The Influence of the Toxin Producing Dinoflagellate, *Alexandrium catenella* (1119/27), on the Feeding and Survival of the Marine Copepod, *Acartia tonsa*

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ABSTRACT

Blooms of harmful algae are increasing globally, yet their impacts on copepods, an important link between primary producers and higher trophic levels, remain largely unknown. Algal toxins may have direct, negative effects on the survival of copepods. They may also indirectly affect copepod survival by deterring feeding and thus decreasing the availability of energy and nutritional resources. Here we present a series of short-term (24 h) experiments in which the cosmopolitan marine copepod, *Acartia tonsa*, was exposed to a range of concentrations of the toxic dinoflagellate, *Alexandrium catenella* (strain 1119/27, formerly *Alexandrium tamarense*), with and without the presence of alternative, non-toxic prey

(*Rhodomonas* sp.). We also present the toxin profile concentrations for *A. catenella*. The survival and feeding of *A. tonsa* was not affected across the range of concentrations recorded for *A. catenella* in the field; increased mortality of *A. tonsa* was only discernible when *A. catenella* was present at concentrations that exceed their reported environmental concentrations by two orders of magnitude. The observed lethal median concentration (LC_{50}) for *A. tonsa* exposed to *A. catenella* was 12.45 ng STX eq L^{-1}. We demonstrate that *A. tonsa* is capable of simultaneously ingesting both toxic and non-toxic algae, but increases clearance rates towards non-toxic prey as the proportional abundance of toxic *A. catenella* increases. The ability to actively select non-toxic algae whilst also ingesting toxic algae suggests that consumption of the latter does not cause physical incapacitation and thus does not affect ingestion in *A. tonsa*. This work shows that short-term exposure to toxic *A. catenella* is unlikely to elicit major effects on the grazing or survival of *A. tonsa*. However, more work is needed to understand the longer-term and sub-lethal effects of toxic algae on marine copepods.

1. INTRODUCTION

Blooms of harmful algae (HA) refer to the high biomass accumulation of toxic or noxious species (Anderson et al., 2012) and can be caused by microalgae such as phytoplankton and cyanobacteria (blue-green algae) or macroalgae (seaweeds). HA blooms can be toxigenic and produce blooms that cause illness

and death in a variety of marine organisms, including fish, seabirds, and mammals. The frequency of HA blooms has increased over the past five decades (Hallegraeff 1993; Anderson et al., 2012) as has the number of toxic blooms, the number of toxins and toxic species reported, and the associated economic losses (Anderson et al., 2012).

Zooplankton grazing plays a vital role in regulating phytoplankton blooms, including those of toxic species (Irigoien, 2005). Toxin-induced mortality may directly affect zooplankton populations (Prince et al., 2006; Jiang et al., 2009) and hence their ability to control blooms of HA. The unpalatability of HA (Teegarden 1999; Costa and Fernández, 2002; Costa et al., 2005; Costa 2012) and physical incapacitation induced by toxicity (Sopanen et al., 2011) can also affect the ability of grazers to control HA blooms. Blooms lacking specific elements or nutrient compounds may further affect zooplankton populations (Breier and Buskey 2007; Cohen et al., 2007; Waggett et al., 2012). All of these mechanisms ultimately result in reduced rates of zooplankton growth and reproduction (Dam, 2013; Turner 2014).

The effects of HA on copepods appear to be species- and/or population-specific. Some copepod species/populations have shown a decrease in feeding and/or survival when exposed to HA (Barreiro et al., 2007; Jiang et al., 2009), whereas others appear to be tolerant to HA (Colin and Dam 2002, 2005, 2007). Several studies have shown that copepods with a history of co-occurrence with HA have higher survival and ingestion rates when exposed to a HA bloom compared to species with no history of co-occurrence (Turner et al., 2000; Turner et al., 2005; Maneiro et al., 2005; Doucette et al., 2006; Deeds et al., 2007), potentially suggesting an evolved tolerance to HA species that co-occur in the

same region (Colin & Dam 2002, 2005, 2007). Some copepods have a discrete toxin-resistant phenotype which suggests evolution of a simple genetic mechanism to adapt and tolerate PSP toxins during bloom conditions (Avery and Dam 2007). For example, the fitness of *Acartia hudsonica* increased after feeding on *Alexandrium fundyense* for over four generations, which shows that adaptation occurred (Finiguerra et al., 2014). Sodium channel mutations have been suggested as a possible mechanism (Avery and Dam 2007; Chen et al., 2015); however, *A. hudsonica* exposure to HA did not induce an increased proportion of mutant isoforms (Finiguerra et al., 2014) which suggests that the mutant isoforms are unlikely to be responsible for adaptations of *A. hudsonica* to toxigenic HA.

Differences between studies may also arise from experimental differences, e.g. concentrations of HA used as food, the level of toxins within the food, and different species of copepod examined (Turner 2014). It is therefore currently difficult to make generic predictions about how copepods will respond to future blooms of HA.

In UK waters, the dinoflagellate, *Alexandrium catenella* (previously recorded in this region as *A. tamarense* Group I, reassigned taxonomically by John et al., 2014 & Fraga et al. 2015, accepted in Prud'homme van Reine 2017), is capable of producing Paralytic Shellfish Poisoning (PSP) toxins and is frequently associated with shellfish toxicity (Bresnan et al., 2005; 2008). Densities of *A. catenella* between 1000-2000 cells L^{-1} typically result in shellfish flesh exceeding the regulatory limit for PSP toxins, and blooms usually last for a few days (Bresnan et al., 2005; Turner et al., 2014). Maximum reported densities of this species around the UK are 5,000 and 18,000 cells L^{-1} in the waters around the Western Isles and Shetland, respectively (Bresnan et al., 2008). These 'blooms' do

not necessarily result in *A. catenella* becoming dominant within the phytoplankton community but can nevertheless result in an environmental problem, depending on the specific levels of toxins present.

The derivatives of PSP toxins are categorized into three main groups: carbamate-, N-sulphocarbamoyl-, and decarbamoyl- toxins. The carbamate toxins saxitoxin (STX) and neosaxitoxin (NEO) are considered to be the most potent compared to other PSP toxins. Other highly potent analogues include gonyautoxins 1 to 4 (GTX1, GTX2, GTX3, GTX4). N-sulphocarbomoyl (C) toxins and some of the decarbomoyl (dc) congeners are considered less potent (Cook et al., 2010). Saxitoxins inhibit nerve transmission by blocking water-soluble sodium channels (Luckas et al., 2015). The consumption of shellfish contaminated with PSP toxins can cause difficulty in breathing, gastrointestinal problems, and a sense of dissociation followed by complete paralysis in humans and other vertebrates (Wang 2008); however, how PSP toxin exposure/consumption affects invertebrate fitness remains unclear.

The composition and concentrations of toxins within any species of HA, including *A. catenella*, can be variable, even when grown under similar conditions (Turner et al., 1998; Loret et al., 2002; Etheridge et al., 2005; Brown et al., 2010; Waggett 2012). Some of the factors that can change the toxin levels within a HA bloom include turbulence (Juhl et al., 2001), salinity (Grzebyk et al., 2003), and nutrient conditions (Turner et al., 1998; John and Flynn 2002; Leong et al., 2004). HA can also significantly increase the production of PSP toxins in response to nutrient stress and chemical cues from copepods (Wohlrab et al. 2010; Griffin et al. 2019). A group of predator cues named copepodamides, which are exuded by

three calanoid copepod species (*Centropages typicus*, *Pseudocalanus* sp., and *Calanus* sp.), were found to trigger increased PSP toxin production in dinoflagellates, and also amnesic shellfish poisoning (ASP) toxins in diatoms (Selander et al., 2015). However, toxin profile concentrations are rarely reported, particularly in the context of copepod grazing studies. This makes it difficult to robustly explain the observed results and hinders comparisons between different studies.

We studied the survivorship and grazing of a globally distributed marine copepod, *Acartia tonsa*, feeding on a toxic strain of *A. catenella* across the range of concentrations above and below those that are typically encountered in UK waters. Additional experiments examined how ingestion rates and prey selection in *A. tonsa* were influenced by the relative abundance of *A. catenella* in the prey field. We present our results alongside a full toxin profile for the *A. catenella* used in our experiments, which allows us to calculate the lethal median concentration (LC_{50}).

2. METHODS

2.1 Culture conditions

A toxic strain of *Alexandrium catenella* (Strain 1119/27; CCAP, Loch Ainort, Scotland) and the non-toxic cryptomonad, *Rhodomonas* sp., were both grown in a culture cabinet at 15 °C with a 16:8 h (Light:Dark) photoperiod. *A. catenella* was cultured in seawater collected from the Western Channel Observatory monitoring site L4 off Plymouth, UK (https://www.westernchannelobservatory.org.uk/) amended with L1 medium (3.5 ml of L1 medium for every 1 Litre). *Rhodomonas*

sp. was cultured using artificial seawater (Tropic Marin seasalt, 31) and F/2 medium (1.5 ml of F/2 medium for every 1 Litre). *Acartia tonsa* (Reefshotz, Cardiff, UK, where they have been cultured for > 10 years) were cultured in artificial seawater (Tropic Marin Seasalt; 31) for 5 months prior to experimentation and fed *Rhodomonas* sp. three times a week at a concentration of 60,000 cells ml^{-1}. All cell counts were undertaken using a Beckman Multisizer 3 Coulter Counter equipped with a 70 μm aperture. The carbon and nitrogen content of phytoplankton and copepods were determined for each experiment using a Carlo Erba EA-1108 elemental analyser.

2.2 Influence of *A. catenella* concentration on the feeding and survival of *A. tonsa*

Experiment 1 simultaneously examined how the ingestion rate and survival of *A. tonsa* changed across a plausible environmental range of *A. catenella* concentrations. A total of 150 adult female *A. tonsa* were carefully transferred via pipette into 500ml of 0.2μm sterile filtered L4 seawater (SFSW hereafter) and incubated for 24 h to clear their guts. The carbon and nitrogen content of *A. catenella* and toxin concentration profiles were determined from the stock culture of *A. catenella* prior to their exposure to copepod grazing. Triplicate 5 ml samples from the *A. catenella* stock culture were collected onto glass fibre filters (Whatmann GF/F) and dried at 40 ^{0}C for subsequent elemental analysis (Carlo Ebra EA-1108 elemental analyser). Triplicate 50ml samples of the *A. catenella* stock culture were also centrifuged at 3000 rpm for 20 mins and the resulting pellets were transferred into 2 ml Eppendorf tubes and stored frozen at -80 °C until toxin analysis. Survival and grazing experiments were conducted in 215 ml

clear bottles containing *A. catenella* at nominal concentrations of 20, 100, 200 and 1000 µg C L^{-1}. These concentrations were achieved by determining the concentration of the *A. catenella* stock culture and subsequently diluting this with an appropriate volume of SFSW. Nine bottles at each of the required concentrations were prepared. Three initial bottles at each concentration were immediately preserved with acidified Lugol's iodine to enumerate the number of cells at the start of the experiment. Ten female *A. tonsa* were added to each of three grazing bottles at each concentration and incubated alongside triplicate control bottles at each concentration on a plankton wheel (2 rpm) for 24 h at 19 °C with a 12:12 h (L:D) photoperiod. At the end of the experiment, the grazing and control bottles were preserved with acidified Lugol's iodine after removing copepods from the grazing bottles using a 200 µm mesh and transferred into SFSW. The number of motile copepods observed after mechanical stimulus was recorded. Surviving animals were transferred onto glass fibre filters (Whatmann GF/F) and dried at 40 ^{0}C for subsequent elemental analysis (Carlo Erba EA-1108 elemental analyser).

Experiment 2 was conceptually similar to experiment 1, but examined a broader concentration range of *A. catenella* and made no attempt to quantify ingestion rates. A total of 210 adult female *A. tonsa* were incubated in SFSW for 24 h prior to experimentation. Triplicate groups of 10 animals were subsequently incubated for 24 h with *A. catenella* at nominal concentrations of 100, 1000, 3000, 5000, 7500, and 10,000 µg C L^{-1}, where *A. catenella* from the stock culture was diluted with SFSW. Samples to determine the elemental content of *A. catenella* and *A. tonsa* were collected at the beginning and end of the experiment,

respectively. The number of animals responding to mechanical stimulus was recorded at the end of the experiment.

2.3 Ingestion and selection for/against *A. catenella* in the presence of non-toxic cells

Experiment 3 examined how, for a given total quantity of food, ingestion rates and food selection in *A. tonsa* changed in response to proportional changes in the availability of toxic and non-toxic food. Experimental females were incubated in SFSW for 24 h prior to experimentation. Experiments were conducted in 215 ml culture bottles, each containing a total of 1250 µg C L^{-1} of algae. The four treatment levels contained 0 %, 25 % 50 %, and 75 % of *A. catenella*-derived carbon, with the remainder being provided via *Rhodomonas* sp. Nine bottles at each level were initially set up, with three being sampled to determine cell numbers and their elemental content at the outset of the experiment. Ten female *A. tonsa* were added to each of three grazing bottles and incubated alongside triplicate control bottles on a plankton wheel for 24 h. At the end of the experiment, samples were collected to determine the elemental content of the copepods and the amount of food ingested (as above).

2.4 Toxin analysis

The frozen pellets of *A. catenella* from each experiment were mixed with 1.5ml of 1% acetic acid before being vortex-mixed for 90 s at 2500 rpm. Solutions were subjected to probe sonication (Sonic Dismembrator, Fisher Scientific) set to 30 % power for 1.5 mins per sample. After sonication was complete, vials were re-mixed, and 400 µL taken for desalting clean-up using carbon solid phase extraction (SPE) as described by Turner et al., 2015. A Gilson (Middleton, WI)

Aspec XL-4 SPE liquid handler was used for automated SPE clean-up processing prior to a 4:1 dilution in acetonitrile and HILIC-MS/MS analysis. Quantitation of individual PSP toxin concentrations was conducted, enabling the assessment of toxin profiles of *A. catenella* for each experiment. An Agilent (Manchester, UK) Infinity II 1290 Ultra-high Performance Liquid Chromatograph (UHPLC) coupled to a 6495B tandem mass spectrometer (MS/MS) was used for acquiring two Multiple Reaction Monitoring (MRM) transitions for each toxin analogue. Certified reference calibration solutions for PSP toxins to allow identification and quantification were sourced from the Institute of Biotoxin Metrology, National Research Council, Canada. These were used to prepare working calibration solutions, which were utilized as external standards for quantitation of individual analogues. PSP toxins incorporated into the method included the gonyautoxins (GTX1, GTX2, GTX3, GTX4, GTX5, GTX6), decarbamoyl-gonyautoxins (dcGTX 1, dcGTX2, dcGTX3, dcGTX4), saxitoxin (STX), neosaxitoxin (NEO), decarbamoyl-STX (dcSTX), decarbamoyl-NEO (dcNEO), (doSTX) deoxydecarbamoyl-saxitoxin and the N-sulfocarbamoyl C toxins (C1, C2, C3, C4). HILIC chromatography and MS/MS detector conditions were those described by Turner et al., 2015 and Turner et al., 2019 respectively. Mobile phase A1 consisted of water + 0.015 % formic acid + 0.06 % of 25 % ammonia. Mobile phase B1 comprised 70 % MeCN + 0.01 % formic acid. For chromatographic separation of PSP toxins, a Waters (Manchester, UK) BEH Amide HILIC column (2.1 x 150 mm; 1.7 μm) together with an equivalent 5 mm guard cartridge was used. The column was held at 60 °C and sample injection volumes were 2 μL.

2.5 Data analyses

Copepod daily ingestion rates were calculated using established equations (Frost, 1972) and expressed relative to the carbon content of the females, giving carbon-specific ingestion rates (CSI, % C day^{-1}). The number of copepods surviving each experiment was expressed as a percentage of the ten that were initially added to each grazing bottle. The influence of increasing concentrations of *A. catenella* on the survival and ingestion of *A. tonsa* were examined using separate one-way ANOVAs in which the concentration of *A. catenella* was treated as a categorical variable. Post-hoc comparisons were conducted using Tukey's test with alpha = 0.05. Feeding selectivity of *A. tonsa* for or against *A. catenella* and *Rhodomonas* sp. was examined by plotting the proportion of these cells in the available food against their proportion in the ingested diet. Values above and below a 1:1 line indicate positive and negative selection, respectively.

The concentration of PSP toxins, expressed in STX equivalence, of *A. catenella* in each experiment was calculated using a six-point calibration curve drawn from the included toxin standards and then converting molar concentrations of each toxin to STX equivalence using the toxin equivalent factors (TEF) for each PSP toxin analogue detected and quantified (EFSA 2009). These same conversion factors are used to standardize data generated in the UK regulatory monitoring programmes (Turner et al., 2014). The lethal median concentration that killed 50 % of the population (LC$_{50}$), expressed in STX equivalents, was determined by applying a non-linear regression using a dose-response model. All statistical analyses were performed using Prism Graphpad software (v.7.0).

3. RESULTS

The average carbon content of the *A. tonsa* used in the experiments was 2.6 µg C Copepod^{-1} (± 0.53, SD). Table 1 presents the nominal and actual concentrations of *A. catenella* offered in the different experiments, along with the cellular carbon quotas. The cell toxicity analysis detected 12 different toxins out of the 19 included in the calibration standard mix, these different congeners showed variability in their concentrations and STX equivalence leading to an average total cellular toxin quota of 2.7 pg STX eq cell^{-1}. (Table 2).

3.1 Influence of *A. catenella* concentrations on the feeding and survival of *A. tonsa*

Survival, and daily rates of clearance, total ingestion and carbon-specific ingestion (CSI) for *A. tonsa* offered *A. catenella* at nominal concentrations between 20-1,000 µg C L^{-1} (experiment 1) and between 100-10,000 µg C L^{-1} (experiment 2) are presented in Figures 1 and 2, respectively. The survival of *A. tonsa* was not affected by the increase in concentration of *A. catenella* between 20 and 1,000 µg C L^{-1} (ANOVA, df = 14, p=0.310; Fig. 1A). Survival was only significantly reduced when offered *A. catenella* at the highest concentration treatment (Experiment 2: Fig. 2A) (ANOVA, df =20, p =0.001). The calculated LC$_{50}$ was 12.45 ng STX eq L^{-1} (Fig 2B), with a 95 % confidence interval between 10.31-14.0 ng STX eq (Fig. 2B). This corresponds to an *A. catenella* concentration of 7707 µg C L^{-1}, or ~4,561,000 cell L^{-1}.

Clearance rates were variable, ranging between 5.1 and 13.5 ml copepod^{-1} day^{-1} and were not significantly affected by prey availability (ANOVA, df = 11, p= 0.52; Fig. 1B). Total ingestion rates ranged between 0.87 and 6.17 µg C copepod^{-1}

day^{-1}, and, by contrast, changed significantly as a function of prey concentration (ANOVA, df = 11, p= x0.0009; Fig. 1C). Post-hoc comparisons revealed that the ingestion rate observed when *A. catenella* was offered at a concentration of 1,000 µg C L^{-1} was significantly higher than the ingestion rates in all other treatments. Daily CSI rates ranged between 0.27 to 2.27 and also increased significantly as a function of the concentration of *A. catenella* (ANOVA, df = 11, p=0.0003; Fig. 1D); the CSI rate in the treatment offering *A. catenella* at 1,000 ug C L^{-1} was significantly higher than in all of the other treatments.

3.2 Ingestion and selection for/against *A. catenella* in the presence of non-toxic cells

Clearance rates of *A. tonsa* were consistently lower (0.0-1.9 ml copepod^{-1} day^{-1}) when fed *A. catenella* than *Rhodomonas* sp. (2.0-11.2 ml copepod^{-1} day^{-1}) (Fig. 3A). The total CSI rate of *A. tonsa* (*Rhodomonas* sp. & *A. catenella*) was not affected by the proportion of *A. catenella* in the available prey field (ANOVA, df=10, p= 0.780). The majority of ingested carbon was always derived from *Rhodomonas* sp. (Fig. 3B & C), but the difference in the amounts ingested of *Rhodomonas* sp. and *A. catenella* was only statistically discernible at 25% toxic algae (Tukey's test, df=11, p= 0.025); with no significant difference between *Rhodomonas* and *A. catenella* at 50 % and 75 % toxic algae (Tukey's test, df=11, p> 0.329 in both cases). Figure 4 shows that the percentage of *Rhodomonas* sp. in the diet was consistently higher than that of the available food, whereas the percentage of *A. catenella* in the diet was consistently lower than available in the prey field.

4. DISCUSSION

This study examined how the feeding and survival of *Acartia tonsa* were influenced by the concentration of the toxin-producing dinoflagellate, *Alexandrium catenella*. It also examined how the presence of alternative, non-toxic prey influenced food ingestion and patterns of prey selection.

4.1 Survival of *A. tonsa* feeding on *A. catenella*

Our study failed to discern any negative effects of a toxic strain of *A. catenella* on the survival of *A. tonsa* across a range of plausible environmental concentrations. Maximum reported densities of *A. catenella* in UK waters range between 5,000-18,000 cells L^{-1} (Bresnan et al., 2008), which equate to between 10-36 μg C L^{-1} (assuming *A. catenella* contains 2.0 ng C $cell^{-1}$; Table 1). Significant mortality was only observed at a concentration of 10,000 μg C L^{-1}, equivalent to ∼5,919,000 cells L^{-1}. These data suggest that *A. tonsa* survival is unlikely to be affected during natural blooms of toxic strains of *A. catenella*. Our experimental animals had been maintained in culture for > 10 years without exposure to HA. We therefore suggest that it is unlikely that they possessed genetic resistance to the toxins of *A. catenella*. Indeed, the observed lethal median concentration (LC_{50}) of 12.45 ng STX eq L^{-1} is equivalent to 7707 μg C L^{-1} or ∼4,561,000 cells L^{-1}. Our lethal median concentration (LC_{50}) was estimated using the toxin concentration profile of *A. catenella* that was determined prior to their exposure to copepod grazing. We cannot exclude the possibility that *A. catenella* increased toxin production during our grazing experiments (Wolhrab et al. 2010; Selander et al., 2015; Griffin et al. 2019) and therefore consider our LC_{50} estimate to be conservative. Nevertheless, this concentration is two orders of magnitude higher

than any of the maximum densities of *A. catenella* recorded in natural systems (Bresnan et al., 2008; Fauchot et al., 2005). Our experiments were not designed to determine which of the toxins present (Table 2) were responsible for the observed effects. We therefore present toxicity as a saxitoxin equivalent (EFSA 2009). Through use of an ultra-sensitive detection method, we have the first reported evidence for the presence of certain PST analogues. For example, *A. catenella* is well known to contain C1&2, STX, NEO and GTX1-4 – but here we also demonstrate the presence of C4, GTX5, dcSTX and doSTX (Table 2). Our findings support the understanding that *Acartia* spp. are able to ingest toxic *Alexandrium* spp. without negative impacts on their survival (Teegarden and Cembella 1996; Dutz, 1998; Colin and Dam 2002). Nevertheless, it is probable that the effects of *A. catenella* are strain-specific; the toxicity of six different strains of *A. catenella* found in Scottish waters ranged between 2.8-10.5 pg STX eq cell^{-1} (Brown et al., 2010).

4.2 Ingestion and prey selection

The ingestion rates observed in this study (μg C Copd^{-1} day^{-1}) agree well with those previously reported for the genus *Acartia* (Colin and Dam 2002; Jiang et al., 2009; Zheng et al., 2011), and demonstrate that *A. tonsa* can readily achieve CSI rates of > 1 % day^{-1} feeding on a toxin-producing species. Indeed, our results suggest that CSI rates observed when feeding on *A. catenella* alone may exceed those when feeding on non-toxic *Rhodomonas* at a similar concentration (compare Figs. 1C and 3C). This result is consistent with the observation that *Acartia grani* ingested more of the toxic dinoflagellate, *Alexandrium minutum,* than the non-toxic *Rhodomonas* controls (Costa et al., 2008). It has been suggested that

copepods increase ingestion in order to compensate for the increased energy required for detoxification (Dutz 1998; Costa et al., 2008). Compensatory feeding may also be required if the nutritional value of *A. catenella* is lower than the control diet of *Rhodomonas* sp. This has been shown to occur when *A. tonsa* was fed the nutritionally insufficient species of toxic algae, *Karenia brevis*, which lacks various fatty acids and other nutritional components, and affected egg production and hatching success in a manner similar to starvation (Collumb and Buskey, 2004; Prince et al., 2006). Unfortunately it is not possible to robustly distinguish between these two non-mutually exclusive mechanisms from the data presented herein.

This study demonstrated that, in the absence of alternative prey, *A. tonsa* achieves similar daily CSI rates when fed either *A. catenella* (Fig. 1) or *Rhodomonas* (Fig. 3). However, when offered both species together, *A. tonsa* consistently selected for *Rhodomonas* and against *A. catenella*, even as the proportional abundance of the latter was increased (Figs. 3 and 4). This agrees with the understanding that *A. tonsa* can discriminate between different species of *Alexandrium* with different toxin contents, showing preference for the least toxic species (Turner and Tester 1989; Teegarden 1999; Colin and Dam 2003; Selander et al, 2006). Similar results have been reported for *A. tonsa* fed on toxic *K. brevis* (Turner and Tester 1989). Exactly how copepods achieve this food selection remains unclear; they may be able to physically distinguish different prey items prior to ingestion, or alternatively, their selection mechanisms may be based on a cell's palatability/toxicity or nutritional content. Physical incapacitation caused by toxicity would have resulted in decreased ingestion rates of the non-toxic algae (Colin and Dam 2003), but the total CSI of *A. tonsa* (*Rhodomonas* sp. & *A. catenella*)

was not statistically different between copepods fed a mixed diet and the control (0 % toxic algae) (Fig. 3C). It is possible that the apparent selection was size-related, as the equivalent spherical diameters (ESD) of *A. catenella* (25 μm*)* and *Rhodomonas* sp. (7 μm) were different. However, we suggest that this is unlikely as previous work has shown that female *A. tonsa* have an optimal prey size of 14.8 μm ESD, but are able to achieve approximately equal clearance rates when fed *Rhodomonas baltica* (ESD = 7 μm) and the dinoflagellate *Scripsiella faroense* (ESD= 19 μm) (Berggreen et al., 1988). Further work is required to determine if the apparent rejection of *A. catenella* is because of its palatability or its nutritional content.

4.3 Future-work

The presented 24 h experiments are an important first step towards understanding how marine copepods respond to toxin-producing algae. However, HA blooms in natural ecosystems last from a few days up to few months (Bresnan et al., 2008) and extended periods of exposure to toxic algae may cause substantial mortality within a population of copepods (Prince et al., 2006; Barreiro et al., 2007; Jiang et al., 2009; Turner et al., 2014). Previous work has shown a time-dependent decrease in the feeding rates of the copepods *Euterpina acutifrons* and *Acartia grani* when exposed to the toxic dinoflagellate *Gymnodinium catenatum* (Costa et al., 2012). The presence of toxin-producing algae can also lead to reduced rates of growth, respiration and egg production in copepods (Dutz 1998; Colin and Dam 2007). However, it is not yet known if these effects arise because of direct toxicity, the costs of detoxification, unpalatability of toxin-producing cells, or the absence of particular elemental and biochemical

components in the HA that are essential for healthy functioning of copepods. Indeed, it is possible for all four of these mechanisms to operate simultaneously, and determining the relative importance of each remains a challenge. We suggest that additional, longer-term experiments are required to better appreciate the population-scale effects of interactions between HA and copepods. Furthermore, a more detailed understanding of the energetic and nutritional requirements of copepods such as *A. tonsa* is required for us to determine if HA are in some way nutritionally inferior to other, non-toxin producing algae.

5. CONCLUSION

Our short-term experiments demonstrate that the dinoflagellate *Alexandrium catenella* does not have a direct impact on *Acartia tonsa* at concentrations typically encountered in natural systems. Decreased survival of *A. tonsa* was only apparent when offered *A. catenella* at a concentration two orders of magnitude higher than previously reported maximum densities. Ingestion rates of *A. tonsa* were not reduced when offered *A. catenella*, suggesting that the concentrations of toxins present in our experiments did not incapacitate *A. tonsa*. However, active selection towards non-toxic cells when presented simultaneously with *A. catenella* suggests that the toxins do act as a feeding deterrent. Further studies are required to understand the longer-term, sub-lethal effects of *A. catenella* on *A. tonsa.*

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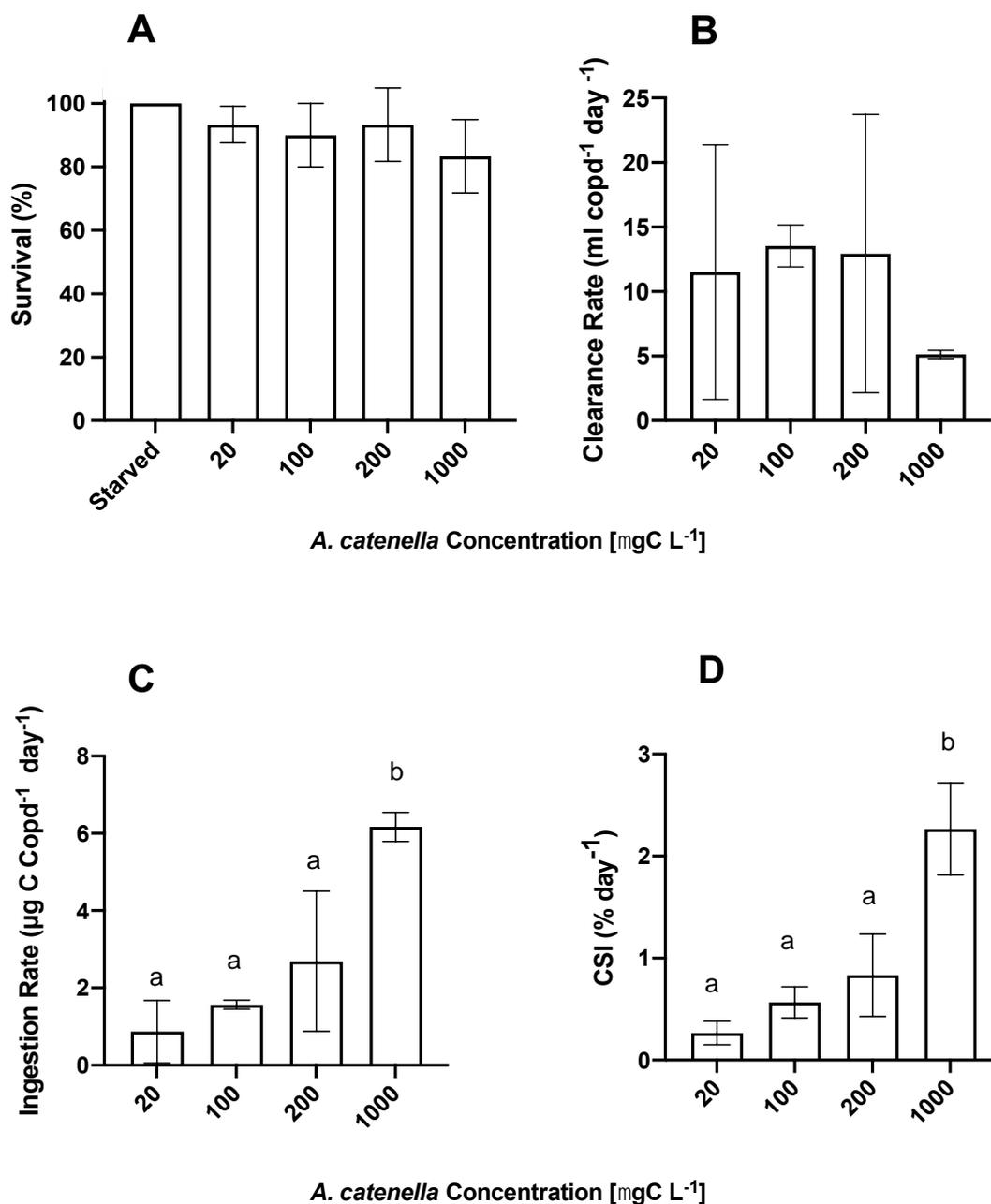
Figure 1. Survival and feeding of *Acartia tonsa* fed toxic algae (*Alexandrium catenella*) at 4 different concentrations. The bars show the mean value ± standard deviation (SD). A: survival (%). B: clearance rates (ml Copd^{-1} day^{-1}). C: Ingestion rate (μg C Copd^{-1}day^{-1}). D: Carbon Specific Ingestion (CSI: %C day^{-1}). Different letters denote significant differences (Tukey's test, p < 0.05).

A

B R^2= 0.6687

LogLC$_{50}$= 4.095 µg STX eq. L^{-1} **LC$_{50}$**= 12452 µg STX eq. L^{-1}

95% CI LogLC$_{50}$= 4.013 - 4.146 µg STX eq. L^{-1} **95% CI LC$_{50}$** =10313 - 13997 µg STX eq. L^{-1}
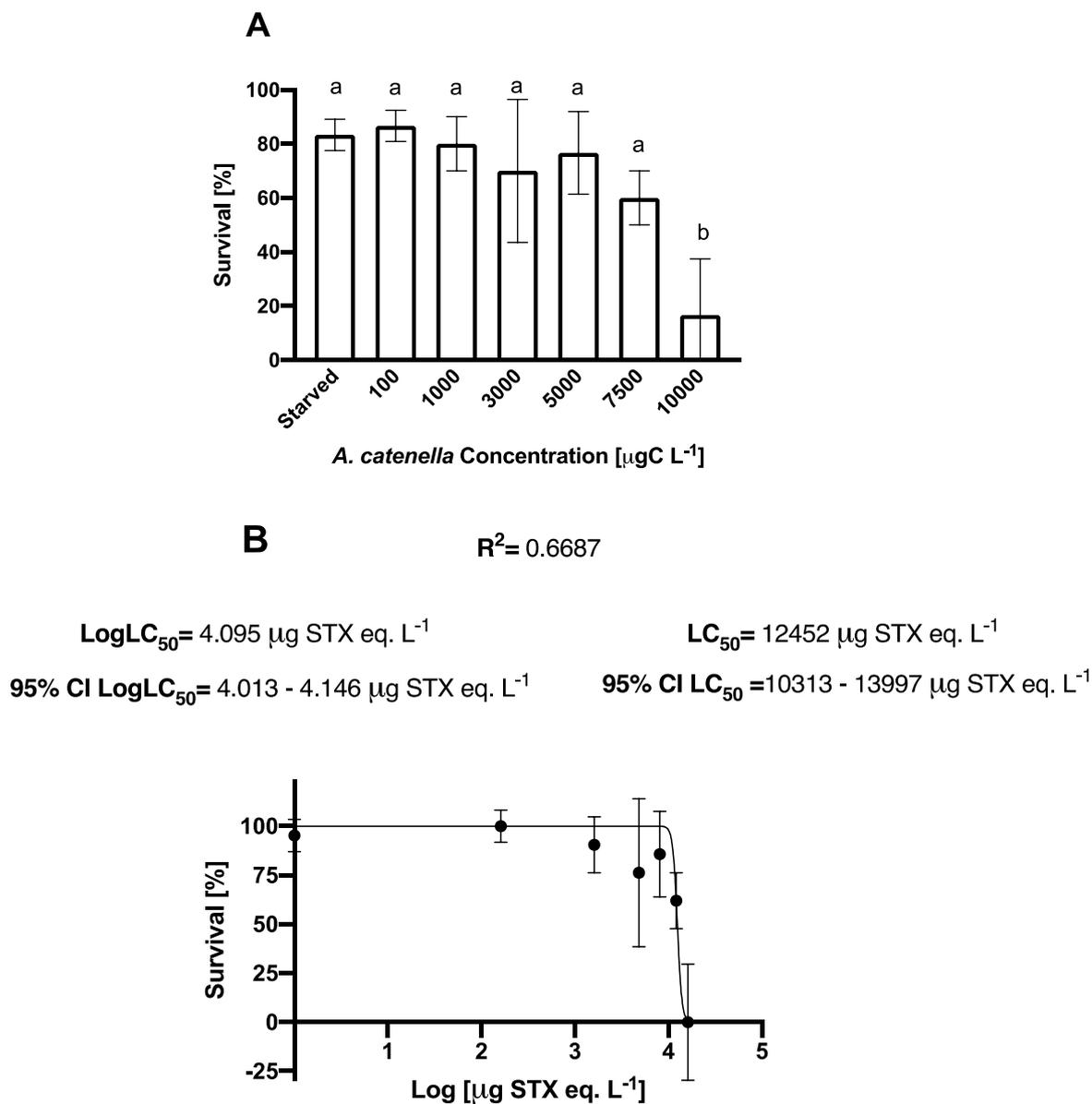

Figure 2. Survival of *Acartia tonsa* females across 7 different concentrations of *Alexandrium catenella*. The bars show the mean ± SD. A: survival. Different letters denote significant differences (Tukey's test, p < 0.05). B: The log$_{10}$ curve of *A. catenella* concentration with normalized % of survival to determine the LC$_{50}$ of the survival experiment. The LC$_{50}$ was calculated as 12452 µg STX eq L^{-1} (7707 µg C L^{-1}; 4561 cell ml^{-1}), with a 95% confidence interval between 10313-13997 µg STX eq L^{-1}.
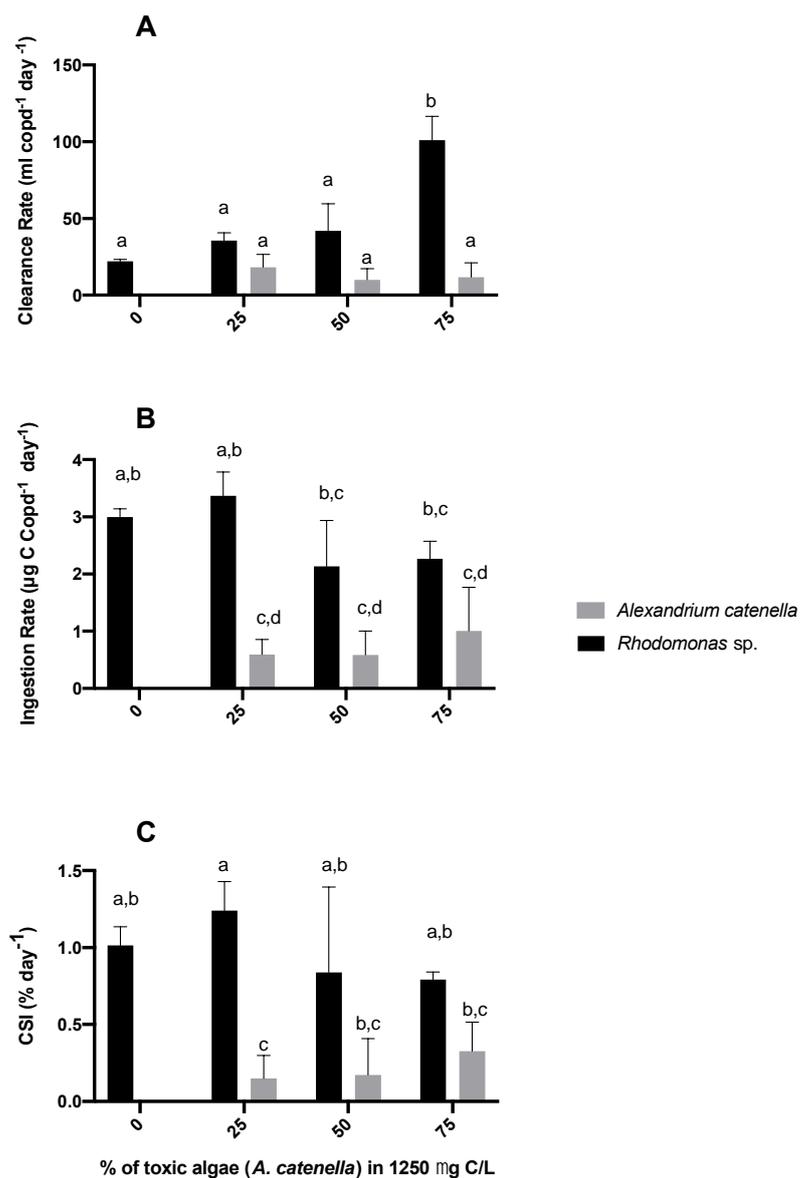
Figure 3. Mean (± SD) clearance and ingestion rates of *Acartia tonsa* fed a mixed diet of *Alexandrium catenella* (grey bars) and *Rhodomonas* sp. (black bars), where the total concentration of carbon in all treatments remained constant (1250 µg C L^{-1}). A: Clearance rates (ml Copd^{-1} day^{-1}). B: Ingestion rate (µg C Copd^{-1} day^{-1}). C: Carbon Specific Ingestion (CSI: %C day^{-1}). Survival was 100% in all the of treatment levels. Different letters denote significant differences (Tukey's test, $p < 0.05$).
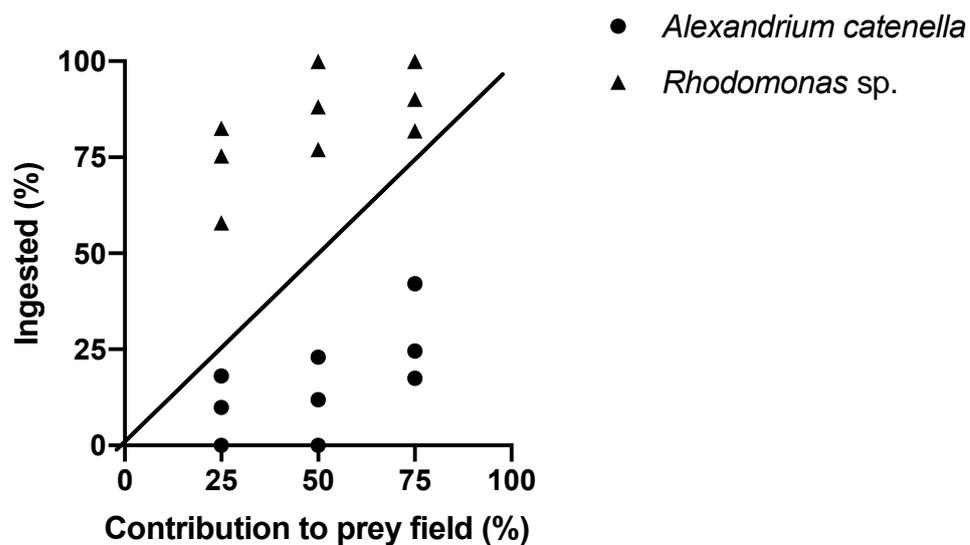
Figure 4. Prey selection of *Acartia tonsa* fed a mixed diet of *Alexandrium catenella* and *Rhodomonas* sp., where the total concentration of carbon in all treatments remained constant (1250 μg C L^{-1}). Percent (%) Carbon *Rhodomonas* sp./*Alexandrium catenella* ingested (based on CSI: % C day^{-1}) versus % carbon of *Rhodomonas* sp./*Alexandrium catenella* in the prey field.

**Table 1. The nominal and actual concentrations of *Alexandrium catenella*
offered in the different experiments, along with the cellular carbon &
nitrogen quotas**

Experiment	*Rhodomonas sp* Carbon content (Nitrogen Content) ng cell^{-1}	*Alexandrium catenella* Carbon content (Nitrogen Content) ng cell^{-1}	Nominal Concentration (Actual concentraton) µg C L^{-1}
1	-	1.62 – 1.75 (0.48-0.52)	20 (22) 100 (112), 200 (224), 1000 (1120)
2	-	2 (0.4)	100 (150) 1000 (1150), 3000 (3450), 5000 (5750), 7500 (8625), 10000 (11500)
3	0.053-0.054 (0.010)	1.96-2.11 (0.05)	*A. catenella*: 25% (30%) 50% (55%) 75% (79%)

Table 2. *Alexandrium catenella* (1119/27-Ainort) toxin profile concentrations (fg Cell^{-1} & fg STX eq. cell^{-1}) cultured in L1 media and analysed using HILIC-MS/MS (Liquid Chromatography Mass Spectrometry) at CEFAS.

Toxin Concentration fg cell^{-1} (fg STX eq cell^{-1})									
C1	**C2**	**C3**	**C4**	**dcGTX2**	**dcGTX3**	**dcGTX1**	**dcGTX4**	**GTX6**	**dcNEO**
18.8 (0.2)	1130.8 (113.1)	nd	44.3 (4.43)	nd	nd	nd	nd	nd	nd
GTX2	**GTX3**	**GTX1**	**GTX4**	**GTX5**	**doSTX**	**dcSTX**	**STX**	**NEO**	**STX eq cell^{-1}**
88.9 (35.6)	307.3 (184.4)	88.8 (88.8)	450.5 (315.4)	42.9 (4.3)	40.2 (2.0)	6.5 (6.5)	1089.7 (1089.7)	888.3 (888.3)	2732.5