Marine Biology (2024) 171:112
https://doi.org/10.1007/s00227-024-04427-0

ORIGINAL PAPER

Interactions between the calanoid copepod *Acartia hongi* and the bloom-forming dinoflagellates *Karenia bicuneiformis* and *K. selliformis*

Jin Hee Ok[1] · Hae Jin Jeong[2,3] · Ji Hyun You[2] · Sang Ah Park[2] · Hee Chang Kang[2] · Se Hee Eom[2] · Moo Joon Lee[4] · Jung-Rae Rho[5]

Received: 13 August 2023 / Accepted: 15 March 2024

Abstract

Copepods are a major component of metazooplankton and important prey for fish and invertebrates such as crabs, shrimps, and flatworms. Certain bloom-forming dinoflagellates can kill copepods, but there is little research on the interactions between copepods and the bloom-forming dinoflagellates *Karenia bicuneiformis* and *K. selliformis*. In this study, the survival and ingestion rates of the calanoid copepod *Acartia hongi* feeding on *K. bicuneiformis* and *K. selliformis* were determined as a function of prey concentration. On day 2, the survival of *A. hongi* incubated with *K. bicuneiformis* was 90–100% at all the tested prey concentrations, while that with *K. selliformis* was 0–20% at ≥ 582 ng C mL^{-1}. Compared to other harmful dinoflagellates from the literature, *K. bicuneiformis* caused low mortality of *Acartia*; however, *K. selliformis* caused almost the highest mortality at similar dinoflagellate concentrations. With increasing mean prey concentration, the ingestion rates of *A. hongi* feeding on *K. bicuneiformis* increased on day 1, but those on *K. selliformis* did not increase. *Acartia hongi* stopped feeding on *K. bicuneiformis* at mean prey concentrations of ≥ 341 ng C mL^{-1} and *K. selliformis* at all prey concentrations on day 2. At the prey concentration of 1000 ng C mL^{-1}, the ingestion rate of *A. hongi* feeding on *K. bicuneiformis* was moderate among the rates of *Acartia* spp. feeding on harmful dinoflagellates; however, that on *K. selliformis* was the lowest. These results indicate that *K. bicuneiformis* and *K. selliformis* differentially affect the survival and ingestion rates of *A. hongi*.

Keywords Grazing · Kareniaceae · Marine · Mesozooplankton · Mortality · Survival · Toxin

Introduction

Copepods are ubiquitous in the world's oceans and play a crucial role as major components of marine ecosystems (Turner 2004; Rombouts et al. 2009). They feed on bacteria, protists, including autotrophic, heterotrophic, and mixotrophic species, and larval fish (Mullin 1963; Turner et al. 1985; Gifford 1991; Turner and Tester 1992; Jeong 1994; Jeong et al. 2001; Calbet et al. 2007; Besiktepe and Dam 2020; Lee et al. 2023) and are suitable prey for diverse metazoans (Turner 1984; Barroso et al. 2013). As a key link in marine food webs, the population dynamics of copepods can thus affect those of diverse marine organisms (Turner 2004; Kim et al. 2013; Zeldis and Décima 2020). Therefore, elucidating the interactions between copepods and other marine organisms is crucial to understanding marine ecosystem balance and fishery production.

Phototrophic dinoflagellates are a core component of marine ecosystems and play diverse ecological roles as

Responsible Editor: S. Shumway.

✉ Hae Jin Jeong
hjjeong@snu.ac.kr

[1] Department of Marine Sciences and Convergent Engineering, College of Marine Sciences and Convergence Engineering, Hanyang University ERICA, Ansan 15588, Republic of Korea

[2] School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Republic of Korea

[3] Research Institute of Oceanography, Seoul National University, Seoul 08826, Republic of Korea

[4] Department of Marine Biotechnology, Anyang University, Incheon 23038, Republic of Korea

[5] Department of Marine Biotechnology, Kunsan National University, Kunsan 54150, Republic of Korea

Published online: 09 April 2024

primary producers and prey for various predators such as mixotrophic and heterotrophic dinoflagellates, ciliates, copepods, and invertebrate larvae (Kleppel 1993; Taylor et al. 2008; Jeong et al. 2010, 2021; Ok et al. 2023a; You et al. 2023; Kang et al. 2023). However, certain phototrophic dinoflagellate species are harmful to diverse marine organisms; they often cause large-scale mortality in fish, shellfish, and mammals (Landsberg 2002; Tillmann and John 2002; Burkholder et al. 2006; Holmes et al. 2014; Turner 2014; Ok et al. 2017; Basti et al. 2018; Broadwater et al. 2018). Thus, although some dinoflagellates can be prey for copepods, they can kill or adversely affect copepods by decreasing their ingestion rates, fecundity, and egg-hatching success (Shin et al. 2003; Turner 2006, 2014). Therefore, the interactions between harmful dinoflagellates and copepods warrant closer inspection. Furthermore, some phototrophic dinoflagellates often cause harmful algal blooms (HABs), and their abundance varies largely in marine environments (e.g., Band-Schmidt et al. 2010; Karlson et al. 2021; Gu et al. 2022). To identify the critical abundances at which bloom-forming dinoflagellate species affect copepods, copepod survival and ingestion rates should be explored as a function of prey concentrations.

Many species of the dinoflagellate genus *Karenia* produce neurotoxins (Hort et al. 2021). There are 10 described species of *Karenia* (Guiry and Guiry 2023), all of which cause blooms (e.g., Chang 1999; Yang et al. 2001; Botes et al. 2003; Davidson et al. 2009; Steidinger 2009; Yamaguchi et al. 2016; Iwataki et al. 2022; Orlova et al. 2022). Several studies exist on the interactions between the common calanoid copepod *Acartia* and two *Karenia* species, *K. brevis* and *K. mikimotoi*, which have been shown to inhibit copepod survival, ingestion, and fecundity (Uye and Takamatsu 1990; Turner et al. 1998; Speekmann et al. 2006; Breier and Buskey 2007; Cohen et al. 2007; Waggett et al. 2012). However, no studies have reported the interactions between other bloom-forming *Karenia* species and *Acartia*.

Karenia selliformis is known to produce brevetoxins, gymnodimines, or brevenal (Miles et al. 2000, 2003; Haywood et al. 2004; Mardones et al. 2020), and its blooms have been associated with the death of vertebrates, including fish and sea birds, and invertebrates, including abalone, chitons, sea anemones, sea urchins, sea stars, snails, limpets, and octopuses, in many countries (Arzul et al. 1995; Mackenzie et al. 1996; Clément et al. 2001; Heil et al. 2001; Uribe and Ruiz 2001; Orlova et al. 2022). Although the lethality of *K. selliformis* has been reported in diverse marine organisms, such as phytoplankton, manila clams, oyster larvae, and juvenile kelp sporophytes (Hégaret et al. 2007; Natsuike et al. 2023; Ok et al. 2023b), no harmful effects have been reported for copepods. Moreover, *Karenia bicuneiformis* (= *K. bidigitata*) contains brevetoxins (Haywood et al. 2004); however, no harmful effects from its blooms have been reported to date when blooms by this species occurred in Gordon Bay, South Africa, and Benguelan current system (Botes et al. 2003; Trainer et al. 2010). Furthermore, the interactions between *K. bicuneiformis* and copepods have not yet been explored.

In this study, the survival and ingestion rates of *Acartia hongi*, feeding on *K. bicuneiformis* CAWD81 and *K. selliformis* NIES-4541, were determined as a function of prey concentration. These observed survival and ingestion rates were compared with those of *Acartia* spp. on other harmful dinoflagellate species based on literature data. Our results provide a basis for understanding the interactions between copepods and harmful *Karenia* species and HAB dynamics.

Materials and methods

Preparation of experimental organisms

A culture of *K. bicuneiformis* CAWD81, initially collected from Fouveaux Strait, New Zealand, and a culture of *K. selliformis* NIES-4541, initially collected from Katsurakoi Port, Japan, were obtained from the Cawthron Institute Culture Collection of Microalgae and the Microbial Culture Collection at the National Institute for Environmental Studies, Japan, respectively (Table 1). All *Karenia* cultures were maintained in 250-mL flat culture flasks containing L1 medium at 20 °C under a 14:10 h light–dark cycle (50 µmol photons m^{-2} s^{-1} produced by a cool-white fluorescent light). Every 3 weeks, they were transferred into identical flasks containing fresh L1 medium (Guillard and Hargraves 1993). The cell volumes of *K. bicuneiformis* (ellipsoid) and *K. selliformis* (prolate spheroid) were calculated following

Table 1 Information on the experimental organisms used in this study

Organism	Strain	Collection site	Time	T	S	References
Karenia bicuneiformis (= *Karenia bidigitata*)	CAWD81	Fouveaux Strait, New Zealand	NA	NA	NA	
Karenia selliformis	NIES-4541	Katsurakoi Port, Japan	Sep 24, 2021	16.5	28.2	Iwataki et al. (2022)
Acartia hongi		Shiwha Bay, Korea	Jun 01, 2023	18.2	32.1	This study

T water temperature (°C), *S* salinity, *NA* not available

Hillebrand et al. (1999). The carbon content of each culture was estimated using the measured cell volumes following Menden-Deuer and Lessard (2000).

Using a 303-µm mesh net, copepods were collected from Shiwha Bay, Korea, in June 2023, when the water temperature and salinity were 18.2 °C and 32.1, respectively. They were transported to the laboratory within a few hours (Table 1). The copepods were acclimatized in a temperature-controlled room at 20 °C. They were fed with the dinoflagellate *Prorocentrum cordatum* (approximately 200 cells mL^{-1}) as prey for 1 day for the *K. selliformis* experiment and additional 2 days for the *K. bicuneiformis* experiment. Adult female copepods collected during the same sampling event were used throughout this study.

Survival and ingestion rates

In Experiments 1–2, the survival and ingestion rates of *A. hongi*, feeding on either *K. bicuneiformis* (Experiment 1) or *K. selliformis* (Experiment 2), were measured as a function of the prey concentration (Table 2). The initial concentrations of *K. bicuneiformis* (or *K. selliformis*) were set up using autopipettes, and the initial copepod densities (10 female copepods per 500-mL bottle) were established by transferring the copepods individually using a Pasteur pipette (Table 2). Triplicate 500-mL polycarbonate experimental (predator plus prey) and prey control bottles (prey only) were established at each predator–prey density combination. Moreover, triplicate 500-mL predator control bottles (predator only) containing 10 female copepod individuals without prey were also established. Subsequently, a 10-mL aliquot was removed from each experimental and prey control bottle, transferred to a scintillation vial, and fixed with 2% Lugol's solution at the beginning of the experiment to determine the actual initial prey concentrations. The bottles were then filled with freshly filtered seawater, capped, placed on plankton wheels rotating at 0.9 rpm (0.00017 g), and incubated at 20 °C under 20 µmol photons m^{-2} s^{-1} illumination with a 14:10 h light–dark cycle. After incubation for 24 h, 10-mL aliquots were extracted from each experimental and prey control bottle and fixed with 2% Lugol's solution. The number of living copepods was enumerated by careful observation through the sides of the capped bottles. The bottles were then refilled to capacity with freshly filtered seawater, capped, placed on rotating plankton wheels, and incubated under the same conditions mentioned above. After the bottles were incubated for 48 h, 10-mL aliquots were extracted from each experimental and prey control bottle and fixed with 2% Lugol's solution. The water in the experimental bottles was filtered through a 100-µm mesh, and the retained copepods were quickly placed into 50-mL beakers. All living and dead copepods were then enumerated under a dissecting microscope (SZX2-ILLB, Olympus, Tokyo, Japan). The prey concentration was measured by counting all the cells, or > 200 cells in 1-mL Sedgewick Rafter chambers under a compound microscope (BX53; Olympus). When Experiment 2 was conducted, the survival and ingestion rates of *A. hongi* feeding on *Prorocentrum cordatum* as a non-toxic prey control were measured at a single high prey concentration (10,000 cells mL^{-1}) in the same manner as described above.

Using the equations described by Frost (1972), the ingestion and clearance rates of the copepods for each target *Karenia* species at day 1 (from day 0 to day 1) and day 2 (from day 1 to day 2) were calculated. The dilution of the cultures associated with refilling the bottles on days 0 and 1 was considered in calculating the ingestion rates. The data on the *K. bicuneiformis* ingestion rates were fitted to a Michaelis–Menten equation:

$$IR = \frac{I_{max}(x)}{K_{IR} + (x)} \quad (1)$$

where x = mean prey concentration (cells mL^{-1} or ng C mL^{-1}); I_{max} = the maximum ingestion rate (cells $predator^{-1}$ day^{-1} or ng C $predator^{-1}$ day^{-1}); and K_{IR} = the prey concentration sustaining ½ I_{max} (cells mL^{-1} or ng C mL^{-1}).

Molecular identification of *Acartia* species used in this study

To identify the copepods used in this study, each of the 10 copepods in the predator control bottle in Experiment 1 (with *K. bicuneiformis*) was transferred to an individual 1.5-mL tube after the experiment had ended. The genomic

Table 2 Experimental design and the actual initial densities of prey and predator species

Expt No	Prey		Predator	
	Species	Actual initial density (cells mL^{-1})	Species	Actual initial density (individuals in 500 mL)
1	*Karenia bicuneiformis*	0, 20, 97,188, 464, 986, 1960	*Acartia hongi*	10
2	*Karenia selliformis*	0, 19, 72, 145, 390, 793, 1555	*A. hongi*	10

DNA of the copepods was extracted using the AccuPrep® genomic DNA extraction kit (Bioneer, Daejeon, Korea). For the amplification of small subunit ribosomal DNA (SSU rDNA), polymerase chain reaction (PCR) was conducted using F-Star Taq DNA Polymerase (BIOFACT Co., Daejeon, Korea) in a total volume of 50 µL containing 5 µL of 10 × Taq buffer, 1 µL of 10 mM dNTP mix, 0.25 µL of DNA polymerase, 5 µL of 5 × Band Helper™, 2 µL each of the forward and reverse primers, and 1 µL of the DNA template. The forward and reverse primers used in this study were 18s1075H (5′-CGA AGG CGM TCA GAT ACC GCC CTA G-3′) and 18s1871Hr (5′-CAC CTA CGG AAA CCT TGT TAC GAC-3′), respectively (Easton and Thistle 2014). The thermocycler (AllInOneCycler™, Bioneer) was run under the following conditions: 95 °C for 2 min, followed by 40 cycles of 95 °C for 20 s, 59 °C for 30 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. The PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) following the manufacturer's instructions. They were then sequenced using an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The obtained sequences were assembled using ContigExpress software (Infomax, Frederick, MD, USA) and compared with the SSU rDNA sequences of *Acartia* species in the NCBI GenBank database based on the BLASTn algorithm. Sequences with > 90% query coverage are shown in the sequence difference comparisons in this study.

For the phylogenetic tree, SSU rDNA sequences of the *Acartia* species were obtained from GenBank and were aligned using the MEGA v4 program (Tamura et al. 2007). A phylogenetic tree based on the Bayesian and maximum likelihood analyses was established following Kang et al. (2010).

Swimming speed of *Karenia*

To explore whether the ingestion rates of *Acartia* species feeding on dinoflagellates were affected by the swimming speeds of the dinoflagellates, the swimming speeds of *K. bicuneiformis* CAWD81 and *K. selliformis* NIES-4541 were measured. A dense clonal culture of *K. bicuneiformis* (or *K. selliformis*) in a 50-mL cell culture flask, incubated at 20 °C under 20 µmol photons m^{-2} s^{-1}, was placed under a dissecting microscope (SZX10, Olympus). The field in which the flask was placed was leveled using a magnetic torpedo level. After 30-min acclimation, the swimming *Karenia* species were recorded in each flask using a video analyzing system (SRD-1673DN; Samsung Techwin, Seongnam, Korea) at a magnification of 20×. The swimming speeds of *K. bicuneiformis* (or *K. selliformis*) were calculated by measuring the travel distance and time of 20 swimming *Karenia* cells showing straight, linear paths.

Statistical analysis

The non-parametric Kruskal–Wallis test was used to examine the effect of *Karenia* concentration on *A. hongi* survival. Pearson's correlations were conducted to examine the relationships between the ingestion rates of *A. hongi* feeding on dinoflagellates, the mortality (%) of *A. hongi* incubated with the dinoflagellates, and the dinoflagellate cell sizes and swimming speeds. The software SPSS 26.0 (IBM-SPSS Inc., NY, USA) was used for statistical analyses. The criterion for statistical significance was set at a P value of 0.05.

Results

Molecular identification of *A. hongi*

The obtained SSU rDNA sequences of the 10 copepod individuals used in this study were 100% identical to each other and to those reported for *A. hongi* (Accession numbers GU969195 and MZ413971; Fig. 1). The *A. hongi* sequences obtained in this study were 13.2–15.8% different from those of other *Acartia* species such as *A. omorii*, *A. clausii*, *A. tonsa*, *A. bifilosa*, *A. negligens*, *A. danae*, *A. steueri*, *A. pacifica*, and *A. erythraea*. The SSU rDNA sequence of *A. hongi* used in this study was deposited in GenBank under accession number OR356145.

Survival of *A. hongi*

On day 1, the survival of *A. hongi* incubated with *K. bicuneiformis* at mean prey concentrations of 0–1352 ng C mL^{-1} (0–1988 cells mL^{-1}) was 87–100% (Fig. 2a). On day 2, they were 90–100% at mean prey concentrations of 0–1394 ng C mL^{-1} (0–2050 cells mL^{-1}) (Fig. 2b). The survival was not significantly affected by *K. bicuneiformis* concentration (Kruskal–Wallis test; $H_6 = 8.97$, $P = 0.175$ for day 1; $H_6 = 9.12$, $P = 0.167$ for day 2).

On day 1, the survival of *A. hongi* incubated with *K. selliformis* at mean prey concentrations of 0–2400 ng C mL^{-1} (0–1690 cells mL^{-1}) was 87–97% (Fig. 3a). On day 2, the survival decreased as a function of mean prey concentrations (Fig. 3b); all the copepods had died at a mean prey concentration of 2682 ng C mL^{-1} (1889 cells mL^{-1}). The survival was not significantly affected by *K. selliformis* concentrations on day 1 (Kruskal–Wallis test, $H_6 = 4.03$, $P = 0.673$); however, they were significantly affected on day 2 (Kruskal–Wallis test, $H_6 = 18.23$, $P = 0.006$).

The survival of *A. hongi* incubated with *P. cordatum* was 95% at a single mean prey concentration of 1452 ng C mL^{-1} (9680 cells mL^{-1}) for 2 days.

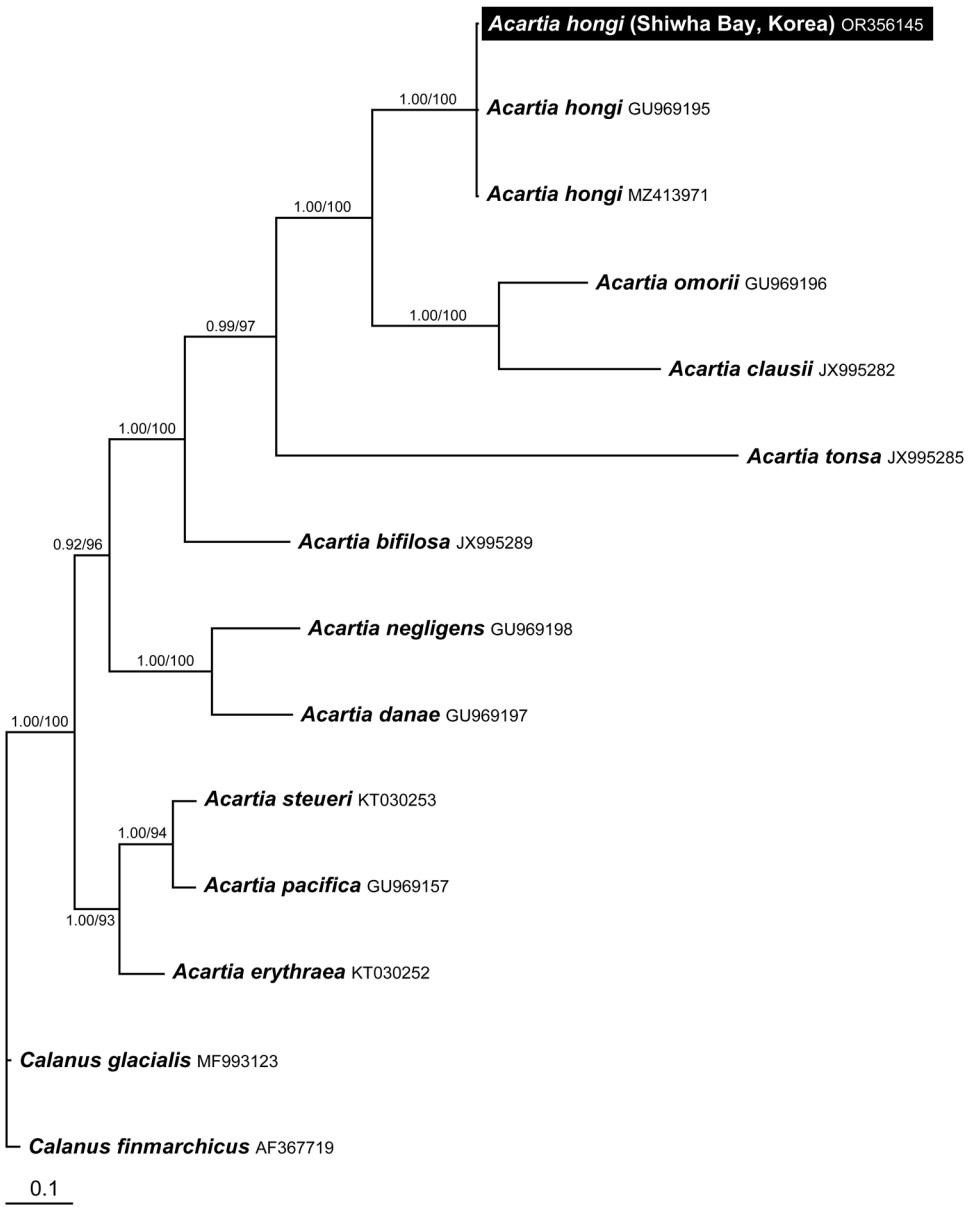

Fig. 1 Bayesian tree showing genetic relationships among the calanoid copepod *Acartia* based on the small subunit ribosomal DNA region (707 bp), using a GTR + G + I model. Numbers near branches represent the Bayesian posterior probability (left) and maximum likelihood bootstrap values (right). A black box represents *A. hongi*, which was used in this study. The assumed empirical nucleotide frequencies of SSU rDNA comprised a substitution rate matrix with A–C substitutions = 0.1152, A–G = 0.2406, A–T = 0.1173, C–G = 0.1114, C–T = 0.2874, and G–T = 0.1281. Rates were assumed to follow a gamma distribution with a shape parameter of 0.3661 for variable sites. The proportion of sites assumed to be invariable was 0.2416

Ingestion rate of *A. hongi*

On day 1, the ingestion rates of *A. hongi* feeding on *K. bicuneiformis* continuously increased with increasing mean prey concentrations (Fig. 4a). The highest ingestion rate of *A. hongi* on *K. bicuneiformis*, 3813 ng C predator^{-1} day^{-1} (5608 cells predator^{-1} day^{-1}), was achieved at a mean prey concentration of 1352 ng C mL^{-1} (1988 cells mL^{-1}). When Eq. (1) was used, the I_{max} of *A. hongi* feeding on *K. bicuneiformis* was 9286 ng C predator^{-1} day^{-1} (13,656 cells predator^{-1} day^{-1}), and the K_{IR} was 1710 ng C mL^{-1} (2515 cells mL^{-1}). The maximum clearance rate of *A. hongi* feeding on *K. bicuneiformis* was 338 µL predator^{-1} h^{-1} at a mean prey concentration of 13 ng C mL^{-1} (19 cells mL^{-1}). On day 2, the ingestion rates of *A. hongi* feeding on *K. bicuneiformis* increased at mean prey concentrations ≤ 116 ng C mL^{-1} (171 cells mL^{-1}); however, they decreased to zero at higher prey concentrations, indicating that ingestion was inhibited at high prey concentrations (Fig. 4b).

On day 1, the ingestion rates of *A. hongi* feeding on *K. selliformis* were positive at mean prey concentrations ≤ 1159 ng C mL^{-1} (816 cells mL^{-1}). However, the rate decreased to zero at a higher prey concentration (Fig. 5a). The highest ingestion rate of *A. hongi* on *K. selliformis*, 1373 ng C predator^{-1} day^{-1} (967 cells predator^{-1} day^{-1}), was achieved at a mean prey concentration of 1159 ng C mL^{-1} (816 cells mL^{-1}). The maximum clearance rate of *A. hongi* feeding on *K. selliformis* was 1612 µL predator^{-1} h^{-1} at a mean prey concentration of 18 ng C mL^{-1} (13 cells mL^{-1}). On day 2, the ingestion rates of *A.*

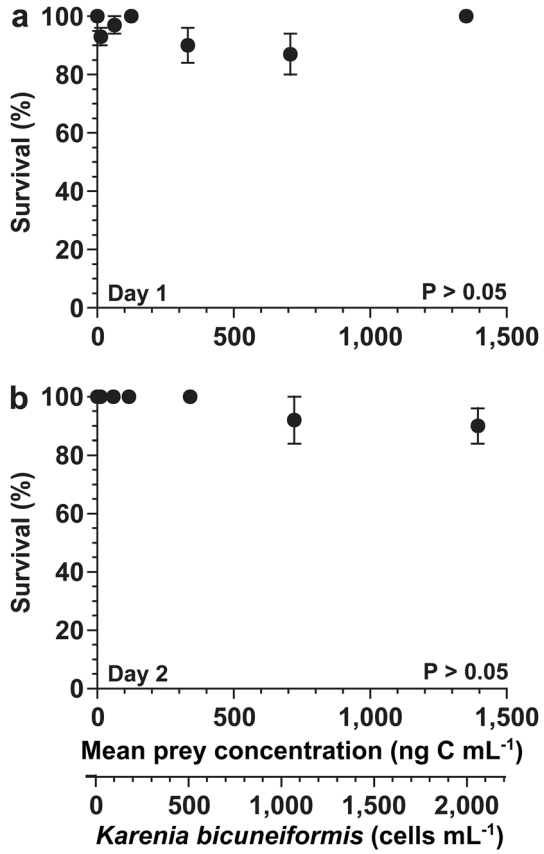

Fig. 2 Survival (%) of *Acartia hongi* as a function of mean *Karenia bicuneiformis* concentration on day 1 (**a**) and day 2 (**b**). Symbols represent treatment means ± standard error

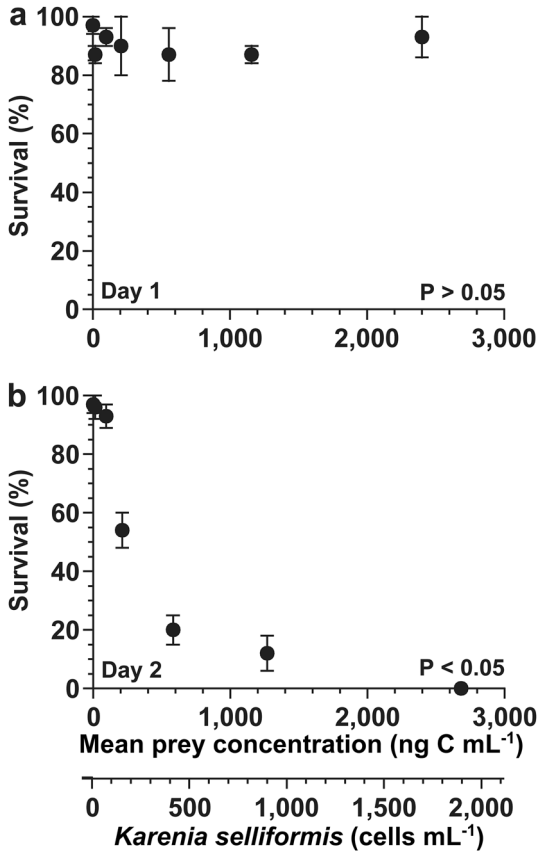

Fig. 3 Survival (%) of *Acartia hongi* as a function of mean *Karenia selliformis* concentration on day 1 (**a**) and day 2 (**b**). Symbols represent treatment means ± standard error

hongi feeding on *K. selliformis* were zero at all prey concentrations (Fig. 5b).

The ingestion rate of *A. hongi* feeding on *P. cordatum* was 4541 ng C predator^{-1} day^{-1} (30,273 cells predator^{-1} day^{-1}) at a single mean prey concentration of 1452 ng C mL^{-1} (9680 cells mL^{-1}) for 2 days.

Swimming speed of *Karenia*

Karenia bicuneiformis and *K. selliformis* showed linear and helical swimming behaviors; however, jumping behaviors were not observed. The average (± standard error) and maximum swimming speeds of *K. bicuneiformis* (n = 20) were 257 (± 17) and 480 µm s^{-1}, respectively. The average and maximum swimming speeds of *K. selliformis* (n = 20) were 232 (± 13) and 340 µm s^{-1}, respectively.

Discussions

The present study reports, for the first time, the interactions between *Acartia* species and the dinoflagellates *K. bicuneiformis* CAWD81 and *K. selliformis* NIES-4541. Previous studies on the interactions between *Acartia* species and *K. brevis*, *Acartia* species and *K. mikimotoi*, and *Temora longicornis* and *K. selliformis* K-1319 have been explored (Uye and Takamatsu 1990; Turner et al. 1998; Breier and Buskey 2007; Cohen et al. 2007; Waggett et al. 2012; Xu and Kiørboe 2018).

This study highlights that *A. hongi* feeds on *K. bicuneiformis* and *K. selliformis*; however, it stops feeding on them depending on the mean prey concentration and incubation time. The highest ingestion rate of *A. hongi* when feeding on *K. bicuneiformis* was higher than that when feeding on *K. selliformis*. Furthermore, *A. hongi* died when exposed to *K. selliformis* but not when exposed to *K. bicuneiformis*. Therefore, *K. bicuneiformis* and *K. selliformis* interact with *A. hongi* differently.

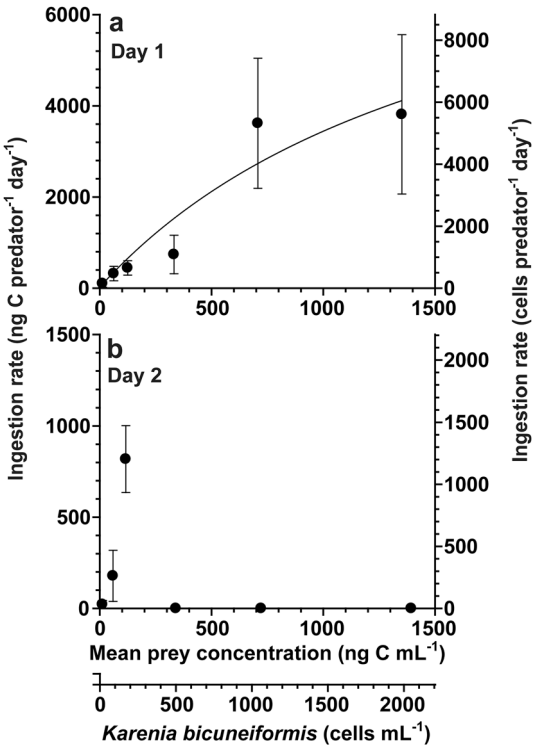

Fig. 4 Ingestion rates of *Acartia hongi* feeding on *Karenia bicuneiformis* as a function of mean prey concentration (x) on day 1 (**a**) and day 2 (**b**). The curve in (**a**) is fitted using the Michaelis–Menten equation. Ingestion rate (ng C predator^{-1} day^{-1}) = 9286 [x/(1710 + x)], $r^2 = 0.525$. Symbols represent treatment means ± standard error

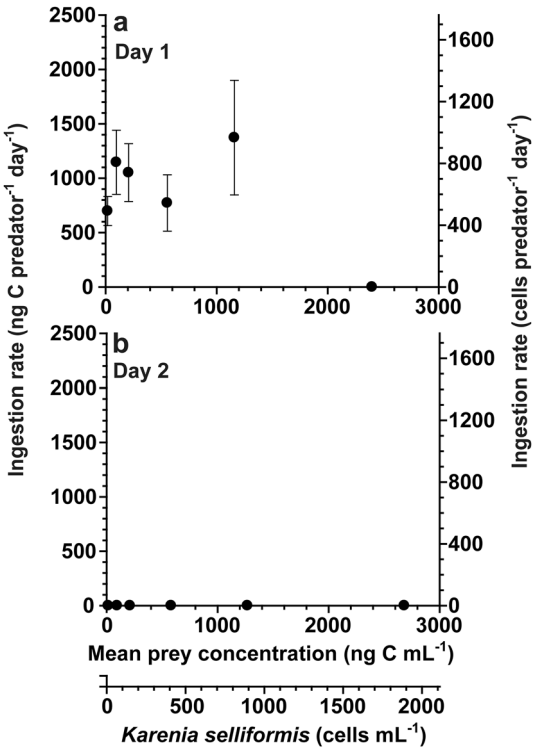

Fig. 5 Ingestion rates of *Acartia hongi* feeding on *Karenia selliformis* as a function of mean prey concentration on day 1 (**a**) and day 2 (**b**). Symbols represent treatment means ± standard error

Effects of two *Karenia* species on the survival of *A. hongi*

Acartia hongi was killed by *K. selliformis* NIES-4541 but not by *K. bicuneiformis* CAWD81. Before this study, in the family Kareniaceae, *K. brevis* CCMP2228, the Nagasaki University strain of *K. mikimotoi*, *Karlodinium armiger* K-0668, and the Alfacs Bay strain of *Karlodinium corsicum* were known to kill *Acartia* spp., whereas *Karlodinium veneficum* K-1385, K-1635, K-1640, and K-1386 does not kill them (Uye and Takamatsu 1990; Delgado and Alcaraz 1999; Waggett et al. 2012; Berge et al. 2012). At similar Kareniacean dinoflagellate concentrations (1100–1500 ng C mL^{-1}) after 2 days of incubation, the mortality (the inversion of the survival) of *A. hongi* incubated with *K. selliformis* NIES-4541 was slightly lower than those of *Acartia* spp. incubated with *Kl. armiger* K-0668 and the Alfacs Bay strain of *Kl. corsicum*; however, much higher than those of *Acartia* spp. incubated with *K. brevis* CCMP2228, *K. bicuneiformis* CAWD81, and *Kl. corsicum* GCORS1 (Table 3; Fig. 6). These comparisons indicate that *K. selliformis* is more harmful to *A. hongi* than *K. bicuneiformis*. To date, there have been no reports on the mortality of marine animals during *K. bicuneiformis* blooms (e.g., Botes et al. 2003; Trainer et al. 2010). However, reports from various countries, such as Chile, Kuwait, New Zealand, and Russia, have detailed the mortality of marine animals during *K. selliformis* blooms (Mackenzie et al. 1996; Clément et al. 2001; Heil et al. 2001; Uribe and Ruiz 2001; Orlova et al. 2022). Thus, the field reports on the relationships between marine animal mortality and *K. bicuneiformis* and *K. selliformis* blooms are similar to the results of this study, which shows that *K. bicuneiformis* and *K. selliformis* have different effects on the survival of *A. hongi*.

Some strains in the genus *Karenia* have diverse toxin compositions such as brevetoxins, brevenal, gymnodimines, and gymnocins (Baden 1989; Seki et al. 1995; Miles et al. 2000, 2003; Satake et al. 2002, 2005; Bourdelais et al. 2005; Rasmussen et al. 2017; Mardones et al. 2020). Therefore, it is difficult to compare the lethality of these Kareniacean dinoflagellate species and strains using the amount of toxins, and a different method of comparing their lethality is needed. The results of this study suggest that comparing the mortality of *Acartia* at similar concentrations of Kareniacean dinoflagellates can be a useful method for understanding the relative harmfulness of these organisms. There may be intraspecific variability in the amount of secondary metabolites produced by each dinoflagellate species (e.g., Burkholder and Glibert 2006), and this amount may also be

Table 3 Mortality (%) of *Acartia* incubated with harmful dinoflagellates at the prey concentrations of 1100–1500 ng C mL^{-1} for 2 days, calculated ingestion rate (ng C $predator^{-1}$ day^{-1}) of *Acartia* species feeding on harmful dinoflagellates at 1000 ng C mL^{-1}, and the cell size (ESD, equivalent spherical diameter, µm) and maximum swimming speed (MSS, µm s^{-1}) of the dinoflagellates

Copepod	Dinoflagellate prey (strain or origin)	Mortality	Ingestion rate	ESD	MSS	References
A. tonsa	*Karlodinium armiger* (K-0668)	100[a]		14.9[f]		Berge et al. (2008, 2012)
A. grani	*Karlodinium corsicum* (from Alfacs Bay)	100		12.6		Delgado and Alcaraz (1999) da Costa and Fernández (2002)
A. hongi	*Karenia selliformis* (NIES-4541)	90	1241[c]	29.7	340	This study
A. tonsa	*Margalefidinium polykrikoides* (CP1)	55[b]	2393[bc]	28.2	1449[h]	Jeong et al. (1999) Jiang et al. (2009)
A. tonsa	*Karenia brevis* (CCMP2228)	42[b]		22.4[g]	417[h]	McKay et al. (2006) Waggett et al. (2012)
A. grani	*Gymnodinium catenatum* (GC19V)	20	1247[c]	33.9	440[h]	Fraga et al. (1989) Jeong et al. (2004) da Costa et al. (2012)
A. hongi	*Karenia bicuneiformis* (CAWD81)	10	3427[d]	21.0	480	This study
A. grani	*Karlodinium corsicum* (GCORS1)	6[b]		12.6		da Costa and Fernández (2002) da Costa et al. (2005)
A. tonsa	*Karenia brevis* (Wilson)		3469[ce]	20.4	417[h]	McKay et al. (2006) Cohen et al. (2007)
A. clausi	*Alexandrium minutum* (A1 IV)		3688[d]	22.0	474[h]	Guisande et al. (2002) Lewis et al. (2006)
A. clausi	*Alexandrium minutum* (BAH-ME 91)		5136[c]	19.4	474[h]	Dutz (1998) Lewis et al. (2006)
A. tonsa	*Alexandrium catenella* (1119/27)		5703[bc]	25.8[fg]	185[h]	Karp-Boss et al. (2000) Abdulhussain et al. (2020)
A. hudsonica	*Alexandrium catenella* (CB-301)		7658[de]	19.2	185[h]	Karp-Boss et al. (2000) Colin and Dam (2007)

[a]The mortality at 45 h

[b]Data estimated from the figure

[c]Data calculated by linear interpolation using two adjacent data points

[d]Data calculated using the equation provided in the reference

[e]The highest ingestion rate of *Acartia* spp. feeding on the same strains in several experiments

[f]The median value of the ranges provided in the reference

[g]Data calculated using the equation in Menden-Deuer and Lessard (2000)

[h]Values of a strain different from the listed strain

affected by the growth phase and culture conditions. Thus, with careful consideration of intraspecific variability and the growth phase of the culture, the relative harm to strains or species should be compared.

Among the dinoflagellates causing mortality of *Acartia* spp., the mortality of *Acartia* spp. incubated with cells of *Kl. armiger* K-0668, the Alfacs Bay strain of *Kl. corsicum*, *K. selliformis* NIES-4541, *Margalefidinium polykrikoides* CP1, *K. brevis* CCMP2228, and *Gymnodinium catenatum* GC19V at the dinoflagellate concentrations of 1100–1500 ng C mL^{-1} for 2 d was significantly higher than that in controls such as being incubated without the dinoflagellate species, with their lowest cell density, or with non-toxic prey species (Fig. 6). Physical contact and chemicals (toxins or inhibitory substances) can cause mortality of *Acartia* spp. incubated with these dinoflagellates. Delgado and Alcaraz (1999) reported that the death of *A. grani* was caused by direct physical contact with the Alfacs Bay strain of *Kl. corsicum*. Berge et al. (2012) also reported that *Kl. armiger* K-0668 attacked, immobilized, and ingested *A. tonsa*. In addition, *Kl. armiger* K-0668, *K. brevis* CCMP2228, and *G. catenatum* GC19V contain karmitoxin, brevetoxins, and gonyautoxins and saxitoxin, respectively, which cause mortality of *Acartia* spp. (da Costa et al. 2012; Wagget et al. 2012; Rasmussen et al. 2017). Moreover, *M. polykrikoides* CP1 has harmful effects on copepods and contains reactive oxygen species-like chemicals (Jiang et al. 2009; Tang and Gobler 2009). Therefore, toxins or inhibitory substances of *Kl. armiger* K-0668, *M. polykrikoides* CP1, *K. brevis* CCMP2228, and *G. catenatum* GC19V are likely to cause mortality in *Acartia* spp. Some strains of *K. selliformis* are known to contain toxins such as gymnodimines or brevenal (Miles et al. 2000,

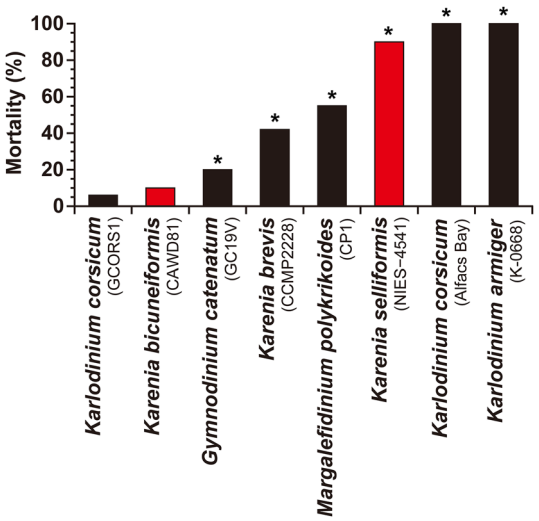

Fig. 6 Mortality (%) of *Acartia* species incubated with harmful dinoflagellates at 1100–1500 ng C mL^{-1} concentrations for 2 days. Red bars indicate the mortality measured in this study. An asterisk on each bar indicates that the value in this figure was significantly or ≥ 20% different from the mortality of *Acartia* species without the target dinoflagellate species (i.e., predator-only control), with the lowest cell density, or with non-toxic prey species in the reference. See Table 3 for details

2003; Mardones et al. 2020), and thus *K. selliformis* NIES-4541 may potentially possess toxins leading to mortality of *A. hongi*. However, the presence of toxins of *K. selliformis* NIES-4541 has not been reported. The Alfacs Bay strain of *Kl. corsicum* at 1100–1500 cells mL^{-1} for 2 days of incubation resulted in high mortality of *A. grani*, while *Kl. corsicum* GCORS1 did not cause significant mortality of *A. grani* at similar *Kl. corsicum* concentrations, implying intraspecific variability (Fig. 6). Therefore, considering potential intraspecific variability, further examination is needed to confirm the presence of toxins of *K. selliformis* NIES-4541.

Effects of two *Karenia* species on the ingestion rate of *A. hongi*

The calculated ingestion rates of *Acartia* spp. on seven reported harmful dinoflagellate species (nine strains) at 1000 ng C mL^{-1} varied, ranging from 1241 to 7658 ng C $predator^{-1}$ day^{-1} (Table 3; Fig. 7a). The calculated ingestion rate of *A. hongi* feeding on *K. bicuneiformis* at 1000 ng C mL^{-1} was lower than those of *Acartia* spp. feeding on *Alexandrium catenalla*, *Alexandrium minutum*, and *K. brevis*; however, it was higher than those of *Acartia* spp. feeding on *M. polykrikoides*, *G. catenatum*, and *K. selliformis*. Thus, the ingestion rate of *A. hongi* feeding on *K. bicuneiformis* was close to the median of those of *Acartia* spp. feeding on harmful dinoflagellates. The calculated ingestion rate of *A. hongi* feeding on *K. selliformis* was lower than that of *Acartia* spp. feeding on the other six harmful dinoflagellates (eight strains) at a prey concentration of 1000 ng C mL^{-1}. The mortality of *A. hongi* incubated with *K. selliformis* for 2 days was higher than those of *Acartia* spp. incubated with *M. polykrikoides*, *G. catenatum*, and *K. bicuneiformis* (Fig. 7b). Thus, this high *A. hongi* mortality may be partially responsible for the low ingestion rate of *A. hongi* feeding on *K. selliformis*.

The calculated ingestion rates of *Acartia* spp. feeding on the nine harmful dinoflagellate strains at a prey concentration of 1000 ng C mL^{-1} showed a negative correlation with the equivalent spherical diameters of the harmful dinoflagellates (19.2–33.9 µm; Pearson's correlation; $r = -0.727$, $P = 0.026$; Fig. 7c). The calculated ingestion rate of *A. hongi* feeding on *K. selliformis* at 1000 ng C mL^{-1} was the lowest, while its equivalent spherical diameter was the second largest. Nival and Nival (1976) reported that modified filtration efficiency could decrease when particle sizes are ≥ 26.2 µm. Thus, the large size of *K. selliformis* may be partially responsible for the relatively low ingestion rate of *A. hongi* feeding on *K. selliformis*.

The calculated ingestion rates of *Acartia* spp. feeding on harmful dinoflagellates at a prey concentration of 1000 ng C mL^{-1} were not significantly correlated with the maximum swimming speeds of the nine harmful dinoflagellate strains (Pearson's correlation; $r = -0.407$, $P = 0.278$; Fig. 7d). The calculated ingestion rate of *A. hongi* feeding on *K. bicuneiformis* was higher than that of *A. hongi* feeding on *K. selliformis*, and the maximum swimming speed of *K. bicuneiformis* was higher than that of *K. selliformis*. Contrary to this trend, the calculated ingestion rate of *A. tonsa* feeding on *M. polykrikoides* was relatively low, but *M. polykrikoides* has the highest maximum swimming speed among the harmful dinoflagellates (Jiang et al. 2009; Jeong et al. 2015). Thus, the fast swimming speed of *M. polykrikoides* may lower the ingestion rate of *Acartia* feeding on it; however, the maximum swimming speed of *K. selliformis* may not be high enough to lower the ingestion rate of *Acartia*.

Difference in the survival and ingestion rates on days 1 and 2

The survival and ingestion rates of *A. hongi* feeding on *K. selliformis* NIES-4541 on day 2 were much lower than those on day 1. Furthermore, the ingestion rates of *A. hongi* feeding on *K. bicuneiformis* CAWD81 at high mean prey concentrations on day 2 were much lower than those on day 1, although the survival on day 2 was not different from those on day 1. Therefore, differences in the survival or ingestion rates of *A. hongi* feeding on *K. bicuneiformis* and *K. selliformis* were observed as the incubation time increased. Thus, if the target dinoflagellate species are harmful, it is

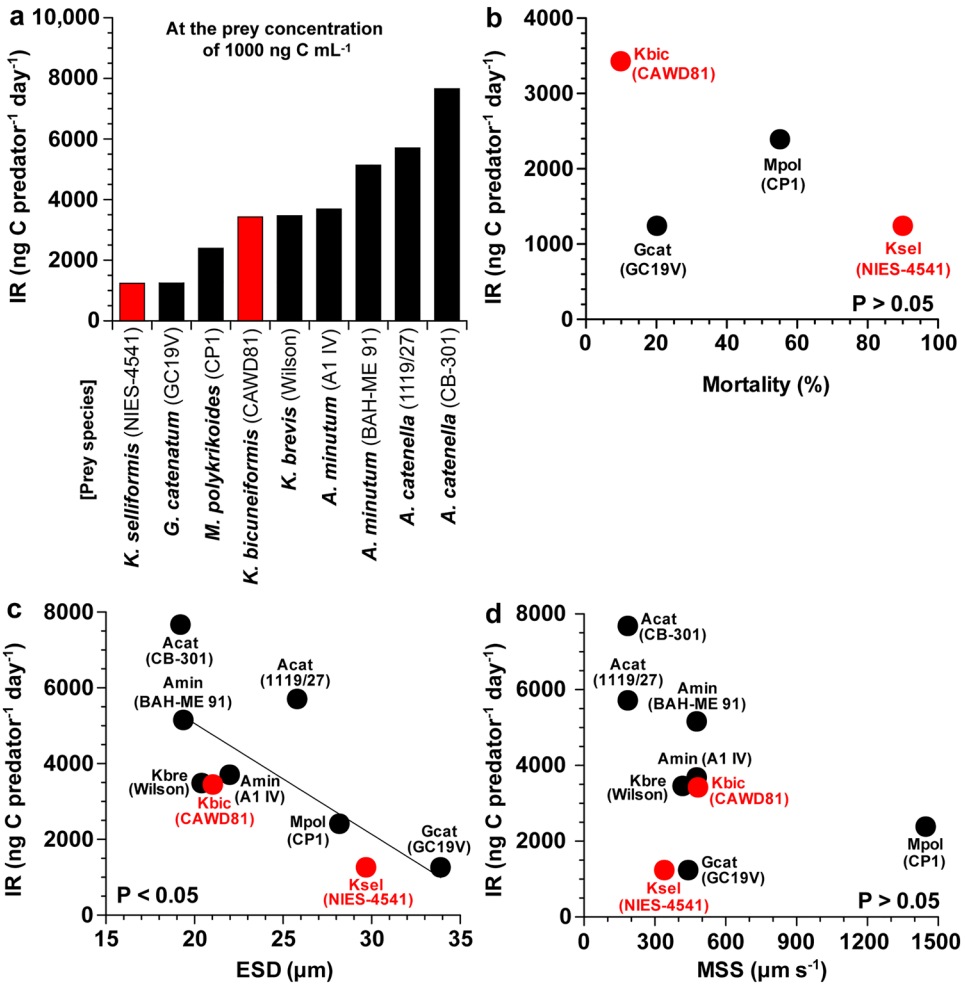

Fig. 7 Calculated ingestion rates (ng C predator^{-1} day^{-1}) of *Acartia* species feeding on harmful dinoflagellates at the prey concentration of 1000 ng C mL^{-1} (IR; **a**). Correlations of IR with mortality (%) of *Acartia* species incubated with the dinoflagellates at 1100–1500 ng C mL^{-1} (**b**) for 2 days, equivalent spherical diameters (ESD) of the dinoflagellates (**c**), and maximum swimming speeds (MSS) of the dinoflagellates (**d**). Red bars or circles indicate the results of this study. See Table 3 for details. Linear equation in (**c**): IR = − 292 (ESD) + 10,900, $r^2 = 0.529$

reasonable to measure the survival and ingestion rates of *Acartia* on days 1 and 2, with subsampling on days 0, 1, and 2.

Ecological implications

Blooms of *K. selliformis* have been reported in many countries, such as Chile, Japan, Kuwait, New Zealand, Russia, and Tunisia (Mackenzie et al. 1996; Clément et al. 2001; Heil et al. 2001; Elleuch et al. 2021; Iwataki et al. 2022; Orlova et al. 2022; Boudriga et al. 2023). During these blooms, the reported *K. selliformis* abundance ranges from 43 to 60,000 cells mL^{-1}; however, it is usually ≥ 140 cells mL^{-1} (Table 4). In the present study, half of *A. hongi* were killed at a *K. selliformis* concentration of 149 cells mL^{-1} on day 2. Moreover, the surviving *A. hongi* did not feed on *K. selliformis* on day 2. Therefore, during *K. selliformis* blooms, *Acartia* spp. may be killed by the inhibiting substances from *K. selliformis* or starvation. Exploring the absence of *Acartia* spp. during *K. selliformis* blooms will be beneficial.

Karenia selliformis is known to lyse several phytoplankton, such as the prasinophyte *Pyramimonas* sp. and the cryptophytes *Teleaulax amphioxeia* and *Storeatula major* (Ok et al. 2023b). Therefore, *K. selliformis* may eliminate alternative prey for the copepods. During *K. selliformis* blooms, *Acartia* spp. may have difficulty maintaining their populations due to the direct (i.e., production of inhibiting substances) and indirect effects of the dinoflagellate (i.e., removal of alternative prey).

The results of this study show that *K. bicuneifomis* was predated by *A. hongi* at prey concentrations of < 500 cells mL^{-1} but not at higher prey concentrations on day 2. Thus, *K. bicuneifomis* cells may be eaten by *A. hongi* at the initial stage of a *K. bicuneifomis* bloom but not during the main bloom.

The interactions between copepods and six of the ten described *Karenia* species (i.e., *K. asterichroma*, *K. brevisulcata*, *K. concordia*, *K. cristata*, *K. longicanalis*, and *K. papilionacea*) have not yet been explored. These *Karenia* species are known to cause HABs (Chang 1999; Yang et al. 2001; Botes et al. 2003; de Salas et al. 2004; Chang

Table 4 Locations and times of *Karenia selliformis* blooms and the highest abundance (cells mL^{-1}) during each bloom

Location	Time	Abundance	References
Magellanic fjords, Chile	Apr, 1999	43	Uribe and Ruiz (2001)
Spaseniya Bay, Russia	Oct, 2020	66	Orlova et al. (2022)
Foveaux Strait, New Zealand	Feb, 1994	140	MacKenzie et al. (1996)
First Kuril Strait, Russia	Oct, 2020	162	Orlova et al. (2022)
Utashud Island, Russia	Oct, 2020	254	Orlova et al. (2022)
Three Sisters Bay, Russia	Oct, 2020	482	Orlova et al. (2022)
Sarannaya Bay, Russia	Oct, 2020	622	Orlova et al. (2022)
Timaru Harbor, New Zealand	Apr, 1994	> 900	MacKenzie et al. (1996)
Ellouza, Tunisia	Jun–Jul, 2020	1604[a]	Elleuch et al. (2021)
Kuwait Bay, Kuwait	Oct, 1999	> 6000	Heil et al. (2001)
Chiloé Archipelago, Chile	Mar–Apr, 1999	8000	Clément et al. (2001)
Hiroo, Japan	Oct, 2021	9600	Iwataki et al. (2022)
Toyokoro, Japan	Oct, 2021	10,560	Iwataki et al. (2022)
Sfax, Tunisia	Sep–Oct, 2019	27,000	Boudriga et al. (2023)
Sfax, Tunisia	Oct, 1994	60,000	Arzul et al. (1995)

[a]qPCR measurement

and Mullan 2012; Yamaguchi et al. 2016). Therefore, to better understand the interactions between copepods and harmful dinoflagellates in marine ecosystems, the survival and ingestion rates of copepods feeding on the six *Karenia* species as a function of the *Karenia* concentration should be explored.

Acknowledgements We are grateful to Eun Ji Kim for technical support.

Author contributions JHO and HJJ designed the study conception and drafted the manuscript. MJL collected copepods. JHO, JHY, SAP, HCK, SHE, and JR conducted experiments including feeding experiments and swimming speed measurement. JHO and HJJ conducted data analyses. All authors discussed the results.

Funding Open Access funding enabled and organized by Seoul National University. This research was supported by the National Research Foundation of Korea funded by the Ministry of Science and ICT (NRF-2021M3I6A1091272; NRF-2021R1A2C1093379; NRF-RS-2023-00291696) award to HJJ and the National Research Foundation of Korea funded by the Ministry of Education (NRF-2022R1A6A3A01086348) award to JHO.

Data availability Data collected and analyzed during this study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of organisms were followed.

Consent to participate Not applicable.

Consent to publish All authors consent to the publication of this manuscript.

References

Abdulhussain AH, Cook KB, Turner AD, Lewis AM, Elsafi MA, Mayor DJ (2020) The influence of the toxin producing dinoflagellate, *Alexandrium catenella* (1119/27), on the feeding and survival of the marine copepod. Acartia Tonsa Harmful Algae 98:101890. https://doi.org/10.1016/j.hal.2020.101890

Alam M, Sanduja R, Hossain MB, Van der Helm D (1982) *Gymnodinium breve* toxins. 1. Isolation and X-ray structure of O, O-dipropyl (E)-2-(1-methyl-2-oxopropylidene) phosphorohydrazidothioate (E)-oxime from the red tide dinoflagellate *Gymnodinium breve*. J Am Chem Soc 104:5232–5234

Arzul G, Turki S, Hamza A, Daniel P, Merceron M (1995) Fish kills induced by phycotoxins. Toxicon 9:1119

Bachvaroff TR, Adolf JE, Place AR (2009) Strain variation in *Karlodinium veneficum* (Dinophyceae): toxin profiles, pigments, and growth characteristics. J Phycol 45:137–153. https://doi.org/10.1111/j.1529-8817.2008.00629.x

Baden DG (1989) Brevetoxins: unique polyether dinoflagellate toxins. FASEB J 3(7):1807–1817. https://doi.org/10.1096/fasebj.3.7.2565840

Band-Schmidt CJ, Bustillos-Guzmán JJ, López-Cortés DJ, Gárate-Lizárraga I, Núñez-Vázquez EJ, Hernández-Sandoval FE (2010) Ecological and physiological studies of *Gymnodinium catenatum* in the Mexican Pacific: A review. Mar Drugs 8:1935–1961. https://doi.org/10.3390/md8061935

Barroso MV, De Carvalho CVA, Antoniassi R, Cerqueira VR (2013) Use of the copepod *Acartia tonsa* as the first live food for larvae of the fat snook *Centropomus parallelus*. Aquac 388:153–158. https://doi.org/10.1016/j.aquaculture.2013.01.022

Basti L, Hégaret H, Shumway SE (2018) Harmful algal blooms and shellfish. In: Shumway SE, Burkholder JM, Morton SL (eds) Harmful algal blooms: a compendium desk reference. Wiley, Singapore. https://doi.org/10.1002/9781118994672.ch4

Berge T, Hansen PJ, Moestrup Ø (2008) Prey size spectrum and bioenergetics of the mixotrophic dinoflagellate *Karlodinium armiger*. Aquat Microb Ecol 50:289–299. https://doi.org/10.3354/ame01166

Berge T, Poulsen LK, Moldrup M, Daugbjerg N, Hansen PJ (2012) Marine microalgae attack and feed on metazoans. ISME J 6:1926–1936. https://doi.org/10.1038/ismej.2012.29

Besiktepe S, Dam HG (2020) Effect of diet on the coupling of ingestion and egg production in the ubiquitous copepod *Acartia tonsa*. Prog Oceanogr 186:102346. https://doi.org/10.1016/j.pocean.2020.102346

Botes L, Sym SD, Pitcher GC (2003) *Karenia cristata* sp. nov. and *Karenia bicuneiformis* sp. nov. (Gymnodiniales, Dinophyceae): two new *Karenia* species from the South African coast. Phycologia 42:563–571. https://doi.org/10.2216/i0031-8884-42-6-563.1

Boudriga I, Abdennadher M, Khammeri Y, Mahfoudi M, Quéméneur M, Hamza A, Haj HmidaZouariHassen NBABMB (2023) *Karenia selliformis* bloom dynamics and growth rate estimation in the Sfax harbour (Tunisia), by using automated flow cytometry equipped with image in flow, during autumn 2019. Harmful Algae 121:102366. https://doi.org/10.1016/j.hal.2022.102366

Bourdelais AJ, Jacocks HM, Wright JL, Bigwarfe PM Jr, Baden DG (2005) A new polyether ladder compound produced by the dinoflagellate *Karenia brevis*. J Nat Prod 68:2–6. https://doi.org/10.1021/np049797o

Breier CF, Buskey EJ (2007) Effects of the red tide dinoflagellate, *Karenia brevis*, on grazing and fecundity in the copepod *Acartia tonsa*. J Plankton Res 29:115–126. https://doi.org/10.1093/plankt/fbl075

Broadwater MH, Van Dolah FM, Fire SE (2018) Vulnerabilities of marine mammals to harmful algal blooms. In: Shumway SE, Burkholder JM, Morton SL (eds) Harmful algal blooms: a compendium desk reference. Wiley, Singapore. https://doi.org/10.1002/9781118994672.ch5

Burkholder JM, Glibert PM (2006) Intraspecific variability: an important consideration in forming generalisations about toxigenic algal species. Afr J Mar Sci 28:177–180. https://doi.org/10.2989/18142320609504143

Burkholder JM, Azanza RV, Sako Y (2006) The ecology of harmful dinoflagellates. In: Granéli E, Turner JT (eds) Ecology of harmful algae. Springer, Berlin, pp 53–66

Calbet A, Carlotti F, Gaudy R (2007) The feeding ecology of the copepod *Centropages typicus* (Kröyer). Prog Oceanogr 72:137–150. https://doi.org/10.1016/j.pocean.2007.01.003

Chang F (1999) *Gymnodinium brevisulcatum* sp. nov. (Gymnodiniales, Dinophyceae), a new species isolated from the 1998 summer toxic bloom in Wellington Harbour. N Z Phycol 38:377–384. https://doi.org/10.2216/i0031-8884-38-5-377.1

Chang FH, Mullan AB (2012) Extended blooms of *Karenia concordia* and other harmful algae from 2009 to 2011 in Wellington Harbour, New Zealand. In: Kim HG, B Reguera, GM Hallegraeff, CK Lee, MS Han, JK Choi (eds) Harmful Algae 2012, Proceedings of the 15th International Conference on Harmful Algae. Maple Design Agency, Busan, pp 199–202

Clément A, Seguel M, Arzul G, Guzmán L, Alarcón C (2001) A widespread outbreak of a haemolytic, ichthyotoxic *Gymnodinium* sp. in southern Chile. In: Hallegraeff GM, Blackburn SI, Bolch CJ, Lewis RJ (eds) Harmful algal blooms 2000. Intergovernmental Oceanographic Commission, UNESCO, Paris, pp 66–69

Cohen JH, Tester PA, Forward RB (2007) Sublethal effects of the toxic dinoflagellate *Karenia brevis* on marine copepod behavior. J Plankton Res 29:301–315. https://doi.org/10.1093/plankt/fbm016

Colin SP, Dam HG (2007) Comparison of the functional and numerical responses of resistant versus non-resistant populations of the copepod *Acartia hudsonica* fed the toxic dinoflagellate *Alexandrium tamarense*. Harmful Algae 6:875–882. https://doi.org/10.1016/j.hal.2007.05.003

da Costa RM, Fernández F (2002) Feeding and survival rates of the copepods *Euterpina acutifrons* Dana and *Acartia grani* Sars on the dinoflagellates *Alexandrium minutum* Balech and *Gyrodinium corsicum* Paulmier and the Chryptophyta *Rhodomonas baltica* Karsten. J Exp Mar Biol Ecol 273:131–142. https://doi.org/10.1016/S0022-0981(02)00132-6

da Costa RM, Franco J, Cacho E, Fernández F (2005) Toxin content and toxic effects of the dinoflagellate *Gyrodinium corsicum* (Paulmier) on the ingestion and survival rates of the copepods *Acartia grani* and *Euterpina acutifrons*. J Exp Mar Biol Ecol 322:177–183. https://doi.org/10.1016/j.jembe.2005.02.017

da Costa RM, Pereira LCC, Ferrnández F (2012) Deterrent effect of *Gymnodinium catenatum* Graham PSP-toxins on grazing performance of marine copepods. Harmful Algae 17:75–82. https://doi.org/10.1016/j.hal.2012.03.002

Davidson K, Miller P, Wilding TA, Shutler J, Bresnan E, Kennington K, Swan S (2009) A large and prolonged bloom of *Karenia mikimotoi* in Scottish waters in 2006. Harmful Algae 8:349–361. https://doi.org/10.1016/j.hal.2008.07.007

de Salas MF, Bolch CJ, Hallegraeff GM (2004) *Karenia asterichroma* sp. nov. (Gymnodiniales, Dinophyceae), a new dinoflagellate species associated with finfish aquaculture mortalities in Tasmania, Australia. Phycologia 43:624–631. https://doi.org/10.2216/i0031-8884-43-5-624.1

Delgado M, Alcaraz M (1999) Interactions between red tide microalgae and herbivorous zooplankton: the noxious effects of *Gyrodinium corsicum* (Dinophyceae) on *Acartia grani* (Copepoda: Calanoida). J Plankton Res 21:2361–2371. https://doi.org/10.1093/plankt/21.12.2361

Dutz J (1998) Repression of fecundity in the neritic copepod *Acartia clausi* exposed to the toxic dinoflagellate *Alexandrium lusitanicum*: relationship between feeding and egg production. Mar Ecol Prog Ser 175:97–107. https://doi.org/10.3354/meps175097

Easton EE, Thistle D (2014) An effective procedure for DNA isolation and voucher recovery from millimeter-scale copepods and new primers for the 18S rRNA and cytb genes. J Exp Mar Biol Ecol 460:135–143. https://doi.org/10.1016/j.jembe.2014.06.016

Elleuch J, Ben Amor F, Barkallah M, Haj Salah J, Smith KF, Aleya L, Fendri I, Abdelkafi S (2021) q-PCR-based assay for the toxic dinoflagellate *Karenia selliformis* monitoring along the Tunisian coasts. Environ Sci Pollut Res 28:57486–57498. https://doi.org/10.1007/s11356-021-14597-9

Fraga S, Gallagher SM, Anderson DM (1989) Chain-forming dinoflagellates: an adaptation to red tides. In: Okaichi T, Anderson D, Nemoto T (eds) Red tides: biology, environmental science and toxicology. Elsevier, Amsterdam, pp 281–284

Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17:805–815. https://doi.org/10.4319/lo.1972.17.6.0805

Gifford DJ (1991) The protozoan-metazoan trophic link in pelagic ecosystems. J Protozool 38:81–86. https://doi.org/10.1111/j.1550-7408.1991.tb04806.x

Gu H, Wu Y, Lü S, Lu D, Tang YZ, Qi Y (2022) Emerging harmful algal bloom species over the last four decades in China. Harmful Algae 111:102059. https://doi.org/10.1016/j.hal.2021.102059

Guillard RRL, Hargraves PE (1993) *Stichochrysis immobilis* is a diatom, not a chrysophyte. Phycologia 32:234–236. https://doi.org/10.2216/i0031-8884-32-3-234.1

Guiry MD, Guiry GM (2023) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org. Accessed 07 July 2023

Guisande C, Frangópulos M, Carotenuto Y, Maneiro I, Riveiro I, Vergara AR (2002) Fate of paralytic shellfish poisoning toxins ingested by the copepod *Acartia clausi*. Mar Ecol Prog Ser 240:105–115. https://doi.org/10.3354/meps240105

Haywood AJ, Steidinger KA, Truby EW, Bergquist PR, Bergquist PL, Adamson J, Mackenzie L (2004) Comparative morphology and molecular phylogenetic analysis of three new species of the genus *Karenia* (Dinophyceae) from New Zealand. J Phycol 40:165–179. https://doi.org/10.1111/j.0022-3646.2004.02-149.x

Hégaret H, da Silva PM, Wikfors GH, Lambert C, De Bettignies T, Shumway SE, Soudant P (2007) Hemocyte responses of *Manila clams*, *Ruditapes philippinarum*, with varying parasite, *Perkinsus olseni*, severity to toxic-algal exposures. Aquat Toxicol 84:469–479. https://doi.org/10.1016/j.aquatox.2007.07.007

Heil CA, Glibert PM, Al-Sarawi MA, Faraj M, Behbehani M, Husain M (2001) First record of a fish-killing *Gymnodinium* sp. bloom in Kuwait Bay, Arabian Sea: chronology and potential causes. Mar Ecol Prog Ser 214:15–23. https://doi.org/10.3354/meps214015

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403–424. https://doi.org/10.1046/j.1529-8817.1999.3520403.x

Holmes MJ, Brust A, Lewis RJ (2014) Dinoflagellate toxins: an overview. In: Botana LM (ed) Seafood and freshwater toxins: pharmacology, physiology and detection, vol 1, 3rd edn. CRC Press, Boca Raton

Hort V, Abadie E, Arnich N, Dechraoui Bottein MY, Amzil Z (2021) Chemodiversity of brevetoxins and other potentially toxic metabolites produced by *Karenia* spp. and their metabolic products in marine organisms. Mar Drugs. https://doi.org/10.3390/md19120656

Iwataki M, Lum WM, Kuwata K, Takahashi K, Arima D, Kuribayashi T, Kosaka Y, Hasegawa N, Watanabe T, Shikata T, Isada T, Orlova TY, Sakamoto S (2022) Morphological variation and phylogeny of *Karenia selliformis* (Gymnodiniales, Dinophyceae) in an intensive cold-water algal bloom in eastern Hokkaido, Japan. Harmful Algae 114:102204. https://doi.org/10.1016/j.hal.2022.102204

Jeong HJ (1994) Predation effects by the calanoid copepod *Acartia tonsa* on the heterotrophic dinoflagellate *Protoperidinium* cf. *divergens* in the presence of co-occurring red-tide dinoflagellate prey. Mar Ecol Prog Ser 111:87–97

Jeong HJ, Shim JH, Kim JS, Park JY, Lee CW, Lee Y (1999) Feeding by the mixotrophic thecate dinoflagellate *Fragilidium* cf. *mexicanum* on red-tide and toxic dinoflagellates. Mar Ecol Prog Ser 176:263–277. https://doi.org/10.3354/meps176263

Jeong HJ, Kang HJ, Shim JS, Park JY, Kim JS, Song JY, Choi HJ (2001) Interactions among the toxic dinoflagellate *Amphidinium carterae*, the heterotrophic dinoflagellate *Oxyrrhis marina*, and the calanoid copepods *Acartia* spp. Mar Ecol Prog Ser 218:77–86. https://doi.org/10.3354/meps218077

Jeong HJ, Yoo YD, Kim JS, Kim TH, Kim JH, Kang NS, Yih WH (2004) Mixotrophy in the phototrophic harmful alga *Cochlodinium polykrikoides* (Dinophycean): prey species, the effects of prey concentration and grazing impact. J Eukaryot Microbiol 51:563–569. https://doi.org/10.1111/j.1550-7408.2004.tb00292.x

Jeong HJ, Yoo YD, Kim JS, Seong KA, Kang NS, Kim TH (2010) Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. Ocean Sci J 45:65–91. https://doi.org/10.1007/s12601-010-0007-2

Jeong HJ, Lim AS, Franks PJ, Lee KH, Kim JH, Kang NS, Lee MJ, Jang SH, Lee SY, Yoon EY, Park JY, Yoo YD, Seong KA, Kwon JE, Jang TY (2015) A hierarchy of conceptual models of red-tide generation: nutrition, behavior, and biological interactions. Harmful Algae 47:97–115. https://doi.org/10.1016/j.hal.2015.06.004

Jeong HJ, Kang HC, Lim AS, Jang SH, Lee K, Lee SY, Ok JH, You JH, Kim JH, Lee KH, Park SA, Eom SH, Yoo YD, Kim KY (2021) Feeding diverse prey as an excellent strategy of mixotrophic dinoflagellates for global dominance. Sci Adv 7:4214. https://doi.org/10.1126/sciadv.abe4214

Jiang X, Tang Y, Lonsdale DJ, Gobler CJ (2009) Deleterious consequences of a red tide dinoflagellate *Cochlodinium polykrikoides* for the calanoid copepod *Acartia tonsa*. Mar Ecol Prog Ser 390:105–116. https://doi.org/10.3354/meps08159

Kang NS, Jeong HJ, Moestrup Ø, Shin W, Nam SW, Park JY, de Salas MF, Kim KW, Noh JH (2010) Description of a new planktonic mixotrophic dinoflagellate *Paragymnodinium shiwhaense* n. gen., n. sp. from the coastal waters off western Korea: morphology, pigments, and ribosomal DNA gene sequence. J Eukaryot Microbiol 57:121–144. https://doi.org/10.1111/j.1550-7408.2009.00462.x

Kang HC, Jeong HJ, Ok JH, Lim AS, Lee K, You JH, Park SA, Eom SH, Lee SY, Lee KH, Jang SH, Yoo YD, Lee MJ, Kim KY (2023) Food web structure for high carbon retention in marine plankton communities. Sci Adv. https://doi.org/10.1126/sciadv.adk0842

Karlson B, Andersen P, Arneborg L, Cembella A, Eikrem W, John U, West JJ, Klemm K, Kobos J, Lehtinen S, Lundholm N, Mazur-Marzec H, Naustvoll L, Poelman M, Provoost P, De Rijcke M, Suikkanen S (2021) Harmful algal blooms and their effects in coastal seas of Northern Europe. Harmful Algae 102:101989. https://doi.org/10.1016/j.hal.2021.101989

Karp-Boss L, Boss E, Jumars PA (2000) Motion of dinoflagellates in a simple shear flow. Limnol Oceanogr 45:1594–1602. https://doi.org/10.4319/lo.2000.45.7.1594

Kim JS, Jeong HJ, Yoo YD, Kang NS, Kim SK, Song JY, Lee MJ, Kim ST, Kang JH, Seong KA, Yih WH (2013) Red tides in Masan Bay, Korea, in 2004–2005: III. Daily variation in the abundance of mesozooplankton and their grazing impacts on red-tide organisms. Harmful Algae 30S:S102–S113. https://doi.org/10.1016/j.hal.2013.10.010

Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99:183–183

Landsberg JH (2002) The effects of harmful algal blooms on aquatic organisms. Rev Fish Sci Aquac 10:113–390. https://doi.org/10.1080/20026491051695

Lee MJ, Jeong HJ, Yoo YD, Park SA, Kang HC (2023) Feeding by the calanoid copepods *Acartia* spp. on the heterotrophic dinoflagellates *Gyrodinium jinhaense*, *G. dominans*, and *G. moestrupii*. Mar Biol. https://doi.org/10.1007/s00227-023-04190-8

Lewis NI, Xu W, Jericho SK, Kreuzer HJ, Jericho MH, Cembella AD (2006) Swimming speed of three species of *Alexandrium* (Dinophyceae) as determined by digital in-line holography. Phycologia 45:61–70. https://doi.org/10.2216/04-59.1

Mackenzie L, Haywood A, Adamson J, Truman P, Till D, Satake M, Yasumoto T (1996) Gymnodimine contamination of shellfish in New Zealand. In: Yasumoto T, Oshima Y, Fukuyo Y (eds) Harmful and toxic algal blooms. Intergovernmental Oceanographic Commission, UNESCO, Paris, pp 97–100

Mardones JI, Norambuena L, Paredes J, Fuenzalida G, Dorantes-Aranda JJ, Chang KJL, Guzmán L, Krock B, Hallegraeff G (2020) Unraveling the *Karenia selliformis* complex with the description of a non-gymnodimine producing Patagonian phylotype. Harmful Algae 98:101892. https://doi.org/10.1016/j.hal.2020.101892

McKay L, Kamykowski D, Milligan E, Schaeffer B, Sinclair G (2006) Comparison of swimming speed and photophysiological responses to different external conditions among three *Karenia brevis* strains. Harmful Algae 5:623–636. https://doi.org/10.1016/j.hal.2005.12.001

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr. https://doi.org/10.4319/lo.2000.45.3.0569

Miles CO, Wilkins AL, Stirling DJ, MacKenzie AL (2003) Gymnodimine C, an isomer of gymnodimine B, from *Karenia selliformis*. J Agric Food Chem 51:4838–4840. https://doi.org/10.1021/jf030101r

Miles CO, Wilkins AL, Stirling DJ, MacKenzie AL (2000) New analogue of gymnodimine from a *Gymnodinium* species. J Agric Food Chem. https://doi.org/10.1021/jf991031k

Mullin MM (1963) Some factors affecting the feeding of marine copepods of the genus *Calanus*. Limnol Oceanogr 8:239–250. https://doi.org/10.4319/lo.1963.8.2.0239

Natsuike M, Kanamori M, Akino H, Sakamoto S, Iwataki M (2023) Lethal effects of the harmful dinoflagellate *Karenia selliformis* (Gymnodiniales, Dinophyceae) on two juvenile kelp sporophytes *Saccharina japonica* and *S. sculpera* (Laminariales, Phaeophyceae). Reg Stud Mar Sci. https://doi.org/10.1016/j.rsma.2023.103094

Nival P, Nival S (1976) Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): Effects on grazing. Limnol Oceanogr 21:24–38. https://doi.org/10.4319/lo.1976.21.1.0024

Ok JH, Jeong HJ, Lim AS, Lee KH (2017) Interactions between the mixotrophic dinoflagellate *Takayama helix* and common heterotrophic protists. Harmful Algae 68:178–191. https://doi.org/10.1016/j.hal.2017.08.006

Ok JH, Jeong HJ, Lim AS, You JH, Yoo YD, Kang HC, Park Sam Lee MJ, Eom SH (2023a) Effects of intrusion and retreat of deep cold waters on the causative species of red tides offshore in the South Sea of Korea. Mar Biol 170:6. https://doi.org/10.1007/s00227-022-04153-5

Ok JH, Jeong HJ, Lim AS, Kang HC, You JH, Park SA, Eom SH (2023b) Lack of mixotrophy in three *Karenia* species and the prey spectrum of *Karenia mikimotoi* (Gymnodiniales, Dinophyceae). Algae 38:39–55. https://doi.org/10.4490/algae.2023.38.2.28

Orlova TY, Aleksanin AI, Lepskaya EV, Efimova KV, Selina MS, Morozova TV, Stonik IV, Kachur VA, Karpenko AA, Vinnikov KA, Adrianov AV, Iwataki M (2022) A massive bloom of *Karenia* species (Dinophyceae) off the Kamchatka coast, Russia, in the fall of 2020. Harmful Algae 120:102337. https://doi.org/10.1016/j.hal.2022.102337

Prasad AK, Shimizu Y (1989) The structure of hemibrevetoxin-B: a new type of toxin in the Gulf of Mexico red tide organism. J Am Chem Soc 111:6476–6477

Rasmussen SA, Binzer SB, Hoeck C, Meier S, de Medeiros LS, Andersen NG, Place A, Nielsen KF, Hansen PJ, Larsen TO (2017) Karmitoxin: An amine-containing polyhydroxy-polyene toxin from the marine dinoflagellate *Karlodinium armiger*. J Nat Prod 80:1287–1293. https://doi.org/10.1021/acs.jnatprod.6b00860

Rombouts I, Beaugrand G, Ibañez F, Gasparini S, Chiba S, Legendre L (2009) Global latitudinal variations in marine copepod diversity and environmental factors. Proc r Soc B: Biol Sci 276:3053–3062. https://doi.org/10.1098/rspb.2009.0742

Satake M, Shoji M, Oshima Y, Naoki H, Fujita T, Yasumoto T (2002) Gymnocin-A, a cytotoxic polyether from the notorious red tide dinoflagellate, *Gymnodinium mikimotoi*. Tetrahedron Lett 43:5829–5832. https://doi.org/10.1016/S0040-4039(02)01171-1

Satake M, Tanaka Y, Ishikura Y, Oshima Y, Naoki H, Yasumoto T (2005) Gymnocin-B with the largest contiguous polyether rings from the red tide dinoflagellate, *Karenia* (formerly *Gymnodinium*) *mikimotoi*. Tetrahedron Lett 46:3537–3540. https://doi.org/10.1016/j.tetlet.2005.03.115

Seki T, Satake M, Mackenzie L, Kaspar HF, Yasumoto T (1995) Gymnodimine, a new marine toxin of unprecedented structure isolated from New Zealand oysters and the dinoflagellate, *Gymnodinium* sp. Tetrahedron Lett 36:7093–7096. https://doi.org/10.1016/0040-4039(95)01434-J

Shin K, Jang MC, Jang PK, Ju SJ, Lee TK, Chang M (2003) Influence of food quality on egg production and viability of the marine planktonic copepod *Acartia omorii*. Prog Oceanogr 57:265–277. https://doi.org/10.1016/S0079-6611(03)00101-0

Speekmann CL, Hyatt CJ, Buskey EJ (2006) Effects of *Karenia brevis* diet on RNA: DNA ratios and egg production of *Acartia tonsa*. Harmful Algae 5:693–704. https://doi.org/10.1016/j.hal.2006.03.002

Steidinger KA (2009) Historical perspective on *Karenia brevis* red tide research in the Gulf of Mexico. Harmful Algae 8:549–561. https://doi.org/10.1016/j.hal.2008.11.009

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599. https://doi.org/10.1093/molbev/msm092

Tang YZ, Gobler CJ (2009) Characterization of the toxicity of *Cochlodinium polykrikoides* isolates from Northeast US estuaries to finfish and shellfish. Harmful Algae 8:454–462. https://doi.org/10.1016/j.hal.2008.10.001

Taylor FJR, Hoppenrath M, Saldarriaga JF (2008) Dinoflagellate Diversity and Distribution. Biodivers Conserv 17:407–418. https://doi.org/10.1007/s10531-007-9258-3

Tillmann U, John U (2002) Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content. Mar Ecol Prog Ser 230:47–58. https://doi.org/10.3354/meps230047

Trainer VL, Pitcher GC, Reguera B, Smayda TJ (2010) The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. Prog Oceanogr 85:33–52. https://doi.org/10.1016/j.pocean.2010.02.003

Truxal LT, Bourdelais AJ, Jacocks H, Abraham WM, Baden DG (2010) Characterization of tamulamides A and B, polyethers isolated from the marine dinoflagellate *Karenia brevis*. J Nat Prod 73:536–540. https://doi.org/10.1021/np900541w

Turner JT (1984) The feeding ecology of some zooplankters that are important prey items of larval fish. NOAA Tech Rep Natl Ma Fish Serv 7:1–28

Turner JT (2004) The importance of small planktonic copepods and their roles in pelagic marine food webs. Zool Stud 43:255–266

Turner JT (2006) Harmful algae interactions with marine planktonic grazers. In: Granéli E, Turner JT (eds) Ecology of harmful algae. Springer, Berlin, pp 259–270

Turner JT (2014) Planktonic marine copepods and harmful algae. Harmful Algae 32:81–93. https://doi.org/10.1016/j.hal.2013.12.001

Turner JT, Tester PA (1992) Zooplankton feeding ecology: bacterivory by metazoan microzooplankton. J Exp Mar Biol Ecol 160:149–167. https://doi.org/10.1016/0022-0981(92)90235-3

Turner JT, Tester PA, Hettler WF (1985) Zooplankton feeding ecology: a laboratory study of predation on fish eggs and larvae by the copepods *Anomalocera ornata* and *Centropages typicus*. Mar Biol 90:1–8. https://doi.org/10.1007/BF00428208

Turner JT, Tester PA, Hansen PJ (1998) Interactions between toxic marine phytoplankton and metazoan and protistan grazers. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. Springer, Heidelberg, pp 453–474

Uribe JC, Ruiz M (2001) *Gymnodinium* brown tide in the Magellanic fjords, southern Chile. Rev Biol Mar Oceanogr 36:155–164

Uye SI, Takamatsu K (1990) Feeding interactions between planktonic copepods and red-tide flagellates from Japanese coastal waters. Mar Ecol Prog Ser 59:97–107

Van Wagoner RM, Satake M, Bourdelais AJ, Baden DG, Wright JL (2010) Absolute configuration of brevisamide and brevisin: confirmation of a universal biosynthetic process for *Karenia brevis* polyethers. J Nat Prod 73:1177–1179. https://doi.org/10.1021/np100159j

Waggett RJ, Hardison DR, Tester PA (2012) Toxicity and nutritional inadequacy of *Karenia brevis*: synergistic mechanisms disrupt top-down grazer control. Mar Ecol Prog Ser 444:15–30. https://doi.org/10.3354/meps09401

Xu J, Kiørboe T (2018) Toxic dinoflagellates produce true grazer deterrents. Ecology 99:2240–2249. https://doi.org/10.1002/ecy.2479

Yamaguchi H, Hirano T, Yoshimatsu T, Tanimoto Y, Matsumoto T, Suzuki S, Hayashi Y, Urabe A, Miyamura K, Sakamoto S, Yamaguchi M, Tomaru Y (2016) Occurrence of *Karenia papilionacea* (Dinophyceae) and its novel sister phylotype in Japanese coastal waters. Harmful Algae 57:59–68. https://doi.org/10.1016/j.hal.2016.04.007

Yamasaki Y, Kim DI, Matsuyama Y, Oda T, Honjo T (2004) Production of superoxide anion and hydrogen peroxide by the red tide dinoflagellate *Karenia mikimotoi*. J Biosci Bioeng. https://doi.org/10.1016/S1389-1723(04)70193-0

Yang ZB, Hodgkiss IJ, Hansen G (2001) Karenia longicanalis sp nov (Dinophyceae) a new bloom-forming species isolated from Hong Kong May 1998. Bot Mar. https://doi.org/10.1515/BOT.2001.009

You JH, Ok JH, Kang HC, Park SA, Eom SH, Jeong HJ (2023) Five phototrophic *Scrippsiella* species lacking mixotrophic ability and the extended prey spectrum of *Scrippsiella acuminata* (Thoracosphaerales, Dinophyceae). Algae 38:111–126. https://doi.org/10.4490/algae.2023.38.6.6

Zeldis JR, Décima M (2020) Mesozooplankton connect the microbial food web to higher trophic levels and vertical export in the New Zealand Subtropical Convergence Zone. Deep-Sea Res I: Oceanogr Res 155:103146. https://doi.org/10.1016/j.dsr.2019.10314