Universidade de Aveiro Departamento de Biologia
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Carnivoria em *Acartia tonsa*: uma perspectiva em larvicultura marinha

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DOCUMENTO PROVISÓRIO

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada, realizada sob a orientação científica do Doutor Ricardo Jorge Guerra Calado, Investigador Principal no Departamento de Biologia (dbio) e Centro de Estudos do Ambiente e do Mar (CESAM) da Universidade de Aveiro.

Dedico este trabalho à minha família, por todo o apoio incondicional e por terem tornado possível esta ótima experiência.

o júri

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palavras-chave

Presas vivas, artémia, *Brachionus plicatilis*, predação, ácidos gordos, copépodes calanoides

resumo

A componente nutricional dos copépodes é um dos fatores cruciais para o seu bom desempenho como presas vivas em larvicultura marinha. A carnivoria é um aspeto crucial na dieta dos copépodes calanoides, sendo as suas implicações relevantes para a aquacultura. Neste estudo foram investigados i) o comportamento predatório entre *Acartia tonsa* (Copepoda, Calanoida) e outros alimentos vivos vulgarmente usados como presas vivas em larvicultura marinha (artémia e rotíferos), tendo em conta a influência da temperatura e da disponibilidade de microalga, e ii) a suplementação da dieta de *A. tonsa* com artémia, em termos de produção de ovos, eclosão a 48 h e composição em ácidos gordos. A presença de *A. tonsa* reduziu significativamente a sobrevivência de artémias e rotíferos. Este comportamento predatório mostrou-se dependente do sexo dos copépodes, sendo maioritariamente atribuído às fêmeas. A predação diminuiu com a diminuição da temperatura e com o aumento da disponibilidade de microalga. A suplementação da dieta de *A. tonsa* com artémia não promoveu diferenças significativas na produção de ovos e na eclosão a 48 h. A análise da composição em ácidos gordos revelou diferenças significativas entre os ovos e os copépodes produzidos com as diferentes dietas testadas, principalmente devido a $C_{18:3}$ (n3) e $C_{18:4}$ (n3). No entanto as percentagens de HUFAs e rácios DHA/EPA mantiveram-se aproximadamente constantes. Em suma, a suplementação de copépodes com artémia não melhora a sua qualidade nutricional nem aumenta a produtividade ou a qualidade dos ovos.

keywords

Live prey, artemia, *Brachionus plicatilis*, predation, fatty acids, calanoid copepods

abstract

The nutritional component of copepods is one of the crucial factors for its good performance as live prey in marine larviculture. Carnivory is a crucial aspect of calanoid copepods diet, being its implications relevant for aquaculture. This study investigated i) the predatory behaviour between *Acartia tonsa* (Copepoda, Calanoida) and other live prey commonly used in marine larviculture (artemia and rotifers), taking into account the influence of temperature and microalgae availability, and ii) the diet supplementation of *A. tonsa* with artemia, in terms of eggs production, 48 h hatchability and fatty acid composition. The presence of *A. tonsa* significantly reduced artemia and rotifers survival. This predatory behaviour was shown to be dependent on copepod sex, being mainly associated with females. Predation decreased with decreasing temperature and with increasing availability of microalgae. The supplementation of *A. tonsa* diet with artemia did not promoted significant differences in egg production and 48 h hatchability. The fatty acid composition analysis revealed significant differences between eggs and copepods produced with the different diets tested, mainly due to $C_{18:3}$ (n3) and $C_{18:4}$ (n3). However, the percentages of HUFAs and DHA/EPA ratios were kept approximately constant. Overall, the supplementation of copepods with artemia does not improve its nutritional quality nor does it enhances egg production or quality.

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List of Acronyms and Abbreviations

DHA	Docosahexanoic Acid
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
GC-MS	Gas Chromatography–Mass Spectrometry
HUFA	Highly Unsaturated Fatty Acid
MS	Mass Spectrometry
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
SSW	Synthetic Salt Water
SFA	Saturated Fatty Acid

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I. General Introduction

The global fish consumption per capita increased from an average of about 9.9Kg in the early 60's to 18.6Kg in 2010, representing 16.6% of the global animal protein intake (FAO, 2012). The global fisheries increased from about 20 million tonnes in the early 50's to about 90 million tons in 2010 in order to keep up with the demand from the increasing world population (FAO, 2012). Aquaculture has therefore emerged in order to fill the gaps that originated from an increased demand for fish, and is one of the greatest expanding industries in the world (FAO, 2006).

Larviculture and larvae nutrition are of great importance for aquaculture and the lack of proper nutritional supply has caused low survivals and high deformity rates in fish larvae (Rajkumar and Kumaraguru Vasagam, 2006). Most marine fish larvae are altricial and its digestive track is functionally immature in the early life stages, making them unable to digest inert food (Chen et al., 2006). Despite the recent advances in inert diets for marine larviculture, the culture of most fish larvae still rely on live foods (Cahu and Zambonino Infante, 2001; Conceição et al., 2010). The swimming movement of live food organisms is visually stimulant for fingerlings (Barroso et al., 2013) and its small size as well as its high water content make them easily digestible for fish larvae (Conceição et al., 2010). The most commonly used live feeds in marine larviculture are microalgae, rotifers, artemia and copepods.

Microalgae is commonly used as food source for filter feeder organisms, such as bivalves and larval stages of some marine gastropods (Yúfera and Lubián, 1990), and for production of other aquaculture live feeds, such as rotifers, artemia and copepods (Conceição et al., 2010). Microalgae is also used when employing the "green water" technique, which has proven to improve fish larval feeding, development and survival (Reitan et al., 1997). This technique showed that microalgae preserve the nutritional profile of live prey (Makridis and Olsen, 1999), microalgae ingestion improve fish larvae nutrition (Moffatt, 1981) and enhances the microflora diversity for both the culture medium and the gut of developing larvae (Naas et al., 1992).

Rotifers are commonly used in marine larviculture mainly due to its continuous high population growth rate, small size, reasonable nutritional profile, high tolerance to salinity and temperature variation; moreover, their capability of feeding on a wide variety

of foods, such as microalgae, yeast, bacteria and organic particles (as they are nonselective filter feeders) also makes them appealing for marine larviculture (Conceição et al., 2010). These features make their production relatively low cost and always available, especially for *Brachionus plicatilis*, which is the most used rotifer in aquaculture. Since rotifers are nonselective feeders they can also be enriched with nutrient rich emulsions in other to correct/improve their nutritional content (Conceição et al., 2010).

Artemia (*Artemia* sp.), popularly known as brine shrimp, is mostly used in aquaculture due to its capability of forming dormant cysts that are extremely resistant to adverse conditions and so, can be stored dry and be viable for long periods of time. When needed, cysts can be rehydrated and hatch in less than 24 h, making artemia the less labour-intensive live feed available in the industry and thus the most convenient for commercial scale larviculture (Lavens and Sorgeloos, 2000). After hatching, artemia nauplii (its first developmental stage) do not feed and rely on its endogenous reserves to thrive (thus being the most nutritious stage for larviculture purposes). After 6 to 8 hours post hatch, the now called artemia metanauplii (second development stage) is already able to feed (Dhont, J. and Van Stappen, 2003). Similarly to rotifers, artemia metanauplii are filter feeders and can also be enriched with nutrient emulsions. Together with rotifers, artemia represents the overwhelming majority of the total number of live feeds used in marine aquaculture (Conceição et al., 2010).

In the natural environment, copepods represent the majority of the diet of marine fish larvae, thus being only logical to use them for marine larviculture (Støttrup, 2000). Copepods nutritional value is considered to be superior to artemia and rotifers, mainly due to its content in high polyunsaturated fatty acids (PUFAs), and especially, highly unsaturated fatty acids (HUFAs) (Bell and Sargent, 2003). Fish larvae development and survival depend on the intake of certain HUFAs, namely $C_{22:6}$ (n3) docosahexanoic acid (DHA), $C_{20:5}$ (n3) eicosapentaenoic acid (EPA) (Pinto et al., 2013) and minor amounts of $C_{20:4}$ (n6) arachidonic acid (ARA) (Bell and Sargent, 2003). Ratios of DHA/EPA >2 and EPA/ARA >20 also observed for copepods (van der Meeren et al., 2008), have shown to be crucial for fish larvae nutrition (Bell and Sargent, 2003). Artemia and rotifers commonly need to be “enriched”, in order to meet the nutritional requirements of marine fish larvae (especially for Artemia which solely shows trace levels of DHA) (Conceição et al., 2010). Nonetheless, the nutritional profile of copepods still outperforms that of enriched Artemia

and rotifers in terms of fish larvae performance (Rajkumar and Kumaraguru Vasagam, 2006; van der Meeren et al., 2008). Furthermore, copepods are also a rich source of antioxidants such as astaxanthine and vitamins C and E (van der Meeren et al., 2008).

A significant amount of marine fish is successfully reared with natural zooplankton (mostly composed of copepods), produced extensively in ponds or collected from the wild (Lee et al., 2007; Støttrup, 2003). Nonetheless, the use of natural zooplankton in intensive aquaculture is impracticable due to the changes in availability caused by seasonality, as well as by sampling difficulty (Støttrup, 2003). Furthermore, natural zooplankton is also known to be a vector for pathogens (Johnson et al., 2006; Su H-M et al., 2007), which limits its use in commercial scale larviculture (as it impairs any efficient biosecurity protocol to be implemented).

In this way, the superior quality of copepods for marine larviculture has already been recognised and the increasing interest for controlled intensive copepod culturing is of little surprise in 21st century aquaculture. The main copepod species selected for production belong to orders Calanoida and Harpacticoida (Støttrup, 2003). Copepods with short life cycles and highly resistant to temperature and salinity changes are preferred for larviculture purposes. Another important trait for the use of copepods on larviculture is its planktonic lifestyle, or planktonic nauplii stages, as fish larvae commonly prey on the water column (Støttrup, 2003). Culture densities are a constraint for the economic feasibility of intensive cultivation, mainly for Calanoid copepods, that can only be maintained at low densities (rarely exceeding 2 copepods per mL) and commonly experience a decrease in fecundity and an increase in cannibalism due to overcrowding (Støttrup, 2003). Harpacticoid copepods can be cultured at higher densities without productivity decline and are able to feed on microalgae, yeast and organic particles (Støttrup, 2006), whereas calanoid copepods require a marine microalgae based diet, rich in n3 PUFA in order to achieve a good culture performance (Jónasdóttir, 1994). Nonetheless, a major setback for the use of most harpacticoid copepod species for marine fish larviculture is its benthic nature (in opposition to the pelagic lifestyle of calanoid copepods) (Støttrup, 2003). Some calanoid species have promoted positive results in the larviculture of a range of marine fish species (Barroso et al., 2013; Kortner et al., 2011; Olsen et al., 2014; Wilcox et al., 2006). Despite the significant breakthroughs already

achieved in copepod cultivation (Drillet and Lombard, 2013; Drillet et al., 2014b, 2006), optimization protocols must still be pursued (Støttrup, 2000).

The calanoid copepod *Acartia tonsa* Dana, 1849 (Figure I) is considered to be a strong candidate for aquaculture and has been strongly investigated in order to pursuit and establish culture protocols (Drillet and Lombard, 2013; Drillet et al., 2014a, 2014b; Ismar et al., 2008; Zhang et al., 2014). The value of *A. tonsa* value resides on its capability to continuously produce eggs over its three week adult life span and the fact that the eggs sink to the bottom, allowing them to be easily sorted from culture tanks (Støttrup et al., 1986). Additionally, eggs can be cold storage for later use (Drillet et al., 2014a; Støttrup et al., 1986; Zhang et al., 2014) and newly hatched nauplii display a high nutritional profile (Støttrup et al., 1999).

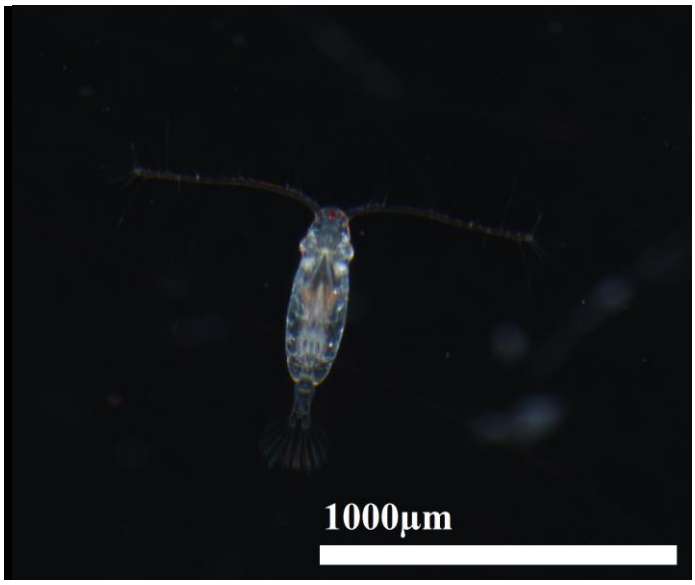

Figure I. *Acartia tonsa* adult female. Photography by P. Aires.

Acartia tonsa is an omnivorous copepod that tends to carnivorous and cannibalistic when overcrowded or exposed to starvation (Boersma et al., 2014; Drillet and Dutz, 2014; Drillet et al., 2014a; Ismar et al., 2008; Lonsdale et al., 1979; Stoecker and Egloff, 1987). Furthermore, its nutritional profile can be adjusted with different microalgal diets (Støttrup, 2000), and it was already revealed that it is possible to increase its PUFAs content with diet supplementation using heterotrophic protists (Veloza et al., 2006). Overall, it is therefore of paramount importance to better understand the carnivorous behaviour of *A. tonsa* and its potential implications for marine larviculture.

Altogether, the aims of this study are to i) evaluate the predation of *A. tonsa* over *Artemia franciscana* nauplii and the rotifer *Brachionus plicatilis*, having in account the potential role of the presence of microalgae and water temperature may have on the copepod feeding behaviour, and ii) evaluate the effect on egg production, hatchability and fatty acid profile (eggs and adults), when *A. tonsa* feeding regime is supplemented with two common "subproducts" present in marine hatcheries: decapsulated artemia cysts (*A. franciscana*) whose hatchability is already compromised and artemia metanauplii (*A. franciscana*) whose nutritional profile makes them inadequate for marine larviculture.

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II. Trophic interactions between the copepod *Acartia tonsa* and other live feeds commonly used in marine larviculture.

1. Introduction

The successful culture of marine fish larvae in captivity is commonly dependent on the use of live prey as primary food source, since the digestive system of marine fish larvae is functionally immature and cannot process inert diets (Chen et al., 2006). In contrast to inert diets, the high water content present in live feeds makes them easy to digest (Conceição et al., 2010) and its motion provides visual stimuli for fish larvae that triggers its predatory feeding behaviour (Barroso et al., 2013). The most commonly used live feeds in marine fish larviculture are rotifers, artemia nauplii and copepods (Conceição et al., 2010). Marine fish larvae require n3 polyunsaturated fatty acids (PUFAs) such as docosahexanoic acid $C_{22:6}$ (n3) (DHA) and eicosapentaenoic acid $C_{20:5}$ (n3) (EPA) for good development and survival (Pinto et al., 2013). Since both rotifers and Artemia exhibit sub-optimal levels of these fatty acids (FA), they commonly need to be enriched to correct/enhance their nutritional profile (Sargent et al., 1997).

Copepods are the natural food for the majority of marine fish larvae in the wild, thus being logical to evaluate their potential as live food in marine larviculture (Støttrup, 2000). Studies showed that copepods are capable of synthetizing highly polyunsaturated fatty acids (HUFAs) from its PUFAs precursors (Nanton and Castell, 1999), consequently presenting a higher DHA and EPA content than the most commonly used live preys in marine larviculture – artemia and rotifers. Several studies demonstrated that copepods enhance larval development and survival when compared to rotifers or artemia (Drillet et al., 2011, 2006; Hamre et al., 2002; McEvoy et al., 1998; Norsker and Støttrup, 1994; Rajkumar and Kumaraguru Vasagam, 2006; Støttrup et al., 1999). Copepods are also rich in antioxidants, such as astaxanthine and vitamins C and E (Barroso et al., 2013), therefor being commonly considered to be a superior food source to marine fish larvae.

Despite the promising results achieved in marine larviculture when employing copepods as live food, their use at a commercial level is somehow impaired due to the difficulty of maintaining high densities of production (Støttrup, 2000). Nonetheless, a number of efforts have already been made towards achieving effective cultivation methods (Drillet and Lombard, 2013; Drillet et al., 2014b, 2006). Several studies have also shown the success of copepod usage in mesocosm feeding approaches (Papadakis et al., 2013;

Papandroulakis et al., 2005) and co-feeding scenarios (Barroso et al., 2013; Kortner et al., 2011; Olsen et al., 2014; Wilcox et al., 2006).

The Calanoid copepod *Acartia tonsa* Dana, 1849 has been pointed as strong candidate for aquaculture, mainly due to the continuous egg laying ability displayed by its females, as well as egg negative buoyancy (making them easy to collect) and egg cold storage properties (Drillet et al., 2014a; Støttrup et al., 1986; Zhang et al., 2014). Despite copepods being traditionally considered as grazers, *Acartia tonsa* is in fact an omnivorous feeder that tends to carnivorous, or even cannibalistic, when overcrowded or exposed to starvation scenarios (Boersma et al., 2014; Drillet and Dutz, 2014; Drillet et al., 2014a; Ismar et al., 2008; Lonsdale et al., 1979; Stoecker and Egloff, 1987).

Since larviculture using mesocosm approaches and co-feeding protocols are often employed for some marine fish species, it is important to understand the potential trophic interactions that may occur between *A. tonsa* and other live feeds. While it is already known that *A. tonsa* feeds on artemia nauplii (Anraku and Omori, 1963) and rotifers (Stoecker and Egloff, 1987), no study has yet been performed on the clearance percentage induced by this copepod on these organisms. Veloza et al., (2006) conducted a study to evaluate FA trophic modifications of *A. tonsa* fed with two heterotrophic protists. However, the effect that dietary shifts may have on the fatty acid profile of this copepod has not been monitored along with the survey of its fecundity and fertility.

In this way, the present study aims to i) evaluate the predation of *A. tonsa* over *Artemia franciscana* nauplii and the rotifer *Brachionus plicatilis*, taking into account the potential role that the presence of microalgae and water temperature may have on the copepod feeding behaviour, and ii) evaluate the effect on egg production, hatchability and FA profile (eggs and adults), when the feeding regime of *A. tonsa* is supplemented with two "subproducts" commonly present in marine hatcheries: decapsulated cysts of *A. franciscana* whose hatchability is already compromised and *A. franciscana* metanauplii whose nutritional profile makes them inadequate for marine larviculture.

2. Material and methods

2.1. Live feed cultures

2.1.1. Microalgae culture

Rhodomonas lens (CCMP 739) and *Isochrysis galbana* (CCMP 1324) were purchased from AQUALGAE and cultured in 2 L and 6 L sterilized glass flasks. Culture medium consisted of GF/C filtered and sterilized synthetic sea water (SSW) with a salinity of 33 (Tropic Marin® Sea Salt), enriched with the commercial medium GOLDMEDIUM (AQUALGAE) and gentle filtered aeration (0.2 μm). Conditions were kept at 18±1 ˚C, 14 h : 10 h light : dark cycle (400 PAR lighting). Cultures were partially renewed every 2 days (50% during late exponential growth phase) and totally renewed approximately every two weeks. Cellular densities (cell mL^{-1}) were calculated based on 575 nm spectrophotometry linear regression equation (R=0.95).

2.1.2. Rotifer culture

Rotifers (*Brachionus plicatilis*) were cultured in a 12 L acrylic tank according to Lubzens, E. and Zmora (2003) using small-scale laboratory cultures and fed *ad libidum* with *I. galbana*. Culture densities ranged from 100 to 150 organisms per mL.

2.1.3. Artemia decapsulation and eclosion

The protocol described by Stottrup and McEvoy (2003) (see Appendix II) for the decapsulation procedure of artemia cysts was followed to remove the corion of brine shrimp eggs (NEW ERA® Artemia cysts). Whenever necessary, due to the logistics of the experimental procedures employed, decapsulated cysts were stored at 4 ˚C in hypersaline solution for a maximum of 10 days for later use. If stored decapsulated cysts were not used in the experimental setups within that time frame (10 days post decapsulation) they were discarded and a new batch of cysts was decapsulated as described above.

Eclosion was performed on conical shaped acrylic tanks containing 1 L of SSW with a salinity of 33, temperature of 26±1 ˚C, continuous 150 PAR lighting and strong aeration. Artemia nauplii were collected after 24 h and both metanauplii and unhatched decapsulated cysts were collected after 48 h.

2.1.4. Copepod culture

Copepods (*Acartia tonsa*, Dana) were obtained from cold preserved eggs provided by IPL-Escola Superior de Turismo e Tecnologia do Mar de Peniche, from a stock population maintained under culture conditions for more than one year. Copepods were cultured in 12 L acrylic cylindrical shaped tanks containing 10 L of SSW with a salinity of 35 and gentle aeration. Tanks were maintained at 18±1˚C and 14 h : 10 h light : dark cycle. Cultures were feed daily with *R. lens ad libidum* (approximately 2.5×10^4 cell mL^{-1}) and cleaned 3 times per week (20% partial water change) plus one weekly total water change. In order to avoid cannibalism, adults, copepodites and nauplii/eggs were sorted in different tanks using a 200 µm, a 125 µm and a 64 µm mesh, respectively whenever water changes were performed.

2.2. Trophic interactions of *A. tonsa* with *A. franciscana* nauplii and *B. plicatilis*

2.2.1. Predatory behaviour test

Artemia nauplii (A) and the rotifer *B. plicatilis* (R) were exposed to female adult copepods (CF), male adult copepods (CM), copepods in the stage of copepodite (Ci) and in the stage of nauplii (Cn), in order to test the predatory behaviour of *A. tonsa* on commonly used live feeds. A total of 14 treatments were performed after single variable combination [(CF), (CM), (Ci), (Cn), (A), (CF+A), (CM+A), (Ci+A), (Cn+A), (R), (CF+R), (CM+R), (Ci+R), (Cn+R)], each with 30 replicates performed in a plastic Petri dish containing 10mL of SSW with a salinity of 35. Animal densities used were: CF and CM − 0.1 organism mL^{-1} (1 per dish); Ci − 1.5 organism mL^{-1} (15 per dish); Cn − 5 organism mL^{-1} (50 per dish); A − 3 organism mL^{-1} (30 per dish); R − 5 organism mL^{-1} (50 per dish), as these values were within the ranges commonly used in fish larviculture studies (Barroso et al., 2013; Ismar et al., 2008; Luizi et al., 1999; Olsen et al., 2014). All animals used in the treatments described above were collected from the lab cultures and softly rinsed with SSW prior to use. CF, CM, Ci and A were manually counted, whereas, due to its smaller size, Cn and R densities were estimated using a given volume pipetted to the plastic Petri dish where experimental trials were performed. All replicates were maintained at 20±1 ˚C and 16 h : 8 h light : dark cycle (85 PAR lighting). After 24 h, each plate was observed under a wide zoom stereo microscope (Olympus SZX16) and live animals counted to estimate survival.

2.2.2. Female:Male proportion test

To investigate if copepod predatory effect on artemia nauplii was gender dependent, 3 female:male (♀:♂) proportions were used: 5♀:0♂, 1♀:4♂ and 0♀:5♂. Seven treatments were assigned [(5♀:0♂), (1♀:4♂), (0♀:5♂), (A), (5♀:0♂+A), (1♀:4♂+A), (0♀:5♂+A)], each with 10 replicates performed in glass flasks containing 50mL of SSW with a salinity of 35. Animal densities per flask were the same as described above (see 2.2.1): CF and CM − 0.1 organism mL^{-1} (5 per flask) and A − 3 organism mL^{-1} (150 per flask). All other conditions and procedures were identical to those previously described (see 2.2.1).

2.2.3. Temperature and food availability test

Based on the data from the experiments described above (see 2.2.1 and 2.2.2), an experimental trial was performed to test the effect of temperature and food availability on the interactions between CF and A. Three densities of the microalgae *R. lens* (Ma) were tested as food source for CF: none, low density − 10^4 cells mL^{-1} ($Ma10^4$) and high density − 10^5 cells mL^{-1} ($Ma10^5$). Four temperatures were also evaluated to determine how copepod predatory behaviour could shift (10±1 ˚C, 15±1 ˚C, 20±1 ˚C and 25±1 ˚C). A total of 9 treatments were performed to ascertain the effect of food availability on the predation of *Artemia* [(CF); (CF+A); (CF+$Ma10^4$); (CF+A+$Ma10^4$); (CF+$Ma10^5$); (CF+A+$Ma10^5$); (A); (A+$Ma10^4$); (A+$Ma10^5$)] and a total of 12 treatments were performed to investigate how this parameter could shift the predatory behaviour of CF [(CF 10 ˚C); (A 10 ˚C); (CF+A 10 ˚C); (CF 15 ˚C); (A 15 ˚C); (CF+A 15 ˚C); (CF 20 ˚C); (A 20 ˚C); (CF+A 20 ˚C); (CF 25 ˚C); (A 25 ˚C); (CF+A 25 ˚C)]. Replicate number, conditions and procedures were identical to those described above (see 2.2.1).

2.3. Effect of feed supplementation on egg production, egg hatchability and fatty acid profile of *A. tonsa*

This experiment was designed to investigate egg production and hatchability in *A. tonsa*, as well as egg and adult copepods fatty acid (FA) profile, when the base food source for A. tonsa (the microalgae *R. lens*) was supplemented with unhatched artemia decapsulated cysts (Ac) and metanauplii (Am).

2.3.1. Egg production and egg hatchability test

Two Ma densities were tested [low density − 10^4 cells mL^{-1} (Ma 10^4) and average density − 5×10^4 cells mL^{-1} (Ma 5×10^4)], with a total of 6 treatments being performed [(Ma 10^4), (Ma 10^4+Ac), (Ma 10^4+Am), (Ma 5×10^4), (Ma 5×10^4+Ac), (Ma 5×10^4+Am)] each with 11 replicates performed in a plastic Petri dish containing 10mL of SSW with a salinity of 35. One adult female copepod and one adult male copepod (both 3-4 day C6 stage) were randomly collected from the culture tank and transferred for each replicate plastic Petri dish. Animals were feed with the respective Ma concentration of the experimental treatment being performed, with treatments A_c and A_m being supplemented with two cysts (2 per female) and one metanauplii (1 per female) per dish respectively. All replicates were maintained at a 18±1 ˚C and 14 h : 10 h light : dark cycle (140 PAR lighting). After 24 h, eggs were counted and copepods were pipetted to new dishes with new food. The eggs were kept in the same dishes under the same conditions and unhatched eggs were counted after 48 h. The number of hatched nauplii after 48 h was accessed by subtracting the number of unhatched eggs from the total egg count (hatched nauplii = total egg count-unhatched eggs). The procedure was repeated along 8 days.

2.3.2. Fatty acid profile test

Three treatments (Ma; Ma+Ac; Ma+Am), each with 6 replicates were designed to investigate FA profile of *A. tonsa* when provided such contrasting feeding regimes. Each replicate was performed in a cylindrical shaped glass tank containing 2 L of SSW with a salinity of 35and soft aeration. Tanks were maintained at 18±1 ˚C and 14 h : 10 h light : dark cycle (140 PAR lighting). Copepod eggs produced were collected from the culture tanks and transferred to the experiment cylindrical shaped glass tanks (~4000 eggs per tank), fed every day *ad libidum* with Ma and grew for 21 days, until they had reached C6 stage. These adult copepods were then fed *ad libidum* with Ma, with tanks from treatments A_c and A_m being supplemented with ~4000 decapsulated cysts per tank and ~2000 metanauplii per tank, respectively for a period of 7 days. All tanks were cleaned daily, with 20% of the culture water being changed during this process. Egg and adult copepod samples for biochemical analysis were collected using a 64 µm mesh and a 200 µm mesh, respectively, 24 h after the last feeding. Food samples (Ma, Ac and Am) were also

collected for biochemical analysis. All samples were rinsed in distilled water, frozen at -20 ˚C and freeze-dried prior to FA analysis.

The Bligh and Dyer, (1959) method was used for total lipid extraction. Samples were resuspended in 2 ml Eppendorf's, using 500 µL of chloroform/methanol (1:2, V/V), incubated on ice for 30 minutes, then added 250 µL of the internal standard solution − heneicosanoic acid methyl ester ($C_{21:0}$) in chloroform (34.92µg mL^{-1}). Samples were centrifuged at 8000 rpm for 10 min at room temperature and the top organic phases transferred to glass centrifuge tubes. The organic phases were dried under a nitrogen stream and preserved at -20 °C for further analysis.

FAs were analysed by gas chromatography–mass spectrometry (GC-MS) after total lipid extracts transesterification. Fatty acid methyl esters (FAME) were prepared using a methanolic solution of potassium hydroxide (2.0 M) according to Aued-Pimentel et al., (2004) method. FAME were resuspended in 40-80 µL of n-hexane, with 2 µL of this solution being used for GC-MS analysis on an Agilent Technologies 6890 N Network (Santa Clara, CA) equipped with a DB-FFAP column with 60 m of length, 0.25 mm of internal diameter, and 0.25 µm of film thickness (J&W Scientific, Folsom, CA). The GC was connected to an Agilent 5973 Network Mass Selective Detector operating with an electron impact mode at 70 eV and scanning the range m/z 50–550 in a 1 s cycle in a full scan mode acquisition. The oven temperature was programmed from an initial temperature of 90 °C, standing at this temperature for 0.5 min, following a linear increase at 14.4 °C min^{-1} to 220 °C, then at 10 °C min^{-1} to 240 °C and 5 °C min^{-1} until 250 °C. The injector and detector temperatures were 220 and 280 °C respectively. Helium was used as carrier gas at a flow rate of 0.5 mL min^{-1}. FAME identification was performed by comparing retention times and mass spectrum, analysed with the MS spectra of commercial FAME standards (Supelco 37 Component FAME Mix) and confirmed by comparison with the Wiley chemical database and the spectral library "The AOCS Lipid Library". FAME quantification was performed by area comparison between identified FAMEs and the internal standard. The identified FAMEs weight was calculated by the equation:

$$W_{FAME} = \frac{A_{FAME} \cdot W_{21:0}}{A_{21:0} \cdot W_{sample}}$$

where W_{FAME} is the identified FAMEs weight (µg mg^{-1} of dry weight); A_{FAME} is the identified FAMEs area; $W_{21:0}$ is the internal standard weight added to the sample; $A_{21:0}$ is the internal standard area; W_{sample} is the sample weight.

2.6. Statistical analysis

Survival (copepod, Artemia and rotifer) and egg hatching percentages were tested using Kruskal-Wallis ANOVA on Ranks and egg production was tested using two-way ANOVA. When differences were found significant ($P < 0.05$), a *post hoc* Tukey's multiple comparison test was used. All statistical analyses were performed using Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA).

FA analysis was performed using Primer 6.0 software (Primer-E Ldt, Lutton, UK) by performing a PERMANOVA to analyse differences in the pool of FA recorded for the different samples analysed. A pre-treatment transformation log(x+1) was performed on the original data and Bray Curtis Similarity resemblance matrixes were later computed and analysed by principal coordinates (PCO) analysis (0.5 Spearman correlation vectors). Due to the small permutation numbers, the Monte Carlo test p-values ($p<0.05$) was used for pairwise comparisons (Anderson J., et al., 2008). Similarity percentages routine (SIMPER) analyses were performed to determine dissimilarities between treatments and the FA that mostly contribute for those differences.

All data presented in this study are expressed as mean ± standard deviation.

3. Results

3.1. Trophic interactions of *A. tonsa* with *A. franciscana* nauplii and *B. plicatilis*

Results from the 24 h predatory test showed that the presence of copepods significantly reduce both artemia nauplii and rotifer survival, as shown on Fig.1. Artemia survival significantly ($p<0.05$) decreased to 78.4±20.7% when exposed to adult male copepods and to 10.7±20.6% when exposed to adult female copepods. Artemia survival did not significantly differ when nauplii were exposed to copepodite and nauplii stage copepods. Rotifer survival significantly ($p<0.05$) decreased to 70.5±14.5%, 59.5±11.7% and 62.5±17.5% when exposed to copepod nauplii, copepodites and adult male copepods, respectively. Nonetheless, the most notable decrease was that recorded for CF (26.7±19.1%).

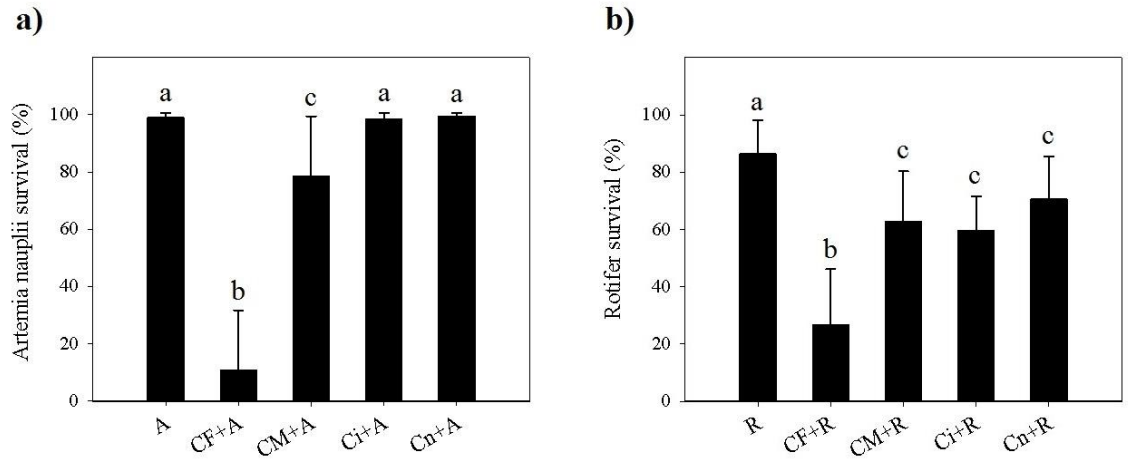

Fig. 1. a) *Artemia franciscana* nauplii survival and **b)** *B. plicatilis* survival concerning predatory behaviour test (A – artemia nauplii control; CF+A – artemia nauplii exposed to adult female copepods; CM+A – artemia nauplii exposed to adult male copepods; Ci+A – artemia nauplii exposed to copepodites; Cn+A – artemia nauplii exposed to copepod nauplii; R – rotifers control; CF+R – rotifers exposed to adult female copepods; CM+R – rotifers exposed to adult male copepods; Ci+R – rotifers exposed to copepodites; Cn+R – rotifers exposed to copepod nauplii). The results are expressed as mean percentages ± standard deviation (n=30). Different letters represent significant differences between treatments ($p<0.05$).

The results from female:male proportion test showed that the presence of adult female copepods significantly reduced artemia nauplii survival, as shown on Fig 2. Artemia survival significantly ($p<0.05$) decreased to 30.7±25.3% and 38.5±21.4% when exposed to 5♀:0♂ and 1♀:4♂ adult female:male copepod ratio, respectively, although no significant difference ($p<0.05$) was found between those two ratios. The decrease recorded in artemia survival to 83.6±9.0% when exposed solely to adult male copepods was not significantly different ($p>0.05$) from that survival recorded in the absence of copepods.

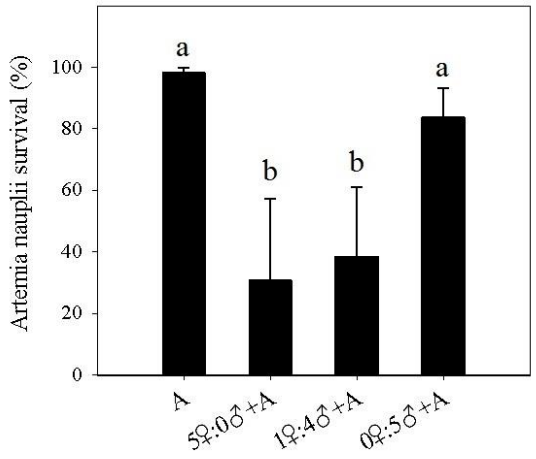

Fig. 2. Artemia nauplii survival concerning female:male proportion test (A – artemia nauplii control; 5♀:0♂+A – artemia nauplii exposed only to adult female copepods; 1♀:4♂+A – artemia nauplii exposed to 1:4 adult female:male copepod ratio; 0♀:5♂+A – artemia nauplii exposed only to adult male copepods). The results are expressed as mean percentages ± standard deviation (n=10). Different letters represent significant differences between treatments ($p<0.05$).

As shown on Fig. 3, results from the temperature and food availability test showed that both tested temperatures and Ma densities significantly affect *A. tonsa* predatory behaviour ($p<0.05$ for both). Artemia survival showed no significant differences ($p>0.05$) between any control when subjected to the tested temperatures (>98.0±2.0%) or tested Ma densities (>97.0±3.0%). *A. tonsa* predatory behaviour increases with increasing temperature and with decreasing densities of Ma. In the temperature test, the highest artemia survival (CF+A) observed was 59.1±22.1% at 10 °C and the lowest was 6.4±13.4% at 25 °C whereas in the food availability test the highest Artemia survival (CF+A) observed was 61.0±28.0% on a density of 10^5 cell mL^{-1} and the lowest was 10.7±20.9% with no Ma.

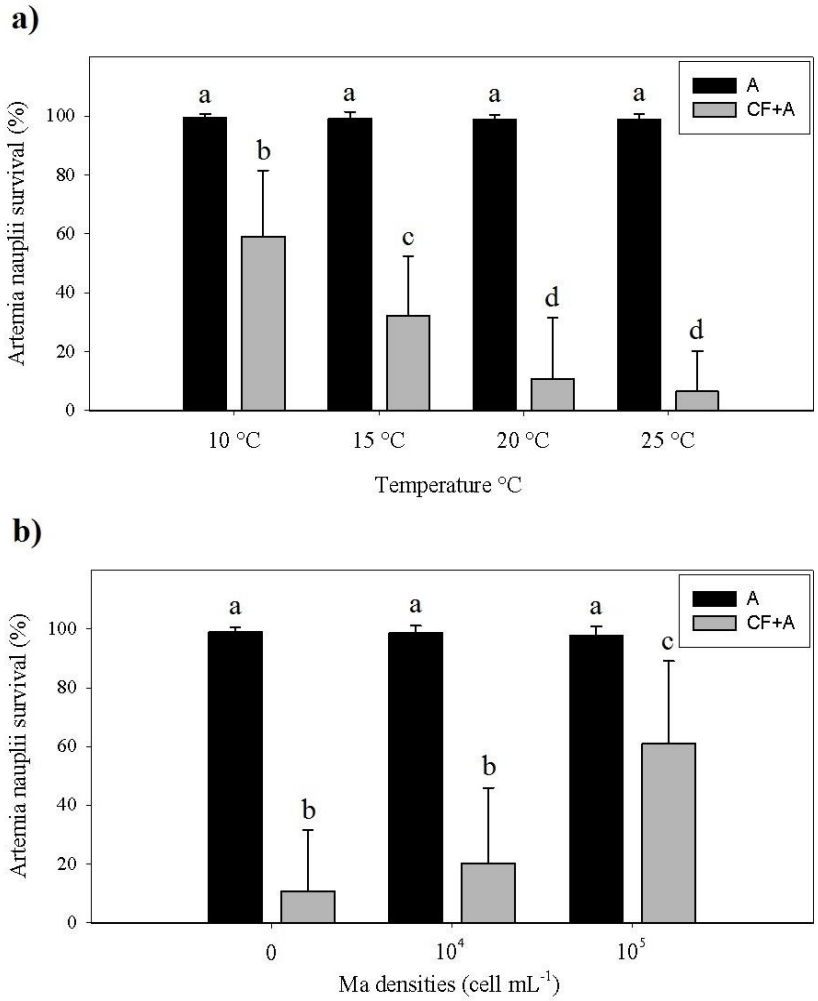

Fig. 3. Artemia nauplii survival concerning temperature and food availability test (A – artemia nauplii control; CF+A – artemia nauplii exposed to adult female copepods); **a)** when subjected to four different temperature conditions (10 °C, 15 °C, 20 °C and 25 °C); **b)** when subjected to three different microalgae (Ma) densities (0 cell mL^{-1}, 10^4 cell mL^{-1} and 10^5 cell mL^{-1}). The results are expressed as mean percentages ± standard deviation (n=30). Different letters represent significant differences between treatments ($p<0.05$).

3.2 Effect of feed supplementation on egg production, egg hatchability and fatty acid profile of *A. tonsa*

As shown on Fig 4. a, significant differences ($p<0.001$) were found between the 2 Ma densities, nonetheless no significant differences ($p>0.05$) were found between Ma, Ma+Ac and Ma+Am diets for both Ma densities. For the highest Ma density the mean 8 days egg production per female was Ma − 325±81, Ma+Ac − 301±94 and Ma+Am − 298±88 whereas for the lowest Ma density was Ma − 167±50, Ma+Ac − 188±49 and Ma+Am − 207±65.

No significant differences ($p>0.05$) were found in the 48 hour hatching percentages (Fig 4. b), between any treatment nor Ma density (percentages ranged from 86.4±18.6% to 96.7±3.1%, mean ± standard deviation).

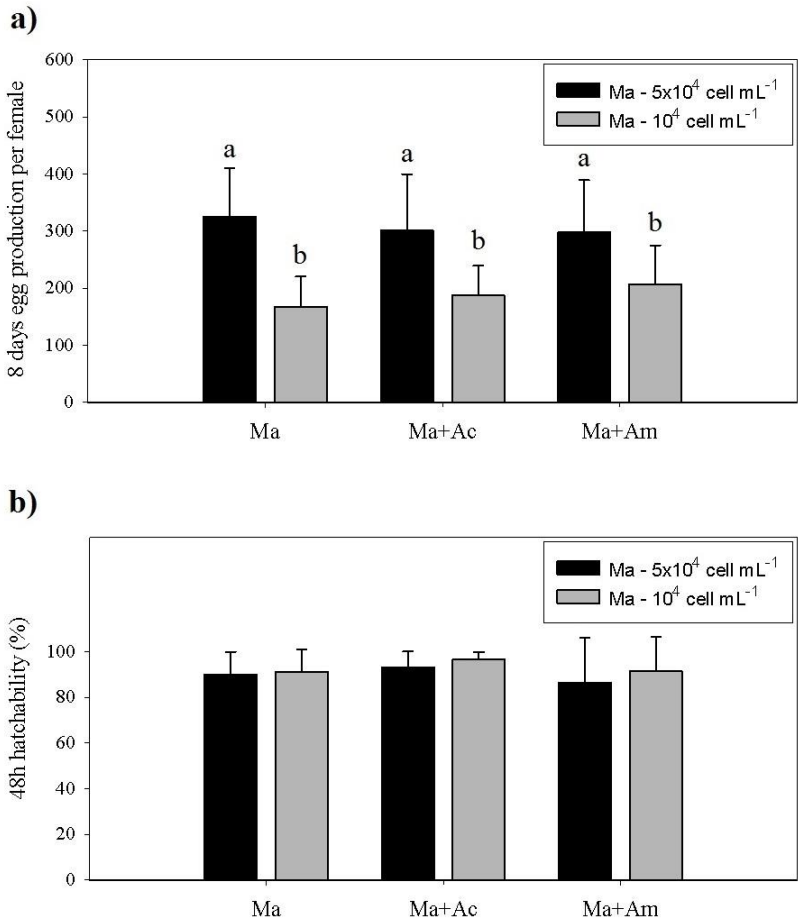

Fig. 4. *Acartia tonsa* **a)** 8 days total egg production per female and **b)** 48 h hatchability concerning egg production and egg hatchability test (Ma – feed only with microalgae; Ma+Ac – feed with microalgae and supplemented with artemia unhatched decapsulated cysts; Ma+Am – feed with microalgae and supplemented with artemia metanauplii) and two base food microalgae (Ma) densities (5×10^4 cell ml^{-1} and 10^4 cell ml^{-1}). The results are expressed as mean number ± standard deviation (n=11). Different letters represent significant differences between treatments ($p<0.05$).

Results from fatty acid profile test are expressed on Table 1. Significant differences ($p<0.05$) were found between the FA contents of the 3 food items. Significant differences ($p<0.05$) were also found in the FA contents of copepod eggs fed the 3 different diets. Regarding adult copepods, significant differences ($p<0.05$) were found between the diet supplemented with artemia metanauplii and the remaining diets, whereas the diet supplemented with decapsulated cysts showed no significant ($p=0.075$) differences with

the Ma control diet. The PCO (Fig. 5) and SIMPER analysis revealed which FA contributed the most for the dissimilarities recorded. These dissimilarities were clearer for copepod eggs (with PCO first two axis explaining 99.0% of all dissimilarities recorded), than for adult copepods (with PCO first two axis explaining 87.3% of all dissimilarities recorded). The SIMPER analysis revealed that the FA that represented > 50% of the dissimilarities recorded in the profile of copepod eggs were: $C_{18:3}$ (n3), $C_{18:1}$ (n7), $C_{20:5}$ (n3), $C_{16:0}$ and $C_{18:4}$ (n3) between Ma and Ma+Ac treatments; $C_{16:0}$, $C_{18:3}$ (n3), $C_{20:5}$ (n3), $C_{14:0}$, and $C_{18:1}$ (n7) between Ma and Ma+Am treatments; $C_{18:1}$ (n9), $C_{18:1}$ (n7), $C_{14:0}$, $C_{18:0}$ and $C_{20:5}$ (n3) between Ma+Ac and Ma+Am treatments. For adult copepods, the SIMPER analysis revealed that the FA that represented > 50% of the dissimilarities recorded were: $C_{18:3}$ (n3), $C_{18:4}$ (n3), $C_{18:1}$ (n7) and $C_{18:1}$ (n9) between Ma and both Ma+Ac and Ma+Am treatments; $C_{18:1}$ (n9), $C_{18:4}$ (n3), $C_{20:5}$ (n3), $C_{16:0}$ and $C_{18:0}$ and between Ma+Ac and Ma+Am treatments.

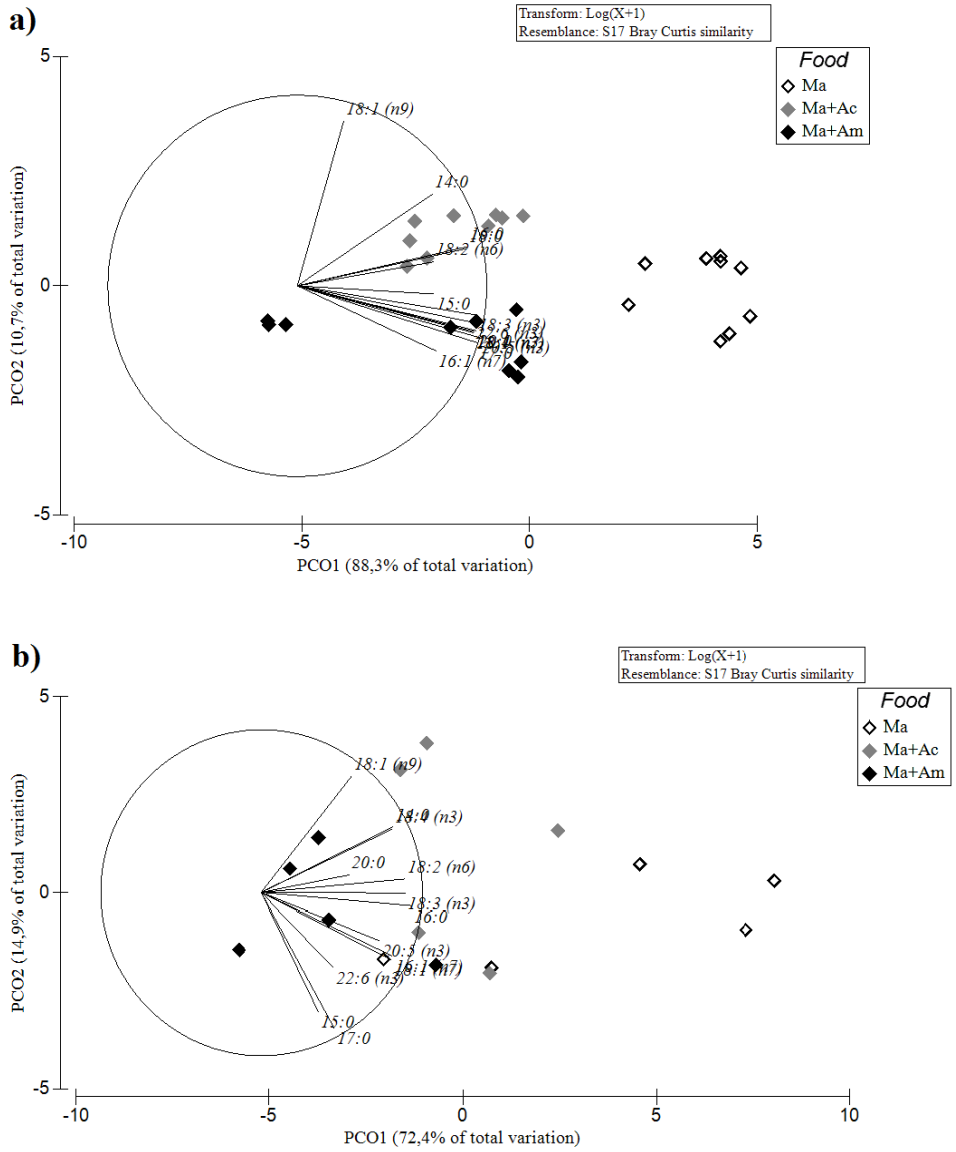

Fig. 5. Principal components analysis (PCO) of FA in copepod **a)** egg samples and **b)** adult samples concerning fatty acid profile test (Ma – feed only with microalgae; Ma+Ac – feed with microalgae and supplemented with artemia unhatched decapsulated cysts; Ma+Am – feed with microalgae and supplemented with artemia metanauplii). Spearman correlation vectors show the coefficients for each FA.

Table 1. FA composition (µg mg^{-1} of dry weight) of *A. tonsa* eggs and adults, feed with different diets (Ma – *R. lens;* Ac – unhatched decapsulated artemia cysts; Am – artemia metanauplii) and food FA composition. Results are expressed as mean values ± standard deviation. Different letters represent significant differences between treatments ($p<0.05$).

	A. tonsa eggs			*A. tonsa* adults			Diets		
	Ma [a] n=9	Ma+Ac [b] n=9	Ma+Am [c] n=9	Ma [a] n=5	Ma+Ac [a] n=5	Ma+Am [b] n=5	Ma [a] n=3	Ac [b] n=3	Am [c] n=3
14:0	2.81±0.21	2.42±0.19	2.01±0.11	1.05±0.29	0.82±0.08	0.67±0.09	0.91±0.06	2.6±0.06	1.53±0.02
15:0	0.14±0.02	0.01±0.01	0.1±0.02	0.07±0.01	0.05±0.01	0.05±0.01	nf	1.01±0.01	0.69±0.01
16:0	12.27±0.81	9.42±0.045	8.77±0.57	6.44±0.57	5.88±0.64	5.25±0.42	5.14±0.19	18.32±0.16	14.11±0.2
16:1 (n7)	1.07±0.14	0.87±0.04	0.93±0.16	0.4±0.08	0.34±0.06	0.27±0.08	nf	17.97±0.38	14.19±0.18
16:2 (n6)	nf	nf	nf	nf	nf	nf	nf	0.66±0.1	0.3±0.04
17:0	0.97±0.07	0.68±0.03	0.73±0.1	0.96±0.1	0.8±0.2	0.83±0.1	0.06±0.01	1.62±0.04	1.66±0.01
17:1 (9)	nf	nf	nf	nf	nf	nf	nf	3.74±0.03	3.53±0.13
18:0	4.24±0.14	3.69±0.41	3.32±0.21	4.22±0.15	4.33±0.27	3.79±0.31	3.36±0.07	9.65±0.07	10.49±0.14
18:1 (n9)	2.52±0.29	2.73±0.14	2.0±0.2	0.98±0.41	1.12±0.31	0.69±0.12	1.45±0.01	28.85±1.36	23.77±0.32
18:1 (n7)	5.11±0.23	3.66±0.12	3.92±0.06	1.97±0.34	1.36±0.26	1.23±0.13	2.24±0.05	12.9±0.25	14.24±0.39
18:2 (n6)	0.96±0.07	0.83±0.12	0.77±0.03	0.3±0.06	0.27±0.05	0.2±0.03	0.53±0.01	7.93±0.16	5.46±0.05
18:3 (n6)	nf	nf	nf	nf	nf	nf	nf	0.95±0.04	0.54±0.01
18:3 (n3)	12.54±0.49	9.27±0.42	9.47±0.69	3.28±0.93	2.08±0.15	1.8±0.16	4.1±0.08	12.85±0.08	10.29±0.15
18:4 (n3)	7.47±0.58	5.85±0.11	5.81±0.58	1.85±0.65	1.23±0.18	0.95±0.15	2.6±0.2	3.36±0.08	2.13±0.02
20:0	0.32±0.01	0.24±0.01	0.26±0.02	0.13±0.02	0.1±0.01	0.09±0.01	nf	nf	nf
20:1 (n9)	nf	nf	nf	nf	nf	nf	0.2±0.02	0.91±0.07	0.75±0.01
20:4 (n6)	nf	nf	nf	nf	nf	nf	nf	1.51±0.08	1.42±0.01
20:5 (n3)	9.65±0.45	7.24±0.26	7.44±0.87	7.23±0.53	7.32±0.85	6.63±0.62	1.51±0.03	16.62±0.09	15.97±0.23
22:6 (n3)	9.734±0.24	7.79±0.17	7.68±0.98	10.75±0.3	10.68±0.98	10.2±0.81	1.31±0.04	nf	nf
∑SFAs[1]	20.75±1.01	16.55±1.05	15.18±0.94	12.86±0.82	11.97±0.72	10.68±0.66	9.47±0.29	33.49±0.57	28.49±0.35
∑MUFAs[2]	8.7±0.5	7.26±0.22	6.86±0.92	3.36±0.78	2.83±0.3	2.19±0.28	3.89±0.04	64.08±1.69	56.48±0.78
∑PUFAs[3]	40.37±1.45	30.99±0.97	31.16±3.07	23.41±1.99	21.58±1.96	19.78±1.52	10.05±0.08	43.89±0.29	36.12±0.43
DHA/EPA	1.01±0.03	1.08±0.02	1.03±0.02	1.5±0.14	1.46±0.04	1.54±0.03	0.87±0.01	–	–
Total	69.82±2.385	54.8±2.05	53.2±4.89	39.62±3.44	36.38±2.81	32.65±2.22	23.4±0.39	141.46±1.61	121.08±1.35

nf – not found; DHA – 22:6 (n3); EPA – 20:5 (n3)
[1] includes 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0
[2] includes 16:1 (n7), 17:1 (n9), 18:1 (n9), 18:1 (n7) and 20:1 (n9)
[3] ncludes 16:2 (n6), 18:2 (n6), 18:3 (n6), 18:3 (n3), 18:4 (n3), 20:4 (n6), 20:5 (n3) and 22:6 (n3)

4. Discussion

4.1 Trophic interactions of *A. tonsa* with *A. franciscana* nauplii and *B. plicatilis*

The present study showed that adult *A. tonsa* copepods predate on both artemia nauplii and *B. plicatilis* rotifers. Our results are in accordance with the study performed by Anraku and Omori (1963), where it was shown that *A. tonsa* can feed on *Artemia* sp.. Our data also is also in agreement with that of Stoecker and Egloff (1987), who reported that *A. tonsa* can predate on rotifers, as well as other zooplankton. Anraku and Omori (1963) also referred that the omnivorous behaviour of *A. tonsa* is likely to be correlated with its mouth parts structure, has it enables them to have both a filter feeder and a raptor feeding behaviour. Results from this study also showed that predation on artemia is higher than the one recorded for rotifers, a feature that can be explained by copepod preference on larger sized prey (Berggreen et al., 1988; Stoecker and Egloff, 1987). Prey detection by copepods is known to be related to velocity and water disturbance caused by prey items (Kiørboe et al., 1999). *A. tonsa* ambush feeding behaviour is more efficient on fast swimming organisms than on smaller and/or slow swimming ones (Jakobsen et al., 2005). Taking this into account, it would be expected that artemia would be more heavily predated than rotifers, as *A. franciscana* nauplii have a faster swimming motion when compared to the smaller *B. plicatilis*, which is also likely to cause a greater water disturbance and thus contribute to a higher predation efficiency by *A. tonsa* on this prey.

In this study, *A. tonsa* copepodite and nauplii stages had a negligible impact on both Artemia and rotifer survival. As documented by Stoecker and Egloff (1987), *A. tonsa* nauplii (N3) show a preference for smaller prey items, such as small ciliates (<40µm) and not for larger preys as the one tested in our study (*A. franciscana* nauplii ~300 µm and *B. plicatilis* ~100 µm).

It is important to highlight that although the high artemia nauplii mortality recorded in this study is considered to be caused by copepod carnivorous feeding behaviour, the majority (>90%) of the dead artemia nauplii were found to be only partially consumed. In this way, the fact that *A. tonsa* kills and only partially ingests artemia nauplii may be considered not only a predatory behaviour, but also a potential agonistic response towards a competitor.

In the present study it was verified that *A. tonsa* predation on artemia and rotifers is significantly higher for adult females than for adult males, especially for artemia. *Acartia*

tonsa adult females impact on artemia nauplii survival was not significantly different from high and low female:male ratios (5♀:0♂ and 1♀4♂ respectively). Stoecker and Egloff (1987) showed that *A. tonsa* females can increase their egg production when fed on zooplankton and suggested that carnivorous feeding can play an important role in the nutrition of this species. As *A. tonsa* uses nearly all the energy intake from food consumption for somatic growth and egg production (Kiørboe et al., 1985), the higher predatory behaviours registered in this study might be related with the females fecundity. The fact that the copepod mainly kills artemia nauplii instead of consuming them, might indicate the existence of a species preservation mechanism that drives the elimination of potential competitors and therefore increase the odds of offspring survival. Lonsdale et al. (1979) already documented this type of behavioural response in *A. tonsa*, as it shows a preference to prey on other copepod species nauplii than their own.

The data collected from the temperature and food availability test revealed that both significantly affected the copepod predatory behaviour. *A. tonsa* preys significantly less on artemia nauplii at lower (10 ˚C and 15 ˚C) than on higher (20 ˚C and 25 ˚C) temperatures. As temperature lowers, the metabolism of both copepod and artemia decreases, thus reducing its swimming motion and therefore decreasing chance encounters and predation. These results are in accordance with those reported by Kleppel (1992). Regarding the food availability test, the copepod prey significantly less on artemia nauplii in the presence of the microalgae *R. lens*, and its predatory behaviour was more significantly reduced at higher densities of microalgae (1×10^5 cell mL^{-1}). While these results are in accordance with those reported by other authors (Anraku and Omori, 1963; Drillet et al., 2014a; Stoecker and Egloff, 1987), previous studies have also suggested that copepod predatory feeding is independent from the availability of phytoplankton (Boersma et al., 2014; Lonsdale et al., 1979). Stoecker and Egloff (1987) suggested that higher densities of microalgae may influence the copepod capability of detecting zooplankton, therefore reducing its predatory behaviour. Considering this, the decrease of *A. tonsa* predatory behaviour at high microalgae densities can be related by the reduced detection of artemia. The decrease in the predatory behaviour may also be explained by the higher availability of food, therefore reducing the need for carnivory.

4.2 Effect of feed supplementation on egg production, egg hatchability and fatty acid profile of *A. tonsa*

The results from the egg production trial showed that the supplementations of both unhatched artemia decapsulated cysts and metanauplii did not promote any significant effect. However, significant differences were observed between the two densities of *R. lens*. The study performed by Jónasdóttir (1994) revealed that egg production in *A. tonsa* increased with the ingestion of $C_{16:1}$ (n7), $C_{18:0}$, DHA and EPA fatty acids, as well as food with high ratios of DHA/EPA and n3/n6 FA. The FA analysis of *R. lens* revealed that this microalgae displays suitable DHA/EPA and n3/n6 ratios (0.87±0.01 and 18.12±0.6, respectively) and has often been pointed as a good food source for copepod culture (Arndt and Sommer, 2014; Ismar et al., 2008; Støttrup and Jensen, 1990). The FA analysis of artemia cysts and metanauplii showed poor n3/n6 ratios (2.97±0.07 and 3.68±0.03, respectively) and an absence of DHA. Artemia cysts consumption in this test showed to be relatively low (14.77% for higher Ma density and 19.32% for lower Ma density). As cysts are motionless, the lack of a sensory stimulus did not trigger the predatory behaviour of copepods. The consumption of artemia metanauplii was relatively low for the highest density of Ma evaluated (47.8%) and significantly increased for the lower Ma density (80.7%) tested; as already reported for the trophic interaction test, the metanauplii were only partially eaten.

While Pan et al. (2012) reported significant differences between *Acartia bilobata* 48 h egg hatching percentages when fed with different algal diets, the lack of differences recorded in the present study can be attributed to the key role played by Ma in the diet of the copepods. The microalgae *R. lens* was therefore the main factor conditioning egg production, as differences in its availability to *A. tonsa* did not affect egg quality.

Støttrup (2000) showed that copepod nutritional profile can be adjusted with different microalgal diets while Veloza et al. (2006) recorded that it is possible to increase the content *A. tonsa* PUFAs by feeding them with heterotrophic protists. Mullin and Brooks (1967) also reported the successful enrichment of the predator copepod *Rhincalanus nasutus* with *Artemia* sp.. In opposition to these previous findings, our results revealed that the supplementation of artemia produced no significant improvement on the fatty acid profile of *A. tonsa*. Thought Artemia cysts and metanauplii display a FA content much higher than that of *R. lens*, the total FA content exhibited by both copepod eggs and

adults actually decreased for specimens supplemented with artemia. The FA $C_{18:1}$ (n9) represented a high percentage of the total pool of FAs in the composition of both *A. franciscana* cysts and metanauplii (20.39±0.01% and 19.63±0.01%, respectively), whereas for *R. lens* was relatively low (6.19±0.01%). $C_{18:1}$ (n9) was found in greater amounts for both copepod eggs and adults when *A. tonsa* was supplied with the artemia cysts. The diet supplemented with artemia metanauplii had the lowest amount of $C_{18:1}$ (n9) for both copepod eggs and adults, revealing a reduced intake of artemia. In the other hand, $C_{18:3}$ (n3) represented a higher percentage of the total pool of FAs displayed by *R. lens* (17.52±0.01%), whereas it was relatively low for both artemia cysts and metanauplii (9.09±0.01% and 8.5±0.01%, respectively). This single FA contributed the most for the dissimilarities recorded between the FA content of copepod eggs and adults supplemented with different diets. A possible explanation for these results, as well as the reduction in total FA content observed in the artemia supplemented diets, is that brine shrimp can compete with the copepods for available microalgae. The stress of contact generated by the continuous swimming of artemia metanauplii and the higher energy consumption promoted by the copepod raptorial jumps might explain the lower FA contents displayed by *A. tonsa* in these treatments.

5. Conclusion

This study showed that *A. tonsa* is able to significantly predate on *A. franciscana* nauplii and *B. plicatilis*, and that its predatory behaviour is reduced at lower temperatures and higher densities of microalgae (*R. lens*). These findings are highly relevant for larviculture trials performed in mesocosm and/or employing the co-feeding of these live preys. *A. tonsa* is often used in marine fish larviculture, mostly in its naupliar stage. As this species is able to reach the adult stage relatively fast (19 days at 20 ˚C), it is highly recommended to remove all remaining copepods from the culture tank prior to the addition of any other live feeds commonly used for more advanced fish larval stages (e.g. artemia and rotifers). This copepod species may not be suitable for mesocosm larviculture, as it may significantly affect the abundance of other live feeds being supplied and bias the perception of ongoing trophic-dynamics during larviculture.

This study also revealed that the supplementation of unhatched decapsulated cysts and *A. franciscana* metanauplii during the production of *A. tonsa* does not significantly enhances its egg production or hatchability, neither does it significantly improve its FAs profile.

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III. Final considerations

The advantage of copepods in marine larviculture is well established, in particular regarding Calanoid copepods. Culture techniques, productivity and nutritional profile are the most investigated aspects of copepod rearing and many studies have recently emerged. Most Calanoid copepods are omnivorous, and carnivory is an essential aspect of its diet, with some studies suggesting the copepod preference for heterotrophic plankton. As shown in this work, the predatory behaviour of the copepod *A. tonsa* on other commonly employed live feeds is not negligible, namely for adult female specimens, and caution is recommended when employing this species for mesocosm and co-feeding larviculture trials. It is also advised to access the predatory behaviour of other commonly used calanoid copepod species (and strains) prior to their use in larviculture and exploit the potential of this behaviour to enhance their nutritional profile.

IV. Annexes

Annex I - Trophic interactions of *A. tonsa* with *A. franciscana* nauplii and *B. plicatilis*: data tables and statistical outputs

Predatory behaviour test (2.2.1.)

Data table I. Artemia nauplii survival percentages from 2.2.1. Predatory behaviour test (A – artemia nauplii control; CF+A – artemia nauplii exposed to adult female copepods; CM+A – artemia nauplii exposed to adult male copepods; Ci+A – artemia nauplii exposed to Copepodites; Cn+A – artemia nauplii exposed to copepod nauplii).

Sample	A	CF+A	CM+A	Ci+A	Cn+A
1	100,00%	0,00%	96,67%	100,00%	100,00%
2	100,00%	0,00%	33,33%	100,00%	100,00%
3	100,00%	6,67%	80,00%	100,00%	100,00%
4	96,67%	0,00%	43,33%	96,67%	100,00%
5	96,67%	0,00%	90,00%	100,00%	100,00%
6	100,00%	56,67%	93,33%	100,00%	100,00%
7	100,00%	0,00%	70,00%	100,00%	100,00%
8	96,67%	0,00%	93,33%	93,33%	100,00%
9	100,00%	0,00%	26,67%	96,67%	100,00%
10	100,00%	0,00%	73,33%	96,67%	100,00%
11	100,00%	0,00%	100,00%	96,67%	100,00%
12	100,00%	0,00%	90,00%	96,67%	100,00%
13	100,00%	33,33%	86,67%	96,67%	100,00%
14	100,00%	40,00%	96,67%	100,00%	100,00%
15	100,00%	13,33%	93,33%	96,67%	100,00%
16	96,67%	0,00%	86,67%	100,00%	100,00%
17	100,00%	93,33%	46,67%	100,00%	100,00%
18	100,00%	0,00%	93,33%	100,00%	100,00%
19	96,67%	0,00%	100,00%	96,67%	100,00%
20	96,67%	0,00%	96,67%	100,00%	100,00%
21	96,67%	13,33%	80,00%	93,33%	100,00%
22	100,00%	0,00%	96,67%	93,33%	100,00%
23	100,00%	0,00%	93,33%	100,00%	100,00%
24	100,00%	16,67%	60,00%	100,00%	100,00%
25	96,67%	0,00%	70,00%	100,00%	100,00%
26	100,00%	0,00%	40,00%	100,00%	100,00%
27	96,67%	6,67%	76,67%	100,00%	100,00%
28	100,00%	23,33%	83,33%	100,00%	100,00%
29	96,67%	16,67%	76,67%	100,00%	100,00%
30	100,00%	0,00%	86,67%	100,00%	100,00%

Statistical output II. Kruskal-Wallis One Way Analysis of Variance on Ranks performed on artemia survival data expressed on Data table I. (statistical differences are shown on

Fig. 1. a.); output from Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA).

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Data 1 in 2.2.1 Predatory behaviour test

Group	N	Missing	Median	25%	75%
A	30	0	1,000	0,967	1,000
CF+A	30	0	0,000	0,000	0,133
CM+A	30	0	0,867	0,700	0,933
Ci+A	30	0	1,000	0,967	1,000
Cn+A	30	0	1,000	1,000	1,000

H = 119,752 with 4 degrees of freedom. (P = <0,001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0,001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0,05
Cn+A vs CF+A	2953,000	12,410	Yes
Cn+A vs CM+A	1949,000	8,190	Yes
Cn+A vs Ci+A	558,000	2,345	No
Cn+A vs A	465,000	1,954	Do Not Test
A vs CF+A	2488,000	10,456	Yes
A vs CM+A	1484,000	6,236	Yes
A vs Ci+A	93,000	0,391	Do Not Test
Ci+A vs CF+A	2395,000	10,065	Yes
Ci+A vs CM+A	1391,000	5,846	Yes
CM+A vs CF+A	1004,000	4,219	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between the two rank sums that enclose that comparison. For example, if you had four rank sums sorted in order, and found no significant difference between rank sums 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed rank sums is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the rank sums, even though one may appear to exist.

Data table III. *Brachionus plicatilis* survival percentages from 2.2.1. Predatory behaviour test (R – rotifers control; CF+R – rotifers exposed to adult female copepods; CM+R – rotifers exposed to adult male copepods; Ci+R – rotifers exposed to Copepodites; Cn+R – rotifers exposed to copepod nauplii).

Sample	R	CF+R	CM+R	Ci+R	Cn+R
1	94,00%	26,00%	74,00%	46,00%	100,00%
2	84,00%	78,00%	100,00%	64,00%	60,00%
3	100,00%	54,00%	88,00%	50,00%	82,00%
4	100,00%	42,00%	88,00%	52,00%	82,00%
5	94,00%	52,00%	64,00%	40,00%	64,00%
6	98,00%	42,00%	38,00%	66,00%	68,00%
7	64,00%	42,00%	64,00%	56,00%	54,00%
8	100,00%	28,00%	54,00%	52,00%	100,00%
9	78,00%	40,00%	56,00%	42,00%	62,00%
10	100,00%	40,00%	68,00%	62,00%	86,00%
11	100,00%	8,00%	46,00%	40,00%	84,00%
12	82,00%	30,00%	40,00%	56,00%	60,00%
13	92,00%	16,00%	62,00%	62,00%	40,00%
14	90,00%	28,00%	54,00%	38,00%	60,00%
15	82,00%	28,00%	44,00%	50,00%	78,00%
16	74,00%	38,00%	64,00%	68,00%	92,00%
17	82,00%	0,00%	88,00%	60,00%	62,00%
18	88,00%	34,00%	40,00%	54,00%	80,00%
19	84,00%	50,00%	62,00%	68,00%	64,00%
20	96,00%	14,00%	60,00%	68,00%	64,00%
21	86,00%	4,00%	82,00%	66,00%	54,00%
22	96,00%	8,00%	58,00%	74,00%	62,00%
23	98,00%	4,00%	42,00%	60,00%	78,00%
24	74,00%	20,00%	70,00%	74,00%	50,00%
25	62,00%	20,00%	70,00%	72,00%	60,00%
26	66,00%	10,00%	38,00%	72,00%	82,00%
27	68,00%	40,00%	96,00%	90,00%	86,00%
28	86,00%	0,00%	58,00%	68,00%	68,00%
29	82,00%	2,00%	40,00%	60,00%	58,00%
30	94,00%	4,00%	68,00%	56,00%	76,00%

Statistical output IIV. Kruskal-Wallis One Way Analysis of Variance on Ranks performed on rotifer survival data expressed on Data table II. (statistical differences are shown on Fig. 1. b.); output from Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA).

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Data 1 in 2.2.1 Predatory behaviour test

Group	N	Missing	Median	25%	75%
R	30	0	0,870	0,820	0,960
CF+R	30	0	0,280	0,0800	0,400
CM+R	30	0	0,620	0,460	0,700
Ci+R	30	0	0,600	0,520	0,680
Cn+R	30	0	0,660	0,600	0,820

H = 86,715 with 4 degrees of freedom. (P = <0,001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0,001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0,05
R vs CF+R	3045,500	12,798	Yes
R vs Ci+R	1652,000	6,942	Yes
R vs CM+R	1460,000	6,135	Yes
R vs Cn+R	985,000	4,139	Yes
Cn+R vs CF+R	2060,500	8,659	Yes
Cn+R vs Ci+R	667,000	2,803	No
Cn+R vs CM+R	475,000	1,996	Do Not Test
CM+R vs CF+R	1585,500	6,663	Yes
CM+R vs Ci+R	192,000	0,807	Do Not Test
Ci+R vs CF+R	1393,500	5,856	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between the two rank sums that enclose that comparison. For example, if you had four rank sums sorted in order, and found no significant difference between rank sums 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed rank sums is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the rank sums, even though one may appear to exist.

Female:Male proportion test (2.2.2.)

Data table VII. Artemia nauplii survival percentages from 2.2.2. Female:Male proportion test (A – artemia nauplii control; 5♀:0♂+A – artemia nauplii exposed only to adult female copepods; 1♀:4♂+A – artemia nauplii exposed to 1:4 adult female:male copepod ratio; 0♀:5♂+A – artemia nauplii exposed only to adult male copepods).

Sample	A	5♀:0♂+A	1♀:4♂+A	0♀:5♂+A
1	99,33%	54,67%	28,67%	60,00%
2	100,00%	49,33%	57,33%	88,67%
3	98,00%	17,33%	6,67%	92,00%
4	98,67%	63,33%	17,33%	85,33%
5	98,67%	0,00%	15,33%	88,00%
6	94,67%	46,67%	50,00%	86,00%
7	100,00%	0,00%	81,33%	91,33%
8	96,00%	14,67%	41,33%	76,00%
9	98,67%	60,67%	51,33%	82,00%
10	98,00%	0,00%	35,33%	86,67%

Statistical output III. Kruskal-Wallis One Way Analysis of Variance on Ranks performed on Artemia nauplii survival data expressed on Data table III. (statistical differences are shown on Fig. 2.); output from Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA). Groups: A – A; A+F – 5♀:0♂+A; A+FM – 1♀:4♂+A; A+M – 0♀:5♂+A.

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Data 1 in 2.2.2 Artemia

Equal Variance Test: Failed (P < 0,050)

Group	N	Missing	Median	25%	75%
A	10	0	0,987	0,980	0,993
A+F	10	0	0,320	0,000	0,547
A+FM	10	0	0,383	0,173	0,513
A+M	10	0	0,863	0,820	0,887

H = 32,205 with 3 degrees of freedom. (P = <0,001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0,001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0,05
A vs A+F	256,500	6,938	Yes
A vs A+FM	239,500	6,478	Yes
A vs A+M	104,000	2,813	No
A+M vs A+F	152,500	4,125	Yes
A+M vs A+FM	135,500	3,665	Yes
A+FM vs A+F	17,000	0,460	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Temperature and food availability test (2.2.3.)

Data table IV. Artemia nauplii survival percentages from 2.2.3. Temperature and food availability test (A – artemia nauplii subjected to four different temperatures − 10 °C, 15 °C, 20 °C and 25 °C).

Sample	A 10 ˚C	A 15 ˚C	A 20 ˚C	A 25 ˚C
1	96,67%	100,00%	100,00%	100,00%
2	100,00%	100,00%	100,00%	100,00%
3	100,00%	100,00%	100,00%	100,00%
4	100,00%	96,67%	96,67%	100,00%
5	100,00%	100,00%	96,67%	96,67%
6	100,00%	100,00%	100,00%	100,00%
7	100,00%	100,00%	100,00%	100,00%
8	100,00%	100,00%	96,67%	100,00%
9	100,00%	100,00%	100,00%	96,67%
10	100,00%	93,33%	100,00%	96,67%
11	96,67%	100,00%	100,00%	100,00%
12	100,00%	100,00%	100,00%	100,00%
13	100,00%	100,00%	100,00%	100,00%
14	100,00%	100,00%	100,00%	100,00%
15	100,00%	100,00%	100,00%	100,00%
16	100,00%	100,00%	96,67%	96,67%
17	100,00%	100,00%	100,00%	100,00%
18	100,00%	90,00%	100,00%	100,00%
19	96,67%	100,00%	96,67%	100,00%
20	96,67%	100,00%	96,67%	100,00%
21	100,00%	100,00%	96,67%	93,33%
22	96,67%	100,00%	100,00%	93,33%
23	100,00%	100,00%	100,00%	96,67%
24	100,00%	100,00%	100,00%	100,00%
25	100,00%	96,67%	96,67%	96,67%
26	100,00%	100,00%	100,00%	100,00%
27	100,00%	96,67%	96,67%	100,00%
28	100,00%	100,00%	100,00%	100,00%
29	100,00%	100,00%	96,67%	96,67%
30	100,00%	100,00%	100,00%	96,67%

Statistical output IV. Kruskal-Wallis One Way Analysis of Variance on Ranks performed on Artemia nauplii survival data expressed on Data table IV. (statistical differences are shown on Fig. 3. a.); output from Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA).

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Data 1 in Notebook2

Group	N	Missing	Median	25%	75%
A 10 °C	30	0	1,000	1,000	1,000
A 15 °C	30	0	1,000	1,000	1,000
A 20 °C	30	0	1,000	0,967	1,000
A 25 °C	30	0	1,000	0,967	1,000

H = 4,095 with 3 degrees of freedom. (P = 0,251)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0,251)

Data table V. Artemia nauplii survival percentages from 2.2.3. Temperature and food availability test (CF+A – artemia nauplii exposed to adult female copepods, subjected to four different temperatures − 10 °C, 15 °C, 20 °C and 25 °C).

Sample	CF+A 10 ˚C	CF+A 15 ˚C	CF+A 20 ˚C	CF+A 25 ˚C
1	73,33%	46,67%	0,00%	0,00%
2	90,00%	10,00%	0,00%	6,67%
3	20,00%	16,67%	6,67%	0,00%
4	76,67%	23,33%	0,00%	13,33%
5	26,67%	63,33%	0,00%	0,00%
6	43,33%	40,00%	56,67%	0,00%
7	80,00%	6,67%	0,00%	0,00%
8	63,33%	63,33%	0,00%	30,00%
9	16,67%	6,67%	0,00%	0,00%
10	53,33%	36,67%	0,00%	0,00%
11	60,00%	36,67%	0,00%	0,00%
12	36,67%	40,00%	0,00%	3,33%
13	60,00%	53,33%	33,33%	3,33%
14	60,00%	46,67%	40,00%	0,00%
15	66,67%	30,00%	13,33%	0,00%
16	36,67%	0,00%	0,00%	0,00%
17	80,00%	0,00%	93,33%	13,33%
18	26,67%	16,67%	0,00%	6,67%
19	90,00%	46,67%	0,00%	0,00%
20	96,67%	50,00%	0,00%	0,00%
21	50,00%	10,00%	13,33%	0,00%
22	100,00%	43,33%	0,00%	66,67%
23	53,33%	56,67%	0,00%	3,33%
24	60,00%	53,33%	16,67%	0,00%
25	56,67%	43,33%	0,00%	6,67%
26	43,33%	30,00%	0,00%	0,00%
27	73,33%	6,67%	6,67%	6,67%
28	66,67%	60,00%	23,33%	6,67%
29	36,67%	10,00%	16,67%	26,67%
30	76,67%	20,00%	0,00%	0,00%

Statistical output V. Kruskal-Wallis One Way Analysis of Variance on Ranks performed on Artemia nauplii survival data expressed on Data table V. (statistical differences are shown on Fig. 3. a.); output from Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA).

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Data 1 in Notebook2

Group	N	Missing	Median	25%	75%
CF+A 10 °C	30	0	0,600	0,433	0,767
CF+A 15 °C	30	0	0,367	0,1000	0,467
CF+A 20 °C	30	0	0,000	0,000	0,133
CF+A 25 °C	30	0	0,000	0,000	0,0667

H = 67,732 with 3 degrees of freedom. (P = <0,001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0,001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0,05
CF+A 10 °C vs CF+A 25 °C	1864,000	9,783	Yes
CF+A 10 °C vs CF+A 20 °C	1775,000	9,316	Yes
CF+A 10 °C vs CF+A 15 °C	741,000	3,889	Yes
CF+A 15 °C vs CF+A 25 °C	1123,000	5,894	Yes
CF+A 15 °C vs CF+A 20 °C	1034,000	5,427	Yes
CF+A 20 °C vs CF+A 25 °C	89,000	0,467	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Data table VI. Artemia nauplii survival percentages from 2.2.3. Temperature and food availability test (A – artemia nauplii subjected to three different microalgae (Ma) densities – 0 cell mL^{-1}, 10^4 cell mL^{-1} and 10^5 cell mL^{-1}).

Sample	A	A+Ma10^4	A+Ma10^5
1	100,00%	100,00%	100,00%
2	100,00%	100,00%	96,67%
3	100,00%	100,00%	93,33%
4	96,67%	100,00%	100,00%
5	96,67%	96,67%	100,00%
6	100,00%	100,00%	100,00%
7	100,00%	100,00%	96,67%
8	96,67%	96,67%	100,00%
9	100,00%	90,00%	93,33%
10	100,00%	96,67%	100,00%
11	100,00%	100,00%	100,00%
12	100,00%	100,00%	96,67%
13	100,00%	96,67%	100,00%
14	100,00%	100,00%	96,67%
15	100,00%	100,00%	100,00%
16	96,67%	96,67%	100,00%
17	100,00%	100,00%	93,33%
18	100,00%	100,00%	100,00%
19	96,67%	93,33%	100,00%
20	96,67%	100,00%	90,00%
21	96,67%	100,00%	96,67%
22	100,00%	100,00%	90,00%
23	100,00%	100,00%	100,00%
24	100,00%	100,00%	96,67%
25	96,67%	100,00%	100,00%
26	100,00%	100,00%	96,67%
27	96,67%	100,00%	96,67%
28	100,00%	96,67%	100,00%
29	96,67%	96,67%	100,00%
30	100,00%	100,00%	100,00%

Statistical output VI. Kruskal-Wallis One Way Analysis of Variance on Ranks performed on Artemia nauplii survival data expressed on Data table VI. (statistical differences are shown on Fig. 3. b.); output from Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA).

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Data 1 in Notebook1

Group	**N**	**Missing**	**Median**	**25%**	**75%**
A	30	0	1,000	0,967	1,000
A+Ma10^4	30	0	1,000	0,967	1,000
A+Ma10^5	30	0	1,000	0,967	1,000

H = 1,994 with 2 degrees of freedom. (P = 0,369)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0,369)

Data table VII. Artemia nauplii survival percentages from 2.2.3. Temperature and food availability test (CF+A – artemia nauplii exposed to adult female copepods, subjected to three different microalgae (Ma) densities − 0 cell mL^{-1}, 10^4 cell mL^{-1} and 10^5 cell mL^{-1}).

Sample	CF+A	CF+A+Ma10^4	CF+A+Ma10^5
1	0,00%	0,00%	40,00%
2	0,00%	3,33%	90,00%
3	6,67%	46,67%	73,33%
4	0,00%	3,33%	66,67%
5	0,00%	0,00%	50,00%
6	56,67%	0,00%	40,00%
7	0,00%	0,00%	46,67%
8	0,00%	40,00%	90,00%
9	0,00%	43,33%	100,00%
10	0,00%	0,00%	36,67%
11	0,00%	83,33%	83,33%
12	0,00%	73,33%	83,33%
13	33,33%	10,00%	66,67%
14	40,00%	0,00%	30,00%
15	13,33%	63,33%	6,67%
16	0,00%	13,33%	70,00%
17	93,33%	10,00%	93,33%
18	0,00%	56,67%	36,67%
19	0,00%	36,67%	46,67%
20	0,00%	0,00%	53,33%
21	13,33%	3,33%	6,67%
22	0,00%	0,00%	96,67%
23	0,00%	0,00%	96,67%
24	16,67%	0,00%	53,33%
25	0,00%	16,67%	70,00%
26	0,00%	3,33%	100,00%
27	6,67%	36,67%	6,67%
28	23,33%	3,33%	43,33%
29	16,67%	53,33%	70,00%
30	0,00%	3,33%	83,33%

Statistical output VII. Kruskal-Wallis One Way Analysis of Variance on Ranks performed on Artemia nauplii survival data expressed on Data table VII. (statistical differences are shown on Fig. 3. b.); output from Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA).

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Data 1 in Notebook1

Group	**N**	**Missing**	**Median**	**25%**	**75%**
CF+A	30	0	0,000	0,000	0,133
CF+A+Ma10^4	30	0	0,0333	0,000	0,400
CF+A+Ma10^5	30	0	0,667	0,400	0,833

H = 39,929 with 2 degrees of freedom. (P = <0,001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0,001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	**Diff of Ranks**	**q**	**P<0,05**
CF+A+Ma10^5 vs CF+A	1211,500	8,467	Yes
CF+A+Ma10^5 vs CF+A+Ma10^4	894,500	6,251	Yes
CF+A+Ma10^4 vs CF+A	317,000	2,215	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Annex II - Effect of feed supplementation on egg production, egg hatchability and fatty acid profile of *A. tonsa*: data tables and statistical outputs

Egg production and egg hatchability test (2.3.1.)

Data table VIII. *Acartia tonsa* 8 days total egg production per female from 2.3.1. Egg production and egg hatchability test (Ma – feed only with microalgae; Ma+Ac – feed with microalgae and supplemented with artemia unhatched decapsulated cysts; Ma+Am – feed with microalgae and supplemented with artemia metanauplii); subjected to two different microalgae (Ma) densities − 10^4 cell mL^{-1} and 5×10^4 cell mL^{-1}).

	Ma - 10^4 cells mL^{-1}			**Ma - 5×10^4 cells mL^{-1}**		
Sample	**Ma**	**Ma+Ac**	**Ma+Am**	**Ma**	**Ma+Ac**	**Ma+Am**
1	342	370	348	107	226	323
2	344	333	136	161	196	245
3	145	272	308	202	134	293
4	343	107	352	78	170	211
5	442	278	337	128	85	170
6	370	449	375	242	159	128
7	405	273	305	209	197	235
8	245	362	453	184	272	232
9	302	330	174	155	239	102
10	388	376	244	237	210	150
11	247	161	242	139	177	187

Statistical output VIII. Two Way Analysis of Variance performed on *Acartia tonsa* eight days total egg production per female, data expressed on Data table VIII. (statistical differences are shown on Fig. 4. a.); output from Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA). Food – Ma, Ma+Ac and Ma+Am; Ma density − 10^4 cell mL^{-1} and 5×10^4 cell mL^{-1}

Two Way Analysis of Variance

Data source: Data 1 in 2.3.1 Fecundity

Balanced Design

Dependent Variable: Fecundity

Normality Test: Passed (P = 0,293)

Equal Variance Test: Passed (P = 0,426)

Source of Variation	DF	SS	MS	F	P
Food	2	757,909	378,955	0,0641	0,938
Ma density	1	239403,409	239403,409	40,486	<0,001
Food x Ma density	2	12636,636	6318,318	1,068	0,350
Residual	60	354798,000	5913,300		
Total	65	607595,955	9347,630		

The difference in the mean values among the different levels of Food is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Ma concentration. There is not a statistically significant difference (P = 0,938).

The difference in the mean values among the different levels of Ma density is greater than would be expected by chance after allowing for effects of differences in Food. There is a statistically significant difference (P = <0,001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Food does not depend on what level of Ma density is present. There is not a statistically significant interaction between Food and Ma density. (P = 0,350)

Power of performed test with alpha = 0,0500: for Food : 0,0500
Power of performed test with alpha = 0,0500: for Ma density : 1,000
Power of performed test with alpha = 0,0500: for Food x Ma density : 0,0584

Least square means for Food :

Group	Mean
Ma	246,136
Ma+Ac	244,364
Ma+Am	252,273

Std Err of LS Mean = 16,395

Least square means for Ma density :

Group	Mean
Ma(10^4)	307,818
Ma($5x10^4$)	187,364

Std Err of LS Mean = 13,386

Least square means for Food x Ma density :

Group	Mean
Ma x Ma(10^4)	324,818
Ma x Ma($5x10^4$)	167,455
Ma+Ac x Ma(10^4)	301,000
Ma+Ac x Ma($5x10^4$)	187,727
Ma+Am x Ma(10^4)	297,636
Ma+Am x Ma($5x10^4$)	206,909

Std Err of LS Mean = 23,186

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Food**

Comparison	Diff of Means	p	q	P	P<0,050
Ma+Am vs. Ma+Ac	7,909	3	0,482	0,938	No
Ma+Am vs. Ma	6,136	3	0,374	0,962	Do Not Test
Ma vs. Ma+Ac	1,773	3	0,108	0,997	Do Not Test

Comparisons for factor: **Ma density**

Comparison	**Diff of Means**	**p**	**q**	**P**	**P<0,050**
Ma(10^4) vs. Ma($5x10^4$)	120,455	2	8,998	<0,001	Yes

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Data table VIV. *Acartia tonsa* 48 h hatching percentages from 2.3.1. Egg production and egg hatchability test (Ma – feed only with microalgae; Ma+Ac – feed with microalgae and supplemented with artemia unhatched decapsulated cysts; Ma+Am – feed with microalgae and supplemented with artemia metanauplii); subjected to two different microalgae (Ma) densities − 10^4 cell mL^{-1} and $5×10^4$ cell mL^{-1}).

	Ma - 10^4 cells mL^{-1}			**Ma - $5×10^4$ cells mL^{-1}**		
Sample	**Ma**	**Ma+Ac**	**Ma+Am**	**Ma**	**Ma+Ac**	**Ma+Am**
1	96,78%	97,84%	95,11%	98,13%	90,27%	82,97%
2	70,93%	96,70%	97,06%	98,76%	97,96%	96,73%
3	97,24%	88,97%	99,03%	76,24%	81,34%	98,98%
4	98,54%	95,33%	94,60%	89,74%	96,47%	88,63%
5	75,34%	98,92%	97,63%	96,88%	81,18%	97,65%
6	93,78%	99,11%	46,40%	97,52%	99,37%	48,44%
7	96,79%	98,90%	95,74%	75,12%	99,49%	98,72%
8	94,69%	99,17%	87,42%	95,65%	95,96%	96,55%
9	97,68%	95,45%	98,28%	98,06%	99,16%	48,04%
10	84,54%	99,73%	96,72%	74,68%	95,71%	96,67%
11	96,76%	93,79%	96,69%	89,21%	89,27%	97,33%

Statistical output VIV. Kruskal-Wallis One Way Analysis of Variance on Ranks performed on *Acartia tonsa* 48 hour hatching percentages, data expressed on Data table VIV. (statistical differences are shown on Fig. 4. b.); output from Sigmaplot for Windows version 11.0 (Systat Software Inc., San Jose, CA, USA).

Kruskal-Wallis One Way Analysis of Variance on Ranks

Data source: Data 1 in Notebook1

Group	N	Missing	Median	25%	75%
Ma(10^4)	11	0	0,968	0,868	0,971
Ma(10^4)+Ac	11	0	0,978	0,954	0,991
Ma(10^4)+Am	11	0	0,967	0,947	0,975
Ma($5x10^4$)	11	0	0,957	0,795	0,979
Ma($5x10^4$)+Ac	11	0	0,960	0,895	0,989
Ma($5x10^4$)+Am	11	0	0,967	0,844	0,976

H = 4,052 with 5 degrees of freedom. (P = 0,542)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0,542)

Fatty acid profile test (2.3.2)

Data table X. Fatty acid composition (µg mg-1 of dry weight) of *Acartia tonsa* eggs (Ma – feed only with microalgae; Ma+Ac – feed with microalgae and supplemented with artemia unhatched decapsulated cysts; Ma+Am – feed with microalgae and supplemented with artemia metanauplii).

Sample	14:0	15:0	16:0	16:1 (n7)	17:0	18:0	18:1 (n9)	18:1 (n7)	18:2 (n6)	18:3 (n3)	18:4 (n3)	20:0	20:5 (n3)	22:6 (n3)
Ma_1	2,42	0,08	12,32	0,86	0,83	4,26	2,60	4,75	1,01	12,36	6,72	0,30	9,12	9,34
Ma_2	2,93	0,12	14,45	0,95	1,00	4,60	2,49	5,16	1,13	13,76	7,60	0,32	9,54	9,55
Ma_3	2,45	0,12	12,04	0,84	0,86	4,16	2,22	4,65	0,99	11,94	6,55	0,30	8,86	9,38
Ma_4	2,82	0,14	12,17	1,18	1,03	4,25	2,19	5,20	0,94	12,62	8,11	0,34	10,15	9,77
Ma_5	2,97	0,15	12,41	1,20	1,04	4,26	2,31	5,39	0,97	12,79	8,27	0,34	10,22	9,93
Ma_6	2,82	0,14	11,95	1,17	1,03	4,23	2,18	5,17	0,86	12,59	8,12	0,32	10,19	9,85
Ma_7	2,89	0,16	11,76	1,15	1,01	4,20	2,88	5,24	0,95	12,30	7,32	0,32	9,70	9,98
Ma_8	2,93	0,16	11,51	1,13	0,96	4,09	2,97	5,17	0,92	12,15	7,20	0,34	9,45	9,85
Ma_9	3,06	0,16	11,86	1,17	0,98	4,09	2,78	5,27	0,91	12,37	7,37	0,33	9,63	10,01
MaAc_1	2,47	0,09	9,61	0,83	0,68	4,01	2,65	3,57	0,98	9,70	5,84	0,24	7,55	8,10
MaAc_2	2,61	0,09	9,91	0,88	0,71	3,98	2,72	3,70	0,98	9,73	5,83	0,24	7,49	7,96
MaAc_3	2,71	0,11	9,92	0,87	0,74	4,16	2,78	3,74	1,01	9,87	5,96	0,26	7,73	7,97
MaAc_4	2,26	0,09	9,12	0,89	0,69	3,07	2,59	3,79	0,80	8,94	5,95	0,23	7,12	7,61
MaAc_5	2,21	0,09	8,80	0,89	0,65	3,15	2,81	3,63	0,79	8,75	5,85	0,23	7,08	7,63
MaAc_6	2,20	0,08	8,75	0,90	0,68	3,16	2,48	3,58	0,79	8,71	5,87	0,23	7,02	7,56
MaAc_7	2,26	0,10	9,15	0,79	0,62	3,76	2,80	3,43	0,68	8,99	5,60	0,24	6,91	7,77
MaAc_8	2,43	0,10	9,61	0,85	0,67	3,87	2,78	3,64	0,70	9,24	5,75	0,24	7,04	7,73
MaAc_9	2,64	0,13	9,90	0,91	0,70	4,05	2,99	3,83	0,76	9,52	5,97	0,24	7,25	7,83
MaAm_1	1,92	0,07	8,01	0,73	0,62	3,08	1,95	3,11	0,78	8,61	5,06	0,22	6,32	6,31
MaAm_2	2,01	0,09	8,04	0,73	0,58	3,01	1,67	3,08	0,76	8,53	5,02	0,23	6,19	6,30
MaAm_3	1,95	0,09	7,91	0,71	0,62	3,02	1,74	3,11	0,75	8,45	5,00	0,23	6,22	6,41
MaAm_4	1,90	0,12	9,01	1,11	0,85	3,51	1,99	4,51	0,81	9,74	6,40	0,29	8,30	8,67
MaAm_5	1,96	0,09	9,17	1,10	0,84	3,47	2,18	4,51	0,79	9,83	6,41	0,29	8,28	8,62
MaAm_6	1,90	0,11	9,03	1,07	0,85	3,46	2,03	4,43	0,79	9,68	6,32	0,28	8,23	8,69
MaAm_7	2,03	0,11	8,98	0,94	0,71	3,31	2,02	4,03	0,72	9,91	5,82	0,27	7,65	8,04
MaAm_8	2,18	0,13	9,21	0,96	0,76	3,38	2,09	4,14	0,75	10,08	5,98	0,24	7,75	8,07
MaAm_9	2,21	0,12	9,54	1,03	0,76	3,59	2,35	4,34	0,79	10,38	6,27	0,26	8,02	7,99

Statistical output X. PERMANOVA analysis performed on *Acartia tonsa* eggs fatty acid composition, data expressed on Data table X. (statistical differences are shown on Table 1.); output from Primer 6.0 software (Primer-E Ldt, Lutton, UK)

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem1
Data type: Similarity
Selection: All
Transform: Log(X+1)
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Unrestricted permutation of raw data
Number of permutations: 999

Factors

Name	Type	Levels
Food	Fixed	3

PAIR-WISE TESTS

Term 'Food'

Groups	t	P(perm)	Unique perms	P(MC)
Ma, Ma+Ac	9,2619	0,001	979	0,001
Ma, Ma+Am	6,5422	0,001	975	0,001
Ma+Ac, Ma+Am	2,5345	0,002	975	0,009

Denominators

Groups	Denominator	Den.df
Ma, Ma+Ac	1*Res	16
Ma, Ma+Am	1*Res	16
Ma+Ac, Ma+Am	1*Res	16

Average Similarity between/within groups

	Ma	Ma+Ac	Ma+Am
Ma	98,255		
Ma+Ac	94,286	98,406	
Ma+Am	93,55	96,556	96,953

Statistical output XI. Similarity percentages routine (SIMPER) analysis performed on *Acartia tonsa* eggs fatty acid composition, data expressed on Data table X.; output from Primer 6.0 software (Primer-E Ldt, Lutton, UK)

SIMPER

Similarity Percentages - species contributions

One-Way Analysis

```
Data worksheet
Name: Data1
Data type: Other
Sample selection: All
Variable selection: All

Parameters
Resemblance: S17 Bray Curtis similarity
Cut off for low contributions: 50,00%

Factor Groups
Sample   Food
Ma_1     Ma
Ma_2     Ma
Ma_3     Ma
Ma_4     Ma
Ma_5     Ma
Ma_6     Ma
Ma_7     Ma
Ma_8     Ma
Ma_9     Ma
MaAc_1   Ma+Ac
MaAc_2   Ma+Ac
MaAc_3   Ma+Ac
MaAc_4   Ma+Ac
MaAc_5   Ma+Ac
MaAc_6   Ma+Ac
MaAc_7   Ma+Ac
MaAc_8   Ma+Ac
MaAc_9   Ma+Ac
MaAm_1   Ma+Am
MaAm_2   Ma+Am
MaAm_3   Ma+Am
MaAm_4   Ma+Am
MaAm_5   Ma+Am
MaAm_6   Ma+Am
MaAm_7   Ma+Am
MaAm_8   Ma+Am
MaAm_9   Ma+Am


...
```

Groups Ma & Ma+Ac
Average dissimilarity = 5,71

Species	Group Ma Av.Abund	Group Ma+Ac Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:3 (n3)	2,61	2,33	0,71	4,99	12,37	12,37
18:1 (n7)	1,81	1,54	0,69	5,97	12,12	24,49
20:5 (n3)	2,36	2,11	0,65	4,78	11,42	35,91
16:0	2,58	2,34	0,62	3,31	10,81	46,72
18:4 (n3)	2,13	1,92	0,54	3,00	9,41	56,13

Groups Ma & Ma+Am
Average dissimilarity = 6,45

Species	Group Ma Av.Abund	Group Ma+Am Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
16:0	2,58	2,28	0,79	3,49	12,33	12,33
18:3 (n3)	2,61	2,35	0,67	3,20	10,42	22,75
20:5 (n3)	2,36	2,13	0,62	1,97	9,57	32,31
14:0	1,34	1,10	0,61	3,50	9,44	41,76
18:1 (n7)	1,81	1,59	0,58	1,63	9,06	50,82

Groups Ma+Ac & Ma+Am
Average dissimilarity = 3,44

Species	Group Ma+Ac Av.Abund	Group Ma+Am Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:1 (n9)	1,32	1,10	0,60	2,75	17,43	17,43
18:1 (n7)	1,54	1,59	0,36	3,51	10,46	27,89
14:0	1,23	1,10	0,35	1,99	10,14	38,03
18:0	1,54	1,46	0,31	1,76	9,07	47,10
20:5 (n3)	2,11	2,13	0,29	2,40	8,32	55,42

Data table XI. Fatty acid composition (µg mg-1 of dry weight) of *Acartia tonsa* adults (Ma – feed only with microalgae; Ma+Ac – feed with microalgae and supplemented with artemia unhatched decapsulated cysts; Ma+Am – feed with microalgae and supplemented with artemia metanauplii).

Sample	14:0	15:0	16:0	16:1 (n7)	17:0	18:0	18:1 (n9)	18:1 (n7)	18:2 (n6)	18:3 (n3)	18:4 (n3)	20:0	20:5 (n3)	22:6 (n3)
Ma1	0,65	0,07	5,69	0,35	0,88	4,24	0,52	1,55	0,24	1,93	0,95	0,10	6,37	11,08
Ma2	1,07	0,06	6,78	0,35	0,80	4,12	1,22	1,78	0,33	3,21	1,98	0,12	7,59	11,14
Ma3	1,39	0,07	7,20	0,56	1,00	4,26	1,53	2,48	0,38	3,98	2,20	0,15	7,71	10,48
Ma5	1,35	0,07	6,64	0,39	1,02	4,01	1,14	2,23	0,35	4,57	2,79	0,15	7,64	10,51
Ma6	0,81	0,07	5,89	0,34	1,07	4,47	0,50	1,81	0,22	2,72	1,30	0,11	6,87	10,52
MaAc1	0,75	0,07	6,23	0,45	1,05	4,32	0,81	1,77	0,29	2,04	1,02	0,09	8,25	11,81
MaAc2	0,83	0,04	6,98	0,33	0,79	4,29	1,43	1,46	0,36	2,22	1,54	0,10	8,30	11,76
MaAc3	0,85	0,04	5,53	0,31	0,56	4,82	1,21	1,04	0,22	1,82	1,27	0,09	6,64	9,96
MaAc4	0,95	0,05	5,36	0,30	0,61	4,18	1,44	1,15	0,24	2,11	1,25	0,10	6,19	9,31
MaAc5	0,72	0,07	5,30	0,32	0,98	4,01	0,72	1,39	0,22	2,23	1,07	0,10	7,19	10,57
MaAm1	0,74	0,06	6,03	0,40	0,94	3,89	0,69	1,47	0,25	1,95	1,03	0,08	7,64	11,34
MaAm2	0,52	0,05	5,22	0,28	0,71	4,27	0,76	1,23	0,19	1,60	0,82	0,10	6,28	9,94
MaAm3	0,76	0,05	4,79	0,28	0,71	3,32	0,83	1,26	0,20	2,01	1,10	0,10	5,85	8,98
MaAm4	0,71	0,04	5,22	0,19	0,88	3,80	0,68	1,10	0,17	1,79	1,06	0,07	7,00	10,80
MaAm5	0,63	0,06	5,01	0,20	0,92	3,65	0,47	1,10	0,16	1,66	0,72	0,08	6,39	9,94

Statistical output XII. PERMANOVA analysis performed on *Acartia tonsa* adults fatty acid composition, data expressed on Data table XI. (statistical differences are shown on Table 1.); output from Primer 6.0 software (Primer-E Ldt, Lutton, UK)

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem3
Data type: Similarity
Selection: All
Transform: Log(X+1)
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Unrestricted permutation of raw data
Number of permutations: 999

Factors

Name	Abbrev.	Type	Levels
Food	Fo	Fixed	3

PAIR-WISE TESTS

Term 'Fo'

Groups	t	Unique P(perm)	perms	P(MC)
Ma, Ma+Ac	1,6792	0,106	126	0,075
Ma, Ma+Am	2,9809	0,019	126	0,007
Ma+Ac, Ma+Am	1,8763	0,041	126	0,032

Denominators

Groups	Denominator	Den.df
Ma, Ma+Ac	1*Res	8
Ma, Ma+Am	1*Res	8
Ma+Ac, Ma+Am	1*Res	8

Average Similarity between/within groups

	Ma	Ma+Ac	Ma+Am
Ma	93,834		
Ma+Ac	93,446	95,175	
Ma+Am	91,697	94,632	95,897

Statistical output XIII. Similarity percentages routine (SIMPER) analysis performed on *Acartia tonsa* adults fatty acid composition, data expressed on Data table XI.; output from Primer 6.0 software (Primer-E Ldt, Lutton, UK)

SIMPER

Similarity Percentages - species contributions

One-Way Analysis

```
Data worksheet
Name: Data5
Data type: Other
Sample selection: All
Variable selection: All

Parameters
Resemblance: S17 Bray Curtis similarity
Cut off for low contributions: 50,00%

Factor Groups
Sample  Food
Ma1     Ma
Ma2     Ma
Ma3     Ma
Ma4     Ma
Ma5     Ma
MaAc1   Ma+Ac
MaAc2   Ma+Ac
MaAc3   Ma+Ac
MaAc4   Ma+Ac
MaAc5   Ma+Ac
MaAm1   Ma+Am
MaAm2   Ma+Am
MaAm3   Ma+Am
MaAm4   Ma+Am
MaAm5   Ma+Am

...

Groups Ma  &  Ma+Ac
Average dissimilarity = 6,55

           Group Ma  Group Ma+C
Species    Av.Abund    Av.Abund  Av.Diss  Diss/SD  Contrib%  Cum.%
18:3 (n3)      1,43        1,13     1,14     1,69     17,42  17,42
18:4 (n3)      1,02        0,80     0,97     1,55     14,88  32,30
18:1 (n7)      1,08        0,85     0,83     1,62     12,64  44,94
18:1 (n9)      0,66        0,74     0,80     1,38     12,21  57,14
```

*Groups Ma & Ma+*Am
Average dissimilarity = 8,30

Species	Group Ma Av.Abund	Group Ma+M Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:3 (n3)	1,43	1,03	1,46	1,79	17,58	17,58
18:4 (n3)	1,02	0,66	1,33	1,61	16,06	33,64
18:1 (n7)	1,08	0,80	1,03	2,29	12,35	45,99
18:1 (n9)	0,66	0,52	0,85	1,84	10,19	56,18

Groups Ma+Ac & Ma+Am
Average dissimilarity = 5,37

Species	Group Ma+C Av.Abund	Group Ma+M Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
18:1 (n9)	0,74	0,52	0,88	1,46	16,44	16,44
18:4 (n3)	0,80	0,66	0,54	1,32	10,12	26,56
20:5 (n3)	2,11	2,03	0,49	1,45	9,11	35,66
16:0	1,92	1,83	0,44	1,32	8,23	43,89
18:0	1,67	1,56	0,43	1,47	8,10	51,99

Data table XII. Fatty acid composition (µg mg-1 of dry weight) of food items (Ma – microalgae *Rhodomonas lens*; Ac – artemia unhatched decapsulated cysts; Am – artemia metanauplii).

Sample	14:0	15:0	16:0	16:1 (n7)	16:2 (n6)	17:0	17:1 (n9)	18:0	18:1 (n9)	18:1 (n7)	18:2 (n6)	18:3 (n6)	18:3 (n3)	18:4 (n3)	20:1 (n9)	20:4 (n6)	20:5 (n3)	22:6 (n3)
Ma1	0,85	0,00	5,23	0,00	0,00	0,05	0,00	3,38	1,47	2,26	0,54	0,00	4,18	2,32	0,17	0,00	1,55	1,37
Ma2	1,00	0,00	5,30	0,00	0,00	0,07	0,00	3,43	1,45	2,28	0,51	0,00	4,13	2,77	0,21	0,00	1,48	1,27
Ma3	0,89	0,00	4,87	0,00	0,00	0,07	0,00	3,26	1,43	2,18	0,53	0,00	3,99	2,69	0,22	0,00	1,50	1,31
Ac1	2,51	1,00	18,45	18,29	0,79	1,57	3,71	9,55	30,71	12,84	8,10	0,99	12,76	3,24	0,84	1,41	16,76	0,00
Ac2	2,64	1,02	18,42	18,19	0,55	1,66	3,73	9,67	28,33	13,23	7,71	0,90	12,83	3,40	0,88	1,53	16,56	0,00
Ac3	2,64	1,01	18,09	17,44	0,65	1,63	3,79	9,71	27,50	12,63	7,99	0,97	12,96	3,42	1,01	1,60	16,56	0,00
Am1	1,52	0,69	14,33	14,36	0,30	1,66	3,36	10,65	23,45	14,35	5,52	0,53	10,51	2,16	0,76	1,41	16,20	0,00
Am2	1,56	0,70	14,17	14,26	0,35	1,66	3,66	10,52	24,20	14,65	5,45	0,56	10,22	2,12	0,74	1,42	16,05	0,00
Am3	1,52	0,69	13,84	13,95	0,26	1,65	3,58	10,30	23,65	13,72	5,40	0,55	10,15	2,10	0,74	1,43	15,66	0,00

Statistical output XIV. PERMANOVA analysis performed on food items fatty acid composition, data expressed on Data table XII. (statistical differences are shown on Table 1.); output from Primer 6.0 software (Primer-E Ldt, Lutton, UK)

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem2
Data type: Similarity
Selection: All
Transform: Log(X+1)
Resemblance: S17 Bray Curtis similarity

Sums of squares type: Type III (partial)
Fixed effects sum to zero for mixed terms
Permutation method: Unrestricted permutation of raw data
Number of permutations: 999

Factors

Name	Abbrev.	Type	Levels
Food	Fo	Fixed	3

PAIR-WISE TESTS

Term 'Fo'

Groups	t	P(perm)	Unique perms	P(MC)
Ma, Ac	66,328	0,086	10	0,001
Ma, Am	67,412	0,094	10	0,001
Ac, Am	11,511	0,105	10	0,001

Denominators

Groups	Denominator	Den.df
Ma, Ac	1*Res	4
Ma, Am	1*Res	4
Ac, Am	1*Res	4

Average Similarity between/within groups

	Ma	Ac	Am
Ma	98,381		
Ac	49,955	99,14	
Am	52,464	95,054	99,428

Statistical output XV. Similarity percentages routine (SIMPER) analysis performed on food items fatty acid composition, data expressed on Data table XII.; output from Primer 6.0 software (Primer-E Ldt, Lutton, UK)

Similarity Percentages - species contributions

One-Way Analysis

Data worksheet
Name: Data4
Data type: Other
Sample selection: All
Variable selection: All

Parameters
Resemblance: S17 Bray Curtis similarity
Cut off for low contributions: 50,00%

Factor Groups

Sample	Food
Ma1	Ma
Ma2	Ma
Ma3	Ma
Ac1	Ac
Ac2	Ac
Ac3	Ac
Am1	Am
Am2	Am
Am3	Am

...

Groups Ma & Ac
Average dissimilarity = 50,04

	Group Ma	Group Ac				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
16:1 (n7)	0,00	2,94	7,00	122,39	13,99	13,99
18:1 (n9)	0,90	3,40	5,95	51,95	11,88	25,87
20:5 (n3)	0,92	2,87	4,64	165,30	9,27	35,14
18:2 (n6)	0,42	2,19	4,20	96,93	8,40	43,54
17:1 (n9)	0,00	1,56	3,70	194,09	7,40	50,94

Groups Ma & Am
Average dissimilarity = 47,54

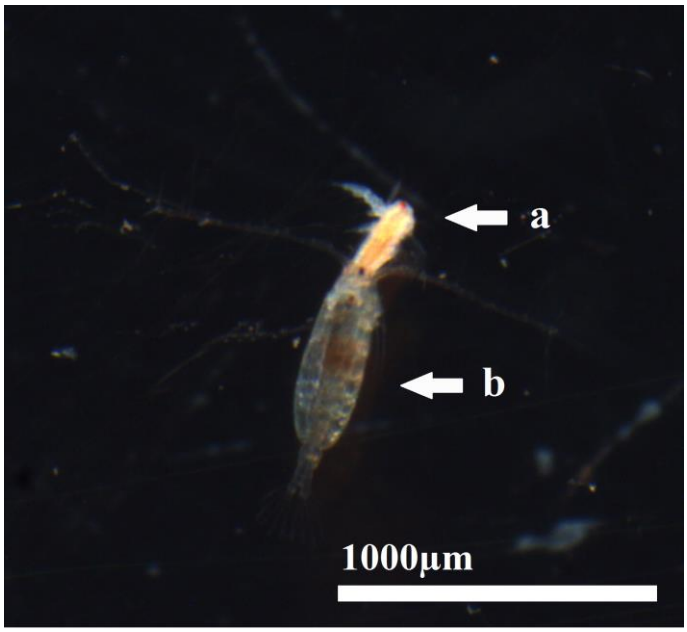
	Group Ma	Group Am				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
16:1 (n7)	0,00	2,72	6,89	293,94	14,49	14,49
18:1 (n9)	0,90	3,21	5,86	165,10	12,33	26,82
20:5 (n3)	0,92	2,83	4,84	133,71	10,18	37,00
18:1 (n7)	1,18	2,72	3,92	54,39	8,25	45,25
17:1 (n9)	0,00	1,51	3,83	50,81	8,05	53,30

Groups C & Am
Average dissimilarity = 4,95

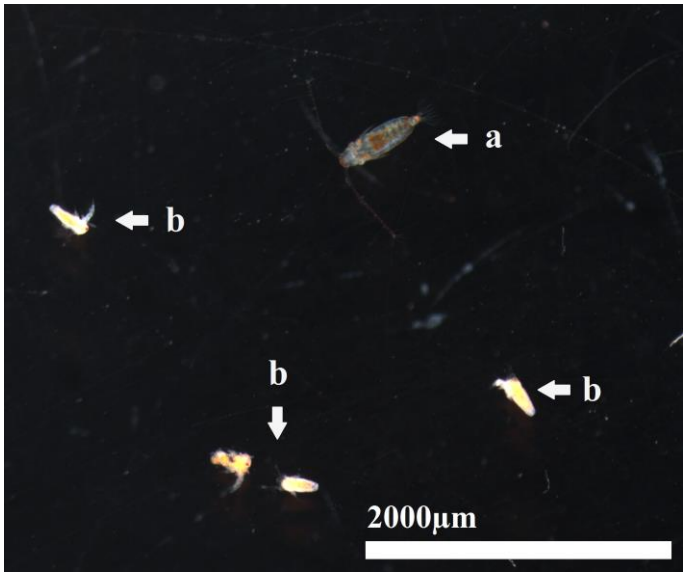
Species	Group C Av.Abund	Group Am Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
14:0	1,28	0,93	0,60	18,04	12,08	12,08
18:4 (n3)	1,47	1,14	0,56	15,51	11,41	23,49
18:2 (n6)	2,19	1,86	0,55	15,49	11,14	34,63
16:0	2,96	2,72	0,42	14,30	8,44	43,08
16:2 (n6)	0,51	0,27	0,41	3,47	8,20	51,28

Annex III – Complementary figures

Complementary figure I. a) Adult female *Acartia tonsa* feeding on b) artemia nauplii. Wide zoom stereo microscope photography of 2.2.1. Predatory behaviour test, treatment CF+A.



Complementary figure II. a) Adult female *Acartia tonsa* and b) partially eaten artemia nauplii. Wide zoom stereo microscope photography of 2.2.1. Predatory behaviour test, treatment CF+A.



Complementary figure III. Chromatography–mass spectrometry (GC-MS) outputs of **a)** *Acartia tonsa* eggs (sample Ma_2 Data table X.), **b)** *A. tonsa* adults (sample Ma2 Data table XI.), **c)** *Rhodomonas lens* (sample Ma2 Data table XII.), **d)** *Artemia franciscana* unhatched decapsulated cysts (sample Ac2 Data table XII.) and **e)** *A. franciscana* metanauplii (sample Am1 Data table XII.).