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Harmful Algae 2 (2003) 247–259
HARMFUL ALGAE
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The potential threat of algal blooms to the abalone (*Haliotis midae*) mariculture industry situated around the South African coast

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Received 27 October 2002; received in revised form 18 January 2003; accepted 26 May 2003

Abstract

Toxic algal blooms are common world-wide and pose a serious problem to the aquaculture and fishing industries. Dinoflagellate species such as *Karenia brevis*, *Karenia mikimotoi*, *Heterosigma akashiwo* and *Chatonella* cf. *antiqua* are recognised toxic species implicated in various faunal mortalities. Toxic blooms of *Karenia cristata* were observed on the south coast of South Africa for the first time in 1988 and were responsible for mortalities of wild and farmed abalone. *K. cristata* and various other dinoflagellate species common along the South African coast, as well as *K. mikimotoi* (Isolation site: Norway, Univ. of Copenhagen) and *K. brevis* (Isolation site: Florida, BIGELOW), were tested for toxicity by means of a bioassay involving *Artemia* larvae as well as abalone larvae and spat. *K. cristata*, like *K. brevis*, contains an aerosol toxin; however, the toxin present in *K. cristata* has not yet been isolated and remains unknown. *K. brevis* was, therefore, used to determine which developmental phase of the bloom would affect abalone farms most, and whether ozone could be used as an effective mitigating agent. Of the 17 dinoflagellate species tested, *K. cristata*, *Akashiwo sanguinea*, *K. mikimotoi* and *K. brevis* pose the greatest threat to the abalone mariculture industry. *K. brevis* was most toxic during its exponential and stationary phases. Results suggest that ozone is an effective mitigation agent but its economic viability for use on abalone farms must still be investigated.

Keywords: Abalone; *Haliotis midae*; Harmful algal blooms; *Karenia cristata*; Mariculture; South Africa

1. Introduction

The global increase of harmful algal blooms (HABs) and their effects on shellfish such as oysters, mussels, surfclams and scallops are well documented (Matsuyama et al., 1998b; Matsuyama, 1999; Nagai et al., 1996; Shumway et al., 1990; Shumway and Cambella, 1993; Shumway, 1995; Shumway et al., 1994; Smolowitz and Shumway, 1997). The consequences of these blooms have developed into a world-wide concern over the past several decades. Not all HABs are directly or even indirectly toxic, but some like the 'brown tide' species *Aureococcus anophagefferens* Hargraves et Sieburth, can interfere with the ability of bivalves to ingest algae, and thus reduce growth rates (Bricelj and Lonsdale, 1997; Probyn et al., 2001). Other than the reported toxic effect of *Heterocapsa circularisquama* Horiguchi and

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doi:10.1016/S1568-9883(03)00044-1

Karenia mikimotoi (Miyake et Kominami ex Oda) G. Hansen et Moestrup on the abalone, *Haliotis discus* Reeve (Matsuyama et al., 1998a), and the two cases of paralytic shellfish poisoning (PSP) toxicity in *Haliotis tuberculata* Linnaeus from north-west Spain (Bravo et al., 1996; Bravo et al., 1999; Martinez et al., 1993; Nagashima et al., 1995) and *Haliotis midae* Linnaeus from South Africa (Pitcher et al., 2001), no published information is available on the effect of HABs on the abalone mariculture industry.

The first aim of this study was to investigate the effect of various microalgal species on abalone larvae and abalone spat (3 mm individuals), and to assess whether these microalgae might pose a threat to the mariculture industry, particularly on the south coast of South Africa. The second aim was to determine the developmental phase of algal cultures most toxic to abalone. Finally, the application of ozone as a potential mitigation agent was investigated. Although the primary objective of this study was to examine the toxicity of various algal species to abalone larvae and juveniles, the bioassay used is that which was originally designed for bioassays with *Artemia* (ARTOXKIT M). The ARTOXKIT M standardised bioassay is routinely used internationally in marine research and aquatic toxicology (Chang and Redfearn, 1999; Demaret et al., 1995; Lush and Hallegraeff, 1996; Medlyn, 1980; Persoone and Wells, 1987) because of the commercial availability of dried *Artemia* cysts, and the simplicity and cost-effectiveness of the kit. We incorporated this bioassay into our study so that our results can be compared to those of other studies using the ARTOXKIT M assay. Because of the difficulty of maintaining *Karenia cristata* Botes, Sym et Pitcher (Botes et al., in press) in culture, *Karenia brevis* (Davis) G. Hansen et Moestrup (which, like *K. cristata*, contains an aerosol toxin (Hemmert, 1975)) was used for the second and third experiments described in this paper.

2. Materials and methods

2.1. Establishment of unialgal cultures

Dinoflagellate species commonly found along the South African coast (Table 1) were isolated into culture vessels containing F/2 (Guillard and Ryther, 1962) and Keller (Keller and Guillard, 1985) growth media. The culture vessels were maintained in incubators at 18 °C under a photon flux density of ca. 200 μE m^{-2} s^{-1} (14:10 h LD photoperiod). Cultures were subsequently sub-cultured into larger containers.

Table 1
List of algal species, and their origin, tested for toxicity

Species	Origin
Akashiwo sanguinea (Hirasaka) G. Hansen et Moestrup	False Bay, RSA
Chatonella cf. *antiqua* (Hada) Ono	False Bay, RSA
Gonyolax polygramma Stein	False Bay, RSA
Gymnodinium aureolum (Hulburt) G. Hansen et Moestrup	Table Bay, RSA
Karenia cristata Botes, Sym et Pitcher	False Bay, RSA
Takayama helix de Salas et al., in press	False Bay, RSA
Gyrodinium cf. *zeta* Larsen	Lamberts Bay, RSA
Gyrodinium cf. *corsicum* Paulmier, Berland, Billard et Nezan	False Bay, RSA
Heterosigma akashiwo (Hada) ex Sourina	False Bay, RSA
Heterocapsa orientalis Iwataki, Botes et Fukuyo	False Bay, RSA
Lepidodinium viride Watanabe, Suda, Sawaguchi et Chihara	False Bay, RSA
Prorocentrum micans Ehrenberg	False Bay, RSA
Prorocentrum rostratum Stein	False Bay, RSA
Prorocentrum triestinum Schiller	False Bay, RSA
Scrippsiella trochoidea (Stein) Balech	False Bay, RSA
Karenia brevis (Davis) G. Hansen et Moestrup	Florida, USA
Karenia mikimotoi (Miyake et Komani ex Oda) G. Hansen et Moestrup	Norway, EUR

2.2. Artemia and abalone bioassay

Hatching of *Artemia* cysts was performed as outlined in the ARTOXKIT M standard operational procedure. The test design of the ARTOXKIT M is based on a disposable multi-well test plate (four rows and six columns) using a control (column 1) and five increasing toxicant concentrations (columns 2–6), each with four replicates (rows). The control contained 2 ml of seawater (20 μm-filtered and UV-treated), while the five treatment concentrations contained 2 ml of dinoflagellate cultures arranged in increasing concentrations from columns 2 to 6. Subsequently, 10 2-day-old *Artemia* larvae (second instar stage) were transferred into each of the four replicate wells of each column. After 24 h, the dead larvae (larvae were considered

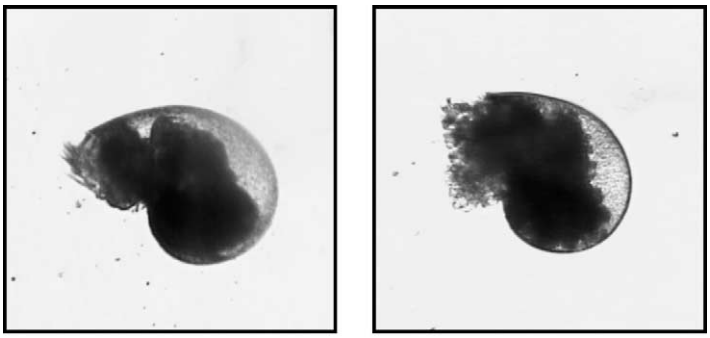

Fig. 1. Two-day-old abalone larvae: (a) before, with intact velum and body, and (b) after, with a flocculated appearance of the velum and body, exposure to toxic dinoflagellate cultures.

dead if they did not exhibit any internal or external movement over 30 s of observation) in each well were counted. The abalone larvae bioassay was similar to the *Artemia* bioassay except that 10 abalone larvae (2-day-old) were placed into each replicate well in place of the *Artemia* larvae. After 24 h, the abalone larvae were considered dead if the larvae inside the shell appeared to have ruptured (Fig. 1). For the abalone spat bioassay, five individuals (3 mm animals) were transferred into each replicate well and were deemed dead after 24 hrs if they were detached from the sides of the container, were unable to right themselves after dropping off the sides, and failed to show tentacle movement after 30 s of observation.

2.3. Assessment of 17 potentially toxic dinoflagellate species

This experiment was included to investigate the potential threat that dinoflagellate species might pose to the developing South African abalone mariculture industry. Cultures of the various microalgal species were isolated and maintained as previously described, with the exception of *K. cristata* which was collected in the field and concentrated by filtration through a 15 μm mesh to increase cell concentration. The toxicity of each species (in exponential phase) to *Artemia* larvae, abalone larvae and abalone spat was investigated in three ways: (a) cultures with intact cells (whole cells), (b) cultures that were sonicated (broken cells) and (c) cultures with cells removed by filtration on Whatman GF/F filter paper (filtrate).

2.4. Mortality associated with different phases of the bloom event

This experiment was designed to determine whether the four developmental phases (lag, exponential, stationary and death phases) of the 'red tide' exhibited different toxicity effects on abalone larvae and spat. Four culture flasks were inoculated with *K. brevis* at the start of the experiment. A Turner Designs fluorometer was used to determine the growth phase of the culture within each culture flask (Parsons et al., 1984). Toxicity of *K. brevis* (whole cells, broken cells and the filtrate) to *Artemia* larvae, abalone larvae and spat was tested for each of the four phases.

2.5. Evaluation of ozone as a mitigation possibility

After a preliminary experiment revealed that using an Aquazone Purifiers ozonizer (output = 250 mg h^{-1}), cells of *K. mikimotoi*, *K. brevis*, *Akashiwo sanguinea* (Hirasaka) G. Hansen et Moestrup, *Heterosigma akashiwo* (Hada) ex Sourina and *Chatonella* cf. *antiqua* (Hada) Ono were killed after 10 min (41.67 mg per 10 min) of ozonation, this ozonation regime was used in subsequent experiments.

K. brevis (7.8×10^6 cell l^{-1}) was used to establish whether ozone could be used as a mitigation agent. Column 1 (control) of the ARTOXKIT M contained 20 μm filtered and UV-treated seawater, column 2 (ozonated control) contained ozonated 20 μm filtered and UV-treated seawater, column 3 (toxicity test) contained *K. brevis* culture with intact cells (exponen-

tial phase), columns 4–6 (tests for mitigation effect of ozone) contained ozonated culture with intact cells (whole cells), ozonated culture with broken cells (broken cells) and ozonated culture where cells have been filtered out (filtrate), respectively. In order to minimise free radicals present during the experiments, the flasks were left to stand for 1 h before proceeding with the experiments.

2.6. Statistical analyses

When assessing the toxicity of the 17 potentially toxic dinoflagellate species, the mortality rate of *Artemia* larvae, abalone larvae and spat as affected by dinoflagellate cell concentration was modelled using a binomial (with probit link) general linear model (GLM). To determine whether larval or spat death at any of the cell concentrations (i.e. treatment concentrations) was significantly greater with respect to that of the control, animal mortality at each dinoflagellate cell concentration was compared with that of the control by treating dinoflagellate cell concentration as a factor in the GLM analysis. The result is a table of *P*-values that indicate the probability of mortality being significantly greater ($P > 0.05$) with respect to the control. To derive 10 and 50% lethal concentrations (LC_{10} and LC_{50}, respectively) and associated standard errors, the 'dose·P' procedure in the 'MASS' package of Venables and Ripley (1999) was used subsequent to fitting the GLM model. Even though it is standard procedure to calculate LC_{50} values, LC_{10} values were also calculated. With dinoflagellate cell concentration as a continuous variable rather than a factor (as in the preceding contrast analysis), LC_{10} or LC_{50} values could not be calculated in situations where the observed proportion of larval or spat mortalities failed to exceed 10 or 50% mortality. As probit analysis requires a consistent trend of mortality with increasing cell concentration, LC-values could not be estimated when mortalities were high (above 10 or 50%) at intermediate cell concentrations but low at low and high cell concentrations, as was sometimes the case with abalone spat. In the few cases where LC-values could not be determined using probit analysis (despite consistent trends in mortality), we used loess smoothing to derive estimates. In such cases standard errors of the estimates are not reported. When assessing the mortality associated with different phases of the bloom event, Student's *t*-tests were used to statistically compare estimates of LC_{50} between growth phases. Statistical analysis of results obtained from the experiment investigating ozone as a possible mitigation agent was similar to that of experiment A where *Artemia* larvae, abalone larvae and spat mortalities in each treatment were compared with that of the control using GLM. All statistical procedures were performed using version 1.6.1 of the statistical package, *R*, an implementation of the S-language (Ihaka and Gentleman, 1996).

3. Results

3.1. Assessment of 17 potentially toxic dinoflagellate species

The toxicity assays (Table 2) suggest that toxicity differed for *Artemia* larvae, abalone larvae and abalone spat. Toxicity also differed when whole or broken cells, or the filtrate of the algal cultures was used, and when the animals were exposed to differing dinoflagellate cell concentrations. As it is unlikely that abalone farmers could afford to lose 50% of the animals, both LC_{50} and LC_{10} values are presented as these could be used to indicate when mitigation actions should be taken. Of the 17 dinoflagellate species tested, *A. sanguinea* (Fig. 2), *K. cristata* (Fig. 3), *K. brevis* (Fig. 4) and *K. mikimotoi* (Fig. 5) (Table 2) could pose a significant threat to abalone mariculture. It is of interest to note, however, that none of these four dinoflagellates species caused *Artemia* larval mortalities >10%, and in most cases mortalities of *Artemia* were not significant.

Akashiwo sanguinea (Fig. 2) caused significant mortality in abalone larvae, resulting in LC_{50} and LC_{10} values of $3.1 \times 10^5 \pm 6.8 \times 10^3$ and $6.2 \times 10^4 \pm 9.7 \times 10^3$ cells l^{-1}, respectively, for whole cells, and LC_{50} and LC_{10} values of ca. 3.6×10^5 cells l^{-1} (no S.E. available) and 5.5×10^4 cells l^{-1} (no S.E. available), respectively, for broken cells. No LC_{50} or LC_{10} values could be determined for the filtrate because of the inconsistent trend of abalone larval mortalities with increasing cell concentrations. Toxicity of *A. sanguinea* to the abalone spat was not as severe as for the larvae, with median mortality rates of above 40% only attained at one concentration of whole cells.

Table 2
Results of toxicity assays on 17 dinoflagellate species (‘whole cells’, ‘broken cells’ and ‘filtrate’) on *Artemia* larvae (ArtL) and abalone larvae (AL) and spat (AS)

Species	Tested on	Whole cells			Broken cells			Filtrate		
		P-value	LC_{10}	LC_{50}	*P*-value	LC_{10}	LC_{50}	*P*-value	LC_{10}	LC_{50}
C. cf. *antiqua*	ArtL	ns	a	a	***	$3.6 \times 10^6 \pm 2.2 \times 10^5$	b	*	a	a
	AL	ns	a	a	ns	a	a	ns	a	a
	AS	***	a	a	***	a	a	***	$5.5 \times 10^6 \pm 2.8 \times 10^5$	a
G. aureolum	ArtL	*	a	a	*	a	a	ns	a	a
	AL	ns	a	a	ns	a	a	ns	a	a
	AS	***	a	a	***	a	a	ns	a	a
K. brevis	ArtL	ns	a	a	ns	a	a	ns	a	a
	AL	ns	a	a	ns	a	a	ns	a	a
	AS	***	$7.5 \times 10^5 \pm 5.6 \times 10^4$	$2.4 \times 10^6 \pm 4.6 \times 10^4$	***	$1.5 \times 10^6 \pm 9.2 \times 10^4$	$4.7 \times 10^6 \pm 2.3 \times 10^5$	***	$8.9 \times 10^5 \pm 6.2 \times 10^4$	$2.7 \times 10^6 \pm 5.9 \times 10^4$
G. cf. *corsicum*	ArtL	*	a	a	ns	a	a	*	a	a
	AL	ns	a	a	ns	a	a	ns	a	a
	AS	ns	a	a	ns	a	a	***	a	a
G. polygramma	ArtL	ns	a	a	*	a	a	ns	a	a
	AL	*	a	a	ns	a	a	ns	a	a
	AS	ns	a	a	***	a	a	***	a	a
T. helix	ArtL	ns	a	a	ns	a	a	ns	a	a
	AL	***	$8.4 \times 10^5 \pm 6.5 \times 10^4$	a	***	$1.1 \times 10^6 \pm 8.3 \times 10^4$	a	***	a	a
	AS	***	a	a	ns	a	a	***	a	a
K. mikimotoi	ArtL	ns	a	a	*	a	a	ns	a	a
	AL	***	$3.3 \times 10^5 \pm 4.1 \times 10^4$	$1.1 \times 10^6 \pm 2.6 \times 10^5$	***	ca. 2.6×10^5[d]	ca. 1.4×10^6[d]	***	ca. 3.4×10^5[d]	ca. 3.2×10^6[d]
	AS	***	c	c	***	c	c	***	c	c
A. sanguinea	ArtL	ns	a	a	*	a	a	ns	a	a
	AL	***	$6.2 \times 10^4 \pm 9.7 \times 10^3$	$3.1 \times 10^5 \pm 6.8 \times 10^3$	***	ca. 5.5×10^4[d]	ca. 3.6×10^5[d]	***	c	c
	AS	***	c	b	***	a	a	ns	a	a
G. cf. *zeta*	ArtL	*	a	a	ns	a	a	*	a	a
	AL	*	a	a	ns	a	a	*	a	a
	AS	***	a	a	***	a	a	ns	a	a
H. akashiwo	ArtL	*	a	a	*	a	a	ns	a	a
	AL	ns	a	a	ns	a	a	ns	a	a
	AS	ns	a	a	***	$6.3 \times 10^6 \pm 3.3 \times 10^5$	a	ns	a	a
H. orientalis	ArtL	ns	a	a	ns	a	a	ns	a	a
	AL	ns	a	a	***	a	a	ns	a	a
	AS	ns	a	a	***	a	a	ns	a	a
K. cristata	ArtL	*	a	a	ns	a	a	*	a	a
	AL	***	$1.1 \times 10^6 \pm 5.8 \times 10^4$	$2.7 \times 10^6 \pm 4.6 \times 10^4$	***	a	a	ns	a	a
	AS	***	$2.5 \times 10^6 \pm 7.4 \times 10^4$	a	ns	a	a	***	a	a
L. viride	ArtL	ns	a	a	ns	a	a	ns	a	a
	AL	ns	a	a	***	a	a	ns	a	a
	AS	ns	a	a	***	a	a	ns	a	a
P. micans	ArtL	ns	a	a	*	a	a	ns	a	a
	AL	*	a	a	ns	a	a	***	a	a
	AS	ns	a	a	***	c	a	**	a	a

Table 2 (*Continued*)

Species	Tested on	Whole cells			Broken cells			Filtrate		
		P-value	LC_{10}	LC_{50}	*P*-value	LC_{10}	LC_{50}	*P*-value	LC_{10}	LC_{50}
P. rostratum	ArtL	*	a	a	ns	a	a	ns	a	a
	AL	*	a	a	ns	a	a	*	a	a
	AS	***	a	a	***	a	a	ns	a	a
P. triestinum	ArtL	*	a	a	*	a	a	***	a	a
	AL	*	a	a	***	a	a	ns	a	a
	AS	ns	a	a	***	a	a	ns	a	a
S. trochoidea	ArtL	*	a	a	*	a	a	*	a	a
	AL	***	a	a	ns	a	a	*	a	a
	AS	ns	a	a	***	a	a	***	c	a

Significance levels (ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.0001$) indicate whether assays resulted in higher mortality rates of larvae or spat when exposed to any of the five cell concentrations tested with respect to mortality rates in controls (in the absence of dinoflagellate cells). LC_{50} values (mean number of cells l^{-1} ± S.E. of estimate) could only be calculated for certain species, and LC_{10} values are thus given for the sake of convenience for some of the 'less toxic' species. Note that statistically significant mortality effects (with respect to the controls) could still be detected even when very low mortality rates eliminated the possibility of calculating LC_{10} values.

[a] Mortality always below 10%.

[b] Mortality below 50%.

[c] Mortality above 10 or 50%, but inconsistent trend of mortality with increasing concentration prevented determination of LC-values.

[d] LC-values predicted from loess curves.

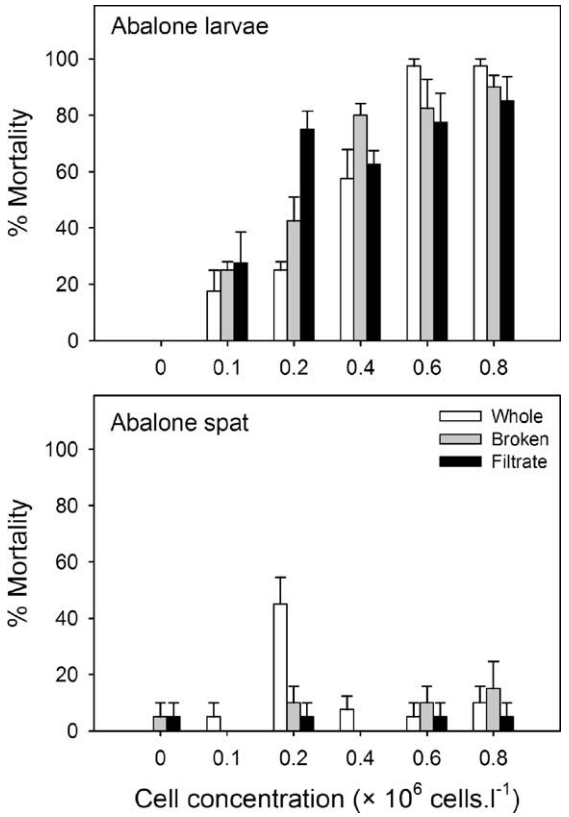

Fig. 2. Graph of percentage mortality of abalone larvae and abalone spat after 24 h of exposure to *Akashiwo sanguinea* (Whiskers = mean + S.E.).

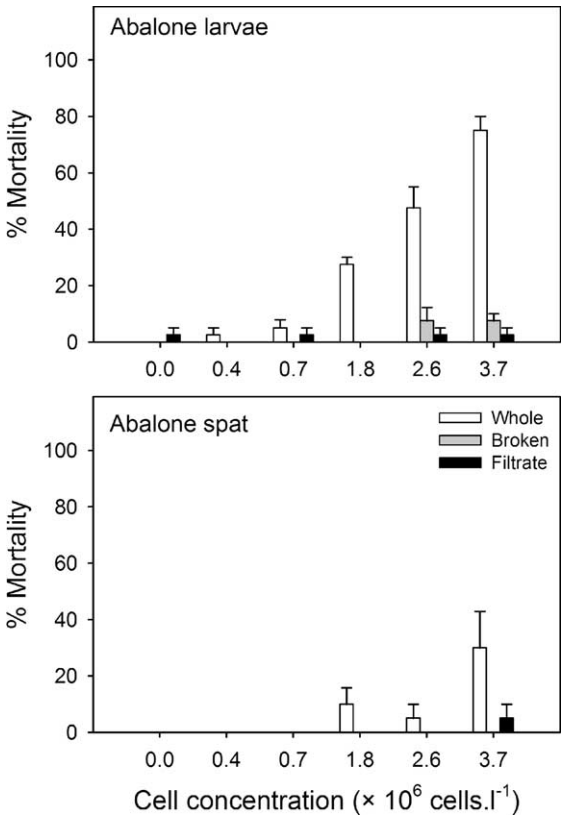

Fig. 3. Graph of percentage mortality of abalone larvae and abalone spat after 24 h of exposure to *K. cristata* (Whiskers = mean+S.E.).

The *K. cristata* (Fig. 3) assay involving whole cells resulted in abalone larval mortalities with an estimated LC_{50} value of $2.7 \times 10^6 \pm 4.7 \times 10^4$ cells l^{-1} and LC_{10} value of $1.1 \times 10^6 \pm 5.8 \times 10^4$ cells l^{-1}. Abalone spat mortality rates of around 30% were observed at the highest cell density tested with a LC_{10} of $2.5 \times 10^6 \pm 7.3 \times 10^4$ cells l^{-1}.

The toxic effect of *K. brevis* was restricted to abalone spat (Fig. 4) with a LC_{50} value of $2.4 \times 10^6 \pm 4.6 \times 10^4$ cells l^{-1} and a LC_{10} value of $7.5 \times 10^5 \pm 5.6 \times 10^4$ cells l^{-1} for whole cells. LC_{50} and LC_{10} values of $4.7 \times 10^6 \pm 2.3 \times 10^5$ and $1.5 \times 10^6 \pm 9.3 \times 10^4$ cells l^{-1}, respectively, were determined for broken cells, while LC_{50} and LC_{10} values for the filtrate were $2.7 \times 10^6 \pm 6.2 \times 10^4$ and $8.9 \times 10^5 \pm 6.2 \times 10^4$ cells l^{-1}, respectively.

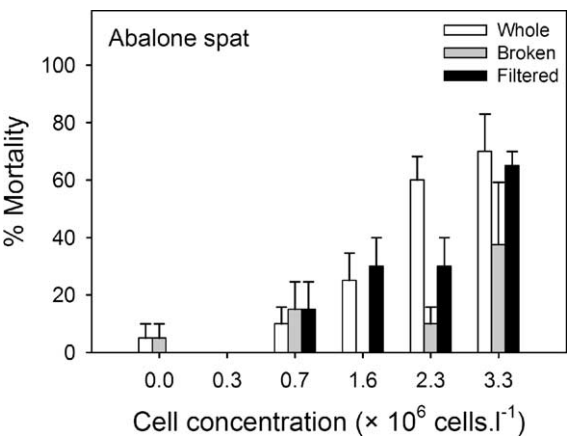

Fig. 4. Graph of percentage mortality of abalone spat after 24 h of exposure to *Karenia brevis* (Whiskers = mean + S.E.).

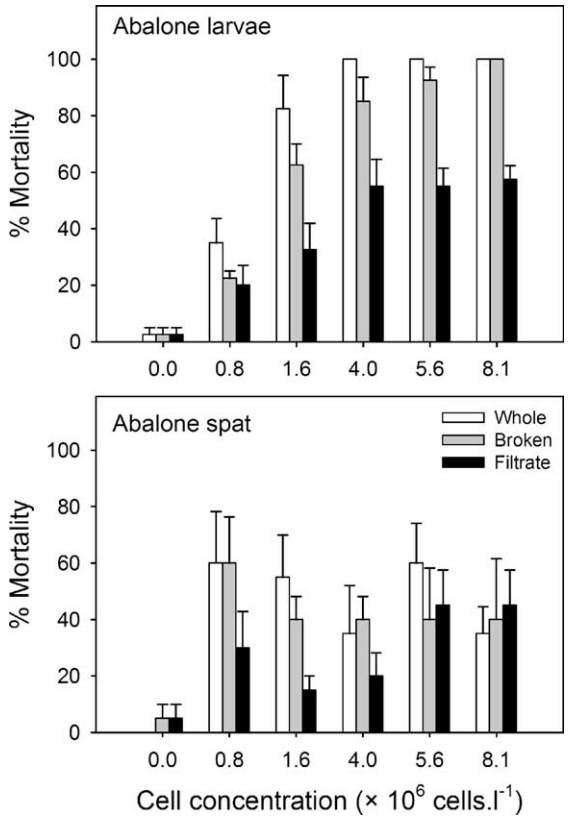

Fig. 5. Graph of percentage mortality of abalone larvae and abalone spat after 24 h of exposure to *Karenia mikimotoi* (Whiskers = mean + S.E.).

K. mikimotoi caused significant abalone larval and spat mortalities (Fig. 5). The LC_{50} for whole cells was estimated at $1.1 \times 10^6 \pm 2.6 \times 10^4$ cells l^{-1} with LC_{10} at $3.3 \times 10^5 \pm 4.1 \times 10^4$ cells l^{-1}. For broken cells, LC_{50} was ca. 1.4×10^6 cells l^{-1} with the lower LC_{10} value at ca. 2.6×10^5 cells l^{-1}. LC_{50} and LC_{10} for the filtrate were ca. 3.2×10^6 and 3.4×10^5 cells l^{-1}, respectively (estimates were obtained from fitting loess curves, and S.E. values could not be estimated). Toxicity effects of this species on abalone spat were also significant, with mortalities of >50% even at the lowest cell concentration tested. However, no clear relationship between *K. mikimotoi* concentration and mortality was evident, with median mortality rates increasing then decreasing with increasing cell density.

C. cf. *antiqua*, *H. akashiwo*, *Gymnodinium* cf. *pulchellum* Larsen, *Prorocentrum micans* Ehrenberg and *Scrippsiella trochoidea* (Stein) Balech resulted in *Artemia* larvae or abalone larvae or spat mortalities of >10% (Table 2). The LC_{10} value for the effect of *C.* cf. *antiqua* (filtrate) on abalone spat is $5.5 \times 10^6 \pm 2.8 \times 10^5$ and $3.6 \times 10^6 \pm 2.2 \times 10^5$ cells l^{-1} for *C.* cf. *antiqua* (broken cells); the LC_{10} for *H. akashiwo* (broken cells) on abalone spat is $6.3 \times 10^6 \pm 3.3 \times 10^5$ and $8.4 \times 10^6 \pm 6.5 \times 10^4$ cells l^{-1} for *G.* cf. *pulchellum* (whole cells) on abalone larvae; and the LC_{10} for *G.* cf. *pulchellum* (broken cells) on abalone larvae is $1.1 \times 10^6 \pm 8.3 \times 10^4$ cells l^{-1}. No LC_{10} values could be obtained for *S. trochoidea*.

Although death rates of *Artemia* larvae, abalone larvae and abalone spat when exposed to *Gymnodinium aureolum* (Hulburt) G. Hansen et Moestrup, *Gyrodinium* cf. *corsicum* Paulmier, Berland, Billard et Nezan, *Gonyolax polygramma* Stein, *Gyrodinium* cf. *zeta* Larsen, *Heterocapsa orientalis* Iwataki, Botes et Fukuyo (Iwataki et al., in press), *Lepidodinium viride* Watanabe, Suda, Sawaguchi et Chihara, *Prorocentrum rostratum* Stein and *Prorocentrum triestinum* Schiller were in some circumstances greater than that of their respective controls, in no case did mortalities exceed 10% (Table 2) and, therefore, in the context of the current study, mortalities were considered to be relatively insignificant.

3.2. Mortality effects associated with different phases of the bloom event

Results of toxicity assays of *K. brevis* (during lag, exponential, stationary and death phase) on abalone spat are presented in Fig. 6 and Table 3. Neither whole cells, broken cells nor filtrate resulted in significant mortality effects on abalone spat at any concentration tested during the lag phase. Significant mortalities were, however, obtained during the exponential and stationary phases with mortalities during the exponential phase occurring at lower cell densities than in the stationary phase. In the death phase, mortalities occurred at much higher cell densities than in the stationary phase (LC_{50} values and Student's *t*-test, $P < 0.005$ in Table 3). *K. brevis*, in the exponential phase is, therefore, significantly more toxic than in stationary phase. Toxicity diminishes once the cultures reach the death phase, and LC_{50} values would most likely

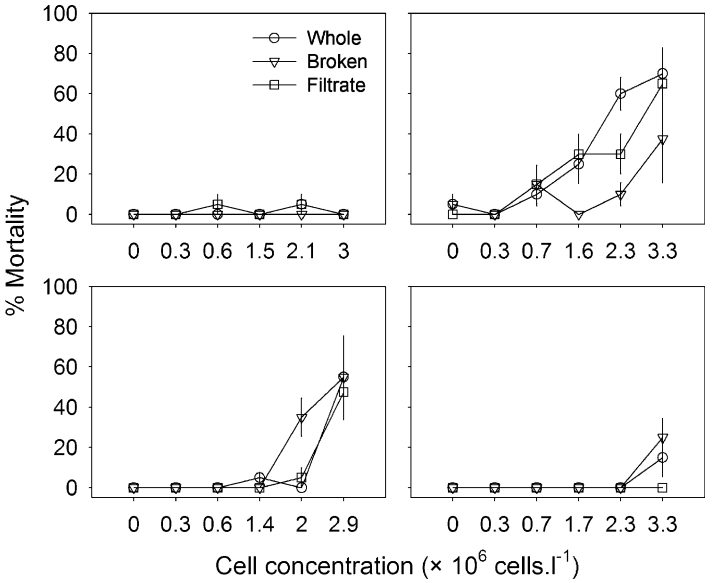

Fig. 6. Graph of percentage abalone spat mortalities during the four growth phases of *Karenia brevis* and various cell concentrations (top left, lag phase; top right, exponential phase; bottom left, stationary phase; bottom right, death phase). Estimated LC_{50} values are presented in Table 3 (symbols represent mean mortalities and vertical bars represent ±S.E.).

Table 3
LC_{50} values (mean number of cells l^{-1} ± S.E. of estimate) showing the effect of *Karenia brevis*, obtained from the exponential and stationary cell growth phases, on abalone spat

Treatment	Exponential phase	Stationary phase	Student's *t*-value	*P*-value
Whole cells	$2.4 \times 10^6 \pm 4.6 \times 10^4$	$2.8 \times 10^6 \pm 3.7 \times 10^4$	−16.56	<0.0001
Broken cells	$4.7 \times 10^6 \pm 2.3 \times 10^5$	$2.6 \times 10^6 \pm 3.8 \times 10^4$	17.25	<0.0001
Filtrate	$2.7 \times 10^6 \pm 5.8 \times 10^4$	$2.9 \times 10^6 \pm 3.3 \times 10^4$	−5.17	<0.005

LC_{50} values could not be estimated for the lag and death phases because at no stage did more than half the animals die within the range of cell concentrations tested (see Fig. 6). The Student's *t*-tests determine whether LC_{50} values of cells obtained from the exponential phase differ from that of the stationary phase for each of the treatment groups.

be at concentration in excess of those tested in this experiment.

3.3. Evaluation of ozone as a mitigation possibility

K. brevis had no effect on *Artemia* larvae or abalone larvae, but the culture with whole cells (7.8×10^6 cells l^{-1}) resulted in 100% mortality of abalone spat. The culture (whole and broken cells, as well as the filtrate) after ozonation for 10 min, had no adverse effect on the assay animals and neither did the control that was ozonated (Fig. 7).

4. Discussion

4.1. Assessment of 17 potentially toxic dinoflagellate species

Although none of the 17 dinoflagellate species tested (at cell concentrations $<10 \times 10^6$ cells l^{-1}) resulted in high mortalities on the 2-day-old *Artemia* larvae used in our assays, several other studies have found evidence to the contrary. For example, death among 4-day-old *Artemia* larvae resulted from exposure to *Alexandrium minutum* Halim and *Heterosigma carterae* (Hulburt) Taylor (syn. *H. akashiwo* (Hada)

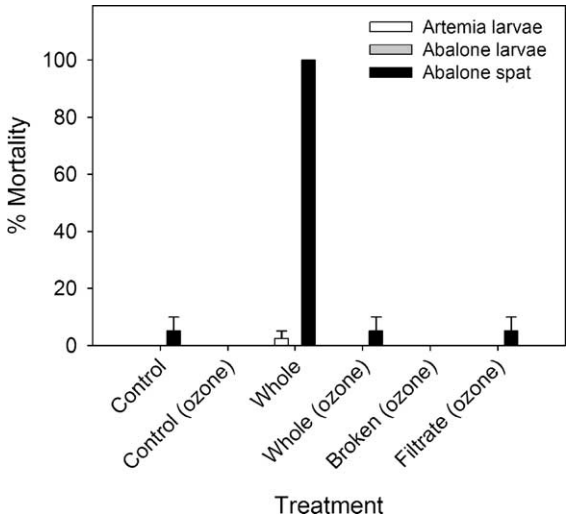

Fig. 7. Graph of percentage mortality of *Artemia* larvae, abalone larvae and abalone spat when exposed to *Karenia brevis* (7.8×10^6 cells l^{-1}) and using various ozonation treatments and controls (Whiskers = mean + S.E.).

Hada ex Y. Hara et Chihara) (Lush and Hallegraeff, 1996) and the *Gymnodinium* sp. which bloomed in Wellington Harbour in 1998 (Chang and Redfearn, 1999). Similarly, Medlyn (1980) found that *K. brevis* was toxic to adult *Artemia*. We incorporated this bioassay into our study for comparative purposes and because the ARTOXKIT M is a standardised bioassay that is routinely used internationally in marine research and aquatic toxicology.

The ARTOXKIT M documentation recommends using instar II larvae, but it is known that different *Artemia* instar stages and adults vary in their susceptibility to toxins. Sorgeloos et al. (1978) demonstrated that instar II larvae are more sensitive than instar I and that instar II and III are equally sensitive. Medlyn (1980), on the other hand, indicated that adults are most sensitive to *K. brevis* toxins. Using adult *Artemia*, however, is often not practical as this requires additional laboratory space, maintenance of the food source and extended time to grow to adult stage. The most likely reason for the low *Artemia* mortalities recorded in our study is that most other studies used much higher dinoflagellate cell concentrations (>10×10^6 cells l^{-1}). What is clear from our studies, however, is that abalone larvae and spat are much more susceptible to dinoflagellate toxins than *Artemia* larvae.

Of the 17 dinoflagellate species tested in the present study, *A. sanguinea*, *K. cristata*, *K. brevis* and *K. mikimotoi* were found to cause the largest mortalities among abalone larvae or spat, and thus may pose a significant threat to the abalone mariculture industry if blooms were to enter farms at concentrations tested in our study. Under certain conditions, these species resulted in mortality rates of more than 50%, with LC_{50} values ranging from 3.1×10^5 cells l^{-1} for *A. sanguinea* to 4.7×10^6 cells l^{-1} for *K. brevis*.

Initially, it was surprising that *A. sanguinea* was found to be toxic to abalone larvae as it is generally considered a non-toxic species. Other studies, however, have found it to be acutely toxic to oyster larvae (*Crassostrea gigas* Thunberg; Cardwell et al., 1979) and juvenile and adult oysters (*Ostrea lurida* Carpenter; Nightlingale, 1936; Woelke, 1961). Whilst our study did not provide evidence that exposure of abalone spat to *A. sanguinea* resulted in direct mortality, observations suggested that it did affect the strength of attachment of the spat to the sides and bottom of the container, and their ability to right themselves after they became dislodged.

The LC_{50} value of *K. cristata* for abalone larvae was estimated at ca. 2.7×10^6 cells l^{-1}, whereas for abalone spat an estimate would most likely result in a LC_{50} value at cell densities greater than those tested in our study. The spat that were still alive after exposure to *K. cristata*, were very weak suggesting that this dinoflagellate species contains a very mild toxin and needs cell concentrations in excess of 10×10^6 cells l^{-1} to kill adult abalone (Horstman et al., 1991).

K. brevis (whole cells, broken cells and filtrate) resulted in mortality of abalone spat only. In all cases, the spat that were alive after 24 h were weak and when dislodged, were unable to right themselves. As with *K. cristata*, *K. mikimotoi* had an adverse effect on both abalone larvae and spat but, whereas only whole cells of *K. cristata* had a toxic effect, broken cells and the filtrate of *K. mikimotoi* cultures also exhibited a toxicity effect. Abalone larvae that were exposed to *K. cristata* and *K. mikimotoi* and which were alive after the 24 h treatment either displayed velum movement with no vertical movement, or shed their shells. It is interesting to note, in comparison, that Matsuyama et al. (1998a) and Sawada and Wada (1983) concluded that *K. mikimotoi* was highly toxic to juvenile *H. discus*

and that *K. mikimotoi* significantly affected adults of this species.

Although no significant mortalities were evident in abalone larvae and spat when exposed to the two highest cell concentrations of *C.* cf. *antiqua* and *H. akashiwo* (whole and broken cells), the abalone larvae displayed velum movement but no vertical migration. Similarly, Nagai et al. (1996) found that *H. akashiwo* had no effect on juvenile pearl oysters (*Pinctada fucata* Dunker) and Nielsen and Strøngrem (1991) showed that *H. akashiwo* had no effect on the mussel, *Mytilus edulis* Linnaeus. Matsuyama et al. (1998a), however, found that *H. akashiwo* (at 10^5 cells ml^{-1}) was responsible for mortalities of juveniles of the abalone, *H. discus* and also juveniles of *Sulculus diversicolor* Reeve. In their assay, however, only one individual at a time was exposed to three different cell concentrations and the statistical validity of their result must, therefore, be seen within this limitation.

4.2. Mortality associated with different phases of the bloom event

Significant abalone spat mortalities were observed during the exponential and stationary growth phases of *K. brevis*. Mortalities during the exponential phase occurred at lower cell densities than during the stationary phase. This was true for whole cell cultures, suspensions of broken cells, and for the filtrate after removal of dinoflagellate cells. *K. brevis*, in the exponential phase is, therefore, significantly more toxic than in stationary phase. Toxicity diminishes once the cultures reach the death phase, then occurring only at very high cell concentrations. Animals that were still alive after exposure to cultures during this phase appeared disorientated and were unable to right themselves when they dropped off the sides of the culture container. For the abalone farmer these results provide an indication of which phase or stage during an algal bloom would pose the greatest risk and may also suggest when mitigating actions, such as recirculation, should be initiated.

4.3. Evaluation of ozone as a mitigation technique

Ozonating (250 mg O_3 h^{-1}) suspensions of *K. mikimotoi*, *A. sanguinea*, *K. brevis*, *H. akashiwo* and *C.* cf. *antiqua* for 10 min killed algal cells and inactivated the toxic effect of *K. brevis* on abalone spat. Similarly, Ho and Wong (2001) found that cells of *P. triestinum*, *S. trochoidea* and *K. digitata* Yang, Takayama, Matuoka et Hodgkiss were broken within 15 min by indirect diffusion of ozone (1 g O_3 m^{-3} seawater h^{-1}). These results suggest that ozone can effectively be used as a mitigation agent to minimize the loss of abalone larvae in the hatchery and early settled spat in the nursery during a red tide event. The economic viability for its use on abalone farms, however, must still be investigated.

An important conclusion of this study is that dinoflagellate species such as *K. mikimotoi*, *K. brevis*, *G.* cf. *pulchellum*, *G.* cf. *corsicum*, *H. akashiwo* and *C.* cf. *antiqua*, listed in the IOC Taxonomic Reference list of Toxic Plankton Algae, are not necessarily toxic to abalone. For the species that this study has shown to be potentially toxic to abalone larvae or spat, it may be appropriate for abalone farmers to undertake mitigating actions following algal blooms. Whether it would be best for farmers to recirculate 'in-house' water during a red tide event, or to utilize mitigation techniques such as ultra-filtration or ozonation, would depend on a range of local circumstances and upon farm design.

Acknowledgements

We wish to thank the personnel of Irvin & Johnson (Ltd.) Abalone Culture Division in particular, Nick Loubser (farm manager) for the use of facilities and valuable insight, Raymond Ferreira and Hennie Otto for assistance and supplying abalone and *Artemia* larvae, Sharon Theart and Michelle Theron for assistance and supplying abalone juveniles and Lilian Viviers for providing accommodation and indispensable advice and discussions. This work was funded by the National Research Foundation (NRF) and the Abalone Farmers Association of South Africa (AFASA).

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