > 0.05$). On the other hand, when the feminine gametes were submitted to different concentrations of crude extract, the rates of unfertilized ova was considerably higher than in control, ranging between 30 and 86%, except for the smallest concentration (15.62 µg mL^{-1}), in which this percentage was similar to control (Table 2).

In all experiments, both pre-fertilization and zygote, our results showed that both crude extract and dolastane induced the appearance of anomalous zygotes, characterized by asymmetric plans of cleavage and atypical proliferations even at low concentrations (Fig. 2g and Fig. 3).

DISCUSSION

Our results indicate that the gametes have become unviable for the process of syngamy during the pre-fertilization experiments, reducing the fertilization rates of 10 to 30% in the tests with sperm and of 20 to 70% in the tests with eggs. Indeed, some of the substances extracted from marine algae have shown to affect fertilization. The fucoidans, for instance, inhibit the penetration of the male gametes into the human eggs (Oehninger *et al.*, 1991; Patankar *et al.*, 1993). Nonetheless, the octadecapentanoic acid, a polyunsaturated fatty acid uncommon in algae, has not demonstrated any capacity of derailing the gametes of the sea urchin *Paracentrotus lividus* in the fertilization processes, yet it has proved to inhibit the embryonic development in the first cleavage (Fériel *et al.*, 2000).

In our experiments, the male gametes showed less resistance to the compounds of *C. cervicornis* than the female. It is relevant to notice that the sperm of the sea urchin are motionless when inside the gonads, only when they get in contact with saline is the motion activated as the intracellular pH is alkalinized and the ATPase, enzyme that promotes the mobility of zygotes in response to the mitochondrial respiration, is activated. Taking this into consideration, it is possible to explain the impossibility of the gametes to realize the fertilization processes since two dolastanes

Table 1. Percentage of zygotes according to development stages in the experiments with raw extract (CE) and dolastane (D): EWC: eggs without cleavage, I – IV: cleavage, and A: abnormal.

CE (µg mL^{-1})	EWC (%)	I (%)	II (%)	III (%)	IV (%)	A (%)
250.0	86.7 ± 5.1	1.0 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	12.3 ± 5.8
125.0	59.7 ± 4.8	0.47 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	39.8 ± 4.3
62.5	52.3 ± 7.7	1.7 ± 0.6	1.3 ± 1.2	0.4 ± 0.6	0.0 ± 0.0	44.3 ± 7.7
31.3	46.9 ± 6.2	0.3 ± 0.5	0.7 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	52.1 ± 6.1
15.62	44.9 ± 2.6	1.3 ± 0.4	2.1 ± 1.1	0.6 ± 1.1	0.0 v 0.0	51.0 ± 4.1
Control	27.9 ± 11.1	0.8 ± 1.4	2.1 ± 2.0	10.3 ± 6.4	59.8 ± 5.9	0.0 ± 0.0
D	EWC	I	II	III	IV	A
50	6.0 ± 3.5	2.6 ± 2.9	5.8 ± 1.7	75.6 v 12.6*	0.8 ± 1.4*	9.1 ± 7.8
25	4.7 ± 4.3	0.0 ± 0.0	0.5 ± 1.0	71.4 ± 7.9*	9.8 ± 8.8*	12.9 ± 1.5
12.5	6.2 ± 2.2	0.0 ± 0.0	1.8 ± 3.1	32.2 ± 12.7	38.7 ± 6.5	21.0 ± 9.1
6.25	2.1 ± 1.9	0.0 ± 0.0	0.41 ± 0.72	23.4 ± 3.3	61.8 ± 6.7	11.4 ± 6.4
3.125	6.5 ± 5.4	0.7 ± 0.6	1.15 ± 0.18	30.1 ± 3.8	48.9 ± 7.4	12.6 ± 9.9
Control	6.5 ± 4.6	0.4 ± 0.6	3.7 ± 1.9	36.1 ± 7.4	53.2 ± 4.2	0.0 ± 0.0

*Proportion overestimated due to the lyses of zygotes.

Table 2. Percentage of unfertilized eggs in the pre-fertilization experiments with raw extract. Average ± standard deviation.

Treatment ($\mu g\ mL^{-1}$)	Experiment Sperm (%)	Ovule (%)
0	14.9 ± 9.0	29.3 ± 21.6
15.62	87.2 ± 3.7	30.3 ± 18.8
31.3	83.0 ± 8.5	57.8 ± 19.3
62.5	71.3 ± 0.6	82.4 ± 6.2
125	73.7 ± 7.8	68.9 ± 4.6
250	90.3 ± 3.6	85.9 ± 2.2

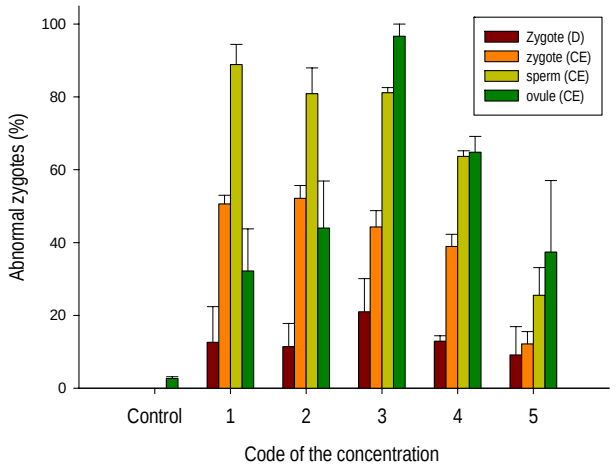

Figure 3. Percentages of abnormal zygotes in experiments with raw extract (CE) and dolastane (D): the code of the concentration 1 to 5 represent at dilutions detailed on material and methods, from less concentration to high concentration.

isolated from *C. cervicornis* inhibited Na^+K^+-ATPase (García *et al.*, 2009).

In the experiments to evaluate the effects of crude extract and dolastane on the embryonic development, the crude extract inhibited the mitotic cycles of the zygotes of *L. variegatus* even at the smallest concentration, while dolastane showed no clear evidences of inhibition. In fact, extracts in acetone from the marine algae *Botryocladia occidentalis*, *Ulva fasciata*, *Gracilaria lemaneiformis* and *Hypnea musciformis* have demonstrated the ability to inhibit the embryonic development of *L. variegatus* in more than 50% at the concentration of 100 $\mu g\ mL^{-1}$ (Torres *et al.*, 2005). Therefore, our results indicate that *C. cervicornis* presents a greater inhibitory effect than other algae, even at lower concentrations.

Given the synchronous divisions and the uniformity at the first cleavages, the tests with zygotes of sea urchins are sufficiently sensitive to identify and differentiate the toxic effects of the antimitotic compounds (Jacobs *et al.*, 1981). In addition, the presence of clear points in the cytoplasm is attributed to the blocking of the coupling of microtubules (Jacobs & Wilson, 1986). In spite of the inhibitory effects of the crude extract on the mitotic cycle, the cytoplasm of the zygotes revealed a homogeneous aspect, which presented no clear points in the cytoplasm. Therefore, the inhibitory action of crude extract can be related to a different mechanism than the blocking of the coupling of microtubules.

On the other hand, both crude extract and dolastane induced the appearance of anomalous zygotes, characterized by asymmetric plans of cleavage and atypical proliferations even at low concentrations (Fig. 2g and Fig. 3). When the gametes were exposed to the highest concentration of crude extract, the inhibition of the cleavages caused an increase in the number of fertilized eggs without cleavage (EWC, Fig. 2b), providing a deceiving impression that the anomalies were reduced. It was at the highest concentration (250 $\mu g\ mL^{-1}$), that the most significant inhibition occurred, more than 80% of the fertilized eggs did not reach cleavage and the cleaved ones became anomalous. In the tests with dolastane, as previously mentioned, it was possible to notice a similar formation of anomalous at all the concentrations tested. Nevertheless, the greatest abnormality rates were found in the experiments with crude extract (Table 1).

During their development, embryos and larvae from marine invertebrates have a more accelerated metabolism when compared to adults. Their metabolic rates might increase according an order of magnitude before the metamorphosis or settlement (Hoegh-Guldberg & Manahan, 1995). In addition, experiments *in vitro* with *L. pictus* revealed that the activity of the enzyme Na^+K^+-ATPase gradually raises in the early life stages of this organism, reaching a peak of 80% (prisma stage) (Leong & Mahanan, 1999). If the inhibition of this enzyme may affect the transportation of ions, the action of *C. cervicornis* on the activity of Na^+K^+-ATPase could provoke disorders in the electrochemical gradient compromising all the cellular processes that depend on ions.

Considering that an antimitotic agent blocks the mitosis whereas a cytotoxic agent damages the cells exposed to it, our results indicate that the crude extract of *C. cervicornis* presents both antimitotic and cytotoxic effects, although the latter was more evident to the tests with dolastane due to the registration of lyses in the highest concentrations (Table 1).

The alterations on the operation of the Na^+K^+ pump might explain the lyses of the embryos, verified when the zygotes were submitted to the highest concentration of the dolastane. As the pump Na^+K^+ is considered the main active transport system in the majority of animal cells, its inhibition causes conditions that favor the intracellular accumulation of Na^+ (Aizman *et al.*, 2001). This fact may create a hypertonic environment that leads to lyses. Still, other experiments are necessary in order to achieve a deeper comprehension of these events.

One of the main challenges in marine biology and ecology is the demographic understanding of the organisms that present planktonic and benthic stages (Pechenik, 1991) and the influence of biological interactions on the size of the population. In this sense, it has recently been discovered that chemical signs of the alga *Delisea pulcra* (Williamson *et al.*, 2000) induce the metamorphosis and the recruiting of the larvae of invertebrates. The same way, it was demonstrated that some species of *Dictyota* present chemical defenses that can facilitate their perpetuation on reefs by competition for space, showing that the early life stages of some corals, may be vulnerable to allelopathic effects (Paul *et al.*, 2011). Nevertheless, the question that remains is: are the capable of avoiding or defending themselves against the new young recruits of their consumers? However, complementary studies must be conducted to elucidate this question. Our results *in vitro* indicated, therefore, that the compounds of the crude extract of *C. cervicornis* act on the sea urchin *L. variegatus* in two ways: 1) reducing fertilization rates, and 2) inhibiting embryonic development.

ACKNOWLEDGEMENTS

FAO-R thanks CAPES for providing a PhD fellowship. DNC thanks CNPq for post-Doctoral degree. We are grateful to CNPq and FAPERJ for financial support and Productivity Fellowships to VLT and finally, we all thank to Dr. Joel Campos de Paula for his help in the identification of the marine alga material.

REFERENCES

Aizman, O., P. Ulhen, M. Lal, H. Brismar & A. Asperia. 2001. Ouabain, a steroid hormone that signals with slow calcium oscillations. Proc. Nat. Acad. Sci., 98(23): 13420-13424.

Bianco, E.M., R. Rogers, V.L. Teixeira & R.C. Pereira. 2009. Antifoulant diterpenes produced by the brown seaweed *Canistrocarpus cervicornis*. J. Appl. Phycol., 21: 341-346.

Bianco, E.M., V.L. Teixeira & R.C. Pereira. 2010. Chemical defenses of the tropical marine seaweed *Canistrocarpus cervicornis* against herbivory by sea urchin. Braz. J. Oceanogr., 58: 213-218.

Blunt, J.W., B.R. Copp, M.H.G. Munro, P.T. Northcote & M.R. Prinsep. 2010. Marine natural products. Nat. Prod. Rep., 27: 165-237.

Cruz-Rivera, E. & M.E. Hay. 2003. Prey nutritional quality interacts with chemical defenses to affect consumer feeding and fitness. Ecol. Monogr., 73: 483-506.

De Paula, J.C., A.G. Pedrini, M.D. Pinheiro, R.C. Pereira & V.L. Teixeira. 2001. Chemical similarity between the brown alga *Dictyota cervicornis* and *D. pardalis* (Dictyotales, Phaeophyta). Biochem. Syst. Ecol., 29: 425-427.

De Paula, J.C., V. Cassano, Y. Yoneshigue-Valentin & V.L. Teixeira. 2007. Diterpenes from Brazilian brown alga *Dictyota crispata* (Dictyotaceae, Phaeophyta). Nat. Prod. Commun., 2: 135-137.

Di Rienzo, J.A., F. Casanoves, M.G. Balzarini, L. Gonzalez, M. Tablada & C.W. Robledo. 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Fériel, S., D. Pesando, G. Bodennec, A. El Abed & J.-P. Girard. 2000. Toxic effects of *Gymnodinium cf. mikimotoi* unsaturated fatty acids to gametes and embryos of the sea urchin *Paracentrotus lividus*. Wat. Res., 34(2): 550-556.

Garcia, D.G., E.M. Bianco, M.C. Santos, R.C. Pereira, M.V. Faria & V.L. Teixeira. 2009. Inhibition of mammal $Na^+ + K^+$ -ATPase by diterpenes extracted from the Brazilian brown alga *Dictyota cervicornis*. Phytother. Res., 23: 943-947.

Harper, M.K., T.S. Bugni, B.R. Copp, R.D. James, B.S. Lindsay, A.D. Richardson, P.C. Schnabel, D. Tasdemir, R.M. Van Wagoner, S.M. Verbitski & C.M. Ireland. 2001. Introduction to the chemical ecology of marine natural products. In: J.B. McClintock & B.J. Baker (eds.). Marine chemical ecology. CRC Press, Boca Raton, pp. 3-70.

Hay, M.E & W. Fenical. 1988. Marine plant-herbivore interactions: the ecology of chemical defense. Ann. Rev. Ecol. Syst., 19: 111-145.

Hoegh-Guldberg, O. & D.T. Manahan. 1995. Coulometric measurement of oxygen consumption during development of marine invertebrate embryos and larvae. J. Exp. Biol., 198: 19-30.

Jacobs, R.S. & L. Wilson. 1986. Fertilized sea urchin egg as a model for detecting cell division inhibitors. In: A. Aszalor & M. Dekker (eds.). Modern analysis of antibiotics. New York, pp. 481-493.

Jacobs, R., S. White & L. Wilson. 1981. Selective compound derived from marine organisms: effects on cell division in fertilized sea urchin eggs. Fed. Proc., 40: 28-31.

Kiselyov, A.S., M.N. Semenova, N.B. Chernyshova, A. Leitao, A.V. Samet, D.G. Weiss, N.N. Ikizalp, S.A. Kuznetsov & V. Semenov. 2010. Novel derivatives of 1, 3, 4-oxadiazoles are potent mitostatic agents featuring strong microtubule depolymerizing activity in the sea urchin embryo and cell culture assays. Eur. J. Med. Chem., 45: 1683-1697.

Leong, P.K. & D.T. Manahan 1999. Na^+/K^+-ATPase activity during early development and growth of an Antarctic sea urchin. J. Exp. Biol., 202: 2051-2058.

Lera, S., S. Macchia & D. Pellegrini. 2006. Standardizing the methodology of sperm cell test with *Paracentrotus lividus*. Environ. Mon. Assess., 122: 101-109.

Magalhães, H.I., P.M. Ferreira, E.S. Moura, M.R. Torres, A.P. Alves & O.D. Pessoa. 2010. *In vitro* and *in vivo* antiproliferative activity of *Calotropis procera* stem extracts. An. Acad. Bras. Cienc., 82(2): 407-416.

Maschek, J.A. & B.J. Baker. 2008. The chemistry of algal secondary metabolism. In: C.H. Amsler (ed.). Algal chemical ecology. Springer, Berlin, pp. 1-20.

Oehninger, S., G.F. Clark, A.A. Acosta & G.D. Hodgen. 1991. Nature of the inhibitory effect of complex saccharide moieties on the tight binding of human spermatozoa to the human zone pellucida. Fertil. Steril., 55: 165-169.

Oliveira, A.S., D.N. Cavalcanti, E.M. Bianco, J.C. De-Paula, R.C. Pereira, Y. Yoneshigue-Valentin & V.L. Teixeira. 2008. Chemical composition of diterpenes from the brown alga *Canistrocarpus cervicornis* (Dictyotaceae, Phaeophyceae). Nat. Prod. Commun., 9: 1468-1472.

Patankar, M.S., S. Oehninger, T. Barnett, R.L. Williams & G.F. Clark. 1993. A revised structure for fucoidan may explain some of its biological activities. J. Biol. Chem., 268: 21770-21776.

Paul, V.J., I.B. Kuffner, L.J. Walters, R. Ritson-Williams, K.S. Beach & M.A. Becerro. 2011. Chemically mediated interactions between macroalgae *Dictyota* spp. and multiple life-history stages of the coral *Porites astreoides*. Mar. Ecol. Prog. Ser., 426: 161-170.

Pechenik, J.A. 1991. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Mar. Ecol. Prog. Ser., 177: 269-297.

Pereira, R.C., A.R. Soares, V.L. Teixeira, R. Vilaça & B.A.P. Da Gama. 2004. Variation in chemical defenses against herbivory in southwestern Atlantic *Stypopodium zonale* (Phaeophyta). Bot. Mar., 47: 202-208.

Pereira, R.C. & B.A.P. da Gama. 2008. Macroalgal chemical defenses and their roles in structuring tropical marine communities. In: C.H. Amsler (ed.). Algal chemical ecology. Springer, Berlin, pp. 25-49.

Pereira, R.C., B.A.P. Da Gama, V.L. Teixeira & Y. Yoneshigue-Valentin. 2003. Ecological roles of natural products of the Brazilian red seaweed *Laurencia obtusa*. Braz. J. Biol., 63(4): 665-672.

Pereira, R.C., M.D. Pinheiro, V.L. Teixeira & B.A.P. Da Gama. 2002. Feeding preferences of the endemic gastropod *Astraea latispina* in relation to chemical defenses of Brazilian tropical seaweed. Braz. J. Biol., 62: 33-40.

Potin, P., K. Bouarab, J.P. Salaun, G. Pohnert & B. Kloareg. 2002. Biotic interactions of marine algae. Curr. Opin. Plant Biol., 5: 308-317.

Semenova, M.N., A. Kiselyov & V.V. Semenov. 2006. Sea urchin embryo as a model organism for the rapid functional screening of tubulin modulators. Biotechniques, 40(6): 765-773.

Steinberg, P.D. 1985. Feeding preferences of *Tegula funebralis* and chemical defenses of marine brown algae. Ecol. Monogr., 55(3): 333-349.

Sun, H.H., O.J. McConnell, W. Fenical, K. Hirotsu & J. Clardy. 1981. Tricyclic diterpenoids of the dolastane ring system from the marine alga *Dictyota divaricata*. Tetrahedron, 37: 1237-1242.

Teixeira, V.L. & A. Kelecom. 1988. A chemotaxonomic study of diterpenes from marine brown algae of the genus *Dictyota*. Sci. Total Environ., 75: 271-283.

Teixeira, V.L., T. Tomassini, B.G. Fleury & A. Kelecom.1986a. Dolastane and secodolastane diterpenes from the marine brown alga *Dictyota cervicornis*. J. Nat. Prod., 49: 570-575.

Teixeira, V.L., T. Tomassini & A. Kelecom. 1986b. Cervicol, a further secodolastane diterpene from the marine brown alga *Dictyota cervicornis* Kutzing Phaeophyceae, Dictyotaceae. Bull. Soc. Chim. Belg., 95: 263-268.

Torres, M.R., A.P. Sousa, E.A. da Silva, C. Pessoa, M.E. Amaral, M.O. de Moraes & L.V. Costa-Lotufo. 2005. Biological activity of aqueous and organic extracts of seaweeds from Ceará State, Brazil. Arq. Ciên. Mar., 38: 55-63.

Vallim, M.A., J.E. Barbosa, D.N. Cavalcanti, J.C. De Paula, V.A.G. Galvão da Silva, V.L. Teixeira & I.C.P. Paixão. 2010. *In vitro* antiviral activity of diterpenes isolates from de Brazilian brown alga

Canistrocarpus cervicornis. J. Med. Plant. Res., 4(22): 2379-2382.

Vallim, M.A., J.C. De Paula, R.C. Pereira & V.L. Teixeira. 2005. The diterpenes from Dictyotacean marine brown algae in the Tropical Atlantic American region. Biochem. Syst. Ecol., 33: 1-16.

Vallim, M.A., V.L. Teixeira & R.C. Pereira. 2007. Feeding-deterrent properties of diterpenes of *Dictyota mertensii* (Phaeophyceae, Dictyotales). Braz. J. Oceanogr., 55(3): 223-229.

Received: 16 May 2011; Accepted: 22 October 2012

Williamson, J.E., R. De Nys, N. Kumar, D.G. Carson & P.D. Steinberg. 2000. Induction of metamorphosis in the sea urchin *Holopneustes purpurascens* by a metabolite complex from the algal host *Delisea pulchra*. Biol. Bull., 198: 332-345.

Zar, J.H. 1996. Bioestatistical analysis. Prentice Hall, New Jersey, 907 pp.