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Growth, egg production and hatching success of *Acartia tonsa* cultured at high densities

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ABSTRACT

Acartia tonsa is a calanoid copepod with high potential as live feed for marine aquaculture. However, its usage remains limited at an industrial scale, with cost effective production being conditional on successful culture at high density. The present study took an integrated approach to provide further insight on the effects of *A. tonsa* stocking density on copepod growth and adult reproduction, specifically egg production and egg hatching success. The effect of stocking density was studied by following the growth and survival of *A. tonsa* copepods, from egg hatching to maturity, on cultures initially stocked with 250, 400, 1000, 3000 and 6000 copepods l^{-1}. Additionally, the effects of high-density rearing, of adults kept at 100, 250, 500 and 2500 copepods l^{-1}, on egg production and hatching success were also evaluated over a 5-day period.

Higher stocking densities were shown to have no unfavourable effect on copepod growth, though mortality significantly increased with density, from ≤2.5% d^{-1} at densities of ≤1000 copepods l^{-1} to 3.5–4.0% d^{-1} at 3000–6000 copepods l^{-1}. Individual egg production decreased with increasing stocking densities, from 28 eggs $female^{-1}$ d^{-1} at 100 copepods l^{-1} to 7 eggs $female^{-1}$ d^{-1} at 2500 copepods l^{-1}. However, total yield still increased with stocking density, with the cumulative egg production at 2500 copepods l^{-1} being 4 times the production at lower densities (from 100 to 500 copepods l^{-1}). Though adult rearing density had no effect on 96-h hatching rate (60 to 69%), a density-dependent late hatching was observed on eggs produced by adults grown in dense cultures (500–2500 copepods l^{-1}), with 48-h hatching success significantly decreasing with increasing densities (from 37% to 1%).

In spite of the negative effect of stocking density on survival during growth and egg production, the magnitude of these effects does not compromise the use of high density cultures. Future research should focus on the improvement of production systems, as the ability to rear calanoid copepod species at large scale would present a major advancement in larviculture of marine fish species.

Supporting statement: The present research focused on the effects of *A. tonsa* stocking density on copepod growth and adult reproduction, specifically egg production and egg hatching success. This is the first study on *A. tonsa* high-density culture from copepod growth to egg production and hatching. It further provides new insight on the viability of using high-density cultures for egg production and its impact on hatching success, essential for the commercial production of this species

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1. Introduction

Hatchery production of fish, molluscs and crustaceans has become of central importance to marine aquaculture and the supply of suitable food for early larval stages is one of the major difficulties during larval rearing, in particular with very small larvae. Current industrial larviculture relies to a great extent on diets of rotifers and brine shrimp for early feeding (e.g. Rainuzzo et al., 1997; Sargent et al., 1997), which besides being of concern in terms of nutritional suitability (e.g. Barclay and Zeller, 1996) and availability (Bengtson, 2003; Lavens and Sorgeloos, 2000), often constitutes a high fraction of the total production costs (e.g. People Le Ruyet et al., 1993; Conceição et al., 2010).

Copepods are a natural prey for the first-feeding of many marine fish larvae in the wild (Hunter, 1981; Pepin and Penney, 1997; Støttrup, 2003) and the nutritional superiority over traditional live food is well established (Drillet et al., 2006a). Their use has been shown to lead to

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enhanced survival, higher growth and weight gain, decreased malpigmentation, higher rates of successful metamorphosis, increased stress resistance and increased feeding response in a number of species (e.g. Doi et al., 1997; Evjemo et al., 2003; Hernandez Molejon and Alvarez-Lajonchere, 2003; Kraul et al., 1992; Luizi et al., 1999; Kuhlmann et al., 1981; Shields et al., 1999). These and other attributes have increased the interest in large-scale culturing of copepods and recent literature has thoroughly discussed culturing techniques and the use of copepods as live prey in marine fish aquaculture (Conceição et al., 2010; McKinnon et al., 2003; Støttrup, 2000, 2003). However, copepod culture for industrial use is mostly done by in situ harvesting or semi-extensive outdoor growth (e.g. Engell-Sørensen et al., 2004; Toledo et al., 1999) and large scale culture is not widespread, in spite of the few published production protocols available for a small number of species (Abate et al., 2015; Payne et al., 2001; Schipp et al., 1999; Sun and Fleeger, 1995).

Acartia tonsa (Dana) is a calanoid copepod that is easily maintained in culture, though its usage in hatcheries remains limited, mainly due to difficulties in attaining reliable and cost efficient production at an industrial scale, associated with a series of technical and biological challenges (Drillet et al., 2011). Extensive efforts have been done towards investigating the differences in the quality of produced eggs, storage effects and the analysis of differences between strains (Drillet et al., 2006a, 2006b, 2008a, 2008b; Hagemann et al., 2016; Hammervold et al., 2015; Hansen et al., 2016; Ohs et al., 2009). Literature has further centred its attention in identifying the main factors influencing *A. tonsa*'s culture performance, with focus on the effects of temperature and diet (Broglio et al., 2003; Kiørboe et al., 1985; Leandro et al., 2006; Støttrup and Jensen, 1990; Zhang et al., 2013, 2014), while photoperiod (Peck and Holste, 2006; Peck et al., 2008), salinity (Holste and Peck, 2006; Peck and Holste, 2006) and density (Drillet et al., 2014a; Jepsen et al., 2007; Medina and Barata, 2004; Peck and Holste, 2006; Zhang et al., 2014) have received less attention. Recent studies have addressed the economic feasibility of production and technical improvements to culture (Abate et al., 2015; Alver et al., 2011; Vu et al., 2014) and focused on gathering existing knowledge towards production (Drillet et al., 2011).

One of the main concerns is the success of production at high-densities (Drillet et al., 2011, 2014a; Støttrup, 2003), as increased copepod density can often decrease water quality and feed availability, while increasing conspecific interactions (Drillet et al., 2014a; Jepsen et al., 2015; Støttrup and Norsker, 1997). For *Acartia* sp., stocking density has been shown to affect not only growth (Medina and Barata, 2004), but also reproduction, namely egg production (Drillet et al., 2014a; Peck and Holste, 2006) and egg hatching (Camus and Zeng, 2009). For *A. tonsa* cultures, 50 to 100 copepods l^{-1} is considered standard stocking density, though a few studies have investigated the possibility of using higher densities (50–400 copepods l^{-1}, Peck and Holste, 2006; 100–600 copepods l^{-1}, Jepsen et al., 2007; 500–2000 copepods l^{-1}, Medina and Barata, 2004; 10–5062 copepods l^{-1}, Drillet et al., 2014a) either for copepod growth or egg production, with small scale experiments using densities as high as 6000 adults l^{-1} (Abate et al., 2015; Drillet et al., 2006b, 2008a). In spite of no reported effects of density on sex ratio (Jepsen et al., 2007; Medina and Barata, 2004) and mortality (Drillet et al., 2014a; Jepsen et al., 2007; Medina and Barata, 2004), negative effects were observed on development time (Medina and Barata, 2004), with conflicting results for egg production and hatching success (Drillet et al., 2014a; Jepsen et al., 2007; Peck and Holste, 2006). While Drillet et al. (2014a) only reported a decrease in the theoretical egg production per female above 2500 copepods l^{-1} (in spite of testing up to 5062 copepods l^{-1}), others reported this effect at much lower densities, of 191–600 copepods l^{-1} (nominal densities of 500 to 2000 copepods l^{-1}; Medina and Barata, 2004) and 65 to 425 copepods l^{-1} (Peck and Holste, 2006). Further to this, Peck and Holste (2006) and Jepsen et al. (2007) observed no differences in 48-h hatching success at densities under 600 copepods l^{-1}, while Drillet et al. (2014a) recorded a significant, though low reduction (1.7% per 1000 copepods) in 72-h hatching success. This variation in densities tested and the disparity between results, likely caused by differences in design and strains used (see Drillet et al., 2014a), further complicates the development of intensive systems. Furthermore, the efforts in investigating the effects of density on copepod growth and reproduction have often been decoupled, with most studies focusing solely on egg production and quality (e.g. Jepsen et al., 2007; Peck and Holste, 2006), but not on high-density growth followed by egg production (e.g. Drillet et al., 2014a).

A clear study of the performance of *A. tonsa* cultured at high-density, from copepod growth to egg production and hatching (see Medina and Barata (2004), for ecotoxicological studies) is therefore essential to inform production using intensive systems. The present study evaluated the effects of stocking density (250 to 6000 copepods l^{-1}) on the development and survival of *A. tonsa*, from egg hatching to maturity, and continued by testing the effects of high-density rearing (100 to 2500 copepods l^{-1}) on egg production and hatching success for a 5-day period.

2. Material and methods

2.1. Acartia tonsa stock cultures

Cultures from *A. tonsa* were started from eggs donated by the Danish Institute for Fisheries and Marine Research (now DTU-Aqua, Charlottenlund, Denmark), belonging to the Danish Sound population (DIFRES strain, DFH-ATI). This strain has been reared for over 70 generations in the laboratory, under constant temperature and light conditions (salinity of 34, 17 °C, dim light), on a diet of *Rhodomonas salina* (Drillet et al., 2008ab; Peck and Holste, 2006; Støttrup et al., 1986).

From the first mixed batch of copepods received (i.e. copepods from different cohorts and life stages), eggs were collected and several new stock cultures were established. The eggs were incubated and the hatched nauplii were cultured to adulthood, repeating the process for several generations. The cultures were kept in 10 to 80 l polyethylene tanks, in UV-treated and 1 μm-filtered seawater (salinity of 33), at 18 ± 1 °C and 24:0 L/D photoperiod in dim light. Cultures were gently aerated and fed daily with *Rhodomonas baltica* and *Isochrysis galbana* (≥50.000 cells ml^{-1} day^{-1}; 1:1). Algal cultures were maintained in 10 and 30 l cultures, in bags, grown in UV-treated and 1 μm-filtered seawater (salinity of 33) with addition of f/2 media (Guillard, 1975) at 18 ± 1 °C and 24:0 L/D photoperiod, and were harvested at their exponential phase. Water quality was measured regularly, with partial daily water exchanges to assure good culture conditions (≥7.0 mg O_2 l^{-1} and pH 7–8). *A. tonsa* cultures were kept at low density (maximum 300 copepods l^{-1}) and were run for maximum period of a month until being discarded. Egg collection was done daily by removing aeration, allowing time for eggs to settle and attracting the copepods to the surface with light, while gently siphoning the settled eggs from the bottom of the tank. A double sieving system (150 μm/50 μm) was used to separate adult copepods from eggs, which were rinsed in sea-water. The eggs were sampled, briefly rinsed in fresh water, covered with Parafilm, excluding air, and stored in 15 ml dark vials (≤50.000 eggs ml^{-1}) at 4 °C, for use in the later experiments.

2.2. Experimental design

2.2.1. Effects of stocking density on copepod growth and survival

The effect of stocking density (250, 400, 1000, 3000 and 6000 copepods l^{-1}) on the growth and survival of *A. tonsa* copepods was followed from egg hatching to maturity (February to April 2009). Due to technical limitations, the experimental design was divided in two experiments, I (12 days) and II (18 days), which tested, respectively, lower initial stocking densities of 250, 400 and 1000 copepods l^{-1} and higher densities of 1000, 3000 and 6000 copepods l^{-1} (3 replicates set per density treatment). The density of 1000 copepods l^{-1} was