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Lethal and Sub-Lethal Effects of the Water-Soluble Fraction of a Light Crude Oil on the Planktonic Copepod *Acartia tonsa*

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ABSTRACT

In this study the acute and sub-lethal effects caused by the Water Soluble Fraction (WSF) of a light crude oil were assessed for the first time on the planktonic copepod *Acartia tonsa*. Chromatographic analysis was also performed to quantify the levels of hydrocarbons (total, aliphatic and polyaromatic hydrocarbons) to which copepods were exposed to. Male and female individuals were exposed to hydrocarbon concentrations varying from 12 to 196 $\mu g.L^{-1}$ (Total hydrocarbons). The LC_{50} was 69.5 (24 hours) and 48.0 $\mu g.L^{-1}$ (48 hours) for females and 84.8 (24 hours) and 70.1 $\mu g.L^{-1}$ (48 hours) for males. Sub-lethal effects were also evaluated by exposing females to the equivalent of LC_{10}. Females showed significant reduction in egg and fecal pellet production, and also a delay in the eggs hatching time. Thus, the toxic effects of WSF of oil may be crucial for the specie population maintenance, possibly influencing the equilibrium of marine ecosystems.

Keywords: Acartia tonsa, acute effect, chronic effect, hydrocarbon, light crude oil, soluble fraction.

RESUMO

Efeitos Letais e Sub-Letais da Fração Solúvel de um Óleo Cru Leve no Copépode Plantônico *Acartia tonsa*

Neste estudo foram avaliados pela primeira vez os efeitos agudos e sub-letais causados pela Fração Solúvel em Água (FSA) de um óleo crú leve no copépode planctônico *Acartia tonsa*. Análises de cromatografia foram realizadas na FSA para determinar as concentrações de hidrocarbonetos (totais, alifáticos e aromáticos) a que foram submetidos os organismos. Machos e fêmeas foram expostos a concentrações que variaram entre 12 e 196 $\mu g.L^{-1}$ (hidrocarbonetos totais). As CL_{50} foram de 69,5 (24 horas) e 48,0 $\mu g.L^{-1}$ (48 horas) para fêmeas e 84,8 (24 horas) e 70,1 $\mu g.L^{-1}$ (48 horas) para machos. Efeitos sub-letais também foram avaliados expondo fêmeas por 24 horas a uma concentração equivalente a CL_{10}. As fêmeas apresentaram uma redução significante na produção de ovos e pelotas fecais, assim como um retardo na eclosão dos ovos. Desta forma, os efeitos tóxicos da FSA do óleo podem comprometer a manutenção da população da espécie no meio ambiente, podendo influenciar o equilíbrio do ecossistema.

Palavras-chave: Acartia tonsa, efeito agudo, efeito crônico, hidrocarbonetos, óleo leve, fração solúvel.

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INTRODUCTION

The fast industrial growth observed worldwide during the last decades, together with a significant growth of human populations in coastal areas, have caused serious environmental problems. Hydrocarbon-related environmental problems are of extreme concern since it is estimated that 6.44 million tons of petroleum contaminate the oceans annually. The contamination comes from many different sources such as sewage, surface drainage, oil tanker accidents, discharge and cleaning operations of oil tankers, drilling activities, refining and consumption of petroleum products (Clark, 2001).

Hydrocarbons derived from oil spills can drastically affect many marine species, causing immediate death by asphyxia and coating, as well as by direct intoxication. Problems regarding bioaccumulation and carcinogenic substance incorporation also occur, although many other effects might be caused due to sub-lethal and chronic exposure, such as alteration in motor perception and reproduction modifications (Kennish, 1997).

Although toxicity associated to the soluble hydrocarbon fractions of oil might be very relevant, due to rapid absorption by biota and potentially high levels nearby impacted areas, little attention has been given to problems caused by the effects of soluble compounds on the plankton (Kennish, 1997). A considerable number of studies about the effects of hydrocarbons concerning different species of marine invertebrates and vertebrates have been performed (Epstein *et al.*, 2000; Long & Holdway, 2002), however little is known specifically on zooplanktonic organisms, such as copepods, which are recognized as the most important primary consumers in marine ecosystems (Lenz, 2000).

Previous studies have shown that oil spills can cause acute damage to the zooplankton community, especially to organisms that occur at the surface. Oil spills can cause acute and chronic effects in holoplanktonic organisms, leading to biomass decrease and structure change at the community level (Samain *et al.*, 1980; Guzmán del Próo *et al.*,1986; Cowles & Remillard, 1983a, b). In addition, deleterious effects have also been recorded for meroplanktonic individuals (Fisher & Foss, 1993; Epstein *et al.*, 2000; Shafir *et al.*, 2003). In Brazil, despite the significant growth in oil-related activities, studies regarding the lethal and sub-lethal toxic effects on zooplanktonic invertebrates are very scarce.

Thus, the objectives of this study were: a) determine the LC_{50} (24 and 48 hours) for the copepod *Acartia tonsa* exposed to the Water Soluble Fraction (WSF) of a light crude oil; b) assess sub-lethal effects, such egg production, fecal pellet production and egg viability, on the *Acartia tonsa* due to exposure to the WSF of a light crude oil.

MATERIAL AND METHODS

Test-species

The planktonic copepod *Acartia tonsa* is a globally distributed species, inhabiting many coastal and estuarine areas of temperate regions (Cervetto *et al.*, 1995). It has a short life cycle of approximately two months and high reproductive potential, producing up to 100 eggs daily (Sedlacek & Marcus, 2005; Holste & Peck, 2006). All these characteristics make *A. tonsa* a potential species for toxicological testing (Tiselius *et al.*, 1995). This copepod species is very important in Southern Brazilian estuaries and coastal areas due to its frequency and high abundance (Montú, 1980).

The specimens of *A. tonsa* used in this study were obtained from a continuous cultivation system from the Zooplankton Laboratory at Universidade Federal do Rio Grande – FURG, Rio Grande – RS, Brazil. The copepods were originally collected from the Patos Lagoon estuary (32° 04' 47" S and 52° 13' 26" W) and cultivated in 500 L tanks, as described by Bersano (2003). In order to assure that all individuals tested were approximately at the same age at the moment of the experiments, the same batch of copepods was reared from eggs to adulthood under controlled conditions of temperature (20 °C), photoperiod (12 hours light:12 hours dark) and salinity (34). Copepods were fed once daily with a mixed diet consisting of *Thalassiosira weissflogii* (20.000 cells.mL^{-1}) and *Isochrysis galbana* (100.000 cells.mL^{-1}).

Extraction of the water soluble fraction

The crude oil Water Soluble Fraction (WSF) was obtained from an Argentinean light crude oil (HYDRA oil), supplied by Ipiranga Refinery (Rio Grande – RS, Brazil), following methodology described by Anderson *et al.* (1974) and Long and Holdway (2002). Briefly, one part of crude oil was gently added to nine parts of filtered (1 μm) natural seawater (S = 34) in a 3 L glass recipient provided with a magnetic bar, which was tightly sealed with an aluminum foil lid. The solution was then stirred for 23 hours with a maximum vortex height of 25% of the solution. This procedure was performed in a temperature controlled room (20 ± 1 °C) under darkness to avoid photodegradation. The solution was left one hour to equilibrate and, then, aqueous phase was siphoned and used as WSF stock solution (100%).

Chemical analysis

Immediately after extraction, 1 L of WSF solution was analyzed to characterize hydrocarbon contents. This solution represents the 100% exposure concentration at 0 hours. The final exposure solution (48 hours) was obtained by exposing the initial solution to the same conditions used for the experiments for 48 hours.

Both aliquots were fortified with 200 μL of surrogate standard priori to concentration using C_{18} cartridges (IST, Jones Chromatography, USA). Cartridges were dried and then eluted three times with 3 mL ethyl acetate and three more times with 3 mL *n*-hexane. After concentrated down to 1 mL, clean-up and fractionation was performed by passing the extract through a silica/alumina column (the silica and alumina were activated at 200 °C for 4 hours and then partially deactivated with 5% Milli-Q water). Elution was performed using 20 mL of hexane to yield the first fraction (which contains the aliphatic hydrocarbons), then 30 mL of hexane/dichloromethane (90:10) followed by 20 mL of hexane/ dichloromethane (50:50) (a combination which contains the polycyclic aromatic

hydrocarbons). Identification and quantification of aliphatic (F1) and polycyclic aromatic (F2) compounds was done using a Perkin Elmer Clarus 500 gas chromatographer equipped with either a flame ionization (GC/FID) or a mass spectrometers detector (GC/MS), respectively.

Quantification was performed against external patterns of aliphatic and polycyclic aromatic hydrocarbons using analytical curves of each analysis and calculation methods by internal standardization using 1-tetradecene and 1-eicosene standards for aliphatic hydrocarbons and deuterated Naphthalene-D_8, Acenaphthene-D_{10}, Phenantrene-D_{10}, Crisene-D_{12} and Perilene-D_{12} standards for polycyclic aromatic hydrocarbons. Methods recovery was evaluated using 1-hexadecene (aliphatics) and *p*-terfenil-D_{14} (aromatics) as surrogate standards, and analytical performance through the analysis of certified reference materials and analytical controls.

Acute exposure experiments

All the tests were performed in triplicate using 200 mL glass flasks for each treatment. The treatment concentrations were obtained diluting the WSF stock solution (100%) into 50, 25, 12.5 and 6.25% exposure solution. Ten females and ten males of *A. tonsa* were added separately to the replicates of these five treatments. Control treatment consisted of five flasks containing 10 copepods each, filled only with filtered (1 µm) natural seawater (S = 34). In order to reduce evaporation of volatile compounds, all flasks were sealed with aluminum foil and maintained without aeration for up to 48 hours in a temperature and light controlled room (T = 20 °C; 12 hours Light:12 hours Dark). Food was not provided during the experiment. To assess the LC_{50}, the number of dead individuals was counted after 24 and also 48 hours. The effects of the WSF on males and females were evaluated separately.

Sub-lethal exposure experiments

The LC_{10} (concentration that caused a mortality of 10% of tested individuals) was chosen for testing sub-lethal effects of the WSF solution on females of *A. tonsa* (Dyer, 2001). Egg production, fecal pellet production and egg viability were evaluated after 24 hours of initial exposure. The LC_{10} used here represented 7.9% of the WSF stock solution, which was prepared at the same way as described above.

The experiment was carried out by transferring hundreds of *A. tonsa* individuals of approximately same age to a 50 L glass aquarium filled with a solution of diluted down to 7.9% WSF (the equivalent to LC_{10}). Copepods were exposed to the LC_{10} for 24 hours without food and aeration. After this first 24 hours, copepods were individually transferred from the aquarium, by using Pasteur pipettes under a dissecting microscope, to a clean 300 mL flasks containing filtered (1 µm) seawater (salinity = 34) and food (*Thalassiosira weissflogii* at 20.000 cells.mL^{-1}). Four previously exposed females and one male were transferred to each one of four glass flasks and then placed in a light and temperature controlled room (T = 20 °C, 12 hours light:12 hours dark) for another 24 hours period. The control was set with 4 glass flasks in the same conditions as described for the treatment flasks, but containing only non-exposed copepods. Flasks were manually rotated every 4 hours in order to avoid algae settling. Following incubation, the survivorship of adults was checked and the contents of the flasks were retained on 20 µm mesh sieves and, then, placed in small beakers. For egg viability analysis, around 30 eggs from each treatment were picked up with a Pasteur pipette and transferred to Petri dishes containing filtered seawater (1 µm). Thus, all the Petri dishes were placed in an incubator (T = 20 °C, 12 hours light:12 hours dark) for another 24-48 hours. The remaining material was preserved in a 4% formaldehyde solution and the numbers of eggs, nauplii and fecal pellets were counted under a dissecting microscope. Hatching success was assessed from number of hatched eggs in relation to total number of observed eggs. Viability of eggs was checked after 24 and 48 hours, in order to determine hatching percentage after these experimental periods.

Data analysis

LC_{50} was estimated using Probit method (Finney, 1971) for both exposure periods (24 and 48 hours), and comparison of LC_{50} values used a 95% Confidence Interval (CI) (APHA, 1976). Mean difference test (*t*-Student) was applied to sub-lethal tests for comparison between control and treatment results (significance level of 95%). Data normality and homogeneity for acute and sub-lethal data was verified with the Levine test. A log (x + 1) transformation was applied on fecal pellet production data to fulfill test requirements. Values are presented as average (n = 4) of number of eggs.$female^{-1}$ and number of fecal pellets $copepod^{-1}$.

RESULTS

Chemical analysis

The overall description of the oil used in this study is presented in Table 1. Total hydrocarbon (THc) concentration present in the water-soluble fraction of the oil was 196 µg.L^{-1} in the initial exposure solution (0 hours) used in the experiments and 133 µg.L^{-1} in the final solution (48 hours), while total aliphatic hydrocarbons (TAl) and total aromatic hydrocarbons (TAr) were 164 and 32 µg.L^{-1} in the initial solution and 122 and 11 µg.L^{-1} in the final solution, respectively (Table 2).

Acute exposure experiments

Based on copepod mortality data, LC_{50} values (expressed as THc, TAl and TAr equivalents) for 24 and 48 hours of exposure to each WSF concentration were calculated for male and female *Acartia tonsa* (Table 3). Total mortality of the individuals tested was observed after 24 hours of exposure to the 100% WSF solution (THc equivalent = 196 µg.L^{-1}). The LC_{50} (THc equivalent) for females was 69.5 (55.8 – 84.5) µg.L^{-1} after 24 hours and 48.0 (38.7 – 58.0) µg.L^{-1} after 48 hours and 84.8 (70.7 – 101.1) µg.L^{-1} and 70.1 (59.1 – 80.1) µg.L^{-1} for males, respectively (Table 3). A significant difference between concentration means was seen for females exposed to 48 hours only.

Table 1 – Physical-chemical characteristics of HYDRA oil (data supplied by Ipiranga Refinery, Rio Grande – RS, Brazil).

Assays	Results		Reference methods
	Minimum	Maximum	
Density at 20/4 °C	0.7661	0.8083	ASTM D1298
API	42.8	46.8	ASTM D1298
Viscosity at 37.8 °C (ssu)	32	43	M8 – 326
Basic Nitrogen (ppm)	93.8	216.3	
Chlorides ($mg.L^{-1}$)	1.6	70.2	M8-298
Carbon Residues (%)	0.89	2.06	ASTM D189
Sulfer (%)	0.038	0.077	ASTM D1552
Water and Sediment (%)	0.05	0.2	ASTM D4007
TAN ($mg\ g^{-1}$ KOH)	0.02	0.17	ASTM D664
Fluidity Point (°C)	0.0	0.0	ASTM D97
Reid Vapor Pressure ($Kgf.cm^{-2}$)	0.21	0.49	ASTM D323
Iron (ppm)	0.35	2.92	Atomic Absorption
Vanadium (ppm)	0.36	0.81	Atomic Absorption
Copper (ppm)	0.12	0.72	Atomic Absorption
Nickel (ppm)	0.44	1.33	Atomic Absorption
Sodium (ppm)	0.67	12.68	Atomic Absorption

ppm – parts per million.

Table 2 – Initial (0 hours) and final (48 hours) concentration of aliphatic and aromatic hydrocarbon ($\mu g.L^{-1}$) in the 100% exposure solution of the petroleum water soluble fraction used in the acute and sub-lethal assays.

Hydrocarbons ($\mu g.L^{-1}$)	Initial (0 hours)	Final (48 hours)
Resolved Aliphatic	148.3	90.0
n-Alkanes ($\Sigma n\text{-}C_{12\text{-}36}$)	25.9	20.4
UCM	15.8	31.8
Total aliphatic (TAl)	164.1	121.8
Total aromatics (TAr)	31.8	10.8
Naphthalene	1.33	0.66
1 Methil Naphthalene	0.32	0.19
2 Methil Naphthalene	0.30	0.16
Biphenyl	0.06	0.24
2,6 Dimethil Naphthalene	0.01	0.05
Acenaphthylene	<LD	0.0004
Acenaphthene	0.0001	0.003
Fluorine	0.004	0.04
Dibenzothiophene	0.003	0.02
Phenanthrene	0.15	0.15
Anthracene	0.22	0.19
Fluoranthene	<LD	0.0004
Pyrene	<LD	0.0005
Benz(a)anthracene	<LD	<LD
Chrysene	<LD	0.0002
Benzo(b)fluoranthene	<LD	<LD
Benzo(k)fluoranthene	<LD	<LD
Benzo(e)pyrene	<LD	<LD
Benzo(a)pyrene	<LD	<LD
Perylene	<LD	<LD
Indeno(1,2,3-cd)pyrene	<LD	<LD
Dibenz(a,h)anthracene	<LD	<LD
Benzo(g,h,i)perylene	<LD	<LD
∑23 ПАН	2.4	1.7
Total hydrocarbons (THc)	195.8	132.6

Sub-lethal exposure experiments

The concentration of total hydrocarbons present in the WSF solution to which copepods were exposed for 24 hours during sub-lethal experiments (LC_{10}) was 15.5 $\mu g.L^{-1}$, with 83.75% of this concentration corresponding to aliphatic and 16.25% to aromatic hydrocarbons.

Significant differences were recorded between 0 hours(Control) and 24 hours exposure for egg (Figure 1a) and fecal pellet production values (Figure 1b). Mean egg production of the Control group was 48.6 ± 6.2 $eggs.female^{-1}$, whereas for the exposed group was 31.4 ± 5.8 $eggs.female^{-1}$ ($p = 0.007$) (Figure 1a). Regarding the number of nauplii produced, the Control presented a mean value of 12.6 $nauplii\ female^{-1}.day^{-1}$, with a minimum of 2 and maximum of 34, while none was produced in the exposed group (not shown).

The egg viability (hatched nauplii) test showed statistical difference only for the eggs hatched after 24 hours of exposure, with a mean value of 27.3 ± 1.5 in the Control group and 24.0 ± 1.0 in the exposure group (Figure 2a). After 48 hours, values did not differ significantly (Figure 2b).

DISCUSSION

The test-species *A. tonsa* has shown to be sensitive to the WSF of the tested oil, since the 24 hours exposure to the 100% WSF was enough to cause mortality to 100% of copepod. Despite the differences in the composition of different WSF of oils, this species has shown to be more sensitive than other organisms, such as embryos of the shrimp *P. pugio*, which have a 20% survival after an exposure of 4 days to 100% WSF obtain from a different oil (Fisher & Foss, 1993). Total mortality of *P. pugio* embryos was observed only after the 12th day of exposure. *A. tonsa* have also shown sensitivity comparable to the mysed *Mysidopsis junie*, which is a standardized toxicity

Table 3 – LC_{50} (µg.L^{-1}) for females and males after 24 and 48 hours of exposure expressed as equivalents of Total Hydrocarbons (THc), total aliphatic hydrocarbons (TAl) and total aromatic hydrocarbons (TAr). CI – Interval of confidence (95%).

	LC_{50} 24 hours (µg.L^{-1})				LC_{50} 48 hours (µg.L^{-1})			
	Female	CI (95%)	Male	CI (95%)	Female	CI (95%)	Male	CI (95%)
THc	69.5	(55.8-84.5)[a]	84.8	(70.7-101.1)[a]	48.0	(38.7-58.0)[b]	70.1	(59.1-80.1)[a]
TAl	58.2	(46.86-71.1)	71.0	(59.2-84.7)	40.2	(32.4-48.7)	58.7	(50.2-67.1)
TAr	11.3	(9.1-13.8)	13.8	(11.5-16.4)	7.8	(6.3-9.5)	11.4	(9.7-13.0)

a and b - different letters were statistically significant (95%).

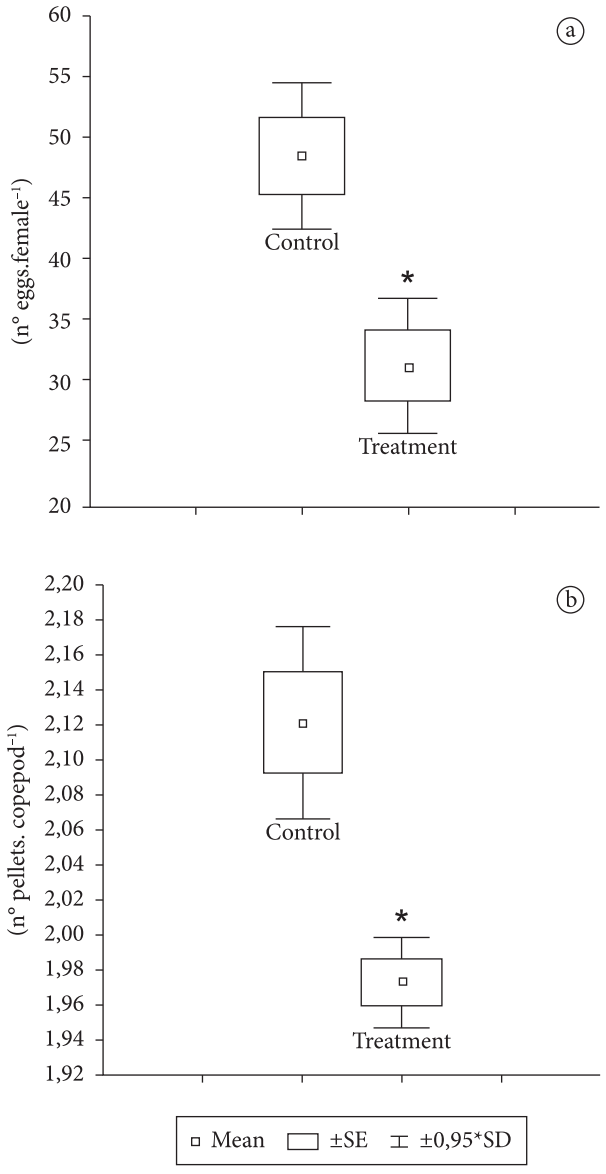

Figure 1 – Sub-lethal effects of exposure to LC_{10} levels of WSF on *Acartia tonsa*. (a) number of eggs produced by each female after 24 hours; (b) number of fecal pellets produced by each copepod (log (x + 1) transformed).

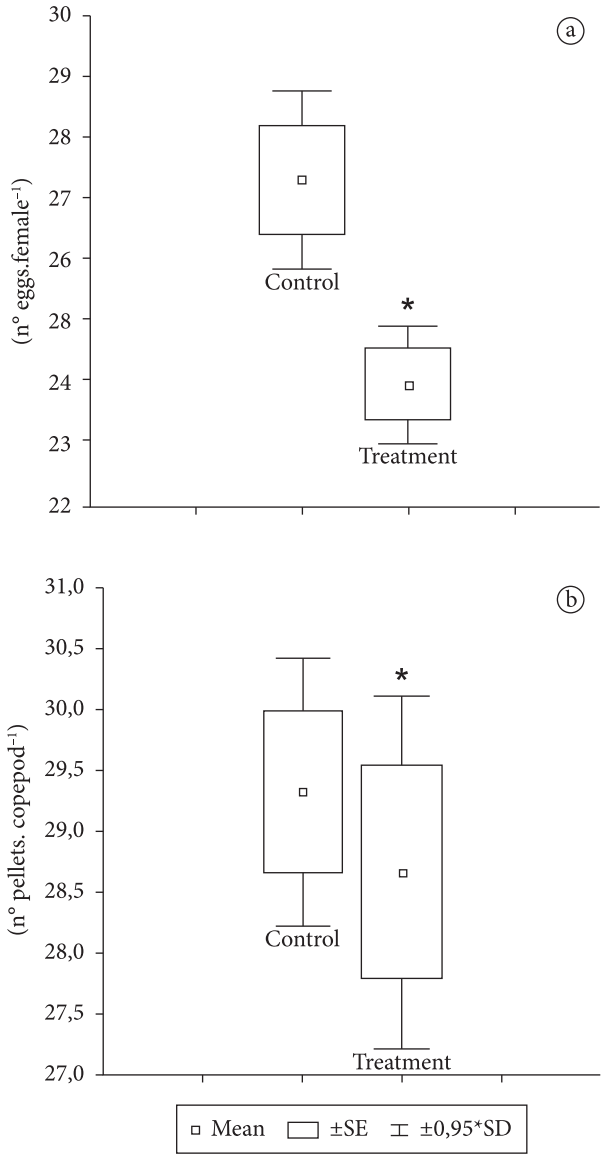

Figure 2 – Number of hatched eggs of *Acartia tonsa* (egg viability) after (a) 24 hours and (b) 48 hours of exposure to LC_{10} levels of WSF.

test species (ABNT, 2005), when exposed to other compounds such as organotins and antifouling herbicides (Perina, 2009).

The results of LC_{50} for exposures of 24 and 48 hours confirm that the longer the exposition of the tested organisms to WSF of oil, the lower the concentration necessary to cause an effect. This general pattern was already observed back in the 70's for the mysid *Mysidopsis almyra* and shrimps (*Palaemonetes pugio* and *Penaeus aztecus*) (Anderson *et al.*, 1974). Therefore, considering that might be also true for many (if not all) planktonic organisms exposed to diffuse or punctual sources of hydrocarbons in the environment (such as oil spills or chronic inputs of urbanized coastal areas), higher effect

rates are expected when exposed to longer periods of time. This might be an issue of concern, especially in low dynamic shallow or enclosure coastal areas and estuaries, where the time (and levels) of exposure will be longer than for open and more dynamic waters.

Although this study does not provide data to elucidate this finding, the higher sensitive shown for females could be related to the amount of energy invested for females in reproduction. Females of *A. tonsa* are free-spawners and show continuous egg production throughout their adulthood, producing up to 100 eggs.day^{-1} (Sedlacek & Marcus, 2005; Holste & Peck, 2006). If females truly invest more energy in reproduction than males, they would probably have less energy available to cope with the WSF contaminants.

With regards to sub-lethal effects associated to WSF, it was observed that the LC_{10} (THc = 15.5 µg.L^{-1}) was enough to cause harmful effects on exposed copepods, reducing the number of eggs and fecal pellets produced. It is well established that for most copepods species, egg production is directly related to female nutritional condition, which is in turn a function of the quantity and quality of food ingested (Kiorboe *et al.*, 1985). Based on this premise, the lower values of egg production observed in this study for females exposed for 24 hours to LC_{10} (THc = 15.5 µg.L^{-1}) may have been caused not only by direct effects of the contaminants on the copepods reproductive system, but also indirectly by inhibiting the mechanism of food ingestion. Considering that fecal pellet production represents an indirect measurement of food ingestion rates, the significantly lower fecal pellet production ($p < 0.05$) for exposed females supports the hypothesis that WSF may have inhibited copepod feeding, which in turn indirectly affected the egg production. In addition, Calbet *et al.* (2007) observed that naphthalene is one hydrocarbon that can cause a decrease in the feeding rates on *Paracartia grani*, supporting the results of the present study.

Although the present study focused on testing the effects of the whole WSF of specific oil, it was already shown the toxicity of different hydrocarbon compounds normally comprised in WSF to copepods. A previous study evaluating the specific effect of naphthalene on copepods found a LC_{50} (48 hours) of 56.1 µmol.L^{-1} for *Oithona davisae*, with narcotic effects caused at lower concentrations (Barata *et al.*, 2005). Moreover, it was noted that long periods of exposition to naphthalene at concentrations of 10 µg.L^{-1} caused both reduction in the prossome length and decrease in egg and nauplii production, and in the life duration of the copepod *Eurytemora affinis* (Ott *et al.*, 1978). For this specific compound, the WSF of oil that killed 50% of female *A. tonsa* had 0.41 µg.L^{-1} (24 hours exposure) and 0.17 µg.L^{-1} (48 hours exposure) of naphthalene, which confirm that toxicity was most probably related to the interaction of different contaminants found in the WSF (other than naphthalene itself).

Concerning the viability experiments, the number of hatched nauplii was only significantly different after 24 hours, indicating that exposure to the LC_{10} of WSF caused just a delay in the hatching of eggs produced by exposed females. Sub-lethal effects were also reported for the copepod *Centropages hamatus*, where a decrease on food ingestion rates (at concentrations up to 10 µg.L^{-1} of dissolved hydrocarbons), egg viability (at concentrations higher than 10 µg.L^{-1}) and activity and swimming (at concentration of 80 µg.L^{-1}) was seen (Cowles & Remillard, 1983a). The same authors did not observe bioaccumulation of hydrocarbons on *C. hamatus*, suggesting that these compounds are metabolized by the organism and their metabolites (more water soluble forms) may have been incorporated in oocyte tissue, affecting egg viability (Cowles & Remillard, 1983b). The same process may have also caused the decrease in egg production and hatching in the present study with *A. tonsa*. However, other studies found evidences of accumulation of some petroleum-derived compounds in copepods. Levels of total polycyclic aromatic hydrocarbon (TPAH) between 0.61 and 1.28 µg.g^{-1} (dry wt) were found in *Neocalanus plumchrus* exposed to environmental levels between 0.010 and 0.024 µg.L^{-1} (water plus particles), and 0.001 and 0.012 µg.L^{-1} (seawater) (Carls *et al.*, 2005).

Despite the scarcity of information concerning the effects of oil spills on planktonic copepod egg production in the environment, field studies demonstrated that there may be considerable decrease in zooplankton biomass as well as changes in community structure after the spill (Samain *et al.*, 1980; Guzmán del Próo *et al.*, 1986). Nonetheless, the zooplankton community seems to reestablish itself after some months of an oil spill, indicating capacity of population recovery (Al-Yamani *et al.*, 1993). However, this recovering might not be so effective under chronic exposures, especially for environments with reduced fluxing time.

As confirmed by the presented results the water soluble fractions of oils can definitely cause acute damage to copepods. In addition, even after losing some toxic compounds though oil weathering, the remaining non-dissolved compounds can be ingested by zooplanktonic organisms (Muschenheim & Lee, 2002), causing toxicity to this and other trophic levels. Thus, the toxicity of WSF under certain conditions may represent a serious risk to zooplanktonic organisms (Cowles & Remillard, 1983; Fisher & Foss, 1993; Gulec *et al.*, 1997) and it is also evidenced in this study, which demonstrates not only the acute effect of WSF, but also sub-lethal effects that, although do not cause death, may be crucial for population maintenance, possibly influencing the equilibrium of marine ecosystems, including fish stocks in coastal areas.

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REFERENCES

ABNT, Associação Brasileira de Normas Técnicas, 2005, *Norma ABNT-NBR15308:2005, "Ecotoxicologia aquática - Toxicidade aguda - Método de ensaio com Misidáceos (Crustacea)"*, ABNT, Rio de Janeiro, 17p.

AL-YAMANI, F. Y., AL-RIFAIE, K. & ISMAIL, W., 1993, Post-spill zooplankton distribution in the NW Gulf. *Mar. Poll. Bull., 27*: 239-243.

ANDERSON, J. W., NEFF, J. M., COX, B. A., TATEM, H. E. & HIGHTOWER, G. M., 1974, Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Mar. Biol., 27*: 75-88. doi:10.1007/BF00394763.

APHA, American Public Health Association, America Water Works Association, Water Pollution Control Federation, 1976, *Standard methods for the examination of water and wastewater*. APHA, Washington, D.C., 1193p.

BARATA, C., CALBET, A., SAIZ, E., ORTIZ, L. & BAYONA, J. M., 2005, Predicting single and mixture toxicity of petrogenic polycyclic aromatic hydrocarbons to the copepod *Oithona davisae*. *Environ. Toxicol. Chem.*, 24: 2992-2999. doi:10.1897/05-189R.1.

CALBET, A., SAIZ, E. & BARATA, C., 2007, Lethal and sublethal effects of naphthalene and 1,2-dimethylnaphthalene on the marine copepod *Paracartia grani*. *Mar. Biol*., 151: 195-204. doi: 10.1007/s00227-006-0468-0.

CARLS, M. G., SHORT, J. W., PAYNE, J., LARSEN, M., LUNASIN, J., HOLLAND, L. & RICE, S. D., 2005, *Accumulation of Polycyclic Aromatic Hydrocarbons by neocalanus copepods in Port Valdez, Alaska*. Prince William Sound Regional Citizens Advisory Council, Anchorage, AK, 56p. Contract 956.04.1.

CERVETTO, G., PAGANO, M. & GAUDY, R., 1995, Feeding behaviour and migrations in a natural population of the copepod *Acartia tonsa*. *Hydrobiol.*, 300/301: 237-248. doi:10.1007/BF00024464.

CLARK, R. B., 2001, *Marine Pollution*, 5th ed., Oxford University Press, Oxford, 237p.

COWLES, T. J. & REMILLARD, J. F., 1983a, Effects of exposure to sublethal concentrations of crude oil on copepod *Centropages hamatus.* 1. Feeding and egg-production. *Mar. Biol.*, 78: 45-51. doi:10.1007/BF00392970.

COWLES, T. J. & REMILLARD, J. F., 1983b, Effects of exposure to sub-lethal concentrations of crude oil on copepod *Centropages hamatus*. 2. Activity patterns. *Mar. Biol.*, 78: 53-57. doi:10.1007/BF00392971.

DYER, S. D., 2001, Determination of the aquatic $PNEC_{0.05}$ for boron. *Chemosphere*, 44: 369-376. doi: 10.1016/S0045-6535(00)00356-8.

EPSTEIN, N., BAK, R. P. M. & RINKEVICH, B., 2000, Toxicity of third generation dispersants and dispersed Egyptian crude oil on Red Sea coral larvae. *Mar. Poll. Bull.*, 40: 497-503. doi:10.1016/S0025-326X(99)00232-5.

FINNEY, D. J., 1971, *Probit Analysis*. Cambridge University Press, Cambridge, England, 31p.

FISHER, W. S. & FOSS, S. S., 1993, A simple test for toxicity of number 2 fuel oil and oil dispersants to embryos of grass shrimp *Palaemonetes pugio*. *Mar. Poll. Bull.*, 26: 385-391. doi:10.1016/0025-326X(93)90186-N.

GULEC, I., LEONARD, B. & HOLDWAY, D. A., 1997, Oil and dispersed oil toxicity to amphipods and snails. *Spill Sci. Technol. Bull*., 4: 1-6. doi:10.1016/S1353-2561(97)00003-0.

GUZMÁN DEL PRÓO, A. S., CHÁVEZ, E. A., ALATRISTE, F. M., DE LA CAMPA, S., DE LA CRUZ, G., GÓMEZ, L., GUADARRAMA, R., GUERRA, A., MILLE, S. & TORRUCO, D., 1986, The impact of the Ixtoc-1 oil spill on zooplankton. *J. Plankton. Res.*, 8: 557-581. doi:10.1093/plankt/8.3.557.

HOLSTE, L. & PECK, M. A., 2006, The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): a laboratory investigation. *Mar. Biol.*, 148: 1061-1070. doi:10.1007/s00227-005-0132-0.

KENNISH, M. J., 1997, *Pollution impacts on marine biotic communities*. CRC Press, Boca Raton, 310p. Marine Science Series.

KIORBOE, T., MOHLENBERG, F. & HAMBURGER, K., 1985, Bioenergetics of the copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar. Ecol. Prog. Ser.*, 26: 85-97.

LENZ, J., 2000, Introduction. *In*: R. P. Harris, P. H. Wiebe, J. Lenz, H. R. Skjoldal & M. Huntley (eds.), *ICES Zooplankton methodology manual*. Academic Press, San Diego, p. 1-32.

LONG, S.M. & HOLDWAY, D. A., 2002, Acute toxicity of crude and dispersed oil to *Octopus Pallidus* (Hoyle, 1885) hatchlings. *Water Res.*, 36: 2769-2776. doi:10.1016/S0043-1354(01)00520-6.

MONTÚ, M., 1980, Zooplâncton do estuário da Lagoa dos Patos I. Estrutura e variações temporais e especiais da comunidade. *Atlântica*, 4: 53-72.

MUSCHENHEIM, D. K. & LEE, K., 2002, Removal of oil from the sea surface through particulate interactions: review and prospectus. *Spill Sci. Technol. Bull.*, 8: 9-18. doi:10.1016/S1353-2561(02)00129-9.

OTT, F. S., HARRIS, R. P. & O'HARA, S. C. M., 1978, Acute and sublethal toxicity of naphthalene and three methylated derivatives to the estuarine copepod *Eurytemora affinis*. *Mar. Environ. Res.*, 1: 49-58. doi:10.1016/0141-1136(78)90013-2.

PERINA, F., 2009, *Avaliação da toxicidade de biocidas utilizados em tintas anti-incrustantes*. Dissertação (Mestrado em Oceanografia Física, Química e Geológica) - Universidade Federal do Rio Grande - FURG, Rio Grande, 138p.

SAMAIN, J. F., MOAL, J., COUM, A., LE COZ, J. R. & DANIEL, J. Y., 1980, Effects of the "Amoco Cadiz" oil spill on zooplankton. A new possibility of ecophysiological survey. *Helgol. Meeresunters*, 33: 225-235. doi:10.1007/BF02414748.

SEDLACEK, C. & MARCUS, N. H., 2005, Egg production of the copepod *Acartia tonsa*: The influence of hypoxia and food concentration. *J. Exp. Mar. Biol. Ecol*., 318: 183-190. doi:10.1016/j.jembe.2004.12.012.

SHAFIR, S., VAN RIJN, J. & RINKEVICH, B., 2003, The use of coral nubbins in coral reef ecotoxicology testing. *Biomol. Eng.*, 20: 401-406. doi:10.1016/S1389-0344(03)00062-5.

TISELIUS, P., HANSEN, B., JONSSON, P., KIORBOE, T., NIELSEN, T.G., PIONTKOVSKI, S. & SAIZ, E., 1995, Can we use laboratory-reared copepods for experiments? A comparison of feeding behavior and reproduction between a field and a laboratory population of *Acartia tonsa*. *J. Mar. Sci*., 52: 369-376. doi:10.1016/1054-3139(95)80052-2.