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Comparison of the functional and numerical responses of resistant versus non-resistant populations of the copepod *Acartia hudsonica* fed the toxic dinoflagellate *Alexandrium tamarense*

Sean P. Colin *, Hans G. Dam

Department of Marine Sciences, University of Connecticut, Groton, CT 06340, USA

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Abstract

The functional and numerical responses of grazers are key pieces of information in predicting and modeling predator–prey interactions. It has been demonstrated that exposure to toxic algae can lead to evolved resistance in grazer populations. However, the influence of resistance on the functional and numerical response of grazers has not been studied to date. Here, we compared the functional and numerical responses of populations of the copepod *Acartia hudsonica* that vary in their degree of resistance to the toxic dinoflagellate *Alexandrium tamarense*. In common environment experiments carried out after populations had been grown under identical conditions for several generations, female copepods were offered solutions containing different concentrations of either toxic *A. tamarense* or the non-toxic green flagellate *Tetraselmis* sp. ranging from ~25 to 500 μgC L^{-1}, and ingestion and egg production rates were measured. Throughout most of the range of concentrations of the toxic diet, copepod populations that had been historically exposed to toxic blooms of *Alexandrium* exhibited significantly higher ingestion and egg production rates than populations that had little or no exposure to these blooms. In contrast, there were no significant differences between populations in ingestion or egg production for the non-toxic diet. Hence, the between population differences in functional and numerical response to *A. tamarense* were indeed related to resistance. We suggest that the effect of grazer toxin resistance should be incorporated in models of predator and toxic prey interactions. The potential effects of grazer toxin resistance in the development and control of *Alexandrium* blooms are illustrated here with a simple simulation exercise.

Keywords: *Acartia hudsonica*; Harmful algal blooms; Toxin resistance; Ingestion; Egg production; Grazer impact

1. Introduction

The functional (ingestion versus food concentration) and numerical (reproduction versus food concentration) responses are essential pieces of information in order to predict both the grazer-induced mortality on a producer, and the effect of the producer on the grazer, and hence in modeling predator–prey interactions. With few exceptions (e.g., Dutz, 1998; Liu and Wang, 2002; Dam and Colin, 2005), there is a lack of knowledge of both the functional and numerical responses of grazers fed harmful dinoflagellates. For instance, models of the population dynamics of harmful algae assume a constant algal mortality coefficient, regardless of the

* Corresponding author. Present address: Environmental Sciences/ Marine Biology, Roger Williams University, Bristol, RI 02809, USA. Tel.: +1 401 254 3142.
E-mail address: scolin@rwu.edu (S.P. Colin).

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ambient cell concentration (Watras et al., 1985; McGillicuddy et al., 2006; Stock et al., 2006). However, because the ingestion rate is linearly or hyperbolically related to food concentration, we know such an assumption is incorrect. Modelers in fact recognize that variability in mortality is a major source of uncertainty in their models (e.g., Stock et al., 2006). Knowledge of the functional response coupled with grazer abundance can go a long way to alleviate uncertainty in mortality estimates used in models.

The impact of harmful algae on grazers appears to be highly species-specific, and even within a single grazer species their impact is site-specific (Turner and Tester, 1997; Colin and Dam, 2003). One factor that contributes to such a disparity of effects is that grazers have been shown to be capable of evolving a resistance to the effects of toxic algae (Hairston et al., 1999; Colin and Dam, 2002a). Populations of the copepod *Acartia hudsonica* historically exposed to toxic *Alexandrium* blooms (henceforth called resistant) exhibit enhanced feeding, egg production and population growth rates relative to non-exposed (henceforth called non-resistant) populations (Colin and Dam, 2002a, 2003, 2004). Further, the potential exists for toxic dinoflagellates to cause rapid evolution on non-resistant copepod populations (Colin and Dam, 2004). However, these studies were done using either a single or a narrow range of algal concentrations. In order to understand the robustness of these studies on evolved resistance and to increase their utility for examining predator–prey interactions, comparisons need to be made at multiple algal concentrations. Therefore, the goal of this study was to measure the effects of toxic *A. tamarense* on the functional and numerical response of resistant and non-resistant populations of the copepod *Acartia hudsonica*.

2. Methods

Following the methods of Colin and Dam (2002a), we used common-environment experiments (Walker et al., 2001) to compare the ingestion and fecundity of female copepods fed toxic *Alexandrium*, from populations that experience recurrent blooms of toxic *Alexandrium* and from those that are never or rarely exposed to those blooms (identified as resistant and non-resistant populations, respectively, according to Colin and Dam, 2002a). Specifically, we exposed five separate populations of the ubiquitous copepod, *Acartia hudsonica*, to toxic *A. tamarense* from Casco Bay, ME, USA (strain CB-301). Two of the *A. hudsonica* populations were from regions that regularly experience blooms of toxic *Alexandrium* (Passamoquoddy Bay, New Brunswick, Canada: 45°04′N, 67°03′W, and Casco Bay: 43°39′N, 70°12′W). One copepod population was from a region where *Alexandrium* blooms less frequently and has lower toxin content (Cape Cod, Massachusetts: 41°34′N, 70°30′W) and two populations were from regions where *Alexandrium* is present, but does not bloom and has very low toxin content (Long Island Sound, Connecticut: 41°19′N, 72°06′W), or *Alexandrium* is not present (Great Bay, southern New Jersey: 39°23′N, 74°47′W; Cohn et al., 1988).

The copepod cultures were maintained at 12–14 °C and 12-h light:12-h dark regime during rearing and experiments. The standard rearing diet consisted of a mixture of the phytoplankters *Thalassiosira weissflogii*, *Isochrysis galbana*, and *Rhodomonas lens*, which was kept at a concentration of 400–500 $\mu gC\ L^{-1}$ by replenishment every other day. This concentration is near the saturation level of the functional and numerical response of *A. hudsonica*. All copepod populations were reared at the same temperature, light and food regimes for several generations to eliminate both maternal effects and environmental variance. This allowed us to attribute the observed differences among populations to genetic variance (Falconer, 1996; Colin and Dam, 2002a)). Phytoplankton cultures were grown in F/2 media (Guillard, 1975) at 14 °C with 12-h light:12-h dark cycle. The cultures were maintained in exponential growth, for use in the experiments, by replacing half of the culture medium with fresh F/2 media each week.

For the ingestion and egg production experiments, adult females were starved for 48 h, twice as long as egg turnover time (Turner and Tester, 1997) to ensure that the measured egg production reflected ingestion of the experimental food. Then, eight replicate pairs of adult females were incubated in 140-mL bottles filled with food solutions of *A. tamarense* (Strain CB-301, Equivalent spherical diameter (ESD) = 19.2 μm, carbon content = $8.9 \times 10^{-4}\ \mu gC\ cell^{-1}$, 40.5 pg STX eq. $cell^{-1}$) or the non-toxic green flagellate *Tetraselmis* sp. (ESD = 7.6 μm, carbon content = $4.1 \times 10^{-5}\ \mu gC\ cell^{-1}$). Five food solutions raging from ∼25 to 500 $\mu gC\ L^{-1}$ were employed for the *A. tamarense* diet (27, 95, 277, 341, 511 $\mu gC\ L^{-1}$) and seven for the *Tetraselmis* sp. diet (44, 101, 149, 237, 329, 449, 493 $\mu gC\ L^{-1}$). Triplicate control bottles contained the diet solution without copepods. The bottles were topped off with the diet solution, sealed to prevent the formation of air bubbles and placed on a plankton wheel, rotating at 1.3 rpm, for 24 h. Initial water samples were taken and preserved in a 0.5% acid Lugols solution for later cell counts. After 24 h, samples were taken and preserved for cell counts.

Algal concentrations for *Alexandrium* were determined from microscopic cell counts using the Utermohl technique (1958). Cell counts for *Tetraselmis* were performed using an Elzone® 280 Particle Counter, where the algal size distribution used to count cells was determined from initial samples and kept constant for final cell counts. Clearance and ingestion rates were calculated using equations from Frost (1972). Female survival was measured and eggs counted to determine the per capita daily egg production. Egg carbon content was estimated assuming 0.045 μgC egg^{-1} (Kiørboe et al., 1985). Female mortality during the experiments was negligible. Toxin content of *A. tamarense* was measured by the receptor binding assay method (Doucette et al., 1997) by S. Morton, NOAA.

The ingestion and egg production rates were log-transformed and compared for each diet using both an ANCOVA analysis (where *y*-intercepts of the response curves were compared after the slopes were found to be the same; Sokal and Rohlf, 1995) and a two-way ANOVA (location versus concentration; Sokal and Rohlf, 1995). The transformation corrected for differences in variances among the food concentrations. A one-way ANOVA was employed to compare the log-transformed rates of the populations at each concentration. If significant, the ANOVA was followed up by a post hoc analysis using Fishers PLSD test. Statistical analyses were performed using SAS software for the ANCOVA™ analyses and Statview™ and SigmaStat™ software for the ANOVA analyses.

3. Results

Ingestion and egg production rates increased with food concentrations for each of the copepod populations on both diets (Table 1; Fig. 1). A comparison of the rates (Fig. 1A and B) shows that the ingestion and egg production of the northern populations (New Brunswick (NB), Maine (ME) and Massachusetts (MA)) fed toxic *A. tamarense* were significantly greater (ANCOVA Comparison of *y*-intercepts, d.f. = 4, $P < 0.0001$, and 2-level nested ANOVA, d.f. = 4, $P < 0.01$; Post hoc results given in Table 2) than that of the southern populations (Connecticut (CT) and New Jersey (NJ)). This pattern was not observed when the experiments were repeated with the non-toxic diet, the green flagellate *Tetraselmis* sp. Ingestion rates on *Tetraselmis* sp. were not significantly different among the populations (Fig. 1C, ANCOVA comparison of *y*-intercepts, d.f. = 4, $P > 0.05$, and 2-level nested ANOVA, d.f. = 4, $P > 0.1$). While the 2-level nested ANOVA analysis

Table 1
Summary of parameters for the functional and numerical response curves shown in Fig. 1 for female *Acartia hudsonica* feeding on toxic *Alexandrium tamarense* (*Alex*) and non-toxic *Tetraselmis* sp. (*Tet*)

Process	Location	Diet	y_o	a	r^2	P
Ingestion	NJ	*Alex*	−2.87 (1.03)	1.04 (0.20)	0.44	<0.0001
		Tet	−13.85 (2.33)	2.73 (0.44)	0.60	<0.0001
	CT	*Alex*	−4.18 (1.08)	1.26 (0.21)	0.51	<0.0001
		Tet	−14.63 (2.65)	3.43 (0.50)	0.61	<0.0001
	MA	*Alex*	−6.07 (1.14)	1.89 (0.22)	0.66	<0.0001
		Tet	−10.85 (1.75)	2.63 (0.33)	0.62	<0.0001
	ME	*Alex*	−6.71 (1.28)	2.08 (0.38)	0.45	<0.0001
		Tet	−8.72 (1.63)	2.09 (0.31)	0.55	<0.0001
	NB	*Alex*	−5.56 (1.69)	1.90 (0.32)	0.51	<0.0001
		Tet	−11.25 (1.58)	2.71 (0.31)	0.67	<0.0001
Egg production	NJ	*Alex*	0.019 (0.011)	1.15E−04 (3.7E−05)	0.20	0.0034
		Tet	0.060 (0.023)	3.94E−04 (8.5E−05)	0.38	<0.0001
	CT	*Alex*	0.015 (0.010)	1.17E−04 (3.3E−05)	0.26	0.0007
		Tet	0.009 (0.027)	7.06E−04 (9.0E−05)	0.68	<0.0001
	MA	*Alex*	0.032 (0.026)	3.94E−04 (8.6E−05)	0.36	<0.0001
		Tet	0.079 (0.022)	4.60E−04 (7.5E−05)	0.50	<0.0001
	ME	*Alex*	0.009 (0.015)	5.02E−04 (5.0E−05)	0.73	<0.0001
		Tet	0.007 (0.022)	5.34E−04 (7.9E−05)	0.53	<0.0001
	NB	*Alex*	0.029 (0.020)	3.33E−04 (6.5E−05)	0.41	<0.0001
		Tet	0.031 (0.025)	4.99E−04 (9.6E−05)	0.45	<0.0001

Ingestion data were fitted using model I linear regression to the logarithmic curve: $y = y_o + a \ln(X)$. Egg production data were likewise fitted to a linear function: $y = aX + y_o$. Standard errors ($n = 10$) are indicated in parentheses. The % explained variance by the regressions (r^2) and the probability that the regression slope was not different from zero, *P*, are shown.

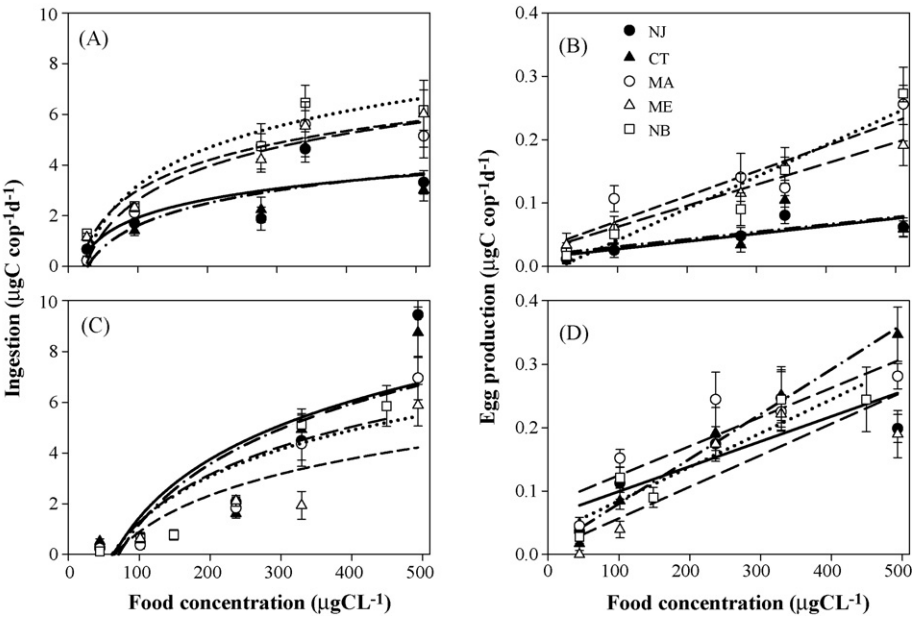

Fig. 1. Results of common environment experiments. Upper panels: Mean (with standard error, $n = 8$) ingestion rates (A) and egg production rates (B) of female *Acartia hudsonica* from five separate populations (Great Bay, New Jersey (NJ, solid line); Mumford Cove, Connecticut (CT, dot-dashed line); Cape Cod, Massachusetts (MA, medium dashed line); Casco Bay, Maine (ME, small dashed line); Passamoquoddy Bay, New Brunswick (NB, dotted line)) fed a toxic strain of *A. tamarense* isolated from Casco Bay, Maine (40.5 pg STX eq. cell^{-1}). Lower panels (C and D): Same experiments with the non-toxic green flagellate *Tetraselmis* sp. as food. Lines are least-square best fits ($P < 0.01$) and are plotted to highlight the differences between the southern vs. northern copepod populations when fed toxic *A. tamarense*. The hyperbolic curves for ingestion represent the least-square linear regression fits after log transformation of the ordinate. Symbols shown in panel B apply to all panels.

revealed no difference in egg production rates among the populations fed *Tetraselmis* sp. (2-level nested ANOVA, d.f. = 4, $P > 0.1$), the ANCOVA revealed differences among the *y*-intercepts of the numerical response curves (ANCOVA comparison of *y*-intercepts, d.f. = 4, $P < 0.003$). Post hoc analyses revealed this was because the northern populations had lower *y*-intercepts than the southern populations (Table 1; ANCOVA comparison of *y*-intercepts, d.f. = 1, $P < 0.05$). Since the northern populations did not feed or reproduce at

Table 2
Results from Fishers PLSD post hoc comparisons of the ingestion and egg production rates of copepod populations (New Jersey (NJ), Connecticut (CT), Massachusetts (MA), Maine (ME), New Brunswick (NB)) vs. diet and algal concentrations

	Alexandrium concentrations (µgC L^{-1})					*Tetraselmis* concentrations (µgC L^{-1})				
	26.6	95.2	277.3	340.8	511.1	44.2	101.4	236.5	329.1	493.4
Ingestion										
NJ								NB	ME	ME, NB
CT	NJ					NJ, MA, ME, NB		NB	ME	ME, NB
MA		NJ, CT	CT	n.s.		ME	n.s.	NB	ME	
ME	NJ, CT, MA	NJ, CT	NJ, CT		NJ, CT			NB		
NB	NJ, CT, MA	NJ, CT	NJ, CT		NJ, CT				ME	
Egg production										
NJ						ME	ME			
CT								NB		NJ, ME
MA	n.s.	NJ, CT	NJ, CT		NJ, CT	ME	ME	NB, ME	n.s.	NJ, ME
ME			CT	NJ, CT	NJ, CT					
NB				NJ, CT	NJ, CT	ME	ME			

The populations indicated under each concentration exhibited a significantly lower ingestion or egg production rate than the populations indicated in the far left-hand column. No significance was observed among the populations at the algal concentrations indicated by 'n.s.' (one-way ANOVA, d.f. = 3, $P > 0.05$).

higher rates when fed the non-toxic *Tetreselmis* sp. diet, we can conclude that their higher feeding and reproductions rates on the toxic dinoflagellate diet were not due to physiological compensation by populations originating from different latitudes grown at a common temperature (Lonsdale and Levinton, 1985).

With the exception of the experiments ran at food concentration of 340 μgC L^{-1}, the effects of resistance on ingestion rate were most evident for the northern-most populations (Table 2). Specifically, the copepods from NB and ME ingested *A. tamarense* at higher rates than the southern populations (CT, NJ) throughout the range of experimental concentrations. Meanwhile, the ingestion rates of the population from MA were greater than the CT and NJ at three of the five tested concentrations. These differences in ingestion rates were not observed when copepods fed on the non-toxic *Tetraselmis* sp. diet (Table 2). In fact, at higher algal concentrations the southern populations exhibited higher ingestion rates than the NB and ME copepod populations.

A similar pattern to ingestion was observed for the egg production rates of the northern versus southern copepod populations (Fig. 1), except that no significant differences were observed between the NB and ME populations relative to the southern populations at *A. tamarense* concentrations below 277 μgC L^{-1}. In addition, the egg production rates of the MA copepods were greater than the southern populations at the same concentrations as ingestion rate and, at the highest concentration of 511 μgC L^{-1}. Again, there was no consistent pattern for egg production rate among populations when the copepods fed on the non-toxic *Tetraselmis* sp. (Table 2).

4. Discussion

The results of this study are consistent with those from previous studies that examined grazer resistance to toxic *Alexandrium* spp. (Colin and Dam, 2002a, 2003, 2004). Taken together, the results of ingestion and egg production in the present study are consistent with the hypothesis of evolved grazer resistance to toxic *Alexandrium* in populations that have been historically exposed to toxic *Alexandrium* blooms. The degree of resistance is also more pronounced in those populations (NB and ME) with the longest history of exposure and higher toxin content of *Alexandrium*.

We observed differences in grazing and egg production rates between the northern and southern copepod populations throughout the range of concentrations examined. The lowest food concentration in the present study of 26.6 μgC L^{-1}, which corresponds to 11 cells mL^{-1}, is within the range (10^2–10^5 cells L^{-1}) of concentrations observed for *Alexandrium* spp. in the Gulf of Maine, USA (Anderson, 1997) and Hiroshima Bay, Japan (Hamasaki et al., 2003; Yamamoto et al., 2002). A true toxic effect does not disappear even if alternative food sources are available to the grazer. In the case of *Alexandrium*, the toxic effect is clearly manifested as an irreversible reduction of ingestion rates in copepods from non-resistant populations, despite the availability of alternative prey (Colin and Dam, 2003). In contrast, no such effect is observed in copepods from resistant populations (Colin and Dam, 2003). Therefore, toxic *Alexandrium* has the potential to suppress grazing and growth in non-resistant copepod populations when present even at the low concentrations observed in nature. We are aware that *Alexandrium* spp. most commonly occurs as part of a mixed assemblage of phytoplankton (Garcés et al., 2005; Turner and Borkman, 2005), and other grazers can select against – but not entirely avoid – *Alexandrium* (Teegardern and Cembella, 1996; Teegarden, 1999). Hence, we cannot generalize the results observed here to other grazers. Furthermore, toxic effects of *Alexandrium* are concentration-dependent (Colin and Dam, 2002b, 2003). Thus, the results of the present study clearly do not apply to all field situations. Nonetheless, the differences in functional and numerical responses between the resistant and non-resistant populations observed here also underscore the importance of being aware of the evolutionary history of grazers when modeling phytoplankton–grazer interactions, particularly in the context of the rapid spread of harmful algal blooms (Hallegraeff, 1993).

Could these observed differences in ingestion rates have implications for grazer control of harmful algal blooms (HABs)? Grazing is hypothesized to be an important factor in the population dynamics of harmful algae (Turner and Tester, 1997; Calbet et al., 2003; Garcés et al., 2005; Dam and Colin, 2005). The grazing impact is determined by the summed impact of both metazoan (e.g., copepods) and protist predators. Often, microplankton are found to be the dominant grazer on toxic *Alexandrium* spp. and their grazing rates (day^{-1}) are often at levels just below or above *Alexandrium* growth rates (Calbet et al., 2003; Garcés et al., 2005). Therefore, at times, their grazing impact alone may be sufficient to suppress the development of HABs. Other times feeding by copepods must make up the difference for grazers to control bloom development. There is no clear consensus on whether copepod grazing contri-

butes significantly to the suppression of HABs. Factors such as low copepod abundances and grazing rates in conjunction with low *Alexandrium* spp. abundances relative to other algal species often results in an insignificant grazing impact by copepods (Turner and Anderson, 1983; Calbet et al., 2003; Turner and Borkman, 2005). However, in the Gulf of Maine the high grazing rates of nearshore copepods on toxic *Alexandrium fundyense* have been found to be sufficient to impact the population growth rate of the toxic dinoflagellate (Campbell et al., 2005). Therefore, copepod grazing, depending on its magnitude, may be sufficient to change the impact of the total grazer community and contribute to the suppression of HAB development.

We illustrate here with a simple simulation exercise the potential effect of grazer resistance on the development and control of *Alexandrium* blooms. We ask if the differences in grazing we observed among the *Acartia hudsonica* populations in the present study are sufficient to change the outcome of grazer community impact on the development of an *Alexandrium* bloom. Fig. 2, represents model predictions of the abundance of the dinoflagellate *Gonyaulax tamarensis* (=*Alexandrium tamarense*) as a function of the grazing pressure observed in a Cape Cod salt pond (after Watras et al., 1985). In the original version of the model, the grazing community only included larvae of the polychaete *Polydora* sp. and the tintinnid *Favella* sp. Here, we have added the grazing pressure of copepods by using in the model the average clearance rates of *A. hudsonica* for the non-resistant copepods from Connecticut (4.8 mL copepod^{-1} day^{-1}) and the historically exposed copepods from New Brunswick (60.0 mL copepod^{-1} day^{-1}). The clearance rates were derived from the ingestion rates from the present study and the originally employed food concentrations in the Watras et al. (1985) model. We also employ the observed copepod abundances in these salt ponds (Turner and Anderson, 1983). The copepod grazing values (derived from Fig. 1) are appropriate for the initial algal density in the model. The different community grazing rates from *Polydora* sp. and *Favella* sp. are compared (low = 0.14, medium = 0.3, high = 0.7 day^{-1}; Watras et al., 1985). In the original model predictions (Watras et al., 1985), the grazer community could only change the fate of the *A. tamarense* bloom at the highest grazing rates (corresponding to the highest recorded grazer densities). The same is true when the grazing pressure from non-resistant *Acartia hudsonica* is added to the grazing community. However, in the model at all community grazing levels, the addition of grazing pressure from resistant copepods changes the fate of the bloom by delaying or stopping it. This exercise was intentionally simplistic (e.g.; constant clearance rate, ignoring selection and using monoculture experiment data) and not intended to suggest these are the outcomes that would occur in a natural setting. Instead, the model was used to illustrate that observed differences in grazing due to evolved resistance can potentially have important consequences on the fate of HABs and should be considered in future studies. Besides differences in feeding rates, observed differences in growth and overall fitness (Colin and Dam, 2004) would further contribute to

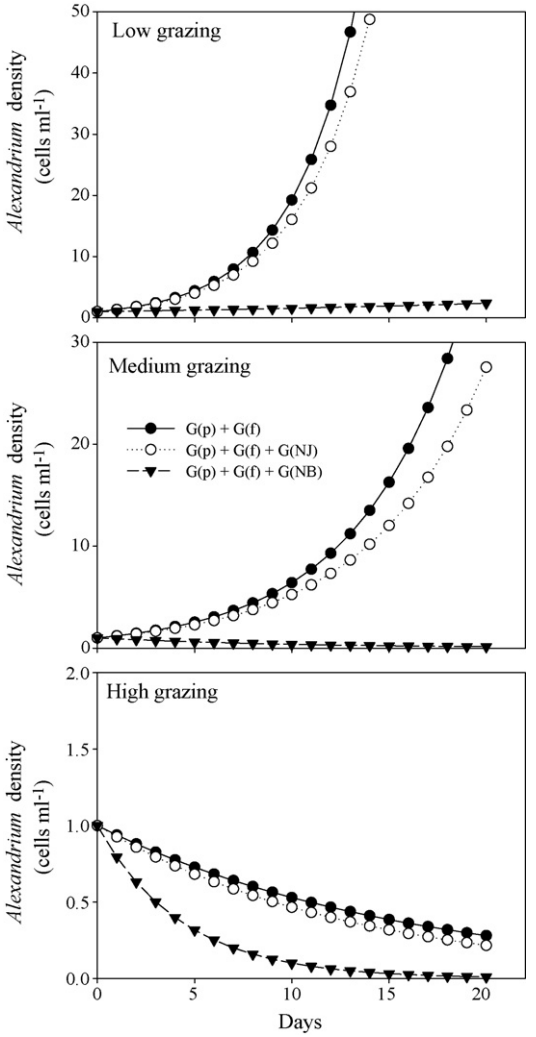

Fig. 2. Model predictions of *Alexandrium* abundance vs. time. Panels show the low, medium and high grazing pressures from *Polydora* sp. (G(p)) and *Favella* sp. (G(f)) reported in Watras et al. (1985). Here, the grazing pressures of non-resistant *Acartia hudsonica* from Connecticut, USA (G(NJ)), and resistant *Acartia hudsonica* from New Brunswick, Canada (G(NB)) have been added to the microzooplankton grazing pressure. The *Alexandrium* growth rate is set to 0.5 day^{-1} (Etheridge and Roesler, 2005).

the impact of grazers on the development of harmful algal blooms.

In addition to potentially impacting the development of HABs, grazers may also serve at vectors of toxins to higher trophic levels. Copepods have been are known to accumulate toxins in relation to how much they ingest regardless of whether the algae were provided as a monoculture or as part of a mixed diet (Teegardern and Cembella, 1996; Teegarden et al., 2003). Our result demonstrate that for *A. tamarense*, copepod ingestion rate is strongly dependent on algal concentration for both resistant and non-resistant populations, but that the slopes of the functional response curves are dependent upon population biogeography and resistance (Table 1). Therefore, the extent to which copepods accumulate HAB toxins, and potentially serve as vectors, will depend not only upon the grazer species (Teegarden et al., 2003) and the concentration of the harmful alga, but also the biogeography of the bloom. Accordingly, for a given toxic alga concentration, we might expect the transfer of harmful algal toxins through the food web to be greatest in regions with resistant grazer populations.

In conclusion, the effect of grazer toxin resistance on the functional and numerical responses of grazers should not be ignored in understanding or modeling the ecological and evolutionary relationships between toxic prey and predators, and particularly in examining the role of grazing in toxic alga bloom control and in the transfer of toxins through the food web.

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