

# Interactions among the toxic dinoflagellate *Amphidinium carterae*, the heterotrophic dinoflagellate *Oxyrrhis marina*, and the calanoid copepods *Acartia* spp.

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**ABSTRACT:** To investigate the interactions among a toxic dinoflagellate *Amphidinium carterae*, the heterotrophic dinoflagellate *Oxyrrhis marina*, and the calanoid copepods *Acartia* spp. (*A. omorii* and *A. hongii*), we measured toxicity of *A. carterae*, the growth and ingestion rates of *O. marina* on *A. carterae*, the ingestion rate of *Acartia* spp. on *A. carterae*, the ingestion rate of *Acartia* spp. on *O. marina* fed a non-toxic strain of *Prorocentrum minimum*, and the ingestion rate of *Acartia* spp. on *O. marina*, the latter originally satiated with *A. carterae* and then starved, as a function of elapsed starvation time. The toxicity of *A. carterae* was 1 MU/1.3 × 10<sup>8</sup> cells when measured using the mouse bioassay. *O. marina* grew well on *A. carterae*. When the data were fitted to the Michaelis-Menten equation, maximum specific growth rate ( $\mu_{\max}$ ) and threshold prey concentration of *O. marina* on *A. carterae* were 1.17 d<sup>-1</sup> and 1.3 ng C ml<sup>-1</sup> (13 cells ml<sup>-1</sup>), respectively. Maximum ingestion and clearance rates of *O. marina* were 2.8 ng C grazer<sup>-1</sup> d<sup>-1</sup> (28 cells grazer<sup>-1</sup> d<sup>-1</sup>) and 2.4  $\mu$ l grazer<sup>-1</sup> h<sup>-1</sup>, respectively. Grazing by *Acartia* spp. on *A. carterae* was undetectable. The ingestion rate of *Acartia* spp. on *O. marina* was very low (maximum = 749 *Oxyrrhis* predator<sup>-1</sup> d<sup>-1</sup>) at Day 0 (*O. marina* starved for 0 to 1 d after satiation with *A. carterae*), but increased with increasing elapsed starvation time. The maximum ingestion rate was 4710 *Oxyrrhis* predator<sup>-1</sup> d<sup>-1</sup> at Day 11 (*O. marina* starved for 11 to 12 d). The average ingestion rates of *Acartia* spp. on *O. marina* fed *P. minimum* were not significantly higher than on *O. marina* fed *A. carterae* at Day 11 at similar mean prey concentrations, but much higher than those fed *A. carterae* at Day 0. This evidence suggests that *O. marina* can reduce its mortality rate due to the predation by *Acartia* spp. if it is satiated with *A. carterae*, and the grazing of *A. carterae* by *O. marina* can sometimes transfer the carbon of *A. carterae* to *Acartia* spp., which cannot feed on *A. carterae*.

**KEY WORDS:** Ecological role · Growth · Grazing · Ingestion · Protist · Toxin

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## INTRODUCTION

Autotrophic (and/or mixotrophic) dinoflagellates, heterotrophic protists, and copepods are the major components of marine plankton communities. Grazing

pressure sometimes plays an important role in the population dynamics of autotrophic dinoflagellates (Watras et al. 1985). In particular, grazing by heterotrophic protists is believed to contribute to the decline of dinoflagellate blooms (Holmes 1967, Eppley & Harrison 1975, Jeong 1995). However, there have been few studies that demonstrate effective grazing by hetero-

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trophic protists or metazooplankton on toxic dinoflagellates, and many studies indicate that most heterotrophic protists or metazooplankton do not feed on toxic dinoflagellates (Hansen 1989, Kamiyama 1997, Turner & Tester 1997).

Toxins may play an important role in reducing grazing pressure if there are no effective grazers on a toxic dinoflagellate, and the materials of the dinoflagellate will not be transferred to higher trophic levels. However, if grazers can feed effectively on a toxic dinoflagellate and do not accumulate the toxin, the grazers may control the toxic dinoflagellate population and transfer their materials to higher trophic level organisms without harming them.

A few heterotrophic protists have been known to feed on the toxic dinoflagellate *Amphidinium carterae* (Blackbourn 1974, Naustvoll 1998). However, there have been no reports of the positive growth of heterotrophic protists on *A. carterae*. *A. carterae* is a bloom organism (Steidinger & Tangen 1997) and can undergo daily vertical migrations (Kamykowski & Zentara 1976, Steidinger & Tangen 1997). Recently we found that the heterotrophic dinoflagellate *Oxyrrhis marina* was able to grow well on *A. carterae*. This strain of *A. carterae* was previously known to be poor prey for the mixotrophic dinoflagellate *Fragilidium cf. mexicanum* (Jeong et al. 1999a), the heterotrophic dinoflagellate *Polykrikos kofoidii* (Jeong et al. 2001), and the naked ciliate *Strombidinopsis* sp. (Jeong et al. 1999b).

The functional and numerical responses of *Oxyrrhis marina* to *Amphidinium carterae* and the functional response of a copepod to *O. marina* fed *A. carterae* are important concerns in understanding trophodynamics related to toxic dinoflagellates. Considering the high growth rate of *O. marina* on *A. carterae*, *O. marina* may be insensitive to the toxin and/or have an ability to detoxify it. If a copepod which does not feed on *A. carterae* can actively prey on *O. marina* fed on *A. carterae*, *O. marina* can play an important role in transferring the materials of *A. carterae* to the copepod.

To understand the interactions among *Amphidinium carterae*, *Oxyrrhis marina*, and *Acartia* spp., we conducted experiments to measure the toxicity of *A. carterae* using the mouse bioassay, the growth and ingestion rates of *O. marina* on *A. carterae*, the ingestion rate of *Acartia* spp. on *A. carterae*, the ingestion rate of *Acartia* spp. on *O. marina* fed a non-toxic strain of *Prorocentrum minimum*, and the ingestion rates of *Acartia* spp. on *O. marina*, originally satiated with *A. carterae* and then starved, as a function of elapsed starvation time. The results of the present study provide a basis for understanding the potential of heterotrophic dinoflagellates to influence the population dynamics of toxic dinoflagellates, and the transfer of carbon from toxic dinoflagellate to larger zooplankton,

as well as the roles of heterotrophic dinoflagellates in toxin dynamics.

## MATERIALS AND METHODS

**Preparation of experimental organisms.** *Amphidinium carterae* (ACKS 0010) were grown at 20°C in enriched f/2 seawater media (Guillard & Ryther 1968) without silicate, with continuous illumination at 100  $\mu\text{E m}^{-2} \text{s}^{-1}$  provided by cool white fluorescent lights. The carbon content for *A. carterae* (0.1 ng C cell<sup>-1</sup>) was measured using a CHN analyzer.

For isolation and culture of *Oxyrrhis marina*, plankton samples collected with a 25 cm diameter, 25  $\mu\text{m}$  mesh plankton net were taken from the mouth of Keum Estuary, Kunsan, Korea, during May 1999, when the water temperature was 16°C. The samples were screened gently through a 154  $\mu\text{m}$  Nitex mesh and placed in 1 l polycarbonate (PC) bottles. A mixture of *Prorocentrum minimum* and *Amphidinium carterae* (8000 cells ml<sup>-1</sup> for each prey) and 50 ml of f/2 media was added as food. Bottles were placed on plankton wheels rotating at 0.9 rpm and incubated at 20°C under continuous illumination at 20  $\mu\text{E m}^{-2} \text{s}^{-1}$  of cool white fluorescent light. Two days later, aliquots of the enriched water were transferred to 6-well tissue culture plates and a monoclonal culture was established by 2 serial single-cell isolations. Once dense cultures of *O. marina* were obtained, they were transferred to 500 or 1000 ml PC bottles of fresh prey every 2 or 3 d. Experiments were conducted when a large volume of *O. marina* culture was available.

Copepods were collected from the mouth of Keum Estuary with a 303  $\mu\text{m}$  mesh net, and acclimated in a 20°C room in the presence of *Prorocentrum minimum*. *Acartia hongii* (Soh & Suh 2000) and *A. omorii*, which co-occur in coastal waters off western Korea, are very similar and are impossible to distinguish from each other when they are alive. Therefore, we had to use a mixture of adult female *Acartia* spp. (*A. hongii* and *A. omorii*).

**Cell volume.** Cell length and maximum width of *Oxyrrhis marina* preserved in 5% acid Lugol's solution were measured with using both a compound and an inverted microscope. The shape of *O. marina* was estimated as a cone (bottom half of the cell) connected to another cone (top half of the cell). The 2 cones joined at the cell equator (= maximum width of the cell). Cell volume of the preserved *O. marina* was calculated according to the equation: volume = 1/3  $\times$  cross-sectional area at equator  $\times$  cell length.

**Toxicity of *Amphidinium carterae*.** To extract toxic materials, dense cultures of *Amphidinium carterae* (100 l in total; 21 000 cells ml<sup>-1</sup> or 2100 ng C ml<sup>-1</sup>) were

harvested by filtration with glass-fibred filters (Whatman GF/C). The harvested cells were extracted with 50% methanol (MeOH)/dichloromethane (DCM) followed by 100% methanol (100 ml each) at 25°C for 7 h, respectively. The combined extract was concentrated under vacuum with a rotary evaporator. The residual water was completely dried under high vacuum. The crude extract thus obtained was dissolved in 50% MeOH/DCM under helium and kept at -80°C until use. The crude extract was used for mouse assay to assess the toxicity of *A. carterae*.

The crude extract was aliquoted in 1.5 ml sterilized Eppendorff tubes, and the solvents completely evaporated under vacuum. The aliquots were suspended in 1% Tween 60 (Aldrich) sterilized-saline solution. The solutions were sonicated for 2 min to give a homogeneous suspension of compounds. Serially diluted samples, equivalent to 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1, 1.2 and 1.5 l each (1 l corresponding to  $1.6 \times 10^8$  cells of *A. carterae*), were prepared in the final volume of 600  $\mu$ l. The solutions were injected intra-peritoneally into 4 wk old male mice of the ICR strain (weight range: 19 to 24 g each). Each solution was injected in duplicate or triplicate, depending upon the reproducibility of injection. The injected mice were observed in a very-well-controlled environment for 48 h.

**Growth and ingestion rates of *Oxyrrhis marina* on *Amphidinium carterae*.** Expts 1 and 2 were designed to measure the growth, ingestion, and clearance rates of *Oxyrrhis marina* on *Amphidinium carterae* as a function of the prey concentration (Table 1). In particular, Expt 2 was designed to measure rates at low prey concentrations because the threshold prey concentration was revealed to be very low in Expt 1.

Dense cultures of *Oxyrrhis marina* growing on *Amphidinium carterae* were transferred, 2 or 3 d before the experiments were conducted, into 1 l PC

bottles containing low concentrations of the prey (50 and 5 cells  $\text{ml}^{-1}$  for Expts 1 and 2, respectively). The bottles were filled to capacity with filtered seawater and placed on rotating wheels to incubate as above, except that illumination was provided on a 12:12 h light:dark cycle. To monitor the condition of and interaction between predator and prey species, cultures were periodically removed from the rotating wheels, examined by looking through the surface of capped bottles using a dissecting microscope, and then returned to the rotating wheels. Once *A. carterae* cells were no longer detectable, three 1 ml aliquots from each bottle were counted using a compound microscope to determine cell concentrations of *O. marina*, and the cultures then used to conduct experiments.

For each experiment, initial concentrations of *Oxyrrhis marina* and *Amphidinium carterae* were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles. Triplicate 270 ml (Expt 1) or 500 ml (Expt 2) PC experiment bottles (mixtures of predator and prey), and triplicate control bottles (prey only) were set up for each predator-prey combination. Triplicate control bottles containing only *O. marina* were also established at 1 predator concentration. Thirty ml (Expt 1) or 50 ml (Expt 2) of f/2 medium were added to all bottles, which were then filled to capacity with freshly filtered seawater and capped. To determine actual predator and prey densities at the beginning of the experiment, a 10 ml aliquot was removed from each bottle, fixed with 5% acid Lugol's solution and examined with a compound microscope to determine predator and prey abundance by enumerating cells in three 1 ml Sedgwick-Rafter counting chambers (SRCs). The bottles were filled again to capacity with freshly filtered seawater, capped, and placed on rotating wheels with the environmental conditions described above. Dilution of the

Table 1. Design of experiments. The numbers in prey and predator columns are the actual initial densities (cells  $\text{ml}^{-1}$  for *Amphidinium carterae* and *Oxyrrhis marina* and ind.  $\text{l}^{-1}$  for *Acartia* spp.) of prey and predator

Expt	Species	Prey		Predator	
		Species	Density	Species	Density
1	<i>A. carterae</i>	0, 40, 71, 235, 727, 1437, 2228, 9807		<i>O. marina</i>	9, 3, 4, 8, 15, 18, 17, 38
2	<i>A. carterae</i>		0, 4, 7, 14, 37, 57, 75	<i>O. marina</i>	5, 2, 3, 3, 5, 5, 5
3	<i>A. carterae</i>	80, 242, 908, 1765, 3798, 11763		<i>Acartia</i> spp.	12
4	<i>O. marina</i> <sup>a</sup>	44, 90, 323, 410, 675, 1540		<i>Acartia</i> spp.	20
5	<i>O. marina</i> <sup>b</sup>	27, 66, 139, 252, 527, 1266		<i>Acartia</i> spp.	20
6	<i>O. marina</i> <sup>b</sup>		522, 1333	<i>Acartia</i> spp.	20
7	<i>O. marina</i> <sup>b</sup>		526, 992	<i>Acartia</i> spp.	20
8	<i>O. marina</i> <sup>b</sup>	22, 47, 102, 209, 573, 923		<i>Acartia</i> spp.	20

<sup>a</sup>Satiated with *Prorocentrum minimum*  
<sup>b</sup>Starved for 0–1 (Expt 5), 3–4 (Expt 6), 8–9 (Expt 7), and 11–12 d (Expt 8) after being satiated with *A. carterae*

cultures associated with refilling the bottles was considered in calculating growth and ingestion rates.

Aliquots of 10 ml (Expt 1) or 20 ml (Expt 2) were taken from each bottle at 24, 48, and 72 h, fixed with 5% Lugol's solution and the abundances of *Oxyrrhis marina* and *Amphidinium carterae* were determined by counting all or >300 cells in three 1 ml SRCs. In Expt 2 10 ml aliquots were transferred into wells of a 6-well plate chamber, the chamber was gently rotated by hand to concentrate most cells near the center of the chamber, and then the cells were counted using an inverted microscope. Prior to taking subsamples, the condition of *O. marina* and its prey was assessed using a dissecting microscope as described above. After subsampling, bottles were again filled to capacity with freshly filtered seawater and placed back on the rotating wheels.

The specific growth rate of *Oxyrrhis marina*,  $\mu$  ( $\text{d}^{-1}$ ), was calculated by averaging the instantaneous growth rates (IGRs) for each sampling interval:

$$\text{IGR} = \frac{\ln(S_{t_2} / S_{t_1})}{t_2 - t_1} \times 24 \quad (1)$$

where  $S_{t_1}$  and  $S_{t_2}$  = the concentration of *O. marina* at consecutive samplings. The final  $t_2$  for calculation was 48 h, which provided the highest specific growth rate.

Data for *Oxyrrhis marina* growth rates were fitted to a Michaelis-Menten equation:

$$\mu = \frac{\mu_{\max}(x - x')}{K_{\text{GR}} + (x - x')} \quad (2)$$

where  $\mu_{\max}$  = the maximum growth rate ( $\text{d}^{-1}$ );  $x$  = prey concentration (cells  $\text{ml}^{-1}$  or ng C  $\text{ml}^{-1}$ ),  $x'$  = threshold prey concentration (the prey concentration where  $\mu = 0$ ),  $K_{\text{GR}}$  = the prey concentration sustaining  $\frac{1}{2}\mu_{\max}$ . Data were iteratively fitted to the model using Delta-Graph® (Delta Point).

Ingestion and clearance rates were calculated using the equations of Frost (1972) and Heinbokel (1978). Incubation time for calculating ingestion and clearance rates was the same as for estimating growth rate. Ingestion rate data were fitted to a Michaelis-Menten equation:

$$\text{IR} = \frac{I_{\max}(x)}{K_{\text{IR}} + (x)} \quad (3)$$

where  $I_{\max}$  = the maximum ingestion rate (cells grazer $^{-1}$   $\text{d}^{-1}$  or ng C grazer $^{-1}$   $\text{d}^{-1}$ );  $x$  = prey concentration (cells  $\text{ml}^{-1}$  or ng C  $\text{ml}^{-1}$ ),  $K_{\text{IR}}$  = the prey concentration sustaining  $\frac{1}{2}I_{\max}$ .

**Ingestion rates of *Acartia* spp. on *Amphidinium carterae*.** Expt 3 was designed to measure the ingestion and clearance rates of *Acartia* spp. on *Amphidinium carterae* as a function of the prey concentration

(Table 1). Adult female *Acartia* spp. (a mixture of *A. omorii* and *A. hongii*) were used.

For the experiment, initial concentrations of *Amphidinium carterae* were established using an autopipette to deliver predetermined volumes of known cell concentrations to the bottles, and those of *Acartia* spp. were obtained by individually transferring the copepods using a Pasteur pipette. Triplicate 500 ml PC experiment bottles (mixtures of predator and prey) and triplicate control bottles (prey only) were set up for each predator-prey combination. Fifty ml of f/2 medium were added to all bottles, which were then filled to capacity with freshly filtered seawater and capped. To determine actual prey densities at the beginning of the experiment, a 10 ml aliquot was removed from each bottle, fixed with 5% Lugol's solution and examined with a compound microscope to determine prey abundance by enumerating cells in three 1 ml SRCs. The bottles were filled again to capacity with freshly filtered seawater, capped, and placed on plankton wheels rotating at 0.9 rpm and incubated at 20°C under an illumination of 20  $\mu\text{E m}^{-2} \text{s}^{-1}$  in a 12:12 h light:dark cycle.

Aliquots of 10 ml were taken from each bottle at 24 and 48 h and fixed with 5% Lugol's solution; the abundances of *Amphidinium carterae* were determined by counting all or >300 cells in three 1 ml SRCs. After subsampling, bottles were again filled to capacity with freshly filtered seawater and placed back on the rotating wheels. After incubation for 48 h, *Acartia* spp. were counted. Mortality of *Acartia* spp. was nil at the end of incubation.

Ingestion and clearance rates were calculated using the equations of Frost (1972).

**Ingestion rates of *Acartia* spp. on *Oxyrrhis marina* fed *Prorocentrum minimum*.** Expt 4 was designed to investigate the ingestion rates of *Acartia* spp. on *Oxyrrhis marina* which had been grown with a non-toxic strain of *Prorocentrum minimum* as food (Table 1). Adult female *Acartia* spp. (*A. omorii* and *A. hongii*) were used for this experiment.

Dense cultures (ca 1100 cells  $\text{ml}^{-1}$ ) of *Oxyrrhis marina* growing on *Prorocentrum minimum* were transferred into 2 l PC bottles containing cultures of *P. minimum* (ca 12 000 cells  $\text{ml}^{-1}$ ). For 24 h, *O. marina* actively eliminated *P. minimum* cells, and the concentration of *P. minimum* decreased to an undetectable concentration. The density of the predator increased to 2030 cells  $\text{ml}^{-1}$ .

For this experiment, initial concentrations of *Oxyrrhis marina* and *Acartia* spp. were established as described above. Triplicate 500 ml PC experiment bottles and triplicate control bottles were set up for each predator-prey combination. All bottles were filled to capacity with freshly filtered seawater and capped. Actual prey

densities at the beginning of the experiment were determined as described above. The bottles were filled again to capacity with freshly filtered seawater, capped, and placed on rotating wheels using the environmental conditions described in the last sub-section.

Aliquots of 10 ml were taken from each bottle at 24 and 48 h and fixed with 5% Lugol's solution; the abundances of *Oxyrrhis marina* were determined by counting all or >200 cells in three 1 ml SRCs. After subsampling, bottles were again filled to capacity with freshly filtered seawater and placed back on the rotating wheels. After incubation, *Acartia* spp. were counted.

Ingestion and clearance rates were calculated using the equations of Frost (1972).

**Ingestion rates of *Acartia* spp. on *Oxyrrhis marina* fed *Amphidinium carterae*.** Expts 5 to 8 were designed to investigate the ingestion rates of *Acartia* spp. on *Oxyrrhis marina* which had been grown with *Amphidinium carterae* as food and then starved (Table 1). Adult female *Acartia* spp. (*A. omorii* and *A. hongii*) were used for these experiments.

Dense cultures (mean  $\pm$  SE = 848  $\pm$  50 cells ml<sup>-1</sup>) of *Oxyrrhis marina* were starved for 2 d and then transferred into four 220 l tanks containing cultures of *Amphidinium carterae* (mean  $\pm$  SE = 10 953  $\pm$  923 cells ml<sup>-1</sup>). In the first 20 h, *O. marina* eliminated the *A. carterae* cells and the concentration of *Amphidinium carterae* decreased to approximately 40 cell ml<sup>-1</sup>. Most *O. marina* cells were observed to contain 6 to 7 ingested *A. carterae* cells at this time. To avoid the interference of remaining *A. carterae*, the *O. marina* culture was held for 20 h until there were no free *A. carterae* cells remaining. The densities of *O. marina* increased to 1402  $\pm$  23 cells ml<sup>-1</sup>, and most *O. marina* cells were observed to contain none or 1 ingested *A. carterae* cells when the first feeding trial with copepods was begun. Therefore, most *O. marina* cells had been starved for 20 h when they were first fed to copepods. Days 0 (Expt 5), 3 (Expt 6), 8 (Expt 7), and 11 (Expt 8) indicate the number of days after Expt 5 started (see Table 1). At Days 0, 3, 8, and 11, 2 l aliquots were taken from each tank and of these, 3 ml aliquots were counted to determine the *O. marina* density in each tank. Cell size was measured. The remaining aliquots were mixed (and slightly concentrated using a 5  $\mu$ m mesh net if necessary) and counted again.

For each experiment, initial concentrations of *Oxyrrhis marina* and *Acartia* spp. were established as described above. Triplicate 500 ml PC experiment bottles and triplicate control bottles were set up for each predator-prey combination. All bottles were filled to capacity with freshly filtered seawater and capped. Actual prey densities at the beginning of the experiment were determined as described above. The bottles were filled again to capacity with freshly filtered sea-

water, capped, and placed on rotating wheels using the environmental conditions described above.

Aliquots of 10 ml were taken from each bottle at 24 and 48 h, fixed with 5% acid Lugol's solution; the abundances of *Oxyrrhis marina* were determined as described in the last sub-section. After subsampling, bottles were again filled to capacity with freshly filtered seawater and placed back on the rotating wheels. After incubation, *Acartia* spp. were counted.

Ingestion and clearance rates were calculated using the equations of Frost (1972).

## RESULTS

**Toxicity of *Amphidinium carterae*.** The minimum lethal dose of *Amphidinium carterae* to mice was the crude whole-cell extract representing toxin present in  $1.3 \times 10^8$  cells ( $1.3 \times 10^7$  ng C). The first symptom of toxicity to mice was prostration. Both hind limbs were paralyzed a few minutes later (usually in 2 min). The paralyzed mice experienced severe dyspnea and a violent paroxysm before death.

**Growth rate of *Oxyrrhis marina* fed *Amphidinium carterae* (Expts 1 and 2).** *Oxyrrhis marina* grew well on unialgal diets of *Amphidinium carterae*. The specific growth rates increased with increasing mean prey concentration up to ca 100 ng C ml<sup>-1</sup>, but were saturated at higher prey concentrations (Fig. 1). When the data were fitted to Eq. (2), the maximum specific growth

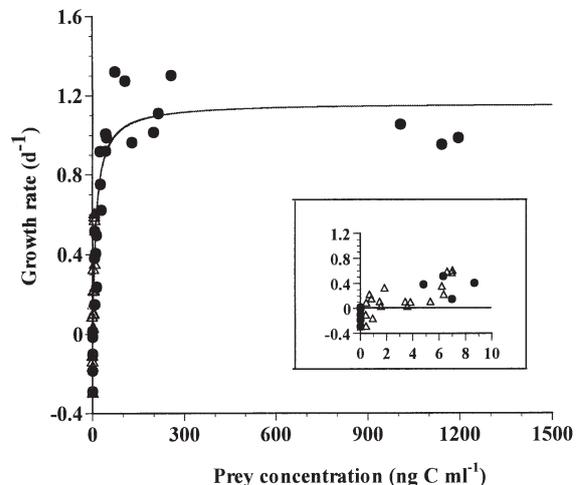


Fig. 1. Specific growth rates of *Oxyrrhis marina* on *Amphidinium carterae* as a function of mean prey concentration ( $x$ ). Symbols represent single treatments. The equation of the regression line was obtained by pooling all treatments from Expts 1 (●) and 2 (Δ). The curves are fitted by a Michaelis-Menten equation (Eq. 2) using all treatments in the experiment. Growth rate (GR, d<sup>-1</sup>) = 1.17{(x - 1.3)/[12.5 + (x - 1.3)]}, R<sup>2</sup> = 0.923. Inset shows values at low prey concentrations

rates ( $\mu_{\max}$ ) and  $K_{GR}$  (prey concentration sustaining  $0.5 \mu_{\max}$ ) were  $1.17 \text{ d}^{-1}$  and  $12 \text{ ng C ml}^{-1}$  ( $120 \text{ cells ml}^{-1}$ ), respectively. The threshold prey concentration (where net growth = 0) was  $1.3 \text{ ng C ml}^{-1}$  ( $13 \text{ cells ml}^{-1}$ ). The result from Expt 2 confirmed this very low threshold prey concentration.

**Ingestion and clearance rates of *Oxyrrhis marina* on *Amphidinium carterae* (Expts 1 and 2).** The ingestion rate of *Oxyrrhis marina* on unialgal diets of

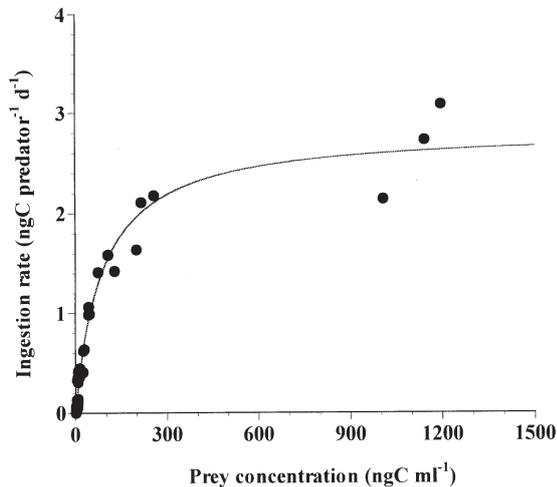


Fig. 2. Ingestion rates of *Oxyrrhis marina* on *Amphidinium carterae* as a function of mean prey concentration ( $x$ ). Symbols represent single treatments. The equation of the regression line was obtained by pooling all treatments from Expts 1 and 2. The curves are fitted by a Michaelis-Menten equation (Eq. 3) using all treatments in the experiment. Ingestion rate ( $IR, \text{ ng C predator}^{-1} \text{ d}^{-1}$ ) =  $2.8 [x/(90 + x)]$ ,  $R^2 = 0.97$

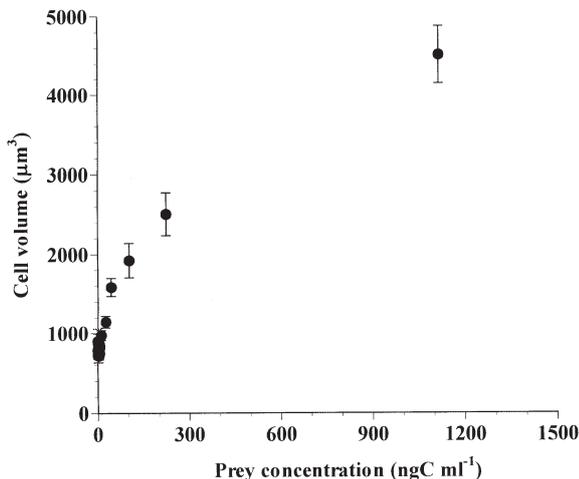


Fig. 3. Cell volume of *Oxyrrhis marina* on *Amphidinium carterae* as a function of mean prey concentration in Expts 1 and 2 (see Table 1). Symbols represent treatment means  $\pm 1$  SE

*Amphidinium carterae* increased rapidly with increasing mean prey concentration up to ca  $200 \text{ ng C ml}^{-1}$  ( $2000 \text{ cells ml}^{-1}$ ), and slowly, but continuously, increased at higher prey concentrations (Fig. 2). When the data were fitted to Eq. (2), the maximum ingestion rate and  $K_{IR}$  (prey concentration sustaining  $0.5 I_{\max}$ ) of *O. marina* on *A. carterae* were  $2.8 \text{ ng C grazer}^{-1} \text{ d}^{-1}$  ( $28 \text{ cells grazer}^{-1} \text{ d}^{-1}$ ) and  $90 \text{ ng C ml}^{-1}$  ( $900 \text{ cells ml}^{-1}$ ), respectively.

The maximum clearance rate of *Oxyrrhis marina* was  $2.4 \mu\text{l grazer}^{-1} \text{ h}^{-1}$ .

**Cell volume of *Oxyrrhis marina* fed *Amphidinium carterae* in Expts 1 and 2 and *O. marina* fed *Prorocentrum minimum* in Expt 4.** In general the cell volume of *Oxyrrhis marina* fed *Amphidinium carterae* did not change markedly with increasing prey concentration  $\leq 7 \text{ ng C ml}^{-1}$  ( $700$  to  $900 \mu\text{m}^3$ ) but cell volume increased at higher prey concentrations and reached a maximum of  $4500 \mu\text{m}^3$  at the prey concentration of  $1114 \text{ ng C ml}^{-1}$  ( $11140 \text{ cells ml}^{-1}$ ) (Fig. 3). *O. marina* appeared to ingest as many prey cells as possible, with 10 *Amphidinium carterae* cells occurring in the protoplasm of some specimens. Accumulation of prey in *O. marina* caused an increase in the length and width.

Cell volume of *Oxyrrhis marina* fed *Prorocentrum minimum* in Expt 4 was  $3018 \mu\text{m}^3$ .

**Ingestion rate of *Acartia* spp. on *Amphidinium carterae* (Expt 3).** In our experiments, grazing by *Acartia* spp. on *A. carterae* was undetectable.

**Ingestion rates of *Acartia* spp. on *Oxyrrhis marina* fed *Prorocentrum minimum* (Expt 4).** The ingestion rates of *Acartia* spp. on *Oxyrrhis marina* grown on *Prorocentrum minimum* increased rapidly with increasing mean prey concentration (Fig. 4). When the data were fitted to Eq. (2), the maximum ingestion rate and  $K_{IR}$  of *Acartia* spp. on *O. marina* fed *P. minimum* were  $22500 \text{ cells predator}^{-1} \text{ d}^{-1}$  and  $1800 \text{ cells ml}^{-1}$ , respectively.

**Ingestion rates of *Acartia* spp. on *Oxyrrhis marina* fed *Amphidinium carterae* (Expts 5 to 8).** The ingestion rates of *Acartia* spp. on *Oxyrrhis marina* grown on *Amphidinium carterae* and then starved were affected by elapsed starvation time of *O. marina*. When the initial prey concentrations were 522 to 573 *O. marina* cells  $\text{ml}^{-1}$ , the ingestion rates of *Acartia* sp. on *O. marina* at Day 0 (*O. marina* starved for 0–1 d after satiation with *A. carterae*) and Day 3 (3 to 4 d after satiation) were 0, but increased with increasing elapsed starvation time and reached a maximum of  $2275 \text{ Oxyrrhis predator}^{-1} \text{ d}^{-1}$  at Day 11 (Fig. 5). When the initial prey concentrations were 923 to 1333 cells  $\text{ml}^{-1}$ , the ingestion rate of *Acartia* sp. on *O. marina* at Day 0 was  $426 \text{ Oxyrrhis predator}^{-1} \text{ d}^{-1}$ , but increased with increasing elapsed starvation time and reached the maximum ingestion rate of  $4710 \text{ O. marina predator}^{-1} \text{ d}^{-1}$  at Day 11.

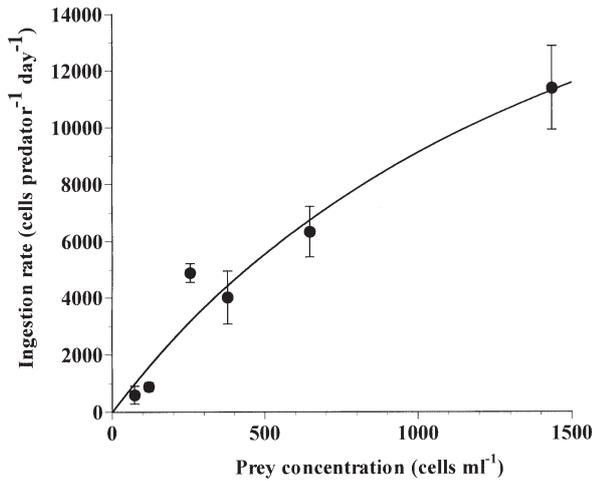


Fig. 4. Ingestion rates of *Acartia* spp. (*A. omorii* and *A. hongji*) on *Oxyrrhis marina* satiated with a non-toxic strain of *Prorocentrum minimum* as a function of mean *Oxyrrhis* concentration ( $x$ ). Symbols represent treatment means  $\pm 1$  SE. The curve is fitted as in Fig. 2. Ingestion rate (IR, cells predator<sup>-1</sup> d<sup>-1</sup>) =  $25\,500[x/(1800 + x)]$ ,  $R^2 = 0.859$

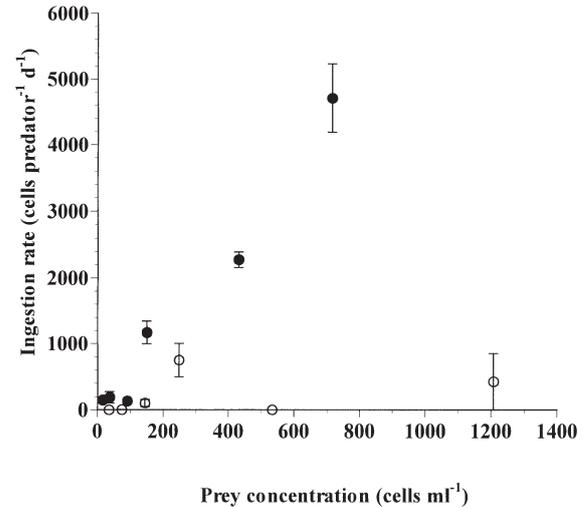


Fig. 6. Ingestion rates of *Acartia* spp. (*A. omorii* and *A. hongji*) on *Oxyrrhis marina* satiated with *Amphidinium carterae* and then starved at Day 0 (○) and Day 11 (●) as a function of the *O. marina* concentration in Expts 5 to 8 (see Table 1). Symbols represent treatment means  $\pm 1$  SE

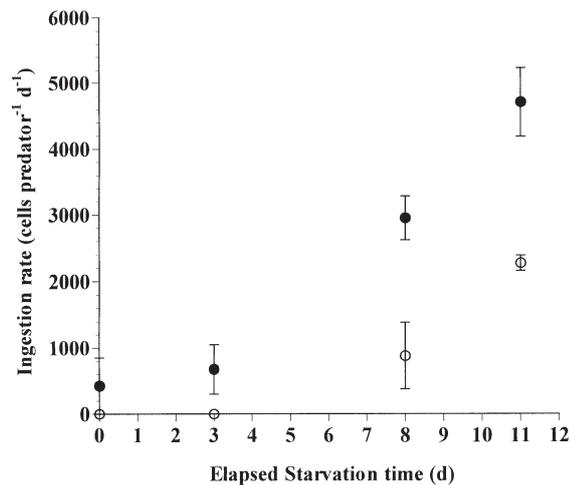


Fig. 5. Ingestion rates of *Acartia* spp. (*A. omorii* and *A. hongji*) on *Oxyrrhis marina* satiated with *Amphidinium carterae* and then starved as a function of elapsed starvation time in Expts 5 to 8 (see Table 1) when the initial prey concentrations were 522 to 573 (○) and 923 to 1333 cells ml<sup>-1</sup> (●). Symbols represent treatment means  $\pm 1$  SE

The functional response of *Acartia* spp. to *Oxyrrhis marina* which had been grown on *Amphidinium carterae* was also affected by starvation time of the *O. marina*. At Day 11 the ingestion rates increased with increasing *O. marina* density, but at Day 0 there was no consistent trend with the maximum ingestion rate of 749 *Oxyrrhis* predator<sup>-1</sup> d<sup>-1</sup> at a mean prey concentration of 252 cells ml<sup>-1</sup> (Fig. 6). The ingestion rates of

*Acartia* spp. on *Oxyrrhis marina* fed *A. carterae* at the mean prey concentrations of 431 and 716 *Oxyrrhis* ml<sup>-1</sup> at Day 11 were not significantly lower than those which had been fed *Prorocentrum minimum* at 377 and 647 *Oxyrrhis* ml<sup>-1</sup>, respectively ( $0.1 > p > 0.05$  for both, 1-tailed *t*-test; Zar 1984). At Day 0 the ingestion rates of copepods for *O. marina* fed *A. carterae* were much lower than those for *O. marina* fed *P. minimum* at all *O. marina* concentrations. These differences do not appear to be due to cell size: at Day 0 *O. marina* fed *P. minimum* were larger ( $3.0 \times 10^3 \mu\text{m}^3$ ) than *O. marina* fed *A. carterae* ( $2.0 \times 10^3 \mu\text{m}^3$ ). However, *O. marina* fed *A. carterae* declined in size by Day 11 ( $1.1 \times 10^3 \mu\text{m}^3$ ).

**Cell density and cell volume of *Oxyrrhis marina* on *Amphidinium carterae* in Expts 5 to 8.** The cell densities of *Oxyrrhis marina* satiated with *Amphidinium carterae* and then starved slightly increased from 1405 cells ml<sup>-1</sup> at Day 0 to 1493 cells ml<sup>-1</sup> at Day 3, but decreased to 863 cells ml<sup>-1</sup> at Day 11 (Fig. 7). That is, more than 60% of *Oxyrrhis marina* were able to survive after an 11 d starvation. Three additional experiments ascertained this resistance to long starvation.

The cell volume of *Oxyrrhis marina* decreased rapidly from 2045  $\mu\text{m}^3$  at Day 0 to 1624  $\mu\text{m}^3$  at Day 2, and then gradually decreased down to 1126  $\mu\text{m}^3$  at Day 11 (Fig. 8). Since *O. marina* had been starved for 20 h before being first fed to copepods (see 'Materials and methods'), their cell volume at Day 0 was smaller than their maximum volume when satiated with *A. carterae* (see Fig. 3).

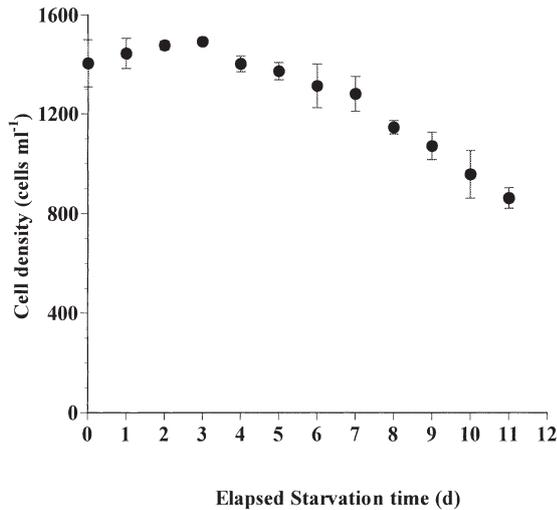


Fig. 7. Cell density (cells ml<sup>-1</sup>) of *Oxyrrhis marina* satiated with *Amphidinium carterae* and then starved as a function of elapsed starvation time in Expts 5 to 8 (see Table 1). Symbols represent treatment means  $\pm$  1 SE

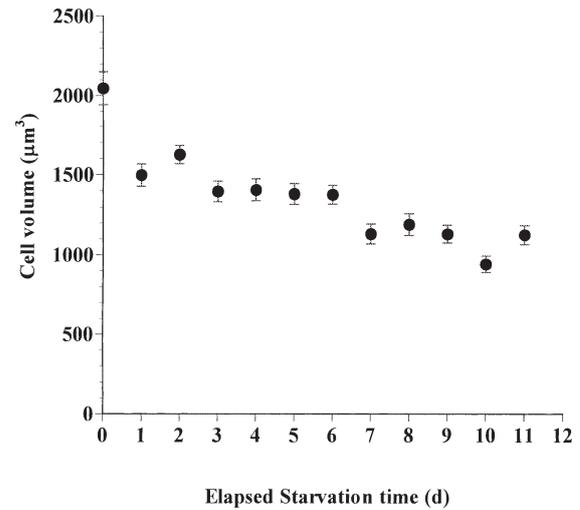


Fig. 8. Cell volume (μm<sup>3</sup>) of *Oxyrrhis marina* satiated with *Amphidinium carterae* and then starved as a function of elapsed starvation time in Expts 5 to 8 (see Table 1). Symbols represent treatment means  $\pm$  1 SE

## DISCUSSION

### Toxicity of *Amphidinium carterae*

The strain of *Amphidinium carterae* used in this study was toxic, although all strains of this dinoflagellate are not of equal toxicity. The toxicity of *Amphidinium carterae* (1 MU/1.3  $\times$  10<sup>8</sup> cells) was lower than that obtained in Nakajima et al. (1981) (4.7 MU/10<sup>8</sup> cells). The exact nature of the toxin produced by the dinoflagellate is not completely known (Ikawa & Sasner 1975, Sasner 1975, Yasumoto 1990, Fessard et al. 1994, Nayak et al. 1997).

### Growth rate of *Oxyrrhis marina* on *Amphidinium carterae*

The growth rate of the mixotrophic dinoflagellate *Fragilidium* cf. *mexicanum* (Jeong et al. 1999a), a naked ciliate *Strombidinopsis* sp. (Jeong et al. 1999b), and the heterotrophic dinoflagellate *Polykrikos kofoidii* (Jeong et al. 2001) on the same *Amphidinium carterae* strain used in this study was 0 or very low. The tintinnid ciliate *Favella ehrenbergii* (Stoecker et al. 1981) did not feed on *A. carterae* either. The heterotrophic dinoflagellates *Diplopsalis lenticula* (Naustvoll 1998) and a tintinnid ciliate *Favella serrata* (Blackbourn 1974) are known to feed on

*A. carterae*. However, these studies did not report positive growth of the grazers on *A. carterae*. Therefore, *O. marina* is the only protistan grazer so far reported to grow well on *A. carterae*.

The maximum growth rate ( $\mu$ ) of *Oxyrrhis marina* on *Amphidinium carterae* (1.17 d<sup>-1</sup>) is slightly lower than that of *O. marina* on *Phaeodactylum tricorutum* (1.3 d<sup>-1</sup>), the optimal prey so far reported, but higher than those on *Dunaliella tertiolecta* or *Isochrysis galbana* (Table 2). Therefore, *A. carterae* is an excellent prey for *O. marina*. This evidence suggests that *O. marina* may be insensitive to the toxin produced by *A. carterae*.

### Ingestion and clearance of *Oxyrrhis marina* and *Acartia* spp. on *Amphidinium carterae*

The maximum ingestion rate (2.8 ng C predator<sup>-1</sup> d<sup>-1</sup>) of *Oxyrrhis marina* on *Amphidinium carterae* obtained in this study is similar to that of *O. marina* on

Table 2. Comparison of growth, ingestion, and clearance rates of *Oxyrrhis marina* on the different phytoplankton species at 20°C.  $\mu_{\max}$ : maximum growth rate as d<sup>-1</sup>;  $I_{\max}$ : maximum ingestion rate in ng C predator<sup>-1</sup> d<sup>-1</sup>;  $C_{\max}$ : maximum clearance rate as  $\mu$ l predator<sup>-1</sup> h<sup>-1</sup>

Prey species	$\mu_{\max}$	$I_{\max}$	$C_{\max}$	Source
<i>Amphidinium carterae</i>	1.17	2.8	2.4	This study
<i>Phaeodactylum tricorutum</i>	1.30	2.6	0.05	Goldman et al. (1989)
<i>Dunaliella tertiolecta</i>	0.79		0.34	Goldman et al. (1989)
<i>Isochrysis galbana</i>	0.79	7	0.36	Goldman et al. (1989)

*Phaeodactylum tricorutum* (2.6 ng C predator<sup>-1</sup> d<sup>-1</sup>), but the maximum clearance rate (2.4 µl predator<sup>-1</sup> h<sup>-1</sup>) on the former prey is much higher than that for the latter prey (Table 2). Therefore, *O. marina* might capture and ingest *A. carterae* more efficiently than *P. tricorutum* at low prey concentrations.

In general, ingestion rates of *Oxyrrhis marina* increased continuously up to and beyond prey concentrations where growth rates were saturated (see Figs 1 & 2;  $K_{IR}$  was higher than  $K_{GR}$ ). The cell volume of *O. marina* also showed a similar increase relative to prey density (Fig. 3). *O. marina* appeared to ingest as many prey cells as possible when prey was plentiful, raising the possibility that rapid ingestion coupled with slow digestion/assimilation of prey may have contributed to observed patterns in cell volume, as for *Strombidinopsis* sp. (Jeong et al. 1999b).

Feeding by *Acartia* spp. (*A. omorii* and *A. hongii*) on *Amphidinium carterae* was undetectable. Huntley et al. (1987) observed that when *A. carterae* was provided as food, the survival of the nauplii of the calanoid copepod *Calanus pacificus* declined abruptly after they reached the first feeding stages (Nauplius III). However, Houde & Roman (1987) found that *Acartia tonsa* fed on a strain of *A. carterae*. These conflicting results may be due to differences in toxicity among the strains of this dinoflagellate.

#### Ingestion rates of *Acartia* spp. on *Oxyrrhis marina* fed *Amphidinium carterae* and *Prorocentrum minimum*

The results from Expts 4 to 8 suggest that the food quality of *Oxyrrhis marina* for *Acartia* spp. is affected by *O. marina*'s prey type and elapsed starvation time. It is not uncommon for food quality of prey to be affected by the prey's diet (Rainuzzo et al. 1989). For example, the egg production of *Acartia tonsa* on *O. marina* is affected by the prey species used to culture *O. marina* (Kleppel & Burkart 1994). However, recovery of ingestion rates of *O. marina* fed *A. carterae* after 11 to 12 d starvation implies that the grazer may have an ability to detoxify the toxin produced by *Amphidinium carterae* and/or excrete the toxin into the ambient water. For further understanding it is worth exploring the structure of the toxin produced by *A. carterae* and the mechanism of reduction of the toxic effect by *O. marina* in the bio-chemical aspects.

#### Ecological importance

The interactions revealed in the present study may be important in marine ecosystems in the following

ways: (1) *Oxyrrhis marina* is an effective grazer on a toxic strain of *Amphidinium carterae*. (2) The mortality of *O. marina* due to predation by *Acartia* spp. (*A. omorii* and *A. hongii*) is reduced if *O. marina* has fed on *A. carterae*, compared to being fed *Prorocentrum minimum*. However, ingestion rates of *Acartia* spp. on *O. marina* can be restored if *O. marina* starved after eating *A. carterae*. (3) *O. marina* can transfer the materials of *A. carterae* to *Acartia* spp., which cannot feed on *A. carterae*. These interactions may influence our conventional view of food webs in marine planktonic community (Jeong 1999). To further understand marine plankton food webs related to toxic dinoflagellates, it would be worthwhile to investigate the interactions among other toxic dinoflagellates, heterotrophic protists, and copepods.

**Acknowledgements.** We are grateful to Drs Michael Mullin and Peter Franks for comments on the manuscript and to Seong Taek Kim, Young Do Yoo and Yoon Jeong Choi for technical assistance. This paper was funded by grants from a Brain Korea 21 Project (1999) in the Ministry of Education, the RRC program of MOST and KOSEF (SERC), MOMAF and KMI, and KOSEF (1999-1-13500-001-5).

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Editorial responsibility: Diane Stoecker (Contributing Editor), Cambridge, Maryland, USA

Submitted: September 1, 2000; Accepted: March 28, 2001  
Proofs received from author(s): July 20, 2001