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Impact of the diatom-derived polyunsaturated aldehyde 2-trans,4-trans decadienal on the feeding, survivorship and reproductive success of the calanoid copepod *Temora stylifera*

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Abstract

Many diatoms, a major class of unicellular algae contributing to over 45% of oceanic primary production, are known to induce deleterious effects on reproductive processes in crustacean copepods. This is mainly due to the production of teratogenic oxylipins, including polyunsaturated aldehydes (PUAs). Here we tested the direct effect of the PUA 2E,4E-decadienal (DD) on feeding activity, survivorship and reproductive success of the calanoid copepod *Temora stylifera*. DD-inoculated cultures induced high mortality at concentrations above 3 µg mL^{-1} compared to controls in both males and females, with males having a higher mortality. Low DD concentrations triggered an increase in female filtration and ingestion rates. Egg production rates and hatching times were also higher in the presence of DD, whereas egg hatching success decreased with increasing DD concentration. Our study shows, for the first time, that the presence of diatom PUAs may increase feeding rates in copepods.

Keywords: Copepod; Feeding; Inhibition; Diatom Polyunsaturated Aldehydes; Mortality; Apoptosis.

1. Introduction

Diatoms constitute an important food source for copepods in marine ecosystems but several studies have reported negative effects of diatom diets on copepod recruitment such as lower egg production rates, egg hatching success and/or naupliar survival (recently reviewed by Ianora and Miralto, 2010). Several mechanisms have been proposed for the observed deleterious effects of diatoms: nutritional deficiency (Jónasdóttir and Kiørboe, 1996; Lacoste et al., 2001), lack of ingestion by nauplii (Koski, 2008) and presence of inhibitory bioactive molecules (Miralto et al., 1999; Pierson et al., 2005). Many diatom species have in fact been shown to produce inhibitory molecules (Carotenuto et al., 2002; Ianora et al., 2004; Poulet et al., 2007), characterized as polyunsaturated aldehydes (see reviews of Pohnert, 2005; Wichard et al., 2005) and other oxylipins (d'Ippolito et al., 2002a; d'Ippolito et al., 2002b; Fontana et al., 2007; Miralto et al., 1999; Pohnert, 2002). Direct effects of these PUAs and oxylipins have been tested on the proliferation of bacteria (Adolph et al., 2004; Ribalet et al., 2008), phytoplankton (Hansen and Eilertsen, 2007; Ribalet et al., 2007a) and other organisms of different phyla (Adolph et al., 2004; Caldwell et al., 2005; Romano et al., 2010). However, very few studies have tested the effects of pure PUAs on copepods (Buttino et al., 2008; Ceballos and Ianora, 2003; Taylor et al., 2007). Since PUAs are released when diatom cells are wounded during copepod grazing ("sloppy feeding") (Pohnert, 2000; Wichard et al., 2007) or lysed from senescent cells during bloom periods (Vidoudez et al., 2011), it should be interesting to determine the direct effects of pure molecules on copepod fitness.

Diatom PUAs are reported to act as repellent compounds to reduce and/or avoid grazing in pelagic freshwater grazers of the genera *Daphnia*, *Cyclops* and *Eudiaptomus* (Jüttner, 2005). However it is unclear whether all copepods are able to discriminate between PUA-producing or non-producing diatoms. Selectivity by copepods on phyto- and microzooplankton has been shown to depend on various factors, such as size, shape, swimming behavior and speed of prey and predators (Kleppel, 1993), as well as sensory perception and palatability (Huntley et al., 1986), but some species also show non-selective feeding behavior (Turner and Tester, 1989). Chemosensory abilities are suggested to be mainly used in the immediate environment around a food particle (Huntley et al., 1986; Strickler, 1982). For example, (Halsband-Lenk et al., 2005) showed that the copepod *Pseudocalanus newmani* did not show a diminution in egg hatching

success during a mixed diatom (toxic and non-toxic species) bloom in Dabob Bay, USA, and suggested that this copepod grazer was capable of discriminating toxic diatoms when nontoxic ones were available. This was further confirmed by grazing experimental results showing that another copepod, *Calanus pacificus*, avoided the most toxic PUA producers (Leising et al., 2005). On the other hand, the copepod *Temora stylifera* was non-selective when diatom species were offered together with the non-toxic dinoflagellate *Prorocentrum minimum* (Turner et al., 2001) suggesting that some grazers are not aware of the toxicity of their food (Barreiro et al., 2011). This is at variance with other phytoplankton toxins, some which can have direct antifeedant effects on copepods, but similar to some other phycotoxins which have no apparent effects (reviewed by (Turner et al., 1998).

To better understand the effects of pure PUAs on copepod feeding, reproduction and behavior, in the present study we conducted: (1) grazing experiments with the copepod *Temora stylifera* and cultures of *Prorocentrum minimum* inoculated with the PUA decadienal (DD), a model aldehyde used in many experimental studies (Ianora and Miralto, 2010), and non-inoculated *P. minimum* cultures, (2) survivorship experiments to reveal whether DD induces copepod mortality and at what concentration, (3) reproduction experiments to reveal whether dissolved DD affects copepod *T. stylifera* reproduction and naupliar survival through the induction of apootosis and 4) two-choice behavioral experiments to investigate the effect of pure DD on *T. stylifera* behavior.

2. Materials and Methods

2.1. Preparation of chemical standards DD preparation

Commercial grade 2E,4E-decadienal (Sigma-Aldrich) was obtained for toxicity testing. Due to low solubility in water 2,4-decadienal (DD) was initially dissolved in methanol and then transferred to filtered seawater (FSW) to give a stock solution of 100 µg mL^{-1}, from which serial dilutions were performed to give the required experimental concentrations.

2.2. Culture of Prorocentrum minimum Pavillard (Schiller)

The dinoflagellate *Prorocentrum minimum* was grown in K-medium on a 12L:12D cycle and a light intensity of 175 µ*e* m^{-2} s^{-1}, at 20° C in a light-temperature controlled chamber. The strain is from the Stazione Zoologica culture collection and does not produce PUAs or other oxylipins (Fontana et al., 2007b).

2.3. Copepod collection

Zooplankton were collected in the Gulf of Naples (Italy) in September 2010 using a 200·µm mesh plankton net, and immediately transported to the laboratory in an insulated box.

2.4. Grazing experiments

Freshly collected (~2 h after collection) healthy mature *Temora stylifera* Dana females were isolated under a Leica stereomicroscope and incubated in flasks containing 100mL of the dinoflagellate *Prorocentrum minimum* at a final concentration of 6000 - 8000·cells·mL^{-1}, in the absence (control = 0 µg mL^{-1} DD) or presence of decadienal (DD) at different concentrations (0.5 and 2 µg mL^{-1} of DD). *P. minimum* was used as the control diet since it does not produce aldehydes or other oxylipins. For each treatment (control, 0.5 and 2 µg mL^{-1} of DD), three flasks were used. Three groups of *T. stylifera* females (*N*=5) were incubated with *P. minimum* in the absence of DD (control treatment). Three groups of *T. stylifera* females (*N*=5) were incubated with *P. minimum* in the presence of DD at the above mentioned concentrations (experimental treatments). After 24 hours of incubation at 20 °C, females were counted again in the experimental treatments and phytoplankton was fixed with Lugol's solution. Samples were counted under a direct microscope in 1 mL Sedgewick–Rafter chambers. Ingestion rates (cells $ind^{-1}h^{-1}$) were calculated following Frost's equations (Frost, 1972) and were then converted into µg C $ind^{-1}h^{-1}$ considering that *P. minimum* carbon content was 274.19 pg C $cell^{-1}$ (Turner et al. 2001).

2.5. Mortality experiments

Freshly-collected (~2 h after collection) healthy mature *T. stylifera* males (*N*=12) and females (*N*=12) were isolated under a Leica stereomicroscope and incubated individually in 5 mL tissue culture wells filled with 0.45-µm filtered seawater (FSW) (control) or DD at different concentrations (0.5, 1.0, 2.0, 3.0, 5.0 and 12 µg mL^{-1}). After 24·hours of incubation at 20°C

without any food, survival of males and females was assessed in the different wells. Dead copepods were counted in each well and the percentage of survivorship was determined for each DD concentration.

2.6. Reproduction experiments

In order to test the biological activity of DD on *T. stylifera* reproduction, freshly collected (~2 h after collection) healthy mature females (*N*=10) with dark gonads (Ianora et al., 1989) were incubated individually in 5 mL tissue culture wells filled with FSW (control) and with DD at different concentrations (0.5, 1.0 and 2.0 µg mL^{-1}). All groups of copepods were incubated in a temperature controlled chamber at 20°C and 12·h:12·h light:dark cycle without any food. *T. stylifera* females were checked under a Leica microscope to detect egg production every half hour. After spawning, females were removed and eggs were left to hatch for 48 hours; percentage egg viability was calculated as described by Ianora et al. (Ianora et al., 1995). Eggs were checked every hour to determine hatching times. After 48 hours nauplii were fixed with formalin and counted under a Leica microscope.

2.7. Fluorescent staining

At the end of the reproduction experiments, all of the nauplii of the different replicates for each treatment (DD and controls) were pooled together for the TUNEL (terminal deoxy-nucleotidyl-transferase-mediated dUTP nick end labeling) analysis to calculate % of apoptotic nauplii with respect to total nauplii. Newly hatched nauplii produced by females incubated in FSW (control) and DD were collected, washed in filtered seawater and fixed in 4% formaldehyde in FSW for 48 hours. Nauplii were rinsed several times in Phosphate-Buffered Saline (PBS) 1x solution and frozen in liquid nitrogen to fracture the carapace and left at -80˚C for one night. Animals were then incubated for 1h 30·min in 0.5·U·mL^{1} chitinase enzyme (EC3.2.1.14; Sigma-Aldrich) to permeabilize the chitinous wall (Buttino et al., 2004). After rinsing in PBS 1x, samples were incubated in 0.1% Triton x-100 for 3 min at room temperature, and then washed twice in PBS and once in PBS+1% Bovine Serum Albumin (BSA) buffer. Animals were incubated in TUNEL for 1.5 hour at 37˚C following the manufacture's instructions. Samples were rinsed again in PBS and observed with the Zeiss fluorescence

microscope using 10x and 20x objectives equipped with Green Fluorescent Proteni (GFP) filter to detect TUNEL green fluorescence which reveals apoptosis.

2.8. Odour choice experiments

Experiments were performed in a transparent PVC vessel 32 cm (length) 13 cm (width) and 10 cm (height), equipped with two 2-cm high vertical bars placed in the middle and separated by a 3-cm wide space. Two agarose gel blocks incorporating DD or methanol (as control), were placed at the opposite sides of the vessel. Agarose gels (0.6%) were prepared by adding 0.3 g of agarose powder (Applichem) to 50 ml of bi-distilled water (BDW), followed by heating. After cooling, 1 ml of DD (Sigma) at 0.5 mg/ml in methanol was added, to obtain a final DD concentration of 10 µg/ml in agarose. One ml of methanol was also added to another agarose gel preparation, which was used as a control. Agarose gels were then poured into two 9-cm wide Petri disks, left to harden and stored overnight at 4°C. Experiments were performed the next day by placing half of each agarose disk (A = 32 cm^2 x h = 0.8 cm) on the bottom of the container, at opposite sides of the vessel. We then identified an area of the vessel with the DD-incorporated agarose block (+), an area with the methanol-incorporated agarose block (-) (control), and an area in the middle (0), where the copepods were released at the beginning of the experiment. The experimental method of using agarose blocks incorporating a known toxin or metabolite is similar to that described in Jüttner et al. (Jüttner et al. 2010) and differs from the Y-shaped choice chambers where copepods are provided with the option of clean seawater or seawater containing test compounds such as in Brooker et al. (Brooker et al. 2013).

T. stylifera specimens were sorted from zooplankton samples collected in the Gulf of Naples from October to November 2012, using routine procedures previously described in the methods section. About 50 ripe females were sorted, incubated into two 1-L stericups containing 50-µm natural filtered seawater, and kept in a temperature-controlled room at 20°C and 12:12 Light:Dark cycle. After 24h, the experiment was started by filling the vessel with 2.5 L of 0.2-µm filtered seawater, and pipetting *T. stylifera* females (N=20) into its central part (0). At fixed intervals of 10,30,60,90,120,180 and 240 min after the start, percentages of copepods in (+), (-) and (0) were assessed by counting the number of females in each area and dividing it by the total number of copepods actually counted in the vessel at that time. Three replicate experiments were performed, every time using freshly prepared agarose gels and changing the orientation of the

vessel with respect to the experimenter and to the light conditions in the room. In two replicates, the vessel was placed vertically with (+) located at the same side or at the opposite side of the observer, whereas in the third replicate, the vessel was placed horizontally with (+) located on the left side of the observer.

3. Results

3.1. Feeding

Filtration and ingestion rates of *T. stylifera* females on *P. minimum* were higher in DD treatments (Fig. 1A, B). On average, filtration rates increased from 0.19±0.12 mL ind^{-1} h^{-1} for controls to 0.40±0.14 and 0.47±0.04 mL ind^{-1} h^{-1} for 2.0 µg mL^{-1} and 0.5 µg mL^{-1} DD, respectively (Fig. 1A). Ingestion rates increased from 0.20±0.11 µg C ind^{-1} h^{-1} for controls to 0.40±0.13 and 0.44±0.03 µg C ind^{-1} h^{-1} for 1.0 µg mL^{-1} and 0.5 µg mL^{-1} DD, respectively (Fig. 1B). Although the differences between the control (DD 0), 0.5 µg mL^{-1} and 2.0 µg mL^{-1} DD were only significant for filtration rate (1-way ANOVA, df = 2, F = 5.368, $p = 0.0461$), but not ingestion rate (1-way ANOVA, df = 2, F = 4.997, $p = 0.0532$), ingestion and filtration rates almost doubled between controls and 0.5 µg mL^{-1} (Student-t test $p<0.05$, for both rates).

3.2. Reproductive success

Egg production rate (EPR) increased with increasing DD concentration, with values ranging from 23.5 eggs $female^{-1}$ day^{-1} (0.0 µg mL^{-1} DD) in controls to 33.8 eggs $female^{-1}$ day^{-1} at 2 µg mL^{-1} DD (Fig. 2A). Egg hatching time (EHT) increased in DD treatments, ranging on average from 19.4 h in controls to 20.7 h at 1.0 µg mL^{-1} DD (Fig. 2B). Egg hatching success (EHS) decreased in DD treatments with values ranging on average from 97% in controls to 54% at 2 µg ml^{-1} DD (Fig. 2C). There was no significant difference between treatments for fecundity (1-way ANOVA, df = 3, F = 1.846, $p = 0.161$) and EHS (1-way ANOVA, df = 3, F = 2.482, $p = 0.081$), but a significant difference for EHT (1-way ANOVA, df = 3, F = 4.603, $p = 0.010$).

3.3. Survivorship

Survivorship was high for both females and males (on average 75-100%) for controls (0.0 DD) and DD concentrations between 0.5 and 2.0 µg mL^{-1} (Fig. 3). Survivorship decreased drastically above 3.0 µg mL^{-1} DD, with values ranging from 0-42% and 0-17% for females and males, respectively

3.4. Observations of apoptotic nauplii

The percentage of apoptotic nauplii increased from 25% in controls to a maximum of 64% at 1.0 µg mL^{-1}. Fifty-seven to 64% of the hatched nauplii from *T. stylifera* females incubated in DD for 24 hours were TUNEL-positive, indicating apoptotic tissues and imminent death (Fig. 4C, Fig. 4C-F) compared to controls (Fig. 4 A,B).

3.5. Odour choice experiments

At the beginning of the experiment (t=0), all copepods were pipetted in the middle part of the vessel (0) (Fig. 5A), but after 10 min, their relative distribution already changed substantially. In particular, 51.8 ± 19.3 % of copepods were located in the area with the DD-containing agarose (+), 37.6 ± 10.9 % were in the middle (0), and 10.6 ± 10.0 % were in the area with the agarose without DD (-). Values in the area with the DD-containing agarose (+) and without DD (-) were significantly different (One-way Anova, $F_{2,6}$=6.644, $p<0.05$, Tukey's Post Test, $p<0.05$), thus suggesting that the copepods were showing a preference for the portion of the vessel that contained the DD. This attraction was more evident at t=30 min, when the copepod distribution increased significantly in (+) (63.7 ± 18.0 %), compared to both (0) (19.2 ± 12.2 %) and (-) (17.0 ± 8.9 %) (One-way Anova, $F_{2,6}$=11.28, $p<0.01$) (Fig. 5B). The relative distribution of *T. stylifera* did not change throughout the experiment, although the highest percentage of copepods in (+) was recorded after 120 min (72.2 ± 10.7 %).

4. Discussion

In this study, female *Temora stylifera* filtration and ingestion rates on *Prorocentrum minimum* increased in the presence of DD, even if the differences were significant only in the case of filtration rates. *P. minimum* is known to be well ingested by *T. stylifera* (Barreiro et al., 2011; Turner et al., 2001) and other copepods (Liu et al., 2010). Our ingestion rates are comparable to those measured in previous studies by Turner et al. (Turner et al., 2001). These authors observed an increase in *T. stylifera* ingestion rates on the diatom *Thalassiosira rotula* in a mixture with *P. minimum*. They are also in agreement with another study using a mixed diet of DD-encapsulated liposomes and *P. minimum* where fecal pellets (an indirect measure of feeding activity) were found to increase in both *T. stylifera* and the copepod *Calanus helgolandicus* (Buttino et al., 2008).

It is unclear why *T. stylifera* fed more on *P. minimum* in the presence of PUAs. PUAs liberated from diatom biofilms have been reported to be repellent to several copepod and cladoceran species (Jüttner, 2005). *Calanus pacificus* seems to avoid the most potent aldehyde producers in nature (Leising et al., 2005). More recently, Michelec et al. (Michelec et al., 2013) have shown that pollutants such as Polycyclic Aromatic Hydrocarbons (PAHs) induced hyperactivity in the estuarine copepod *Eurytemora affinis*, with an increase in swimming speed and activity resembling an escape reaction permitting copepods to evade stressful conditions. Further studies testing the effects of DD on the three-dimensional swimming behavior in *Pseudodiaptomus annandalei* indicated that males and ovigerous females swam faster at higher concentrations, suggesting a complex mode of action of this toxin (Michalec et al. in preparation).

T. stylifera is reported as being non selective in its feeding behavior and, according to Barreiro et al. (Barreiro et al., 2011), seems to be unaware of the toxicity of its food since *T. stylifera* is unable to discriminate against toxic oxylipins in intact diatom cells. Our current results indicated that it is however able to detect DD when absorbed onto ingested *P. minimum* cells and that DD stimulates increased feeding on this dinoflagellate. Furthermore, our choice experiments indicate that *T. styilfera* was attracted to DD also when it was incorporated into an agarose gel, suggesting that it recognized this Volatile Organic Compound (VOC) as a food-related signal. Not much is known about food finding cues in copepods even if a recent study by Steinke et al. (Steinke et al., 2006) found that females of *Temora longicornis* were attracted to plumes of the biogenic gas dimethyl sulfide (DMS), showing characteristic behavioral (tail

flapping) and somersault-type movements that are generally associated with search and food-finding behavior in copepods. It is possible that both DMS and PUAs produced during zooplankton grazing are used by predators to detect, locate and capture their prey. Since grazers are involved in the cell disintegration that triggers both the production of DMS (Wolfe, 2000) and PUAs (Pohnert, 2000) this process could attract herbivores to patches with high food concentrations. VOCs - lipoxygenase products released upon cleavage of polyunsaturated fatty acids, e.g., 1-penten-3-one, 1-penten-3-ol, (Z)-2-pentenal, (E)-2-pentenal, (E,Z)-2,4-heptadienal, and (E,E)-2,4-heptadienal - from damaged green algae serve as a food-finding cue for freshwater benthic herbivores (Fink et al., 2006). Food choice experiments performed on 17 animal species associated with the Mediterranean seagrass *Posidonia oceanica* indicated that these grazers recognized the presence of VOCs such as unsaturated aldehydes with chain lengths from C5 to C10, exhibiting complex patterns of reactions from attractant for some invertebrates that need to maximize the search for food, to repellent for other invertebrates (Jüttner et al., 2010). Hence our conclusion that the unsaturated aldehyde PUA 2-trans,4-trans16 decadienal (DD) may serve as a food-finding cue or feeding stimulant for some planktonic copepods would be in accordance with other studies on VOCs in benthic invertebrates.

Many authors report low survivorship of post-embryonic stages when adult females feed on diatoms in both natural and experimental conditions (Barreiro et al., 2011; Buttino et al., 2008; Carotenuto et al., 2002; Carotenuto et al., 2011; Halsband-Lenk et al., 2005). Our results indicate high mortality rates of *T. stylifera* at DD concentrations above 3.0 $\mu g\ mL^{-1}$. Interestingly, males are more sensitive than females to high concentrations of DD. Taylor et al. (Taylor et al., 2007) reported similar findings for the harpacticoid copepod *Tisbe holothuriae*, with a higher sensitivity of males (LD_{50} value of 18.7 µM) compared to females, with values that were almost half those of both pre-ovigerous (39.2 µM) and ovigerous females (34.5 µM). For *T. stylifera*, this seems to be in accordance with the findings of Carotenuto et al. (Carotenuto et al., 2011) who suggested a possible effect of diatom PUAs or other oxylipins on copepod sex ratio. Indeed, these authors observed that there were no males in cohorts reared on pure diatom diets of *T. rotula* and *Skeletonema maronoi*, or with a mixture of *S. marinoi+P. minimum*.

The enzymes involved in PUA synthesis have already been shown to remain active for 45 minutes after cell-wounding (Fontana et al., 2007), and DD can remain relatively stable for days unless it reacts with other organic molecules present in the environment (Romano et al., 2010).

The implications are that local concentrations of PUAs may be high enough to potentially impact fertilization success and embryonic fitness of marine organisms. In freshwater environments, PUAs are commonly released by diatoms and chrysophytes (see Jüttner, 2005 and references therein) through cell lysis, independently from grazing, conferring rancid smells to source drinking water. Much less is known about the presence of these molecules at sea. Vidoudez et al. (Vidoudez et al., 2011) reported up to 0.1 nM of dissolved PUAs in the Adriatic Sea during a bloom of the PUAs-producing diatom *S. marinoi*, and suggested that these compounds can persist long enough in the water to cause effects on plankton. The concentration of DD used in our incubation experiments was much higher than those measured at sea, ranging from 0.5 µg mL^{-1} to 12 µg mL^{-1}, corresponding to 3-77 nM. However, during diatom blooms, Ribalet et al. (Ribalet et al., 2007b) calculated that the PUAs concentration in the immediate surroundings of each single diatom cell may vary from 1.25 to 0.01 µM at a distance of 1 to 100 µm, respectively. Therefore, a combination of this high local concentration of PUAs and the sloppy feeding behavior of copepods may have strong ecological consequences for zooplankton behavior.

High EPR for *T. stylifera* were observed at all DD concentrations tested (maximum of 34 eggs $female^{-1}$) compared to controls (24 eggs $female^{-1}$ day^{-1}). Our results may be due to higher ingestion rates, and therefore higher EPR, in the presence of DD denoting a stimulatory effect of this metabolite on copepod feeding behavior. We also observed that the presence of DD significantly affected egg hatching times. To our knowledge, very few studies have reported egg hatching times in copepods, which are known to decrease with increasing temperature (Arendt et al., 2005) but not in the presence of toxins or other metabolites (Ueda, 1981). On the other hand, our results support observations by previous studies that hatching success is reduced when eggs are incubated in diatom extracts compared to filtered sea water, *P. minimum* and/or natural phytoplankton mixtures (Ianora et al., 1996; Uye, 1996). Thus, our findings suggest that inhibition of egg hatching by diatoms may not (exclusively) be due to feeding but (also) to direct effects of PUAs released in the environment. We also observed a high percentage of apoptotic nauplii in DD treatments which is in accordance with previous studies reporting naupliiar mortality in the presence of diatoms or diatom extracts (Ianora et al., 2004). This could explain the decrease in copepod recruitment during diatom blooms reported at times in the field (Ianora et al., 2004).

5. Conclusions

This study confirms that pure molecules of diatom PUAs can be directly responsible for deleterious effects on copepods. They induce high mortality of adults with highest sensitivity of males. PUAs reduce copepod reproductive success and recruitment by affecting egg hatching success and by provoking high naupliar apoptosis. The consequence is that although egg production rates are higher in the presence of DD, recruitment is low. Another interesting finding in this study is that at low DD concentrations, filtration and ingestion rates increased, and that copepods were able to detect DD in odor choice experiments indicating the possibility that these compounds may act as food finding cues or feeding attractants for some copepods.

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Conflict of interest

Authors declare that they do not have any conflict of interest.

Author contribution

Conceived and designed the experiments: SK, YC, GR, IB, J-SH, AI. Performed the experiments: SK, YC, GR. Analyzed the data: SK, YC. Contributed reagents/materials/analysis tools: J-SH, AI. Wrote the paper: SK, YC, IB, AI.

All authors have approved the final article.

Figure Legends

Fig. 1. Filtration (A) and ingestion (B) rates of *Temora stylifera* females fed with the dinoflagellate *Prorocentrum minimum* inoculated with 2,4-decadienal at different concentrations (0.5 µg mL^{-1}, 1 µg mL^{-1} and 2 µg mL^{-1}) (Mean ± SD).

Fig. 2. Variations in the egg production rates (A), egg hatching time (B) and hatching success (C) of *Temora stylifera* (N=12) exposed to different concentrations of 2,4-decedienal (from 0.5 µg mL^{-1} to 2 g mL^{-1}). For Fig. 2C, EHS and % of apoptotic nauplii have the same vertical axis.

Fig. 3. Variations in the survivorship of *Temora stylifera* (N=12) males and females (N=12) exposed to different concentrations of 2,4-decadienal (from 0.5 µg mL^{-1} to 12 µg mL^{-1}).

Fig. 4. *Temora stylifera* nauplii hatched from females incubated in seawater (a, b) and decadienal (c, d, e, f) and subsequently labeled with the fluorescent probe TUNEL specific for the detection of apoptosis. TUNEL-positive fluorescent regions (in green) indicate apoptosis. a, c and e are images in transmitted light and b, d and f are the same images in fluorescent light, respectively.

Fig. 5. Odor choice experiments whereby *Temora stylifera* females (N=20) were placed initially into the central part (0) of a vessel filled with filtered seawater. Two agarose gel blocks incorporating DD or methanol (as control) were placed at the opposite sides of the vessel and copepod presence in (+), (-) and (0) areas was assessed by counting the number of females in each area and dividing it by the total number of females in the vessel after 10,30,60,90,120,180 and 240 min from the start. Upper panel shows results of three replicates and lower panel shows the experimental setup.

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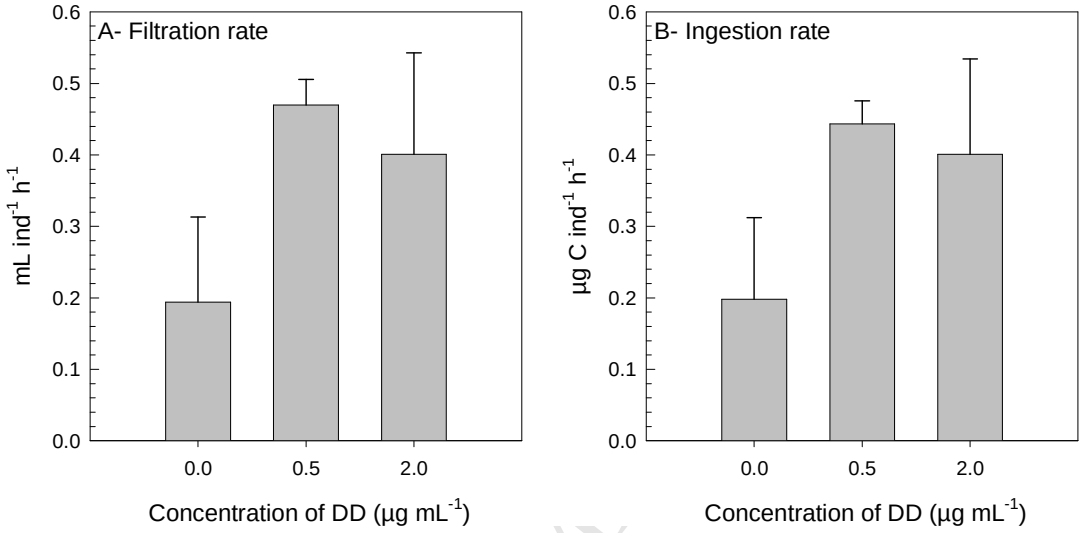


Fig.1

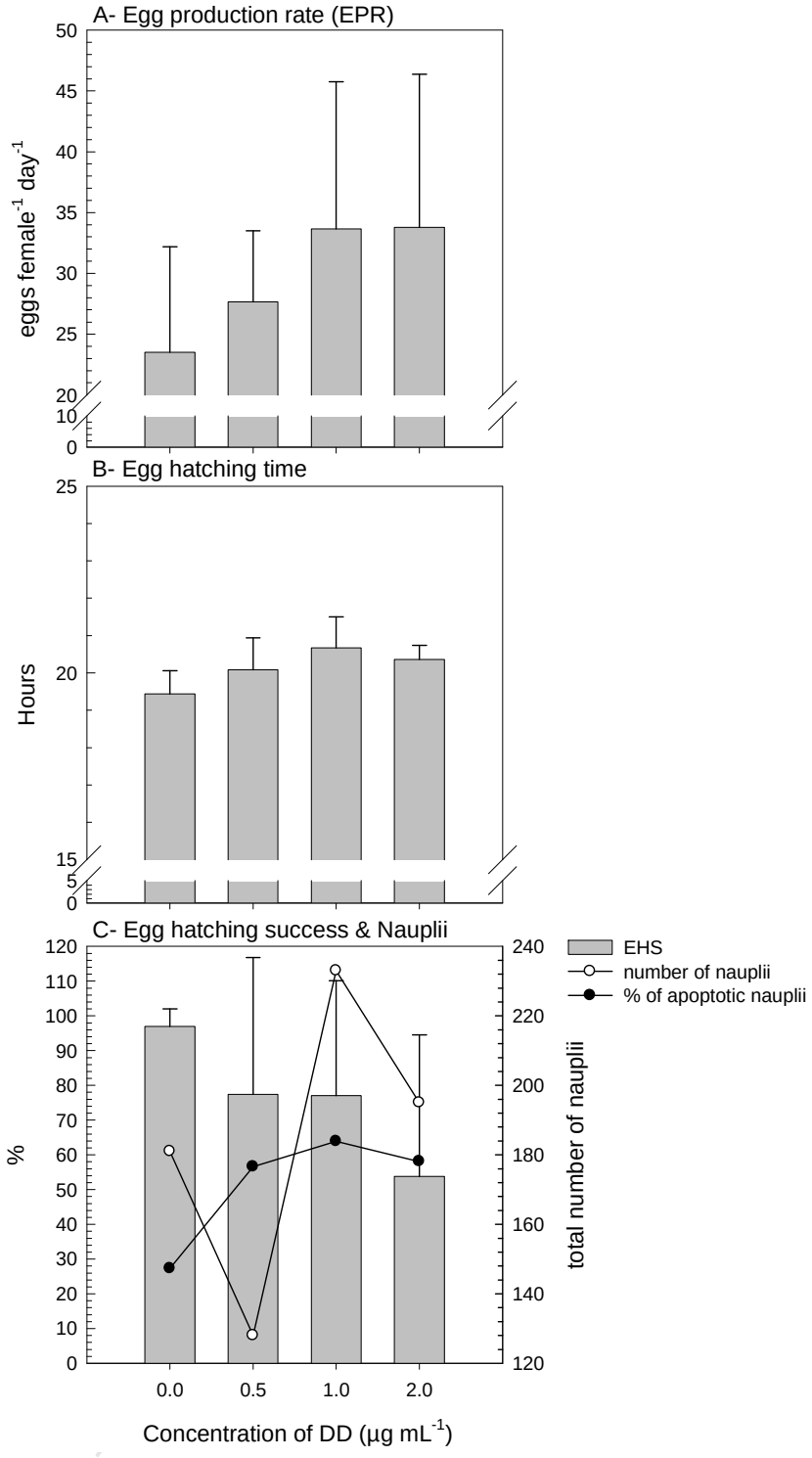

A- Egg production rate (EPR)
eggs female-1 day-1
B- Egg hatching time
Hours
C- Egg hatching success & Nauplii
%
total number of nauplii
EHS
number of nauplii
% of apoptotic nauplii
Concentration of DD (µg mL-1)

Fig. 2

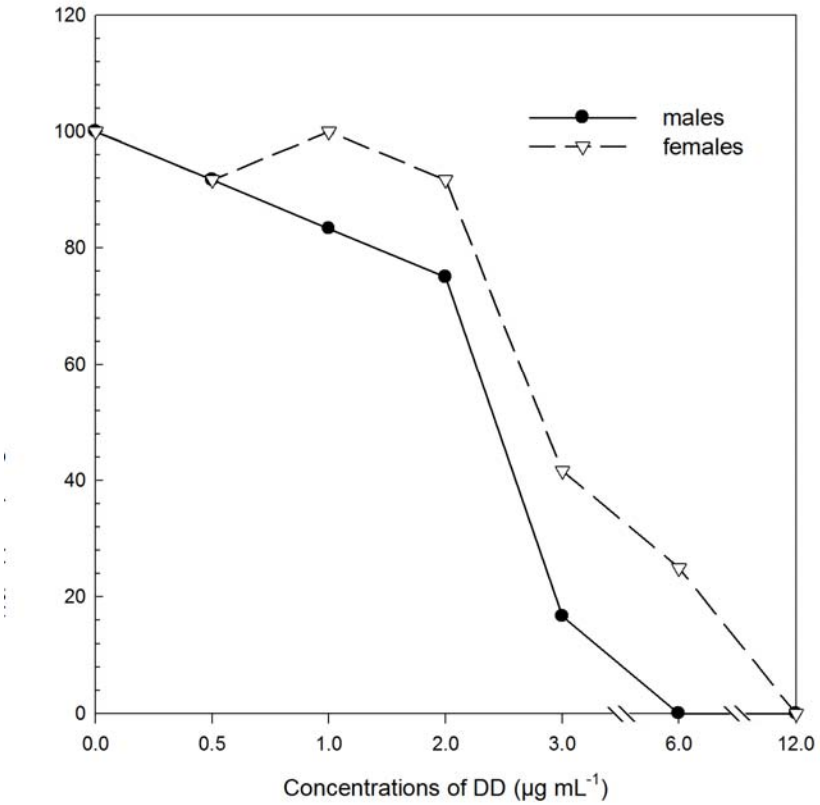


Fig. 3

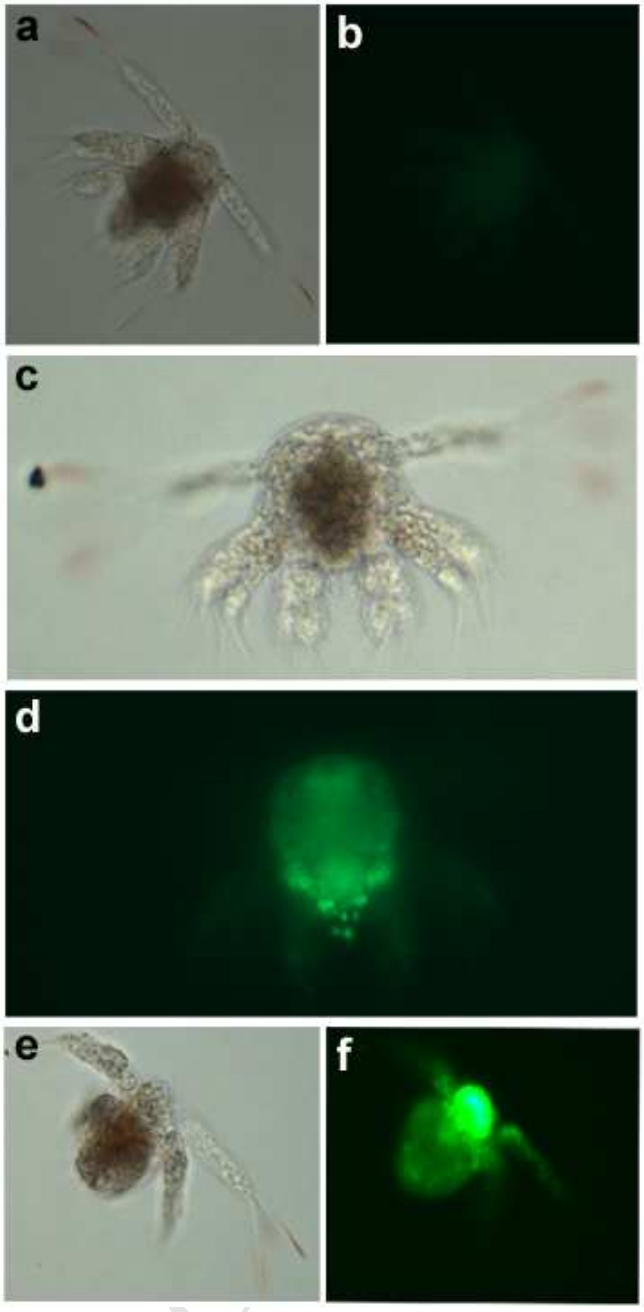
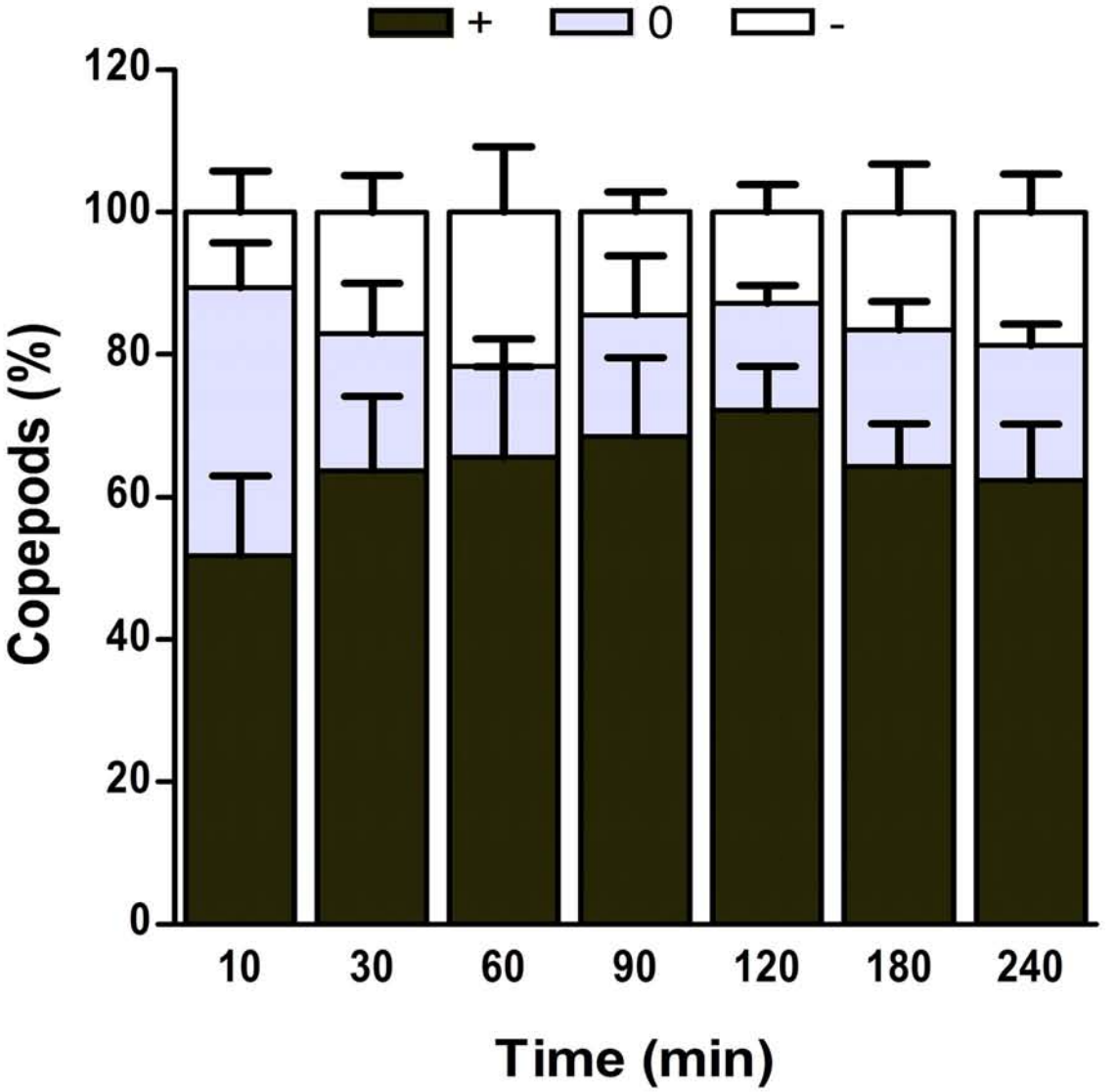
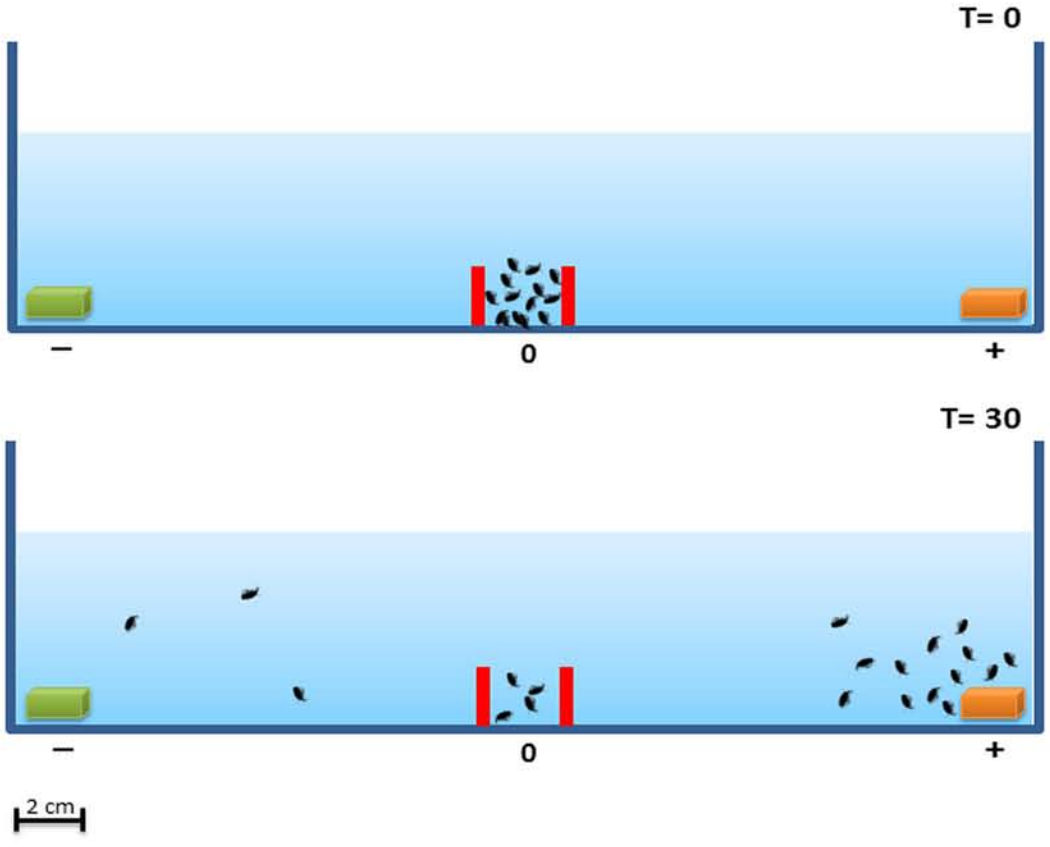


Fig. 4


+
0
-
120
100
80
60
40
20
0
Copepods (%)
10
30
60
90
120
180
240
Time (min)


T= 0
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We test the effects of the polyunsaturated aldehyde (PUA) decadienal (DD) on copepod fitness

DD induced high mortality of *Temora stylifera* adults with highest mortality in males

DD affected egg hatching success and provoked high naupliar apoptosis

At low concentrations ingestion rates increased and DD was detected in odor choice experiments

This is the first study showing that PUAs may act as feeding attractants for some copepods