



The Influence of the Toxin-Producing Dinoflagellate, *Alexandrium catenella* (1119/27), on the Survival and Reproduction of the Marine Copepod, *Acartia tonsa*, During Prolonged Exposure

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Copepods can feed on, and may regulate, the blooms of harmful algae (HA), and may also facilitate dinoflagellate blooms by inducing toxin production and through selective grazing. However, exposure to HA may also cause mortality and reproductive impairment in copepods, with detrimental effects at the population-scale. Here we present the toxin profile of the dinoflagellate, *Alexandrium catenella* (formerly *Alexandrium tamarense*), and examine how it affects the survival and reproduction of the cosmopolitan marine copepod, *Acartia tonsa*. Healthy adult copepods were exposed to mono-specific diets of toxic and non-toxic strains of *A. catenella* (1119/27 and 1119/19, respectively) and non-toxic *Rhodomonas* sp. for 10 days alongside unfed controls to examine how their survival was influenced by likely HA bloom conditions. Additional 2-day experiments examined how their egg production rate and hatching success were affected by food deprivation, toxic *A. catenella*, a non-toxic alternative and a mixture of toxic and non-toxic prey, at high and low concentrations. Survival of *A. tonsa* declined over the 10-day experiment in all treatments but was not significantly lower in the toxic *A. catenella* treatment; mortality was only significantly enhanced in the unfed animals, which showed 100% mortality after 9 days. Egg production rates and hatching success from females in the unfed and toxic *A. catenella* treatments were all significantly lower than values observed in females fed *Rhodomonas* sp. or non-toxic *A. catenella*. Animals offered 1,000 $\mu\text{g C L}^{-1}$ of *Rhodomonas* sp. and a 50:50 mixture of toxic *A. catenella* and *Rhodomonas* sp. produced significantly more eggs than animals fed toxic *A. catenella* alone. These results were not apparent at prey concentrations of 100 $\mu\text{g C L}^{-1}$. The percentages of eggs to successfully hatch from

females offered mono-specific diets of toxic *A. catenella* were always close to zero. Collectively, our results indicate that adult female *A. tonsa* can acquire sufficient energy from toxic *A. catenella* to survive, but suffer reproductive impairment when feeding on this prey alone.

Keywords: phytoplankton, harmful algal bloom, egg production, paralytic shellfish poisoning, saxitoxin, copepod survival

INTRODUCTION

In the past five decades, harmful algal (HA) blooms, which are caused by toxic or noxious species, have increased in frequency, duration, and distribution, resulting in major environmental and economic impacts (Hallegraeff, 1993; Anderson et al., 2012). Regardless of whether or not these increases are due to changing observational frequencies, HA blooms can be toxigenic, creating blooms that cause disease and death in a number of marine species including fish, seabirds, and mammals (Hallegraeff, 1993; Anderson et al., 2012). The impacts of HA on marine life and humans due to consuming intoxicated shellfish might result in illness or mortality (Wang, 2008). However, the interactions between toxic algae and potential grazer species are complex, inconsistent, and difficult to compare between studies due to different concentrations of HA used as food, the level of toxins in the food and the various responses of the species examined.

The dinoflagellate, *Alexandrium catenella* [formerly reported in this area as *Alexandrium tamarense* Group I, reassigned taxonomically by John et al. (2014) and Fraga et al. (2015), acknowledged in Prud'homme van Reine, 2017] can be found in the United Kingdom waters and some strains (such as 1119/27) are associated with shellfish toxicity and produce paralytic shellfish poisoning toxins (PSP), while others can be found naturally non-toxic (such as 1119/19). PSP toxin derivatives are divided into three main groups: carbamate-, N- sulfocarbamoyl-, and decarbamoyl- toxins. Compared to other PSP toxins, the carbamate toxins saxitoxin (STX) and neosaxitoxin (NEO) are considered as the most potent. Gonyautoxins 1 to 4 (GTX1, GTX2, GTX3, and GTX4) are other highly potent analogs, whereas N-sulfocarbamoyl (C) toxins and some of the decarbamoyl (dc) are considered less potent (Cook et al., 2010). Saxitoxins have mainly neurological effects through the blockage of sodium channels (Luckas et al., 2015). Consumption of shellfish contaminated with PSP toxins can cause breathing difficulties, gastrointestinal problems, and a sense of dissociation followed by complete paralysis in humans and other vertebrates (Wang, 2008). However, it remains unclear how exposure to and/or consumption of PSP toxins affect invertebrate fitness.

Zooplankton, and particularly copepods, are major grazers of phytoplankton and can influence phytoplankton dynamics (Runge, 1988; Mann, 1993; Mitra and Flynn, 2006). Copepods dominate the zooplankton communities in most marine water bodies worldwide, and play an important role in the transfer of carbon from phytoplankton to higher trophic levels such as fish and marine mammals (Mann, 1993; Dam, 2013). Toxic algae may affect the survival, feeding and fitness of copepods, and

lead to lower growth and reproduction rates in their populations (Barreiro et al., 2007; Jiang et al., 2009; Waggett et al., 2012). These impacts would ultimately result in less food available to higher trophic levels, and may therefore have negative ecological effects (Dam, 2013; Turner, 2014).

In some cases, copepods are reported to show negative effects when feeding on neurotoxic dinoflagellates, with symptoms consistent with toxicity (Dutz, 1998; Xu and Kiørboe, 2018; Xu et al., 2018; Prevett et al., 2019) and decreased fitness (Cohen et al., 2007; Jiang et al., 2009). In other cases, neurotoxic dinoflagellates were consumed by copepods without obvious adverse effects (Teegarden and Cembella, 1996; Abdulahussain et al., 2020). Dinoflagellates that are not known to produce neurotoxins may also adversely affect copepod fitness, e.g., by reducing egg production and hatching success (Ianora et al., 2004). Copepods may produce detoxification enzymes to reduce the impact of toxins (Dutz, 1998; Costa et al., 2008). However, a recent gene expression study that examined the impact of the toxic dinoflagellate, *A. catenella* (reported as *Alexandrium fundyense*), on the marine copepod, *Calanus finmarchicus*, showed that detoxification is not the main component of the response (Roncalli et al., 2016). Rather, the main genes that responded were related to digestion, suggesting that toxins may reduce the absorption of food and therefore the reproductive output without an impact on survival. It is generally recognized that there is no single obvious relationship between phytoplankton neurotoxicity and effects on plankton grazers, and that experiments involving sympatric grazers and prey species are necessary to understand trophic interactions in the plankton (Smayda, 1997; Turner and Tester, 1997).

The resistance of marine copepods to HA has been well documented (Colin and Dam, 2002, 2005, 2007; Jiang et al., 2011; Zheng et al., 2011). Copepods with a history of co-occurrence with HA were found to have higher survival and ingestion rates when exposed to HA blooms (Turner et al., 2000, 2005; Maneiro et al., 2005; Doucette et al., 2006). They may have evolved tolerance to HA species that co-occur in the same region. Some copepods have a discrete toxin-resistant phenotype that suggests a simple genetic mechanism evolving to adapt and tolerate Paralytic Shellfish Poisoning (PSP) toxins during HA blooms (Avery and Dam, 2007). For example, the fitness of *Acartia hudsonica* increased for several generations after feeding on *A. catenella* (reported as *A. fundyense*), which indicates that adaptation occurred (Finiguerra et al., 2014). Mutations of the sodium channels have been suggested as a possible mechanism (Avery and Dam, 2007; Chen et al., 2015). However, exposure of *A. hudsonica* to HA did not induce an increased proportion

of mutant isoforms (Finiguerra et al., 2014), which suggests that mutation in the sodium channel was not responsible.

The majority of studies examining how copepods respond to HA exposure have been conducted over approximately 1 day (Turner, 2014). This duration is sufficient to quantify grazing and discern patterns of feeding selectivity, but may be insufficient to determine any deleterious effects of the HA on grazers since there is a considerable delay in the effect of grazers' response to toxins, and acclimatization may also take time to occur (Ianora and Miralto, 2010; Turner et al., 2012), e.g., due to the time required to produce detoxification enzymes (Kozłowski-Suzuki et al., 2009). Short-term exposure to toxic algae might not result in significantly increased mortality compared to non-toxic algae. However, extended periods of exposure to toxic algae may cause substantial mortality within a grazers' population (Prince et al., 2006; Barreiro et al., 2007; Jiang et al., 2009; Drillet et al., 2015). Results from short-term, e.g., 24 h, experiments may therefore hinder our ability to understand how copepods are affected by exposure to natural blooms of HA, which can last from a few days up to a few months (Bresnan et al., 2008).

Here we exposed adult female *A. tonsa* to mono-specific diets of toxic and non-toxic strains of *A. catenella* and non-toxic *Rhodomonas* sp. for 10 days alongside unfed controls to examine how their survival was impacted by probable conditions experienced during a HA bloom. In addition, 2-day experiments examined how their egg production rate and hatching success were influenced by the type of food available and its concentration. Our results are presented alongside a full toxin profile for *A. catenella* in each of the experiments conducted.

MATERIALS AND METHODS

Culture Condition

All phytoplankton were grown in a culture cabinet at 15°C with a 16:8 h (Light:Dark) photoperiod, using seawater collected from the Western Channel Observatory monitoring site L4 off Plymouth, United Kingdom¹, amended with either F/2 (*Rhodomonas* sp.) or L1 (*A. catenella*) medium (Guillard and Ryther, 1962; Guillard and Hargraves, 1993). *Acartia tonsa* were purchased from Reefshotz, Cardiff, United Kingdom, where they have been cultured for > 10 years, and were subsequently cultured in artificial seawater (Tropic Marin Sea salt, Classic Meersalz; salinity 31) at room temperature (21 ± 2°C) for > 15 months prior to experimentation. Details of the culturing conditions for all phytoplankton species and for *A. tonsa* can be found elsewhere (Abdulahussain et al., 2020). The toxic *A. catenella* cultured was strain 1119/27 (CCAP, Loch Ainort, Scotland), and the non-toxic *A. catenella* was strain 1119/19 (CCAP Port Ellen, Islay, Scotland). *A. tonsa* cultures were fed *Rhodomonas* sp. (59.0 ± 7.8 pg C cell⁻¹) before experiments 1 and 2 and *Isochrysis galbana* (25.5 ± 0.3 pg C cell⁻¹) before experiment 3 at a concentration of 60,000 cells ml⁻¹ (2,000 ± 1,000 μg C L⁻¹) three times a week.

¹<https://www.westernchannelobservatory.org.uk/>

Influence of Toxic *A. catenella*, Starvation, and Non-toxic Prey Alternatives on the Survival of *A. tonsa*

Experiment 1 examined how the survival of *A. tonsa* was affected by a 10-day exposure to either food deprivation (starved) or one of three different types of food: *Rhodomonas* sp. (RHO), toxic *A. catenella* (TAC) and non-toxic *A. catenella* (NAC). Prey cells were offered at a nominal concentration of 1,000 μg carbon (C) L⁻¹ to ensure that feeding conditions were always saturating (Berggreen et al., 1988; Abdulhussain et al., 2020). Individual adult females were incubated in 15 ml glass dram vials with 10 ml of sterile filtered L4 seawater (0.2 μm; SFSW hereafter) for 10 days at 19°C with a 12:12 h (L:D) photoperiod. Each of the four treatment levels was replicated fifteen times (total = 60 bottles). Nominal prey concentrations were achieved by determining the algal stock culture concentration using a Beckman Multisizer 3 Coulter Counter equipped with a 70 μm aperture and subsequently diluting it with an appropriate volume of SFSW. Each copepod was carefully transferred into a petri dish at the end of every 24 h incubation period and examined for survival, determined by movement after mechanical stimulus. Surviving copepods were subsequently pipetted into clean 15 ml vials of fresh SFSW containing the same species and concentration of prey cells that they had previously been exposed to. Algal concentrations in the stock cultures were counted every other day using the Coulter Counter. Triplicate 5 ml samples from the stock cultures of each of the three prey types (RHO, TAC, and NAC) were collected onto pre-combusted 13 mm glass fiber filters (Whatman GF/F) on the start of days 1 and 9 of the experiment, dried overnight at 40°C and stored in a desiccator prior to determining their carbon and nitrogen content (Carlo Erba EA-1108 elemental analyzer). Triplicate 30 ml samples of the TAC stock culture were collected at the outset of the experiment, centrifuged at 3,000 rpm for 20 min, and the resulting pellets were transferred into 2 ml Eppendorf tubes and stored frozen at -80°C until toxin profile analysis (see section "Toxin Analysis").

Influence of Toxic *A. catenella*, Starvation, and Non-toxic Prey Alternatives on the Egg-Production and Hatching Success of *A. tonsa*

Experiment 2 examined how the egg production rate of *A. tonsa* was affected by different types of food: RHO, TAC, and NAC. Prey cells were offered at a nominal concentration of 1,000 μg C L⁻¹. Four adults (three females and one male) were incubated in 150 ml LPDE plastic bottles with an inner mesh compartment of 120 μm (to separate the adult from the eggs produced during the experiment) filled with 100 ml of SFSW for 2 days at 19°C with a 12:12 h (L:D) photoperiod. Each of the four treatment levels (starved, RHO, TAC, NAC: 12 bottles) was replicated three times. The experiment was run over 2 days, with the first day being considered as an acclimation period (Breier and Buskey, 2007; Cohen et al., 2007; Jiang et al., 2009; Waggett et al., 2012; Abdulhussain et al., 2020). Hatching success was therefore only calculated for eggs produced on day 2. After the first day, the

mesh compartments containing copepods were transferred to another LPDE plastic bottle with fresh SFSW containing the same species and concentration of prey cells that they had previously been exposed to. The eggs left in the LPDE plastic bottles from day 1 were counted under a stereomicroscope (to observe the response of *A. tonsa* to TAC during the acclimation period: see **Supplementary Material**). At the end of day 2, copepods in each treatment were carefully transferred into a petri dish and examined for survival (see section “Influence of Toxic *A. catenella*, Starvation, and Non-toxic Prey Alternatives on the Survival of *A. tonsa*”). Eggs produced on day 2 were counted and then transferred into petri dishes for 48 h at 19°C with a 12:12 h (L:D) photoperiod before counting the eggs hatched for each group, and calculating percentage hatching success (eq. 1):

$$\% \text{ hatching success} = \frac{\text{total number of eggs hatched from day 2}}{\text{total number of eggs produced on day 2}} \times 100 \quad (1)$$

Samples to determine the toxin- and elemental-content of the algal cultures were collected on the start of day 2 (see section “Influence of Toxic *A. catenella*, Starvation, and Non-toxic Prey Alternatives on the Survival of *A. tonsa*”).

Influence of Toxic *A. catenella*, Starvation, and Mixed Diet Prey Alternatives on the Egg-Production and Hatching Success of *A. tonsa* at High and Low Concentrations

Experiment 3 was conceptually similar to experiment 2, but examined how the egg production rate and hatching success of *A. tonsa* was affected by low (100 µg C L⁻¹) and high (1,000 µg C L⁻¹) concentrations of: RHO, TAC, and a diet where the carbon content was derived from 50% RHO to 50% TAC (MIX hereafter). Each of the seven treatment levels (starved, low RHO, high RHO, low TAC, high TAC, low MIX, and high MIX) was replicated three times (two times for low MIX; total = 20 bottles). Samples to determine the toxin- and elemental-content of the algal cultures were collected at the start of the experiment (after the acclimation period) (see section “Influence of Toxic *A. catenella*, Starvation, and Non-toxic Prey Alternatives on the Survival of *A. tonsa*”).

Toxin Analysis

The frozen pellets of *A. catenella* from each experiment were mixed with 1.5 ml 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 min per sample. After sonication, vials were re-mixed, and 400 µL taken for desalting clean-up using carbon solid phase extraction (SPE). Details of the toxin extraction, column, mobile phases, chromatographic separation, and HILIC-MS/MS analysis conditions are described elsewhere (Turner et al., 2015, 2019; Abdulhussain et al., 2020).

Data Analyses

The effects of 10 days of exposure to food deprivation, RHO, TAC, and NAC on the survival of *A. tonsa* were examined using Kaplan Meier survival analysis:

$$S_{t+1} = S_t \times \frac{N_{t+1} - D_{t+1}}{N_{t+1}} \quad (2)$$

Where *S* is the Survival probability; *t* is time in days; *N* is the number of *A. tonsa* that survived; and *D* is the number of *A. tonsa* that died. Curve comparisons were achieved using the Log-rank test. Standard errors are presented to show the deviation from the population mean.

The influence of feeding conditions (starved, RHO, TAC, and NAC) on the egg production of *A. tonsa* in experiment 2 was examined using 1-way ANOVA. Egg hatching success was examined using a 1-way ANOVA. The influence of food type (starved, TAC, MIX) and food concentration (100 or 1,000 µg C L⁻¹) on the egg production of *A. tonsa* in experiment 3 were examined using 2-way ANOVA. Egg hatching success was examined using a 2-way ANOVA. All explanatory variables were treated as categories. *Post hoc* comparisons were conducted using Tukey's test with alpha = 0.05. Standard errors are presented for the egg production and hatching experiments to show the variation within each treatment. All statistical analyses were carried out using Prism Graphpad (v.8.4) software.

RESULTS

The carbon and nitrogen contents of the prey cells offered in the different experiments are presented in **Table 1**. TAC toxin profile concentrations, and their saxitoxin (STX) equivalent, are presented in **Table 2**.

Influence of Toxic *A. catenella*, Starvation, and Non-toxic Prey Alternatives on the Survival of *A. tonsa*

The survival of female *A. tonsa* declined significantly across the 10-day duration of the experiment (**Figure 1**; Log-rank test, Chi square = 29, df = 3, *p* < 0.001). However, survival was only significantly reduced in the starving group, where there was 100% mortality by day 9 (**Figure 1**; Log-rank test, Chi square = 29, df = 3, *p* < 0.001). Survival of the females offered RHO (73%), TAC (80%), and NAC (60%) at nominal concentrations of 1,000 µg C L⁻¹ were statistically indistinguishable, with an average survival of 71 ± 10% across these treatments (**Figure 1**; Log-rank test, Chi square = 1, df = 2, *p* = 0.525).

Influence of Toxic *A. catenella*, Starvation, and Non-toxic Prey Alternatives on the Egg Production and Hatching Success of *A. tonsa*

The egg production rate of *A. tonsa* was affected by the type of food they received (**Figure 2A**; 1-way ANOVA, *F*(3,8) = 13.24, *p* ≤ 0.0115). The females offered RHO and NAC produced

TABLE 1 | The concentrations of carbon (C) and nitrogen (N) (pg cell^{-1}) \pm standard error (SE) in the different prey cells offered to *Acartia tonsa* in the three experiments (Exp.).

Species	Exp.	Day	C \pm SE (pg cell^{-1})	N \pm SE (pg cell^{-1})	C:N	ESD (μm)
<i>Rhodomonas sp.</i>	1	1	57 \pm 0.03	14 \pm 0.43	4.1	6–8
	1	9	63 \pm 0.21	8 \pm 0.06	7.9	6–8
	2	NA	67 \pm 0.36	19 \pm 0.58	3.5	6–8
	3	NA	49 \pm 0.97	9 \pm 0.36	5.4	6–8
Non-toxic <i>Alexandrium catenella</i> -1119/19	1	1	3552 \pm 57.3	732 \pm 15.5	4.9	20–40
	1	9	2980 \pm 524	609 \pm 84	4.9	20–40
	2	NA	1488 \pm 9.0	400 \pm 31.5	3.7	20–40
<i>Alexandrium catenella</i> -1119/27	1	1	4950 \pm 30	1100 \pm 53.3	4.5	20–40
	1	9	3336 \pm 168	1042 \pm 50.9	3.2	20–40
	2	NA	4640 \pm 30	1070 \pm 32.5	4.3	20–40
	3	NA	2103 \pm 34.21	410 \pm 19.53	5.1	20–40

NA, not applicable; ESD, mean equivalent spherical diameter (μm). See section "Materials and Methods" text for further details.

TABLE 2 | *Alexandrium catenella* (1119/27-Loch Ainort) toxin profile concentrations (fmol cell^{-1}).**(A) Toxin concentration, fmol cell^{-1}**

C1	C2	C3	C4	dcGTX2	dcGTX3	dcGTX1	dcGTX4	GTX6	dcNEO
0.35	1.08	nd	nd	nd	nd	nd	nd	nd	nd
GTX2	GTX3	GTX1	GTX4	GTX5	doSTX	dcSTX	STX	NEO	STX eq cell $^{-1}$ (fg STX eq cell $^{-1}$)
0.76	0.55	nd	0.41	0.18	nd	nd	0.26	nd	501.1

(B) Toxin concentration, fmol cell^{-1}

C1	C2	C3	C4	dcGTX2	dcGTX3	dcGTX1	dcGTX4	GTX6	dcNEO
0.27	3.06	nd	0.15	nd	nd	nd	nd	nd	nd
GTX2	GTX3	GTX1	GTX4	GTX5	doSTX	dcSTX	STX	NEO	STX eq cell $^{-1}$ (fg STX eq cell $^{-1}$)
0.43	1.49	nd	2.06	0.15	nd	nd	3.74	3.36	3575.5

Cell toxicity of saxitoxin equivalent concentrations are calculated in (fg STX eq. cell $^{-1}$). (A) Toxin profile concentrations for experiment 1 and 2. (B) Toxin profile concentrations for experiment 3. Saxitoxin (STX), neosaxitoxin (NEO), Gonyautoxins 1 to 4 (GTX1, GTX2, GTX3, GTX4), N-sulfocarbamoyl (C1, C2, C3, C4) toxins, decarbamoyl (dc) toxins.

significantly more eggs (17.7 ± 4.9 and 14.1 ± 5.7 eggs female $^{-1}$ day $^{-1}$, respectively) than those offered TAC (2.4 ± 2.3 eggs female $^{-1}$ day $^{-1}$) and starved (1.0 ± 0.6 eggs female $^{-1}$ day $^{-1}$) (Figure 2A). The hatching success of eggs produced on day 2 was influenced by the maternal feeding conditions (1-way ANOVA, $F(3,7) = 11.53$, $p = 0.0042$). Hatching success of the eggs from females that had received RHO ($60 \pm 9.7\%$) and NAC ($56 \pm 23.9\%$) was higher than the eggs from females fed TAC (0%) or starved ($7 \pm 11.5\%$) (Figure 2B).

Influence of Toxic *A. catenella*, Starvation, and a Mixed Diet on the Egg-Production and Hatching Success of *A. tonsa* at High and Low Food Concentrations

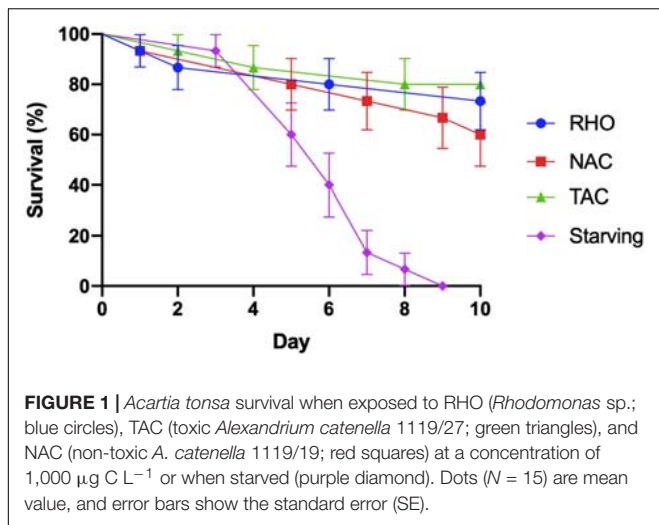
The egg production rate of *A. tonsa* was affected by food type and food quantity (Figure 3A, 2-way ANOVA, food type: $F(3,15) = 40.0$; food quantity $F(1,15) = 25.0$, $p \leq 0.002$ in both cases). Copepods that were fed on MIX at $1,000 \mu\text{g C L}^{-1}$ produced significantly fewer eggs (4.7 ± 1.2 eggs female $^{-1}$ day $^{-1}$) than in the RHO treatment at the same concentration

(9.0 ± 1.3 eggs female $^{-1}$ day $^{-1}$) but significantly more than the females that received TAC (0.3 ± 0.3 eggs female $^{-1}$ day $^{-1}$) (Figure 3A). Egg production rates in the $100 \mu\text{g C L}^{-1}$ treatments were all similarly low (Figure 3A). The hatching success of the eggs produced was influenced by the food type and food quantity (Figure 3B, 2-way ANOVA, $F(3,15) = 15.39$ and 6.32 , respectively, $p \leq 0.0238$ in both cases). The hatching success of eggs from females that were fed $1,000 \mu\text{g C L}^{-1}$ of RHO ($58 \pm 14\%$) and MIX ($56 \pm 25\%$) were both higher than TAC (0%) and starved (0%) (Figure 3B), whereas the hatching success of eggs from females fed at $100 \mu\text{g C L}^{-1}$ of RHO ($67 \pm 18\%$), MIX ($60 \pm 28\%$) and TAC ($62 \pm 28\%$) were all similar.

DISCUSSION

A. catenella Toxin Composition and Concentrations

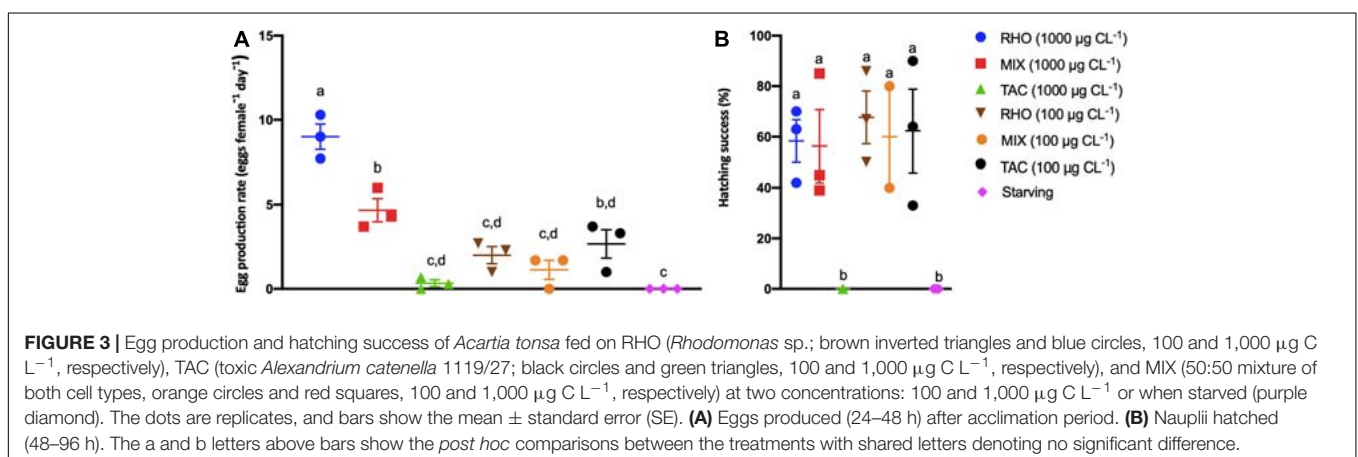
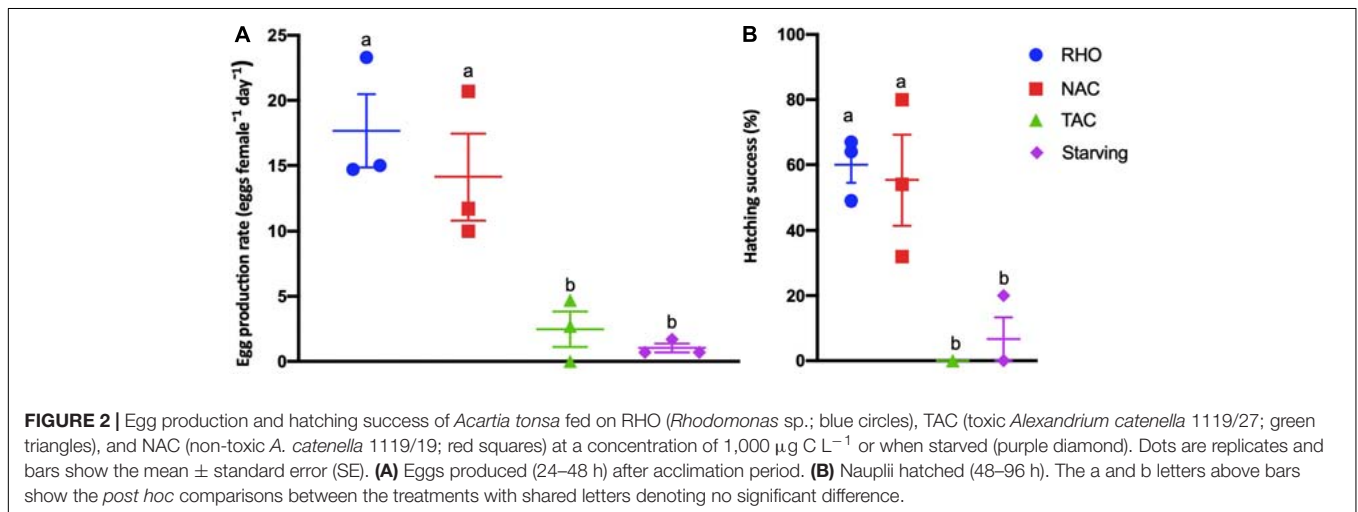
The elemental content, and composition and concentrations of toxins, of *A. catenella* (1119/27) were all variable (Tables 1, 2), despite being grown under near identical conditions. For example, the total toxin concentration was 501.1 STX eq



salinity (Grzebyk et al., 2003), and nutrient conditions (Turner et al., 1998; John and Flynn, 2002; Leong et al., 2004). Toxin levels can also vary with growth phase in cultured algae (Hamasaki et al., 2001; Cook et al., 2010). We suggest that the differences in toxicity in our laboratory-based study were caused by differences in the nutrient concentrations of the seawater that was used for culturing TAC: Experiment 1 and 2 were conducted during January 2020, and experiment 3 during July 2019, when surface nitrate and phosphate concentrations were close to their highest and lowest, respectively². Moreover, in response to nutrient stress and chemical signals from copepods, HA can dramatically increase the development of PSP toxins (Wohlrab et al., 2010; Selander et al., 2015; Griffin et al., 2019). A group of predator cues called copepodamides (also known as taurine-containing lipids; Mayor et al., 2015), which are exuded by three species of calanoid copepods (*Centropages typicus*, *Pseudocalanus* sp., and *Calanus* sp.) have been found to trigger increased PSP toxins in dinoflagellates, as well as amnesic shellfish poisoning (ASP) toxins in diatoms (Selander et al., 2019). It is therefore possible that the toxin content of the prey cells increased during our incubations

cell⁻¹ for experiments 1 and 2, and 3575.5 STX eq cell⁻¹ for experiment 3. Some of the factors that can change the toxin levels within a HA bloom are turbulence (Juhl et al., 2001),

²https://www.westernchannelobservatory.org.uk/l4_nutrients.php



and the concentrations presented in **Table 2** should be considered minimal. These differences highlight the importance of reporting toxin profile concentrations, particularly in the context of copepod survival, grazing, and egg production studies. This makes it easier to robustly compare between different studies.

Survival of *A. tonsa* Exposed to Toxic *A. catenella*

Exposure of *A. tonsa* to a toxic strain of TAC for 10 days did not significantly affect their survival relative to the NAC treatment. Rather, increased mortality was only observed in the starved treatment (**Figure 1**). These findings suggest that *A. tonsa* is able to feed and survive on TAC over the likely duration of a typical bloom of TAC (Bresnan et al., 2008), regardless of their toxicity. Previous 24 h experiments showed that *A. tonsa* can feed upon TAC and survive blooms two orders of magnitude higher than the maximum densities of *A. catenella* recorded in natural systems (Abdulhussain et al., 2020), possibly by producing detoxification enzymes (Dutz, 1998; Costa et al., 2008) or by reducing food absorption efficiency (Asselman et al., 2012; Roncalli et al., 2016). Bloom concentrations of TAC are generally low and typically last for only a few days (Bresnan et al., 2005), although more persistent blooms are reported (Bresnan et al., 2008). Our findings therefore support the understanding that *Acartia* sp. tolerate exposure to TAC for the typical duration of naturally occurring blooms without negative impacts on the survival of adults (Liu and Wang, 2002; Estrada et al., 2008). However, exposure to high densities of HA for an extended period may cause substantial mortality within *Acartia* spp. populations (Prince et al., 2006; Barreiro et al., 2007; Jiang et al., 2009). Thus, the response of *Acartia* spp. appears to vary between different studies, possibly because of different toxin concentrations (section “*A. catenella* Toxin Composition and Concentrations”). Higher survival rates of *A. tonsa* in this study compared to other survival experiments could also be a result of reduced encounter rates between individuals as each experimental vial contained only one female per treatment replicate. However, in denser cultures, elevated encounter rates between individuals may increase levels of stress and cannibalism, potentially resulting in higher rates of mortality (Drillet et al., 2015).

Egg Production and Hatching Success of *A. tonsa* Exposed to Toxic *A. catenella*

The average egg production rate of *A. tonsa* feeding on RHO in our 2-day exposure experiments, ~ 17 eggs female⁻¹ day⁻¹ (**Figure 2**), agrees with values previously reported for the genus *Acartia* sp. (range = 9–76 eggs female⁻¹ day⁻¹; Prince et al., 2006; Barreiro et al., 2007; Breier and Buskey, 2007; Jiang et al., 2009; Waggett et al., 2012). Our results reveal that egg production and hatching success were affected by the types of food available and the concentration at which it was offered (**Figures 2, 3**), with the observed values being significantly lower in the TAC treatments relative to those in the NAC and RHO treatments (**Figure 2**). Egg production

rates by females fed RHO, NAC and MIX (50% RHO, 50% TAC) at 1,000 $\mu\text{g C L}^{-1}$ were also significantly higher than those fed TAC alone. By contrast, the rates of females fed TAC were not statistically different to those of the starved animals (**Figures 2, 3**). Furthermore, the percentages of eggs to successfully hatch from females offered mono-specific diets of toxic *A. catenella* at concentration of 1,000 $\mu\text{g C L}^{-1}$ were always close to zero. These results are consistent with the observation that reproduction in *Acartia clausi* is negatively affected when consuming toxic *Alexandrium minutum*, and that these effects can be alleviated by offering mixed diets (Barreiro et al., 2006, 2007).

Acartia tonsa can discriminate between different species of *Alexandrium* with different toxin contents, showing preference for the least toxic species (Xu et al., 2018; Abdulhussain et al., 2020). Copepods are thought to be capable of rejecting toxic prey cells (Cook et al., 2010; Abdulhussain et al., 2020) or consume them at lower rates than a similarly sized non-toxic cells (Xu and Kiørboe, 2018). It is therefore possible that the reduced egg production rate in the TAC treatment could be caused by starvation. However, we know that *A. tonsa* is capable of surviving and feeding on TAC when alternative prey are not available (Abdulhussain et al., 2020). This understanding is further supported by the current study, where survival of *A. tonsa* in TAC treatment was significantly higher than in the starved treatment (section “Survival of *A. tonsa* Exposed to Toxic *A. catenella*”). The significantly lower egg production rates observed in the TAC treatments of both experiments is therefore unlikely attributable to a starvation effect. The negative effects of TAC on the observed egg production rates and hatching success may have occurred because (a) the females were directly impeded by the toxins, (b) the females needed to produce detoxification enzymes, and therefore had to channel energy and other resources away from producing viable eggs, (c) the presence of toxins reduced the efficiency with which food is absorbed, and therefore reduced the amount of energy and other resources available for producing viable eggs, or (d) TAC were in some way nutritionally inferior to the other diets offered.

Nutritionally deficient food and the presence of toxins have both been suggested to cause reduced egg production in *Acartia* sp. (Dutz, 1998; Collumb and Buskey, 2004; Prince et al., 2006; Costa et al., 2008). The survival of females exposed to TAC was not significantly lower than females exposed to the non-toxic prey treatments, apparently suggesting that TAC toxins do not necessitate additional metabolic costs associated with the production of detoxification enzymes to the extent that they have a discernible, negative effect on the overall physiological performance of *A. tonsa*. However, copepods may increase their ingestion rates when feeding on toxic dinoflagellates in order to compensate for the increased energy required for detoxification (Dutz, 1998; Costa et al., 2008) or reduce the efficiency with which they absorb food (Asselman et al., 2012; Roncalli et al., 2016). For example, ingestion rates of *A. tonsa* were higher when fed on toxic *A. catenella* relative to animals fed on non-toxic *Rhodomonas* sp. at a food saturating concentration of 1,000 $\mu\text{g C L}^{-1}$

(Abdulahussain et al., 2020). Compensatory feeding may also be necessary if the nutritional value of HA is lower than other non-toxic food (Collumb and Buskey, 2004; Prince et al., 2006). Our survival experiments were conducted at a food saturating concentration of $1000 \mu\text{g C L}^{-1}$ (Berggreen et al., 1988; Abdulahussain et al., 2020) and we cannot therefore exclude the possibility that the females increased their ingestion rates to offset any additional metabolic costs. Nevertheless, even if compensatory feeding did occur, it was clearly insufficient to mitigate the negative effects on reproduction associated with ingesting TAC. Egg production rates of females fed NAC, which we assume to have a highly similar nutritional make up to TAC, were indistinguishable from those fed on RHO, a food source that is widely assumed to support optimal reproductive output in *Acartia* spp. (Støttrup et al., 1986; Berggreen et al., 1988). We therefore suggest that the reduced rates observed in the TAC treatment (Figure 2) were not attributable to the absence of nutritive compounds; they occurred because of the presence of toxins, although the specific mechanism cannot be elucidated here.

The negative effects of consuming TAC were less apparent when prey cells were offered at $100 \mu\text{g C L}^{-1}$, where egg production rates were similarly low across all treatments (Figure 3A). This agrees with the understanding that lower food concentrations limit growth, reproduction, and survival (Berggreen et al., 1988). Interestingly, the hatching success of the few eggs produced by females fed TAC at $100 \mu\text{g C L}^{-1}$ was similarly high when compared to the values observed in the other, non-toxic treatments. This may suggest that there is a toxin-threshold level, beyond which eggs do not hatch.

Prey cell size can influence the outcome of copepod feeding experiments. However, in our study, differences between *Rhodomonas* (6–8 μm ESD) and *I. galbana* (4–6 μm ESD) were trivial, suggesting that these are unlikely to cause differences in the feeding rates of our experimental animals. Indeed, previous work has shown that female *A. tonsa* have an optimal prey size of 14.8 μm ESD, but are able to achieve high clearance rates when feeding on both *Rhodomonas* and on the dinoflagellate *A. catenella* (ESD = 20–40 μm) (Berggreen et al., 1988; Abdulahussain et al., 2020). Moreover, despite differences in the feeding histories of the animals in experiments 2 and 3, the outcomes of these two experiments were qualitatively and quantitatively the same: EPR and hatching success in the TAC and starved treatments in both experiments were similarly low (not different to zero); EPR and hatching success in the RHO treatments in both experiments were always higher than in the TAC and starved treatments (Figures 2, 3); ERP in the RHO treatments were not statistically different between the two experiments (Paired *t*-tests: $t = 3.965$, $df = 2$, $p = 0.058$). These results would not have occurred if any feeding history effects had not been accounted for within the 24 h acclimation period.

Finally, in the real environment, there are usually more than one species of algae available for copepods to feed on. The availability of alternative prey suggests that the impact of HA on copepod egg production may be very low due to availability of other algae. Nevertheless, it is clear that further work is required to understand the energetic and nutritional requirements of

A. tonsa and their developing embryos, and how these are directly and indirectly affected when ingesting toxin-producing prey species.

SUMMARY

The survival of *A. tonsa* exposed to monospecific diets of either toxic and non-toxic *A. catenella* or *Rhodomonas* sp. declined over a 10-day incubation experiment. However, mortality only increased significantly in unfed copepods, which showed 100% mortality after 9 days. Egg production and hatching success rates of *A. tonsa* exposed to $1,000 \mu\text{g C L}^{-1}$ toxic *A. catenella* were not significantly different from starved animals, but were significantly lower than animals fed $1,000 \mu\text{g C L}^{-1}$ of non-toxic *A. catenella*, *Rhodomonas* sp., or a 50:50 mix of toxic *A. catenella* and *Rhodomonas* sp. Egg production and hatching success rates of eggs produced by females that were fed either *Rhodomonas* sp., toxic *A. catenella* or a 50:50 mix of toxic *A. catenella* and *Rhodomonas* sp. at $100 \mu\text{g C L}^{-1}$ were not significantly different. Further studies are required to understand the physiological basis of why female *A. tonsa* can feed and survive on toxic *A. catenella*, but display reproductive impairment as a result.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AA, DM, and KC contributed to conception and design of the study. AA conducted the experiments, performed the statistical analyses, and wrote the manuscript. AA, AT, and AL contributed to toxin extraction and analyses. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.652225/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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