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Assessment of *Acartia tonsa* effect on *Necora puber* zoeal stages

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Título: Efeito da administração de *Acartia tonsa* no desenvolvimento larvar de *Necora puber*

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Resumo

A navalheira (*Necora puber*) é uma das espécies mais consumidas em Portugal, sendo que a maioria dos organismos consumidos é atualmente importada de países do Norte da Europa. Para o sucesso da sua produção em aquacultura, é indispensável a administração de presas adequadas à dimensão das fases larvares e que respondam às suas necessidades nutricionais. Os benefícios da administração de copépodes têm vindo a ser reconhecidos, uma vez que os calanóides possuem um largo espectro de tamanhos, movimentos naturais que promovem a sua captura e elevados níveis de ácidos gordos essenciais. *Acartia tonsa* é uma das espécies de copépodes mais recomendadas para administrar nos primeiros estágios larvares, mas os seus protocolos de cultivo em grande escala ainda requerem otimização. Neste enquadramento, o presente estudo teve como objetivo a seleção da microalga mais adequada para o cultivo de *A. tonsa*, possibilitando a avaliação do seu efeito no desenvolvimento larval de *N. puber*, quando comparado com a administração de *Artemia franciscana*. Inicialmente, os copépodes foram submetidos a duas dietas distintas: *Rhodomonas lens* e *Tetraselmis chuii,* a uma densidade de $1x10^4$ cell.mL^{-1}, tendo sido avaliadas as taxas de eclosão, sobrevivência e produção de ovos. A microalga que promoveu uma melhor performance foi administrada nos cultivos contínuos de *A. tonsa*. Em relação ao desenvolvimento larvar de *N. puber*, foram avaliadas as taxas de crescimento, percentagem de estágios ao longo do tempo, bem como a correlação entre o peso e o comprimento das larvas, quando alimentadas com *A. tonsa* e *A. franciscana*. Adicionalmente, foi avaliado o perfil de ácidos gordos de ambas as espécies de microalgas e de zooplâncton. *R. lens* permitiu um melhor desempenho enquanto alimento para cultivos de *A. tonsa*, promovendo uma taxa de eclosão perto de 90%, enquanto que, com *T. chuii* os copépodes apenas sobreviveram até ao oitavo dia. Tais resultados poderão dever-se à dimensão celular de *R. lens*, bem ao seu elevado conteúdo de DHA. Em relação ao desenvolvimento larvar de *N. puber*, *Acartia tonsa* promoveu uma taxa de sobrevivência de 89 ± 1,63% em zoea V e o encurtamento da fase larvar. Com ambas as dietas foi demonstrada uma forte correlação entre o peso o tamanho. Foi observada uma melhor performance larvar a partir de zoea II, quando as larvas foram alimentadas com *A. tonsa*, devido à elevada presença de C 20:5 n3 e C 22:6 n3 e movimentos naturais que promovem a sua captura. Assim, foi possível concluir que as *A. tonsa* é uma presa adequada para larvas de pequenas dimensões, promovendo elevadas sobrevivências e redução da fase larvar.

Palavras-chave: Navalheira, *Rhodomonas lens, Tetraselmis chuii*, copépodes, crustáceos decápodes, desenvolvimento larvar.

Abstract

In Portugal, *Necora puber*, is one of the most appreciated crustacean specie but the almost of organisms consumed are import from Nordic countries. To prevent this situation, it is indispensable the optimization of *N. puber* larviculture through the administration of suitable preys to first zoeal stages small mouth-gapes. The present study aimed to select the most suitable microalgae specie to *Acartia tonsa* feeding and to assess the effect administration during *N. puber* larval development, when compare with *Artemia franciscana*. To *A. tonsa* feeding optimization, copepods were fed on to $1x10^4$ cell.mL^{-1} *Rhodomonas lens* and *Tetraselmis chuii*, and it were assess hatching rate, survival rate and egg production rate. The most suitable microalgal diet was used to maintain copepods continuous cultures. *N. puber s*urvival rate, Larval Stage Proportion (%) and correlation between length and weight was assessed when larvae were fed on *A. tonsa* and *A. franciscana*. R. lens, *T. chuii, A. tonsa* and *A. franciscana* fatty acids were analysed in a gas chromatograph. In the present study was proved that *R. lens* allows a continuous *A.tonsa* production due to their cell size and high content of DHA. This suitable microalgal diet promote a 90,12 ± 1,79 % of hatching rate and with *T. chuii* copepods only survival until 8^{th} day. To *N. puber* larviculture, *A. tonsa* allow higher survival rate (89 ± 1,63%) and larvae achieving zoea V faster. With both diets, larvae weight and length seems to have a strong correlation. *A. tonsa* is a suitable prey to *N. puber* larval development after zoea II, due its natural movements and C 20:5 n3 and C 22:6 n3 content. With these characteristics, *A. tonsa* is a suitable prey to early larval stage with small mouth-gape sizes, promoting high survival rates and shorter larval stages duration.

Keywords: Swimming crab, *Rhodomonas lens*, *Tetraselmis chuii*¸ copepods, decapod crustaceans, larval development.

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ARA – Arachidonic acid

DPH – Days Post hatching

DHA – Docosahexaenoic acid

EPA – Eicosapentaenoic acid

HUFA's – High Unsaturated Fatty Acids

PUFA's – Polyunsaturated Fatty Acids

SGR – Specific growth rate

CLG – Carapace length gain

DWG – Dry Weight gain

1. Introduction

1.1 Aquaculture of decapod crustaceans: trends and bottlenecks

Marine ecosystems are currently under threat, resulting in some unstables food webs and part of marine biological stocks are almost depleted (Nellerman and Corcoran, 2010). This is a direct consequence of human activities such as pollution, destructive fishing methods or overfishing. To counteract these trends, and taking into account the exponential anthropological growth, aquaculture must be assumed as one of the main source of marine protein for human nutrition.

The correct management of stocks still requires a considerate amount of studies and positive results depends on the biology of species and on its life cycle complexity. For instance, crustaceans have great commercial demand, but the lack of studies about their stocks suggests that might be overexploited (Ungfors, 2008).

In the last few years, the aquaculture of decapod crustaceans has fairly increased. In 2012, only 6,4 million tonnes of crustaceans were produced in aquaculture, from a total of 100 million tonnes of farmed species. These 9,7% of the global production represents 22,4% of the global aquaculture production value (FAO, 2014). Fisheries are currently not enough to supply consumer demands, especially in Mediterranean countries where it is verified a great demand for live crustaceans, thus promoting an intensive importation of crabs and shrimps (Barrento et al., 2009).

To achieve the success in decapod crustaceans aquaculture, it is necessary to overcome some bottlenecks. The need to develop rearing protocols for crustacean species, as well as the reduction of the time needed to achieve commercial size, are some of the main reasons for the present unviability of aquaculture production of some valuable crustaceans species.

During its life cycle, a crustacean undergoes a series of moulting events that contributes for a high mortality rate. In fact, moulting or ecdysis is considered the main stressful event that occurs during crustacean life period. In this cyclical process, the old exoskeleton is replaced by a new recently formed. Due to the fragility of the new cuticle, crustaceans are exposed to many external critical factors (Wickins and Lee, 2002), such as predation or mechanical stress. The recover to a normal stage can takes several days, and during that period numerous layers of chitin, protein and calcium carbonate are accumulated on

epidermis. Crustacean ecdysis allows growth and reproduction and the moult cycle can last days or months, depending upon the age, species and size of the animal.

The majority of decapod crustaceans have a complex life-cycle. Larvae are pelagic, coexisting with some other planktonic organisms, being exposed to several biotic and abiotic critical factors (Wickins and Lee, 2002). During this phase, larvae go through plural planktonic larval stages until achieved the benthonic juvenile stage. (Ernest and Mykles, 2011).

To overcome the high mortality imposed by moulting events and during larval development, some authors (Calado et al., 2003) suggests that is indispensable the use of rearing tanks that optimizes food ingestion. Other point to take into account is the administration of a suitable nutritional value.

Still, nutrition can represent up to 50% of production costs in crustaceans aquaculture. This cost can be recovered by high survival rates during molt cycle (Becerra-Dórame et al., 2012).

Nowadays, the optimization of early larval stages rearing relies on the administration of natural feed, as zooplankton, contributing to a right larval nutrition, with digestive enzymes that allows a full range of food to be exploited (Wickins and Lee, 2002).

1.2 Decapod crustacean larviculture and nutritional value of the diet

Cholesterol, vitamins, carotenoids and proteins are indispensable on the composition of a suitable diet for crustacean larvae. Such diets will promote a well-succeed metamorphosis process and have a positive effect on growth performance (Gebauer et al., 1999).

When a suitable diet is administrated, lipids can provide an important source of energy instead of the use of proteins. The good performance of early larval stages depends on diet fatty acids composition, as well as the adequate diet of the broodstock, once that maternal nutrition influence the composition of the yolk (Cunha et al.,2008).

High mortality rate in crustacean larvae is assumed to be directly linked with the scarcity of lipids on diets and the use of available energy reserves (Hien et al., 2005). The presence of lipids, especially fatty acids, on crustacean diet is extremely important to achieve high

survival rates, once crustaceans have limited or no ability to synthetize long chain poly and highly unsaturated fatty acids (PUFAs and HUFAs).

The correct development of marine larvae requires the presence of docosahexaenoic acid (C22:5 6n-3, DHA) in the diet, in adequate amounts. At relatively low levels, DHA can be a limiting factor, reflecting its shortage in structural flaws and on low survival rates (Torres et al., 2007). The administration of diets with a specific lipid profile is a right approach to overcome these problems (Conlan et al., 2014).

Phyto and zooplankton administrated to larvae need to shine through a high fatty acid profile. *Artemia* and rotifers have very low levels of HUFA and have a short ability to produced long chain DHA and eicosapentaenoic acid (C20:5 n3, EPA) through short-chain poly unsaturated fatty acids (PUFA) (Liu and Xu, 2009). Copepod are known to naturally have high levels of DHA and EPA, and a DHA/EPA ratios higher than 1 (Conceição et al., 2010).

1.3 Live feed for decapod crustacean larviculture

One of the most limiting factor for larviculture development is the administration of suitable live preys. Recent studies had demonstrated that wild plankton can promote a better larval growth and survival than traditional live preys (Ajiboye et al., 2011, Olivotto et al., 2010).

One of the most used live prey for larval rearing is *Artemia* sp. Such option is based on its easy cultivation, suitable size and natural movements. Additionally, crustacean nutritional composition can be enriched with specific microalgae or commercial products (Boglino et al., 2012).

Nowadays, the number of species produced in marine hatcheries are limited due to the scarce of available live preys. Based on research studies performed on potential live feeds, copepods have been constantly highlighted given its numerous benefits on rearing marine fish and crustaceans (Sampey et al., 2007).

In marine ecosystems, copepods are consider the most important consumer of phytoplankton, having a relevant role on marine food webs (Freese et al., 2012). Many studies have verified that nauplii of calanoid copepods represent almost 50% of the stomatch content of fish and crustaceans, supporting its importance as natural food.

Additionally, when copepods are offer simultaneously with *Artemia*, gut content tends to be dominated by copepods presence (Chesney, 2005).

Copepods are a valuable asset for marine hatcheries development due to its range of body size between nauplii and adults, natural movements which stimulate the predatory activity of larvae and the high content of highly unsaturated fatty acids (Delbare et al., 1996). Some species of calanoid copepods such as *Acartia* spp., *Eurytemora* spp. and *Parvocalanus* spp., had been demonstrated good results on experiments related with larval development. (Oliviotto et al., 2009).

A boundary linked with copepods cultivation lies on its economic feasibility, but it is essential for aquaculture future given the benefits that can be attained. On general, copepods contribute to a good pigmentation, increment of growth and survival rates, development of digestive tract and to a decrease of deformities frequencies on larval stages (Hansen, 2011). Marques et al., 2014 suggest that these benefits can be only achieved by adjusting the prey size during larval rearing.

A great demand supposes a great offer, but there are several bottlenecks on calanoid copepods continuous production. Copepods trade is mainly ensured by companies that harvest live copepods from their natural habitats instead of its cultivation. Such approach dependent absolutely upon wild primary production and can compromise the sustainability of marine ecosystems.

To overcome this approach, it is necessary to solve several production handicaps, such as reproduction. Traditional live feeds can reproduced by parthenogenesis, in contrast to copepods, that produce their eggs by sexual reproduction (Camus, 2012), increasing the time in culture to achieve high densities. After fecundity, some copepods species release eggs on the water, other keep them during their development, preventing its storage.

Currently, there are very few companies around the world that produce copepods and all of them are facing the same bottlenecks: optimization of copepod feeding and implementation of protocols for large-scale production at a reasonable final price (Camus, 2012).

Acartia tonsa (Dana, 2012) is a calanoid copepod species that is extremely interesting to fed fish and crustacean larvae due to its high tolerance to temperature and salinity ranges (Mauchline, 1998). *A. tonsa* is an estuarine species implicated in the flux of energy and

matter to higher trophic levels, having a high abundance on zooplankton samples (Leandro et al., 2006).

In wild, this omnivorous copepod is a source of food to many estuarine and marine fish and crustacean larvae due to their large range of dimensions: nauplii range approximately 0.1 to 2.7 mm and copepodites can grow until 3 mm (Leandro et al., 2006).

As a crustacean organism, copepods grow by making a multiple moults, allowing a large spectrum of sizes (Gammel and Buskey, 2011). This calanoid species is a free spawner, releasing their eggs to the water, an advantage, once it allow the collection of eggs and their storage at low temperatures. *A. tonsa* produce a large number of eggs, which hatch into a nauplii and take 13 days to grow on mature adults – stage V of development (Leandro et al., 2006).

1.4 Biological model: *Necora puber* (Linnaeus, 1767)

The swimming crab *Necora puber* can be found in the coastal shelf of the northeast Atlantic, from Norway to North Africa including the Mediterranean Sea. Its geographical distribution comprises a high range of temperatures, suggesting that is prepared to subsist against extreme conditions in shallow waters of the intertidal zone.

In natural environment, adults feed on crustaceans, bivalves and on brown algae (Norman and Jones, 1992). Adults can live up to 4 years and achieve sexual maturity one year after the settlement, displaying a fecundity up to 450 000 eggs (Hearn, 2004). During reproductive period, the female moults and the male display agonistic behaviour towards other males. Couples keep together up to 3 days after copulation, while females recover from moulting.

As observed in other species of crustacean decapods, after the first copulation, females can store viable sperm that can be used in successive spawns. Females carry eggs until the release of larvae into the water column (Lee et al, 2004). *N. puber* has five zoeal stages and a megalopa, all of them planktonic. At high temperatures (24º C), Zoea V can be reached in 12 days (Jackson et al., 2014).

Five stages of embryonic development can be identified on *N. puber*. At 17ºC and a salinity of 35, the complete embryonic development is achieved on 33 days. In relation to the

larval development, recent studies demonstrated that high survival rates (up to 60%) could be reached (Jackson et al., 2014).

Along the Portuguese coast, reproduction occurs from February until the end of summer, with a strong presence of zoeal stages on samples of plankton of March to April (Santinho, 2009). After larval settlement, juveniles demonstrate a preference for algae as substrate, increasing their survival rate (Lee et al., 2004).

N. puber is one of the most appreciated decapod crustacean species. The majority of organisms consumed in Portugal are import from North Europe, where captures have been reduced, probably as consequence of over exploration. To invert this situation it is indispensable to increase the knowledge about captive rearing of this species, creating conditions for its aquaculture production (Jackson et al., 2014).

1.5 Aim of study

The present study aimed to assess the effect of *Acartia tonsa* as live feed on the larval development of *N. puber.* The experimental design consisted on two complementary methodological approaches:

(i) Culture optimization of *A. tonsa*, which included:
   a. Appraisal of suitable microalgae;
   b. Determination of hatching, survival and egg production rate.

(ii) Effect of *A. tonsa* ingestion on *N. puber* larvae, which included:
   a. Survival rate;
   b. Larval stage Proportion;
   c. Length-weight relation;
   d. Specific growth rate, carapace length gain and dried weight gain.

2. Material and methods

2.1 Live feed production

2.1.1 Microalgae cultures

Red flagellate microalgae *Rhodomonas lens* (Pascher and Rutther, 1913) and green flagellate microalgae *Tetraselmis chuii* (Butcher, 1959) were scaled-up and produced on 5L plastic carboys, under 20±1 ºC, 35±1 salinity and photoperiod 12 h light: 12 h dark. Modified F/2 medium was used as culture medium (Guillard & Ryther, 1962) and provided in fed-batch mode. Salt water involved on microalgae growth was previously filtered through 0,2 µm and sterilized by temperature and pressure (120ºC, 15 min, 1 atm).

Before administration to *Acartia tonsa* cultures, *R. lens* and *T. chuii* were manually counted.

2.1.2 *Artemia franciscana* (Kellog, 1906)

Artemia franciscana was obtained by commercial cysts (Ocean Nutrition®) and submitted to decapsulation process. *A. franciscana* was collected with a 200µm sieve, after a 24h growth, at 23±1ºC, counted and administrated to *Necora puber* larvae.

2.1.3 Stock culture of *A. tonsa*

Stock culture of *A. tonsa* used on this study, was establish at CETEMARES research facility in the end of September 2015. Adult copepods were collected from Mondego estuary (Portugal) by demersal trawling with a 200 µm mesh WP2 net.

Collected zooplankton samples were transported to the laboratory with permanent aeration. Adults of *A. tonsa* were sorted out at a stereomicroscope, taking advantage of the positive phototaxis exhibited by these individuals. Sorted organisms were placed on large buckets containing 20L of pre-filtrated seawater and aeration.

After pure culture of *A. tonsa* was established, copepods were frequently separated by developmental stage (copepodites and nauplii) through successive sieving's. Meshes sizes were selected according to Leandro et al., 2006 - 200 µm to separate adults from juveniles and 64 µm to collect nauplii and eggs.

Copepods separated by length were transferred to eight 15L cilindroconic tanks without water recirculation. Cultures were maintained under a photoperiod of 12 h light: 12 h dark, at 20±1ºC and 30±1 salinity.

Every three days, the entire seawater volume was sieved in order to separate by development stage. Eggs were easily separated from nauplii, since it tends to set down on the bottom of containers and nauplii exhibits positive phototaxis. After collection, eggs were placed on tanks for hatching or stocked on dark flasks at a temperature of 4±1ºC.

Stock cultures of *A. tonsa*, were daily fed with *R. lens*, adjusted at a final density of $1x10^4$ cell.mL^{-1}.

2.2 Copepod feeding optimization

Most suitable microalgae (*T. chuii* or *R. lens*) for copepod production was identified through hatching rate, survival rate and egg production rate of copepods when subjected to the different treatments.

2.2.1 Hatching rate

Hatching rate experiment comprised three replicates of three different feeding treatments: R*. lens*, *T. chuii* and Starvation.

Initially, copepod eggs were placed in 2L culture vessel (diameter = 12 cm; height = 21.5 cm), at a density of 1000 egg.L^{-1}, with seawater and constant aeration, at 20±1ºC, 30±1ºC salinity and a photoperiod of 12 h light: 12 h dark. Microalgae were added at a density of $1x10^4$ cell.mL^{-1}. Water was daily changed and microalgae adjusted to initial density. Eggs and nauplii were counted at 24h and 48h, allowing to determine hatching rate at these set points (equation 1).

Equation 1 $$Hatching\ rate\ (\%) = \frac{Number\ of\ nauplii}{Initial\ number\ of\ eggs} \times 100$$

2.2.2 Survival rate and egg production rate

A complementary assay (Fig. 2.1) was done to estimate the effect of the diet on the survival rate (equation 2) and on egg production rate (equation 3).

Equation 2 $$Survival\ rate\ (\%) = \frac{Number\ of\ surviving\ individuals}{Initial\ number\ of\ individuals\ under\ test} \times 100$$

Equation 3 $$Egg\ production\ rate\ (\%) = \frac{Number\ of\ eggs}{Number\ of\ femeals} \times 100$$

Nauplii were placed on 2L culture vessels, at a density of 1000 eggs.L^{-1}, with seawater and constant aeration. Vessels were also kept at 20±1ºC, 30±1 salinity and photoperiod 12h light : 12h dark. Organisms were daily fed with two different treatments: *T. chuii* and *R. lens*, at a final concentration of 1x10^4 cells.mL^{-1}. Nauplii was counted from 3 Days Post Hatching (DPH) and, when eggs production began, eggs were daily collected, counted and stocked at previous described conditions (section 2.1.3). Water change and food adjustment were daily performed and assays were followed until survival rated achieved less than 20%. Treatments were performed in triplicate, to ensure statistical relevance.

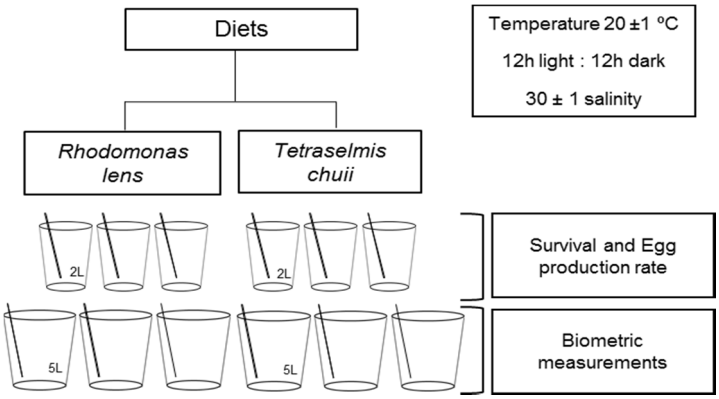

Figure 2.1 Experimental setup to evaluate the effect of the diet on survival and egg production rate of *Acartia tonsa*.

2.2.2 Biometric measurements

Mean individual copepods length is relevant to determine the required time to achieve a particular live prey size. In the present study, it was also assessed the mean legth of all development stages identified. To allow that assay, eggs were placed for incubation on 5L culture vessels (diameter = 17 cm; height = 22.5 cm), at a density of 1000 egg.L^{-1}. Vessels were mantained at 20±1ºC, 30±1 salinity and a 12h light: 12h dark photoperiod. Organisms were daily fed with $1x10^4$ cell. mL^{-1} of microalgae species that promoted a higher hatvhing, survival and egg production rate, in privious assay.. Everyday, 5 individuals were collected from each vessel and photographed with a high precision camara, promoting a total of 15 copepods measeured for treatment. Body length (nauplii) or prosome length (copepodites) were estimated from image analysis through the *ZEISS Microsoft Software ZEN 2 lite* (Fig. 2.2). Treatments were performed in triplicate, to ensure statistical relevance.

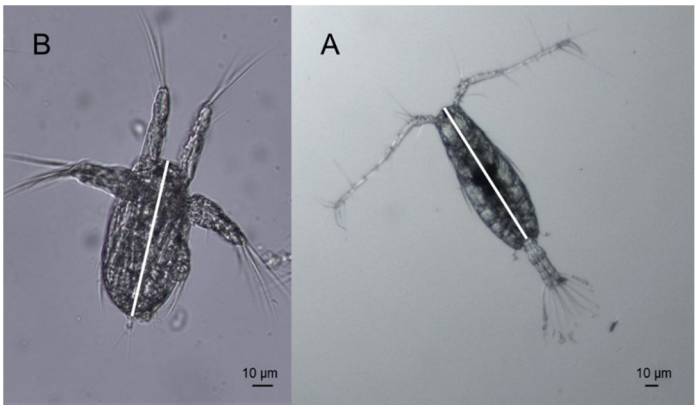

Figure 2. 2 Body length of nauplii (A) and prosome length of copepodit (B) represented by white line.

2.3 Administration of *Acartia tonsa* to *Necora puber* larvae

2.3.1 *Necora puber* female maintenance

Five ovigerous females of *Necora puber* were collected at Peniche coastal zone (Portugal) and transported to the laboratory under controlled temperature. Upon arrival, individuals was measured, weighed and placed on 40L tank. The water tank was fitted at a water-recirculation system, equipped with a protein skimmer (TMC, United Kingdom), a fluidized sand filter and an 36 W UV lamp (TMC, United Kingdom). Nitrogen compounds were kept at

minimal levels of 0,1 mg.$L^{-1}$and temperature was kept at 18±1ºC and salinity at 35 ±1, pH 8.2±0.1 and photoperiod was 12 h light: 12 h dark. *N. puber* was switched fed on frozen mussel, squid and cod roe, twice a day, until spawning. Larvae of one spawn were used to carry out larval development assays.

2.3.2 Larval development

Spawning event occurred at night and larvae were collected by positive phototaxis (Gregati et al., 2010). Larvae were counted and divided at a density of 50 larvae.L^{-1} and distributed by constantly gently aired vessels, filled with 2L of seawater and placed on a water bath with temperature control. Seawater used for larval development was removed from ovigerous female system, including water changes. Nitrogen compounds were kept at minimal levels of 0,1 mg.$L^{-1}$and temperature was kept at 18±1ºC and salinity at 35±1, pH 8.2±0.1 and photoperiod was 12 h light: 12 h dark.

Assays was carried in triplicate and 3 diets were tested: *A. franciscana*, *A. tonsa* and Starvation. Diets were offered with similar lengths and at a density of 10 individuals.mL^{-1} (Fig. 2.3). Daily, larvae were counted, water renewed, and food replaced by new one, to avoid nitrogen compounds accumulation. Larval stages were also daily identified, according to Santos et al. (2004). It was considered that replicates achieved a new larval stage, when more than 50% of larvae moulted to the next development stage.

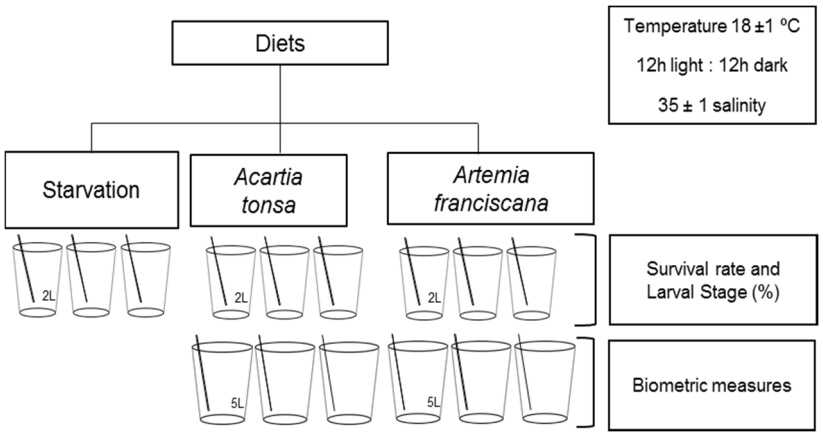

Figure 2.3 Experimental design for *Necora puber* larval development assays.

2.3.3 Biometric measurements

Larvae were similarly displayed at a density of 50 larvae.L^{-1}, on 5L vessels to ensure a large production of larvae to biometric measurements. Three vessels were fed with *A. franciscana* and other three with *A. tonsa,* repeating feed density previously mentioned. To maintain constant temperature, vessels were placed on water of adults maintenance tanks. Water changes were daily performed with breeder system water, to maintain the same parameters. Nitrogen compounds were kept at minimal levels of 0,1 $mg.L^{-1}$ and temperature was kept at 18±1ºC and salinity at 35±1, pH 8.2±0.1 and photoperiod was 12 h light: 12 h dark.

Two days after each zoeal stage were achieved (from zoea I to V), 15 larvae were collected to biometric measurements. Larvae were individually photographed with a high precision camera and analysed with *ZEISS Microsoft Software ZEN 2 lite*. For dry weight estimation, 40 larvae were filtrated through pre-combusted and weighted GFC filters, cleaned with distilled water and submitted to 110±1ºC, during 72h (Durán et al., 2011). The same procedure was performed to both administrated diets, in triplicates.

It was also evaluated the effect of different diets on growth rate (SGR) (equation 4), carapace length growth (CLG) (equation 5) and dry weight gain (DWG) (equation 6) (Durán et al., 2011).

Equation 4
$$\%\, SGR = \frac{ln\, final\ Dry\ Weight - ln\, initial\ Dry\ Weight}{days\ between\ stages} \times 100$$

Equation 5
$$\%\, CLG = \frac{final\ Carapace\ Length - initial\ Carapace\ Length}{initial\ Carapace\ Length} \times 100$$

Equation 6
$$\%\, DWG = \frac{final\ Dry\ Weight - \quad initial\ Dry\ Weight}{initial\ Dry\ Weight} \times 100$$

2.4 Fatty acid profile of live feed

To assess the effect of *R. lens* and *T. chuii* on *A. tonsa* ($1x10^4$ $cell.mL^{-1}$) development, lipid and fatty acid profile were tested. Biomass was recovered by several centrifugation cycles

at v=3500 rpm (Eppendorf 5810R, German), during 5 minutes, T=environment, of 1L of each microalgae. Pellet was recovered and frozen at -80ºC, until further analysis.

To validate the effect of two both diets on larval development, *A. franciscana* with 24h, *A. tonsa* with 7 days were sieved (200 µm), in order to remove the excess of water. Samples were maintained at -80±1ºC until biochemical analysis.

2.4.1 Total lipid content

Total lipid content was quantified following Folch method (Folch et al., 1957). 3 mg of macerate biomass were homogenised, using a high-pressure homogenizer (Yastral politron, Germany), with chloroform:methanol solution (2:1), using a proportion of 1:20 (biomass :solution). Mix was again homogenised in a vortex and centrifuged (Eppendorf 5810R, German) by spin-down. Lipid phase was recovered and washed with 0.2 volume of 0.9% NaCl solution. After vortex homogenization, mix was centrifuged (Eppendorf 5810R, German) at v=2000 rpm, during 10 minutes, at T=environment. Then, the upper phase was removed for a new pre-weight tubes. Chloroform was evaporated in a rotary evaporator (T=40ºC) and lipids content was gravimetrically calculated through Equation 7. Analysis were performed in triplicates to ensure statistical relevance.

Equation 7 $$Total\ lipid\ content\ (\%) = \frac{Inicial\ weight - Final\ weight}{Dry\ biomass\ weight} \times 100\%$$

2.4.2. Direct transesterification

In glass tubes, it was precisely measure 100 mg of each sample, in triplicates. It was add 1,5 mL of methanol:acetil chlorid solution (20:1), followed by 30 seconds vortex homogenization. 1 mL of hexane and 20µl of internal standard was added and homogenised along 30 seconds. With glass tubes covered with Parafilm M ®, it were placed in water bath during 1 hour, at 80ºC. After that, biomass was immediately cooled in ice, and 1 mL of MiliQ water added. Tubes were subjected to 1 minute of homogenisation in vortex, preceded by a centrifugation (Eppendorf 5810R, German) cycle (V=2000 rpm), during 10 minutes, at 4ºC. The organic phase was collected, passed through to an anidro sodium column, previously prepared and placed into vials (D'oca et al., 2011).

2.4.3. Fatty acids quantification

Vials with fatty acids samples were analysed in a Finnigan TRACE GC Ultra (Thermo Electron Corporation ®) gas chromatograph, equipped with fitted with flame ionization detector and an auto-sampler AS 3000 Thermo Electron Corporation. It was used helium (flow rate of 1,5 $mL.min^{-1}$) as carrier gas in a column TR-FRAME (60mx0.25mm IDx0.25m). Detector were programmed to 260ºC and were fed with air and hydrogen at a flow rate of 350 and 35 $mL.min^{-1}$, respectively. Temperature of injector was programmed to 250ºC and it is operating in split less mode. Oven temperature was slowly increased to 60ºC for 1 minute, amplifying to 150ºC at 15ºC min^{-1} and held in 150ºC during 1 minute. Then, temperature rose up to 180ºC at 5ºC min^{-1}, keeping during 3 min. The last increased takes place to 220ºC at 10ºC min^{-1}, held for 1 minute.

Acid methyl esters identification of was made by comparison of retention time with chromatogram of marine PUFA standards. Peak areas were manually integrated, except the peaks with lower relevance (less than 1%).

2.5 Statistic analysis

All data were checked for normality and homoscedasticity.

Regarding the optimization of *A. tonsa* feeding, a two-way analysis of variance (ANOVA) was performed in order to study the effect of diet (*Rhodomonas lens, Tetraselmis chuii* and Starvation) and time in the hatching rate and the effect of diet (*R. lens*, and *T. chuii*) and time in survival rate (Zar, 2009). To determine significant differences between treatments (diets and/or time), and whenever appropriate, the analysis was followed by the multiple comparison tests of Dunnett or Tukey (relatively to control treatment, Starvation) (Zar, 2009).

The same procedure was performed for the larval survival of *N. puber,* in order to evaluate the effect of diet (*A. tonsa, A. franciscana* and Starvation) and time. Subsequently it was performed a one-way ANOVA (Zar, 2009) to evaluate differences on survival, comparing the same diets per each zoeal stage (ZI, ZII, ZIII, ZIV and ZV). In addition, the two-way ANOVA was applied to study the differences in SGR, DWG and CLG when comparing previous referred diets and zoeal stages. Finally, the measuring of the relationship between weight and length was conducted by simple linear regression analysis followed

by calculating the linear correlation coefficient of Pearson (Zar, 2009). Fatty acids results were assess with one-way ANOVA.

When applicable, results are presented as mean ± standard deviation (SD). For all statistical tests, the significance level was set at $p<0.05$. All calculations were performed with SigmaPlot software 136 12.0.

3. Results

3.1 Copepod feeding optimization

3.1.1 Hatching rate

Egg hatching rate of *Acartia tonsa* was higher at 48h, for both treatment (Table I). However significant differences between 24h and 48h were only verified for the Starvation treatment (two-way ANOVA, p-value<0,05). Using *Rhodomonas lens,* the number of nauplii was significantly higher at 24 and 48h, when comparing to *Tetraselmis chuii* and Starvation treatment (two-way ANOVA, , p-value<0,05) (Table I). Maximum hatching rate (91,20 ± 4,31 %) was achieved with *R. lens* administration at 48h.

Table I. Hatching rate at 24 and 48h, when eggs were subjected to 3 different treatments: *Rhodomonas lens*, *Tetraselmis chuii* and Starvation. Values are expressed as mean ± SD.

Time (h)	Hatching rate (%)		
	Rhodomonas lens	*Tetraselmis chuii*	Starvation
24	90,12 ± 1,79	69,37 ± 9,83	44,95 ± 3,64
48	91,20 ± 4,31	72,78 ± 9,06	69,18 ± 1,33

3.1.2 Survival rate and egg production

Survival rate presented a decreasing tendency along days post hatching (DPH). Nauplii fed with *T. chuii* did not achieve copepodite stage and survival rate declined to 12%, at 8 DPH. With *R. lens*, copepods also had a progressive decreased survival rate, achieving 17,5% at 28 DPH day. However, the decrease was less pronounced when compared with the verified in *T. chuii* treatment. Despite of the exhibited differences promoted by the administration of these two microalgae species, significant differences were only verified after 5 DPH day (two-way ANOVA, p-value<0,05), with *R. lens* presenting highest survival rate (Figure 3.1).

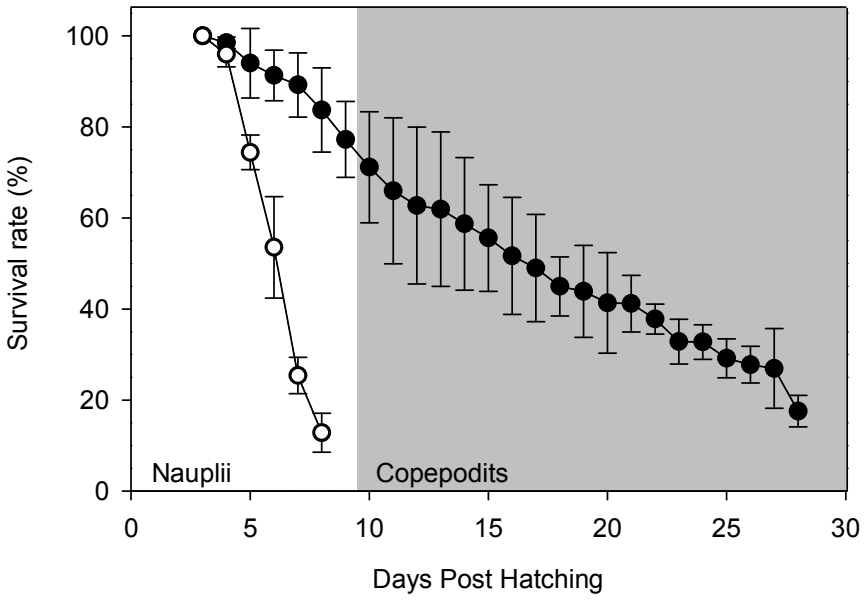

Figure 3.1 Survival rate (%) of *Acartia tonsa* nauplii and copepodits, when fed on *Rhodomonas lens* (●) and *Artemia franciscana* (○), along days post hatching. Values are expressed as mean±SD.

Eggs were only produced by copepodits fed on *R. lens*. Females began egg production at 12 day, producing 1 egg per female. The egg production rate increased until 24 DPH with a maximum value of 34 eggs per female. Afterwards, a significant decrease occurred until the end of assay (Figure 3.2).

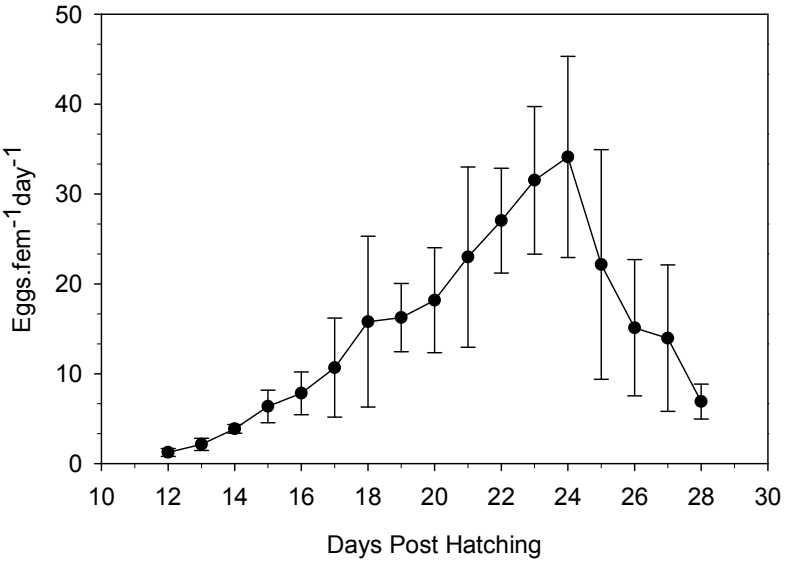

Figure 3. 2 *Acartia tonsa* egg production per female per day, when fed on *Rhodomonas lens*. Values are expressed as mean ± SD.

3.1.3 Prosome and total body length

Body length and prosome length increase along development stages. At 18±1ºC, nauplii achieve the highest values of body length at N VI (0,25 mm). Copepodits, at C VI, presented a prosome length of 1,2 mm (Figure 3.3).

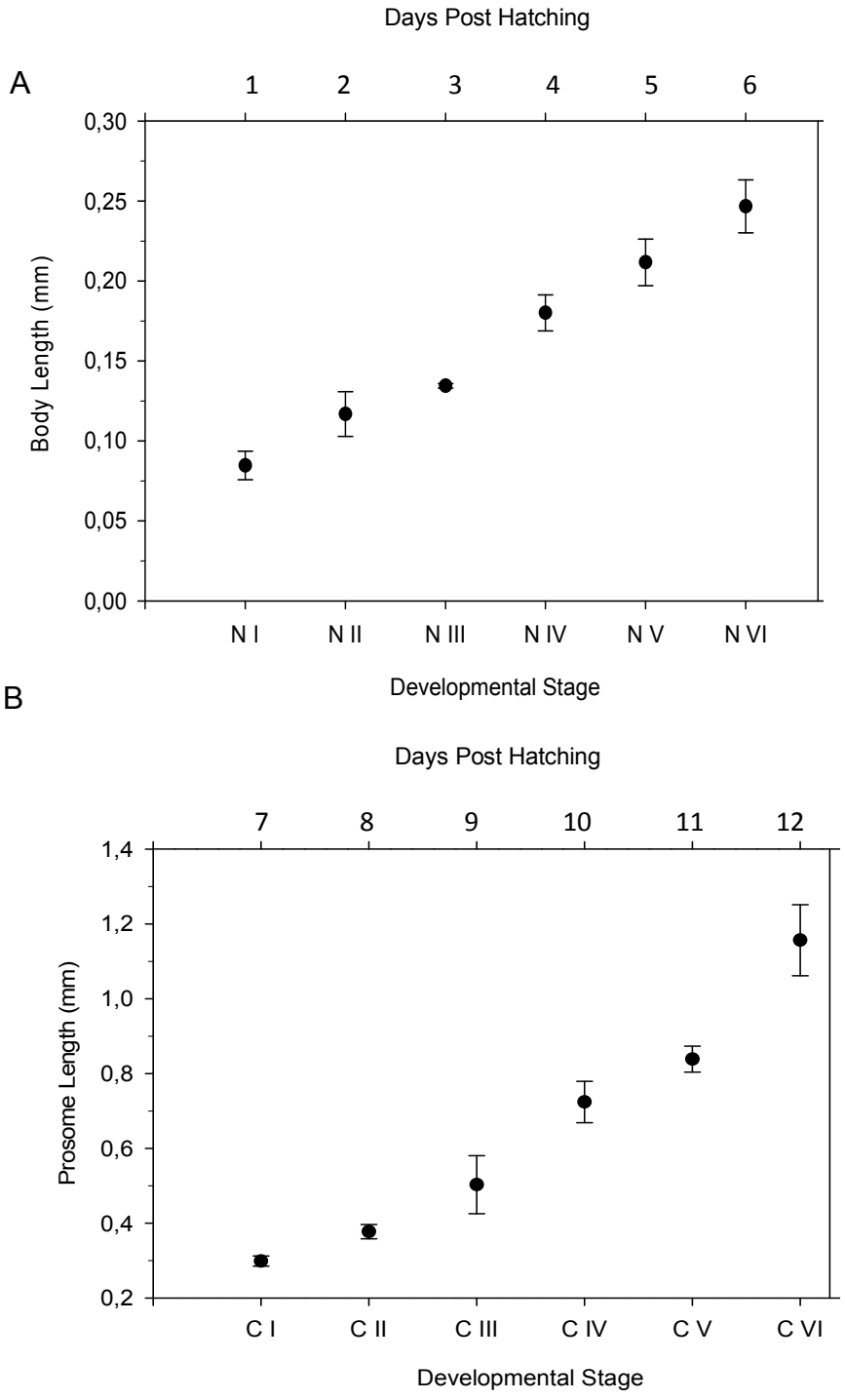

Figure 3.3 Relation between body length and prosome length with developmental stage of nauplii (A) and copepodites (B), respectively. Values are expressed as mean ± SD.

3.2 Administration of *Acartia tonsa* to *Necora puber* larvae

3.2.1 Larval development of *Necora puber*

Survival rate decreased along larval development. There were no differences on survival rate, during the first three days post hatching (two-way ANOVA, p-value<0,05). From 4 DPH, survival rate of larvae subjected to Starvation were significant lower than treatments fed zooplankton. Starved larvae did not survive longer than 8 DPH. From 9 DPH until the end of the assay, *A. tonsa* was the diet that promoted higher survival rate, when compared to *A. franciscana* (two-way ANOVA, p-value<0,05) (Figure. 3.4).

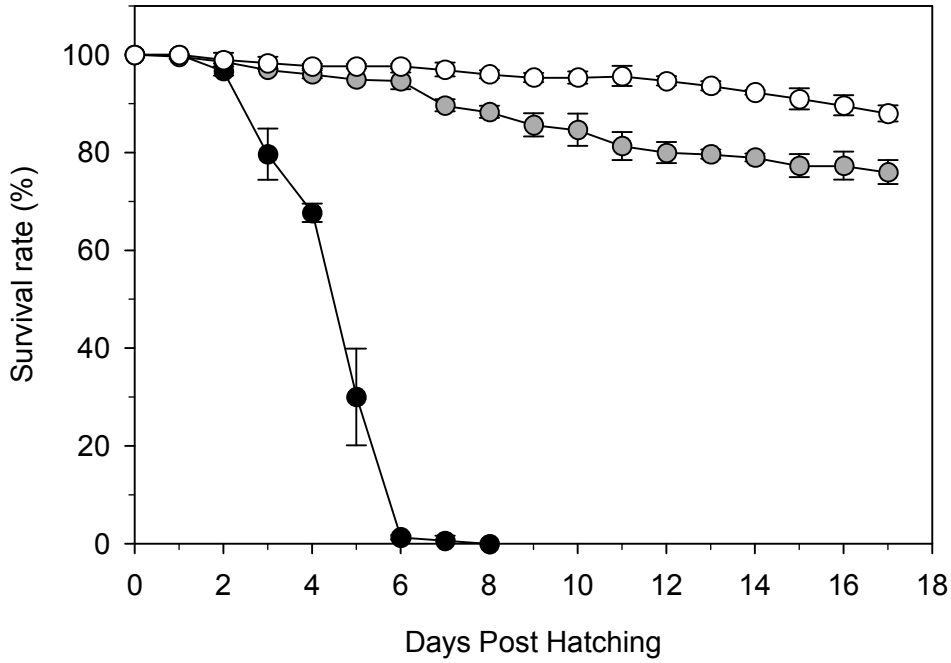

Figure 3.4 Survival rate (%) along Days Post Hatching of *Necora puber* larvae, under Starvation treatment (●) and fed on *Acartia tonsa* (○) and *Artemia franciscana* (◉).Values are expressed as mean ± SD.

Obtained results suggest that survival rate decrease along larval development. At Zoea I, there were no differences among *A. tonsa* and *A. franciscana* (one-way ANOVA, p-value<0,05), and both diets promoted about 97% of survival rate. Larvae submitted to Starvation did not molt to zoea II. From ZII to ZV, survival rate was significant higher when larvae were fed on *A. tonsa* rather than with *A. franciscana* (one-way ANOVA, p-value<0,05). Survival rate was significantly higher on the last day of larval development (89 ± 1,63%), when copepods were used as live feed (one-way ANOVA, p-value<0,05) (Figure 3.5).

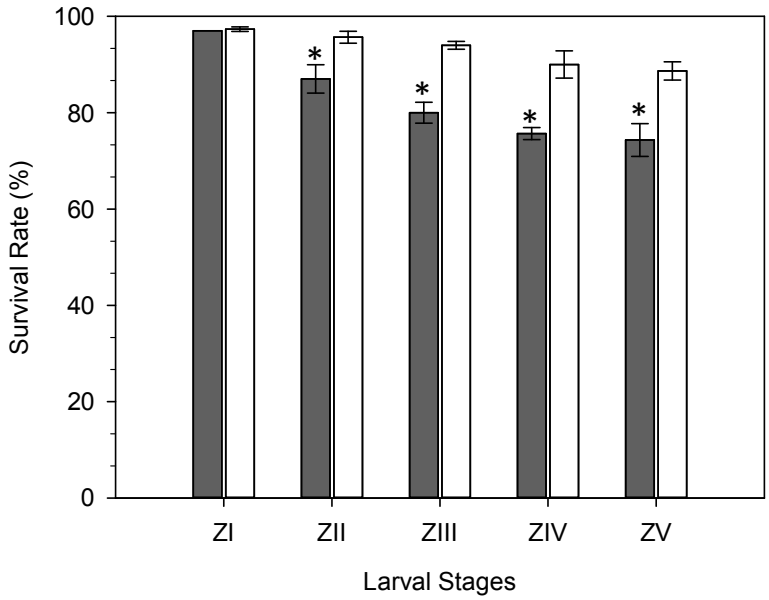

Figure 3. 5 Survival rate (%) by larval stages, when larvae were fed on *Acartia tonsa* (○) and *Artemia franciscana* (●).Values are expressed as mean ± SD and symbols over the bars indicate significant differences.

Concerning to larval development, individuals fed on *A. franciscana* changed to ZII at 4 DPH and to ZIII at 8 DPH. Larvae fed on *A. tonsa* took more days to achieve ZII and ZIII - 6 and 4 days, respectively. Although larvae submitted to *A. franciscana* achieved earlier ZIV and stays longer in ZIII, when compared with *A. tonsa* diet. When larvae were subject to copepod diets, ZIV stage duration was shorter and ZV was achieved one day before with *A. franciscana* diet (Figure 3.6).

A linear regression between length and weight of *N. puber* larvae that fed on *A. tonsa* and *A. franciscana*, was defined taking in account all larval stages. There were no significant differences among length and weight of larvae fed on *A. tonsa* or fed on *A. franciscana*. On each diet, *Pearson's r* suggested a strong correlation of both variables (Figure 3.7), obtaining r = 0,9931 with *A. tonsa* and r = 0,9929 with *A. franciscana*.

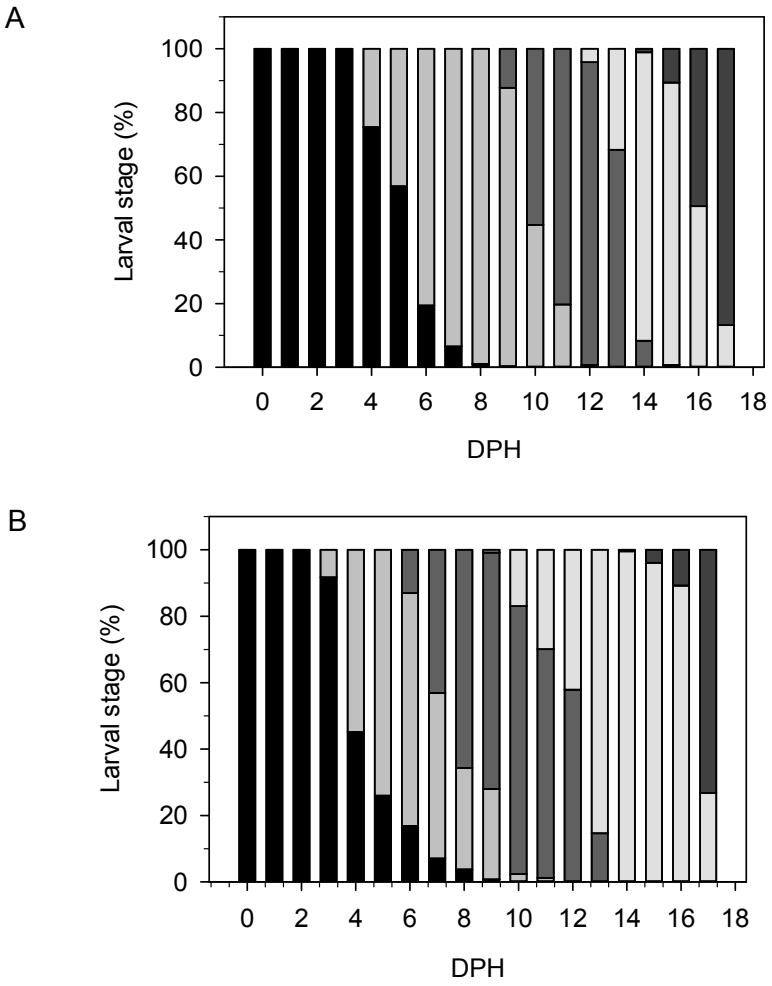

Figure 3. 6 Zoeal stage proportion (%) (Zoea I ■, Zoea II ■, Zoea III ■, Zoea IV ■; Zoea V ■), along Days Post Hatching, when larvae were fed on *Acartia tonsa* (A) and *Artemia franciscana* (B).

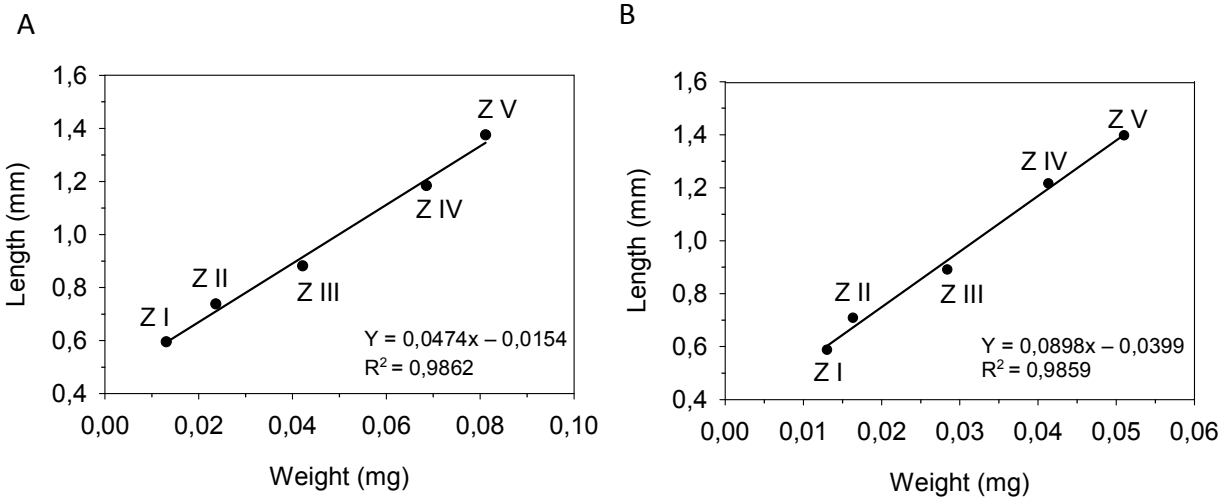

Figure 3. 7 Correlation of lenght and weight when larvae were fed on *Acartia tonsa* (A) and *Artemia franiciscana* (B).

There were no significant differences between live feed diets, for all larval stages concerning to growth rate (SGR), carapace length growth (CLG) and dry weight gain (DWG), along five zoeal stages (Figure 3.8).

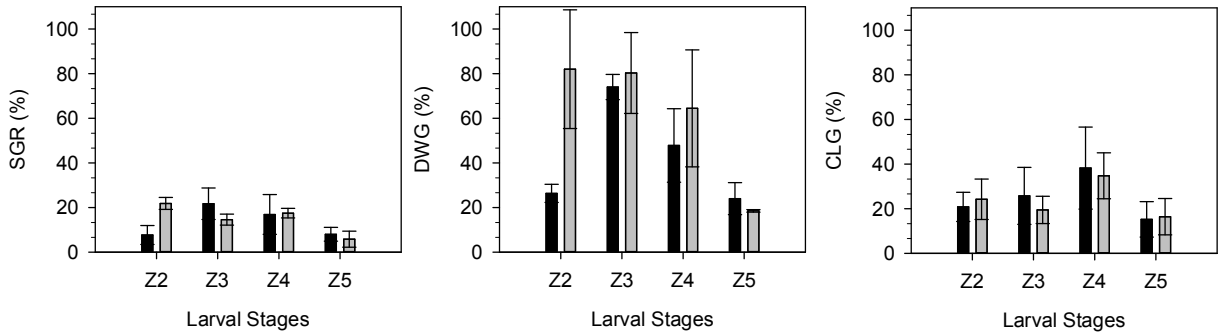

Figure 3. 8 Results of Specific Growth Rate (SGR), Carapace length gain (CLG) and Dry weight gain (DWG), along different stages, when larvae were fed on *Acartia tonsa* (■) and *Artemia franciscana* (■).Values are expressed as mean ± SD.

3.3 Fatty acid profile of live feed

Relating to fatty acid composition (saturated and monounsaturated), no significant differences were verified between *R. lens* and *T. chuii*. *R. lens* presented higher levels of poliunsaturated fatty acids when compared with *T. chuii* (one-way ANOVA, p-value<0,05). The main difference lies in the abcence of DHA on *T. chuii*, resulting in a lower Total PUFA content (one-way ANOVA, p-value<0,05) (Tabel II).

A. tonsa presented a higher poliunsaturated fatty acids level, when compared with *A. franciscana* (one-way ANOVA, p-value<0,05). The major difference between zooplankton species analysed was found in EPA and DHA content, that was significantly higher in *A. tonsa* (one-way ANOVA, p-value <0.05). Copepods exhibit a EPA/DHA ratio close to 1, in contrast to *A. franciscana* (Table II).

Table II. Fatty acid composition of microalgae species, *Rhodomonas lens* and *Tetraselmis chuii*, and zooplankton spicies, *Acartia tonsa* and *Artemia franciscana*. Values are expressed as mean ± SD.

Fatty acids		*Rhodomonas lens*	*Tetraselmis chuii*	*Acartia tonsa*	*Artemia franciscana*
Saturated	C14:0	0,649 ± 0,205	0,359 ± 0,016	0,875 ± 0,000	0,306 ± 0,153
	C16:0	2,915 ± 0,038	1,982 ± 0,152	4,773 ± 0,253	2,519 ± 0,034
	C18:0	1,120 ± 0,126	0,585 ± 0,084	1,855 ± 0,196	1,549 ± 1,132
	Total SFA	**4,684 ± 0,369**	**2,926 ± 0,252**	**7,503 ± 0,449**	**4,374 ± 1,319**
Monosaturated	C 18:1 n9	1,179 ± 0,234	1,075 ± 0,449	0,985 ± 0,092	2,679 ± 0,597
	C 18:1 n7	0,221 ±0,029	0,276 ± 0,077	0,456 ± 0,084	0,647 ± 0,196
	Total MUFA	**6,085 ± 0,632**	**4,277 ± 0,778**	**8,944 ± 0,625**	**7,701 ± 2,111**
Polysaturated	C 18:2 n6	0,739 ± 0,061	1,008 ± 0,120	0,643 ± 0,035	1,314 ± 0,267
	C 18:3 n3	2,809 ± 0,296	1,534 ± 0,162	3,171 ± 0,172	4,661 ± 0,929
	C 18:4 n3	1,539 ± 0,192	0,179 ± 0,043	1,231 ± 0,016	0,793 ± 0,108
	C 20:4 n3	0,313 ± 0,063	-	0,464 ± 0,081	0,145 ± 0,033
	C 20:5 n3 (EPA)	1,516 ± 0,210	0,521 ± 0,085	3,6335 ± 0,193	0,337 ± 0,021
	C 22:6 n3 (DHA)	0,498 ± 0,039	-	3,286 ± 0,004	0,124 ± 0,019
	Total PUFA	**7,413 ± 0,861**	**3,241 ± 0,410**	**12,428 ± 0,500**	**7,375 ± 1,377**
DHA/EPA		0,328 ± 0,056	-	0,907 ± 0,049	0,368 ± 0,025
Total lipid		15,500 ± 8,500	10,667 ± 2,625	22,667 ± 4,028	19,000 ± 13,952

4. Discussion

The present study proved that diets are extremely important for the success of larviculture. Culture conditions such as microalgal quality and species can influence the copepod culture productivity (Camus, 2012), but this parameter still lacks researched. For example, *Calanus* sp. productivity is dependent on high nutritional requirements (Castro-Longoria, 2003), specially concerning fatty acids as eicosapentaenoic acid (C20:5 n3, EPA), docosahexaenoic acid (C22:6 n3, DHA) and other n-3 polyunsaturated fatty acids (PUFAs). PUFAs influence embryonic development, having an important role on hatching rate, and are essential to central nervous system, cell membrane structure and visual system (Camus, 2012). A possible relation between DHA concentration and high hatching rate is supported by the present study, since *Tetraselmis chuii*, which does not present DHA, resulting in a poor hatching rate, when compared with *Rhodomonas lens* (Zhang et al., 2013). *T. chuii* promote similar results when were administrated to another copepods species, as *Acartia sinjiensis*, providing lower hatching rate (Camus, 2012).

When *A. tonsa* nauplii were submitted to *R. lens* or *T. chuii*, there were no differences on survival rate, until 5 DPH, suggesting that initial energy source are nutritional reserves accumulated on yolk. After 6 DPH, survival rate of nauplii fed on *T. chuii* suffer a progressive decrease. Camus (2012) suggested that the poor performance observed on copepods when fed on *T. chuii* can likewise be attributed to nauplii incapacity to ingest large cells size. Microalgae cell sizes must be adapted to the predator mouth and it can be the reason for the significant differences found on survival rate, once *R. lens* have a cell size around 8 µm (Camus, 2012) and *T. chuii* is slightly bigger – 12 to 14 µm (Mohammadi et al., 2015). This could also justify why *T. chuii* did not promote copepodite stage, dying on naupliar stage and not producing any eggs.

In the present study, the administration of monoalgal diet based on *R. lens* promoted a maximum production of 34 eggs female^{-1}. day^{-1}, which coincide with adults number decrease. Teixeira et al. (2010), administrating *Thalassiosira weissflogii*, *Isochrysis galbana* and *Chaetoceros muelleri* as monoalgal diet in *Acartia* spp., obtained a maximum of 28 eggs.female^{-1}. day^{-1}. Egg production optimization play an important role in cost-effective culturing of copepods.

Submitting *Temora stylifera* to several plurialgal diets, Buttino et al. (2009) prove that *Rhodomonas* can have the same benefits as the mix, or even more. Although reported as the most effective microalgae for copepods intensive production, *Rhodomonas* sp. is described as unstable in culture (Knuckey et al., 2005). In the present study, there was no

issues in *R. lens* semi-continuous cultures establishment, achieving maximum densities of $9{,}18x10^6$ cell.mL^{-1}. Feeding protocols with life food increase on copepods cost production. Nevertheless, is indispensable for success, once frozen feed do not promote optimal conditions to higher development rates (Woods, 2003; Payne and Rippingale, 2000). One of the most valuable characteristics of *R. lens* lies on its fatty acids profile, that seems to fulfil copepods nutritional needs. This microalgae species have high EPA and DHA content, indispensable for healthy immune system and phospholipid production. As fatty acids analysis demonstrated, DHA/EPA ratio of *R. lens* was close to 1, preventing larval neural dysfunctions and poor growth and survival exhibited when EPA higher than DHA (Copeman et al., 2002).

Up until now, copepods had been collected from the wild, at places with natural zooplankton blooms or produced at a pilot scale (Conceição et al., 2010). With the increased of copepods demand, the zooplankton blooms and pilot scales do not produce enough biomass and with that were created the firsts intensive culture protocols. Nowadays, calanoids copepods as *A. tonsa*, *Gladioferenses imparipes* and *Calanus helgolandicus* have already a developed protocols for intensive cultivation (Camus T., 2012). Although it is possible their rearing production, the majority of protocols do not reflect the need of economic viability process, being required an optimization, as the feeding methods. The major problem involved on the administration of copepods as live feed on aquaculture relies on a routine that meets production needs and stays economic feasible (Conceição et al., 2010). Low densities, adapted tanks and feed quality needed are some of copepods production bottlenecks (Holt, 2003).

Improvements on inert microdiet technology were not enough to promote high survival rates on first days post hatching of fish and crustacean larvae. Life feeds continue to be the main administrated feed during larviculture processes, however preys as *Artemia* sp. and rotifers are no longer enough to ensure high productivity in aquaculture (Conceição et al., 2010). Research of new life preys can help to overcome one of the major larviculture bottlenecks: the transition from a phase dependent on yolk, to exogenous feeding (Oliviotto et al., 2010).

Studies with decapod species suggest that the administration of enriched preys was a way to deliver the adequate lipid profile, fulfilling nutritional requirements. It was proved in previous studies, that crustacean larvae fed on life preys enriched with *T. chuii* have a greater survival rate, grow faster and moulting process occurs with a higher success. This happened due to this green microalgae improve digestive larval capacity. Using the

digestive enzymes, *T. chuii* promoted a better assimilation of proteins, justifying the need of its introduction on crustacean larval diet (Cunha et al., 2008).

In this study, and concerning *A. tonsa* survival rates with the green flagellate microalgae, it was impossible to administrate *A. tonsa* enriched with *T. chuii*. Results obtained with *Necora puber* larvae development were promoted by copepods enriched with *R. lens*. Larval assays did not last until megalopa stage, once it should require a type feed change (Lee et al., 2004) and it would fall out of the present study aim. Using feed with a most benthonic features, as brown macroalgae, (Norman and Jones, 1992) it is possible reached juvenile stage. Some improvements on dry and frozen diets based on copepods and microalgae can be made in order to increase survival rate of juvenile.

According to survival rates obtained on each zoeal larval stages, percentage decreases along larval development, maybe due to the fragility involved at moulting process. At zoea I, administrated diets did not promote any differences at survival rate, pointing to larvae still catabolize yolk energetic reserves (Simões et al., 2002). In addition, the absence of differences can be explained by larvae not being yet prepared to catch fast preys, as copepods, that have greater swimming abilities than *Artemia* (Jackson et al., 2014). Due to this absence of diet influence on survival rate, some authors begin assays just after two or three days after hatching, collecting only active larvae (Harms and Seeger, 1989).

From 4 DPH, survival of Starvation treatment were significant lower than treatments in which larvae were fed on zooplankton, and died at 8 DPH, not moulting to ZII. This dependence of administrated diets to survive reveal an *N. puber* obligate planktotrophism (Jackson et al., 2014). Harms and Seeger (1989) had similar results, once starved larvae dyed at 10th DPH. In the present study, from 9 DPH to the end of assay, *A. tonsa* promoted a significant higher survival rate, when compared with *A. franciscana.* Previous studies only submite *N. puber* larvae to *Artemia* sp. or phytoplankton species, not contemplating copepods as a possible prey. When *Artemia* sp. was administrated by Harms and Seeger (1989), larvae achive zoea V with 64% of survival rate, being the suitable diet. Heretofore, at 18ºC, the 76 ± 2,45 % obtained was higher than earliar studies, where larvae were fed on *Artemia*. High survival rates obtained in the present study, can be related to broodstock diets. Cunha et al. (2008) had demonstrate that broodstock diets have extreme influence on larval quality and quantity. High survival rates attained in this study could also be due to larvae being caught, counted and separated immediately after hatching - a correct course of action when the objective is achieve high larval performances (Gregati et al., 2010). Consequently, these benefits enhance the growth and survival rate of cultivated species, contributing to a sustainable and feasible aquaculture industry. Survival rate were

even higher with larvae fed on *A. tonsa*, about 88 ± 1,63 %, proving this to be a more suitable diet to *N. puber* early larval stages. Copepods, as a natural live prey to fish and crustacean larvae, have a motion pattern, “stop and go” (Buskey et al., 1993), that promote stronger feeding responses, when compared to *Artemia* sp. Copepods have a greater nutrional profiles that promote best larvae performances, as digestive enzymes, micronutrientes, vitamins and fatty acids (Camus T., 2012).

The administration of *A. franciscana* fostered a faster change to ZII and ZIII, with lower survival rate. As previous referred, it is possible that larvae until ZIII do not have feeding ability to caught copepods, ingesting just some preys during random encounters. As proved by Jackson et al. (2014), with limited access to prey, larvae tend to take more time until change to the next zoea, having more time to growth on the same zoeal stage.

Jackson et al., 2014, with the administration of *A. franciscana* to *N. puber* larvae, obtained zoea V at 16.5ºC, at 18 DPH, similar to obtained results in the present study. *A. tonsa* promoted a shorter zoea III and IV, suggesting that copepods come to be a suitable prey to be consume by *N. puber* larvae in later zoeal stages. With this change, larvae submitted to a copepod diet achieve ZV faster, being flagrant the difference once larvae show the ability to capture this faster live prey.

Beside differences fostered by administrated diets on survival and intermoult period, there were no differences between length and weight for larvae at the same zoea, between diets. There were no studies about the effect of copepods on crustaceans growth, but with fish species, as *Gadus morhua and Labrus bergylta* larvae fed on copepods have a greater growth (Karlsen, et al, 2015).

Copepods, besides the characteristic movement that stimulate predatory and side range of size, also present highly PUFAs content. As demonstrated in the present study, *A. tonsa* have more DHA and EPA, when compared to *A. franciscana*. EPA and DHA is related with marine larval survival and growth, being known as essential in larval diets (Olivotto et al., 2008), once fish and crustacean marine larval cannot synthesize it (Olivotto et al.,2009). Addictionally, deficiencies in EPA and DHA promote longer intermoults (Pina et al., 2006) due to larvae decreased health, poor growth and anemia (Olivotto et al., 2006). Also EPA, when its presence is much higher than DHA, can promote an imbalance in the composition of phospholipids, decreasing the larval quality and growth rates (Bell et al., 2003). This highlight the importance of DHA/EPA ratio close to 1, being similar to results obtained by Seixas et al. (2009). Regarding monosaturated fatty acids, C16:0 had a strong presence in *A. tonsa* and *A. franciscana* and, according to other studies, is related to energy sources

and can be bioconverted to stearic acid (C18:0). This last fatty acid have an extremely important structural role, as demonstrated to *Macrobrachium rosenbergii* larvae (Roustaian et al., 1999).

The lack of works regarding the effect of copepods administration on crustacean larval development do not allow any link to the results obtained in the present study. Still, when authors approach its effect on marine larval fish, the major of studies had proved that copepods promote greater survival and growth rate when compared with plankton array. *Hippoglossus hippoglossus* (Shields et al., 1999), *Gadus morhua* and *Labrus bergylta* (Karlsen et al, 2015) when fed on copepods had a significant higher survival and growth rate, comparing with *Artemia* and rotifers, even enriched. *Hippocampus subelongatus* grew more and had a greater survival rate with copepod nauplii (Payne and Rippingale, 2000); *Elacatinus figaro* had a greater growth rate with copepod nauplii rather than with ciliate or enriched rotifer diet (Côrtes and Tsuzuki, 2012); *Paralichthys olivaceus* fed with copepods had a superior content in DHA, EPA and arachidonic acid (C20:4 n6, ARA) (Liu and Xu, 2009).There are many other studies that highlight the beneficial effects of copepods on marine fish larvae development.

The majority of human population consumes marine species that in their natural habitat do not fed on *Artemia* sp., and rotifers are only ate by estuarine and coastal species. The major of rotifers can be found in freshwater, as for *Artemia* sp. is restrict to high salinity locals, as salt pans. These unnatural feed, do not have a suitable size nor nutritional value for a correct development of most marine species (Olivotto et al., 2010). Besides that, ecological studies suggest that copepods can represent more than 80% of total zooplankton biomass (Kleppel et al., 2005), representing a natural prey to crustacean and fish larvae.

At 1992, Léger and Sorgeloos proved that newly hatched nauplii cannot provide all necessary nutrients for complete larval development, creating the need of other types of live feeds. Rotifers and *Artemia* sp., even enriched, are not suitable for new species that requires demanding nutritional profiles (Ajiboye et al., 2011).

It is possible that improvements on size of administrated preys can promote a higher survival rate. In the present study, to promote a fair comparison between *A. tonsa* and *A. franciscana*, larvae were submitted to similar size preys. With that detail, any changes between larvae survival and growth came from nutritional value and biological characteristics of administrate preys.

5. Conclusion

Although the association with cost production increase, future larviculture protocols should consider the inclusion of copepods as live feed for the success of aquaculture production. Despite of *Rhodomonas lens* being commonly reported as an unstable microalgae to be cultured, is the most suitable diet to the continuous production of *Acartia tonsa*. Having the best nutritional profile, copepods can be classified as the optimal live feed to crustacean and fish larvae.

A. tonsa is a suitable prey to *Necora puber* larval stages, promoting a best larval performance when comparing with *Artemia franciscana*. This calanoid species is a suitable prey to early larval stage with small mouth-gape sizes, promoting high survival rates and shorter larval stages duration.

In future studies, it could be interesting to adjust the size preys to the increment of the larvae mouth-gape. Besides that, the evaluation of the *A. tonsa* effect on other crustacean species is crucial once copepods may reduce larval stage duration. Administration of copepods on later developmental stages, could have benefits in survival and growth rate. By overcoming the lack of studies on this research area, in near future will be possible to decrease the time that organisms take to achieve commercial sizes and the associated cost production.

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