ANNA C. PATI, GENUARIO BELMONTE

Department of Biological and Environmental Sciences and Technologies,
University of Salento - ECOTEKNE, I-73100 Lecce.

EFFECT OF AQUACULTURE DISINFECTANTS ON THE GERMINATION OF A DINOPHYTA CYST

SUMMARY

Cysts of a Dinophyta species collected in a brackish water pond were exposed to different doses of the most common aquaculture disinfectants (formalin, sodium hypochlorite, potassium permanganate, copper sulphate, and organic iodine). Effects of chemicals were evaluated in terms of reduction of the cyst-germination rate. Disinfectant administrations, at the dose usually applied in aquaculture, did not completely inhibit cyst germination. As already demonstrated for other resting stages, common aquaculture treatments are possibly ineffective also against Dinophyta cysts present in breeding systems. Doses of lethal treatments (= doses determining full inhibition of cyst germination) are reported, suggesting a better use of chemicals in order to eradicate from aquaculture systems disturbing organisms.

INTRODUCTION

About 13–16% of known marine Dinophyta produce resting cysts during their life cycle (HEAD, 1996). Information about factors which affect viability of Dinophyta cysts is therefore essential to the understanding of bloom dynamics and their mitigation in managed environments.

Resting stages are produced by a wide range of marine coastal organisms. Generally they accumulate in confined environments (BELMONTE *et al.*, 1995) where they could represent the most important fraction of the viable organic matter (PATI *et al.*, 1999). They can survive dozens of years in the sediments (see MARCUS *et al.*, 1994, for copepods; BELMONTE *et al.*, 1999, for dinoflagellates) and show a high resistance to biological, physical, and chemical factors (CLEGG *et al.*, 2000; TSUJINO *et al.*, 2002; MONTRESOR *et al.*, 2003; PATI and BELMONTE, 2003). Studies on the passive immunity (*sensu* STABILI *et al.*, 1999) of cysts could be of huge interest for the managing of their presence in the environment.

In aquaculture farms, disturbing organisms (viruses, bacteria, fungi, and protists) are eliminated by administering chemicals to the system (GHITTINO, 1985;

MADSEN *et al*., 2000; COSTELLO *et al*., 2001). Different typologies of treatments exist depending on both doses used and exposure times: disinfestation of breeding system structures needs short washing times, whereas disinfestation of industrial and natural basins needs longer exposure to chemicals (GHITTINO, 1985; WHEATON, 1993; DOUILLET, 1998; KECK and BLANCK, 2002).

For the majority of aquaculture farms, however, disinfectant treatments represent a notable expense and they are often not effective in eradicating disturbing organisms (e.g., NÆSS, 1991).

In a previous study, resistance of Crustacea, Rotifera, and Ciliophora cysts to chemical treatments has been tested (PATI and BELMONTE, 2003). In the present study, the resistance of dinoflagellate cysts has been tested.

MATERIALS AND METHODS

Cysts tested in the present study were extracted from sediments of an extensive aquaculture pond (Cabras lagoon; 39°55'N, 08°30'E; Gulf of Oristano, Sardinia, Italy). Sediment was collected in April 2001 and kept at room temperature and in the dark, without chemical preservation, until December 2002. Preliminary analyses revealed the presence of 72 cyst types in the sediment. The cyst type tested in the present work (Figure 1 a, b) represents the more abundant cyst type (11%) of all the assemblage. Only this cyst, when apparently healthy, was isolated and submitted to germination tests. The vegetative stage was obtained with germination (Figure 1c) allowing the tentative attribution of cysts to the genus *Alexandrium* (Dinophyta). A more precise identification was not possible, due to the difficulty of obtaining flagellate populations from single germinated cells (they did not reproduce). This notwithstanding we consider that the experiment could be equally useful in defining tolerance of a Dinophyta representative to disinfectants commonly used in aquaculture.

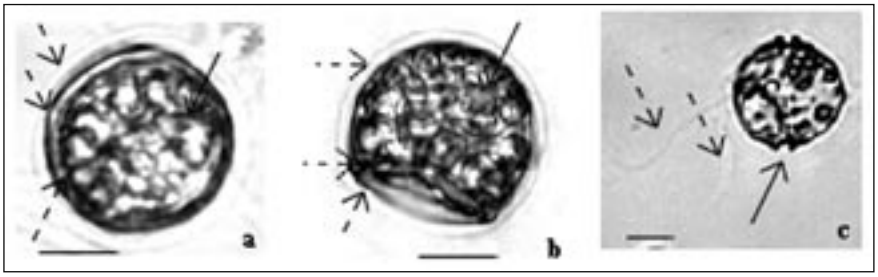

Fig. 1 - Resting cyst (a: apical view, b: lateral view) and motile cell (c) of *Alexandrium* sp. In (a) and (b): arrows indicate the cyst drops, stroke-arrows indicate the three layers of the cyst envelope. In (c) the arrow indicates the cingulum, stroke-arrows indicate the flagella (either flagella are outstretched due to anaesthetic administration).
Scale bars, 10 μm.

For a massive extraction of chosen cysts, sediment aliquots of 35-50 g were suspended in 0.45 μm vacuum-filtered sea water (vfSW) and sieved through plankton nets to obtain the sediment size fraction between 10 and 63 μm. The selected fraction was centrifuged in a mixture of sugar-distilled water 1:1 at 3000 rpm for 3 minutes (according to QUARTA *et al.*, 1992). The supernatant was removed, washed in 0.45 μm vfSW, and observed under an inverted light microscope. Cysts were collected under the microscope by a hand pipette.

Toxicological experiment procedure

Incubation of cysts was carried out at optimal salinity (36‰), temperature (24°C), and photoperiod (14hL:10hD) regimes for germination, as established by germination tests.

Toxicological tests were carried out in parallel with controls (cysts incubated at optimal culture conditions without chemicals in solution) in triplicate (30 cysts/replicate). Cysts were singularly incubated in multi-well plates (well volume, 0.4 cm^3). For all the experiments, natural sea water (from the Ionian Sea, Gulf of Taranto, Italy), 0.45 μm vacuum-filtered, was utilised. All tests were performed without nutrient supply.

Five chemicals were tested: formaldehyde 37% (= formalin), NaOCl, $KMnO_4$, $CuSO_4$, and organic iodine (administered by K30, CIBA-GEIGY, containing organic iodine at 1.2%).

For each chemical two dose-response tests, with different exposure periods, were carried out. In the short exposure test (acute treatment; Ta), cysts were incubated in chemical solution for 2 hours, then rinsed three times, and incubated in fSW until the end of the experiment (27 days). In the continuous exposure (chronic treatment; Tc), cysts were incubated in chemical solutions through the entire experiment (27 days).

The doses utilised in industrial aquaculture for disinfection treatments are greatly variable, depending on both species bred and their pathologies (in therapeutic baths) and type of washing (in farming disinfestation) (see GHITTINO, 1985; for a review). However, it has been possible to establish a medium "ordinary dose" (o.d.), that is a disinfectant concentration usually employed in aquaculture farm disinfestation. The o.d. corresponds to the following concentrations: formalin = 25 mg l^{-1}, NaOCl = 5 mg l^{-1}, $KMnO_4$ = 0.2 mg l^{-1}, $CuSO_4$ = 1 mg l^{-1}, and organic iodine = 0.18 mg l^{-1}. For each disinfestant and exposure, a concentration series was tested (in geometric progression of 4), including concentrations equal to 1, 4, 16, and 64 o.d., so identifying the higher dose at which germination still occurs. Then doses of two and three times the above concentration were applied, in order to better individuate the lethal treatment (that is, the dose at which the complete inhibition of germination, or full cyst inactivation, occurred). In particular, the following additional doses were tested: in acute test, 8 and 12 o.d. for NaOCl and $KMnO_4$, 32 and 48 o.d. for formalin and organic iodine, 2 and 3 o.d. for $CuSO_4$. In chronic test: 8 and 12 o.d. for formalin, NaOCl, organic iodine, and $KMnO_4$. Test subdivision in two steps was necessary, because of the difficulty in collecting a large amount of cysts from the sediment.

To avoid concentration variations, doses were prepared on the same day as starting the testing.

Evaporation (and consequent salt concentration) was avoided by capping culture-plates with parafilm during the whole test period.

Germination was checked daily under an inverted light microscope at 200x; detailed examination and photographs were made at 400x.

Statistical analyses

Results are graphically expressed as germination percentages (average ± SD). A two-factors ANOVA was applied to determine whether the dose and/or exposure affected germination percentage. Due to greater effect of disinfectant doses compared with exposure times (Table 1), the one-factor ANOVA test, taking into account all replicates, was performed to see if different doses affected germination rates. Differences were considered significant for $P < 0.05$.

Tab. 1 - Results of two-factors ANOVA to determine if dose and/or exposure affected cyst germination. Bold indicate the most significant *P* values for each disinfectant test. ANOVA is applied only to doses of the geometric series (see text). Legend of table: SS = Sum of Squares; Df = Degrees of freedom; MS = Mean Square; F = calculated F-ratio (variance among treatments/variance within treatments ratio); P = level of significance; F crit = critical value of F.

Chemical	*Source of variation*	*SS*	*Df*	*MS*	*F*	*P*	*F crit*
Formalin	Dose	55.86	4	13.96	13.96	**$1.35*10^{-5}$**	2.86
	Exposure	9.63	1	9.63	9.63	0.005	4.35
	Interaction	7.86	4	1.96	1.96	0.138	2.86
NaOCl	Dose	71.2	4	17.8	26.7	**$8.98*10^{-8}$**	2.86
	Exposure	0.3	1	0.3	0.45	0.510	4.35
	Interaction	0.53	4	0.13	0.2	0.935	2.86
Organic iodine	Dose	89.53	4	22.38	15.61	**$6.03*10^{-6}$**	2.86
	Exposure	4.8	1	4.8	3.34	0.082	4.35
	Interaction	28.2	4	7.05	4.91	0.006	2.86
$CuSO_4$	Dose	61.33	4	15.33	41.81	**$1.91*10^{-9}$**	2.86
	Exposure	0.3	1	0.3	0.81	0.376	4.35
	Interaction	1.2	4	0.3	0.81	0.528	2.86
$KMnO_4$	Dose	55.2	4	13.8	25.87	**$1.16*10^{-7}$**	2.86
	Exposure	0	1	0	0	1	4.35
	Interaction	$1.42*10^{-14}$	4	$3.55*10^{-15}$	$6.66*10^{-15}$	1	2.86

RESULTS

1. Description of the cyst/motile stage

The tested cyst was a hemisphere, and it was enveloped by a three-layered capsule (Figure 1a). The outer layer appeared to be transparent and smooth; the cyst con-

tained green, yellow, orange, and red drops (Figure 1a,b). The diameter was 25 μm (N = 20; SD = 0.795). The concave part (Figure 1b) was evident in all cysts, and it was not considered an adulteration (e.g. a pole introversion of a sphere) caused by lab conditions (because all the other 71 cyst types did not show this feature).

Vegetative forms, germinated from cysts, were biflagellate; they had a diameter of 25-27 μm and consisted of large, nearly spherical, solitary cells (cell-chains were never observed) (Figure 1c). The epitheca was broad and convex-conical with apparent plate sutures; the hypotheca was hemispherical with flattened antapex; the cingulum was deeply excavated, displaced, with apparent lists.

Cysts showed a germination rate of 12.2 ± 3.8 % without any chemicals in solution (controls) (Figure 2). All vegetative stages came out from the cyst capsule without evident morphological alterations. The lifespan of motile cells was 5-6 days at maximum.

2. Exposure to chemicals

In all treatments, chemical dose affected germination rate more than exposure time (Table 1).

- Formalin. In the acute test, at 1 o.d. (25 mg l^{-1}), an increase of the germination rate (not significant) occurred. Significant germination inhibition occurred at 100 mg l^{-1} (4 o.d.) in the chronic test (Tc) (Table 2). Complete inhibition of cyst germination occurred at 800 mg l^{-1} (32 o.d.) in Ta and at 200 mg l^{-1} (8 o.d.) in Tc (Figure 2a). Individual lifespan was always very short.

Tab. 2 - Germination differences between treated and control cysts, evaluated by ANOVA.
When $P<0.05$, values are reported. ns = not significant. -- indicates no germination.
Ta= acute test; Tc= chronic test. Italics indicates P values of additional tests (see text). Stroke-lines indicate no cyst germination. § indicates presence of aberrant vegetative forms.

Chemical	Exposure	Doses of chemicals									
		1 o.d.	2 o.d.	3 o.d.	4 o.d.	8 o.d.	12 o.d.	16 o.d.	32 o.d.	48 o.d.	64 o.d.
Formalin	Ta	n.s.			n.s.			n.s.	--	--	--
	Tc	n.s.			0.01	--	--	--			--
NaOCl	Ta	n.s.			n.s.	*n.s.*	*0.03*	--			--
	Tc	n.s.			n.s.	--	--	--			--
Organic iodine	Ta	n.s.			n.s.			n.s.	*0.03*	*0.01*	--
	Tc	n.s.			0.03	*0.03*	*0.01*	--			--
$CuSO_4$	Ta	0.03	*0.03*§	--	--			--			--
	Tc	--			--			--			--
$KMnO_4$	Ta	n.s.			0.03	*0.03*	--	--			--
	Tc	n.s.			0.03	--	--	--			--

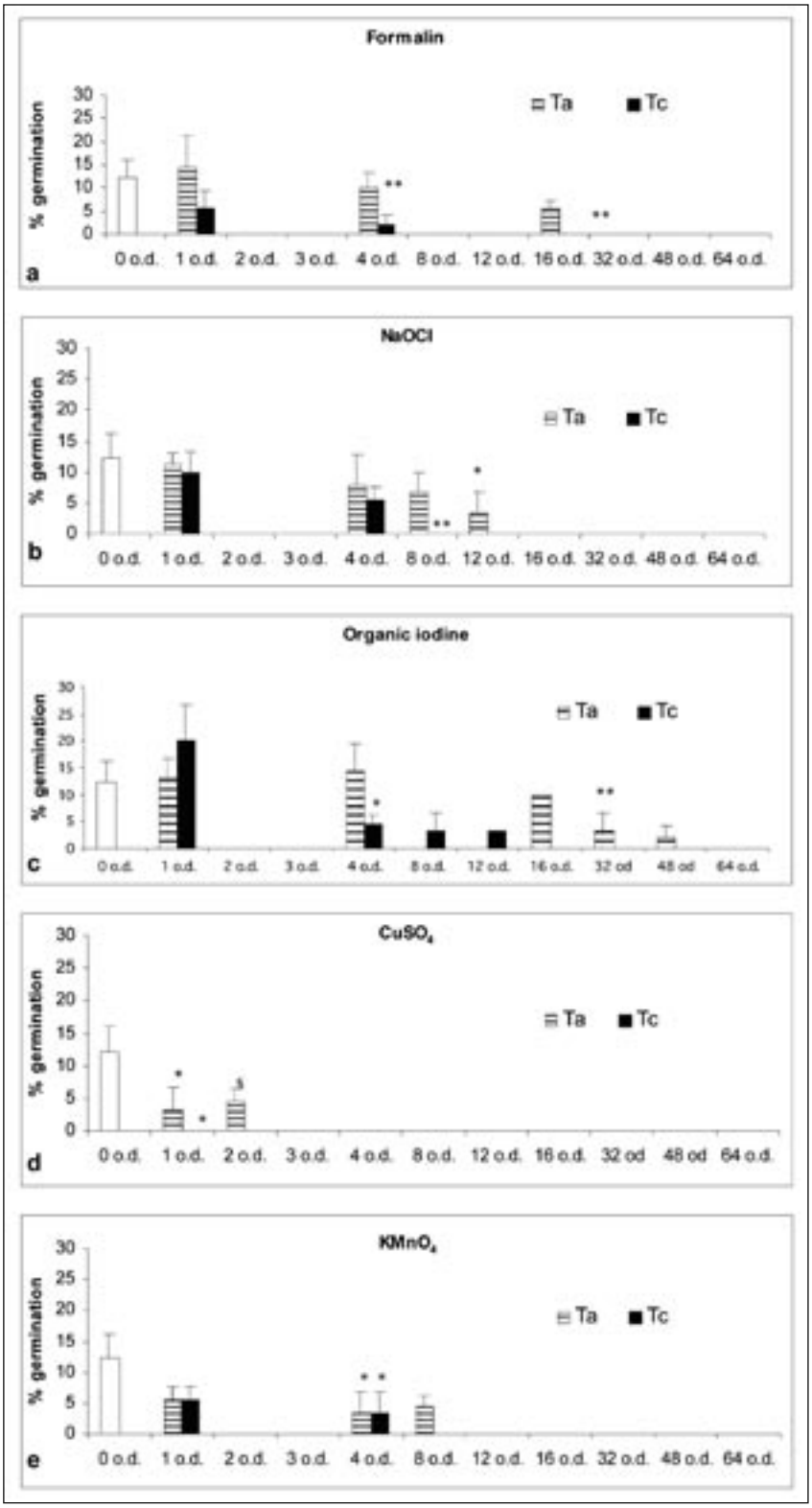

Fig. 2 - Percentage (average ± SD, n=3) of cyst germination in formalin (a), NaOCl (b), organic iodine (c), $CuSO_4$ (d), and $KMnO_4$ (e) treatments. For each test, only tested doses are reported. 0 o.d. indicates the control. Asterisks indicate the lowest doses where germination differences between treated and control cysts are significant (*P<0.05; **P<0.01). § indicates anomalous germinations.

- Sodium hypochlorite. In chronic tests (Tc), at 1 and 4 o.d. (5 and 20 mg l^{-1}) germination percentages were similar to those of controls (Table 2, Figure 2b). In the acute test there were no significant differences of germination between treated and control cysts up to 8 o.d. (40 mg l^{-1}) (Table 2). At the same dose, instead, germination decreased to zero in Tc. Lifespan of motile cells was shorter (3-4 days) than in controls at 4 o.d. and decreased to less than 1 day at higher doses.

- Organic iodine. In Ta, at 1 o.d. a slight germination increase (not significant) occurred (Figure 2c) and the germination percentage was not different from the control up to 16 o.d. (2.88 mg l^{-1}) (Table 2). In Tc, a significant germination decrease occurred at 4 o.d. (0.72 mg l^{-1}). The complete inhibition of cyst germination occurred at 64 o.d. (11.52 mg l^{-1}) and 16 o.d. (2.88 mg l^{-1}) in Ta and Tc respectively (Figure 2c). Lifespan of motile cells was similar (5-6 d) to that of controls up to 4 o.d. in either treatments, but it was reduced to <1 day at 8 o.d. (1.44 mg l^{-1}) in Tc and at 2 and 1 day at 32 and 48 o.d. (8.64 mg l^{-1}) in Ta.

- Copper sulphate. In Tc, the complete inhibition of germinations occurred at 1 o.d. concentration (1 mg l^{-1}) (Figure 2d; Table 2). In Ta, at 2 o.d. (2 mg l^{-1}) all hatched individuals were anomalous, and complete inhibition of germination occurred at 3 o.d. concentration (3 mg l^{-1}) (Figure 2d). In Ta, the lifespan was lower (<1 day) than that of controls.

- Potassium permanganate. Significant inhibition of germination occurred at 4 o.d. (0.8 mg l^{-1}) both in Ta and Tc (Table 2). Complete germination inactivation occurred at 12 o.d. (2.4 mg l^{-1}) and 8 o.d. (1.6 mg l^{-1}) in Ta and Tc, respectively (Figure 2e). Lifespan of individuals was short (1-2 days) in all treatment conditions.

The toxicity scale of chemicals for Dinophyta cysts was: $CuSO_4$ > $KMnO_4$ > NaOCl > formalin > organic iodine, in the acute treatment, and: $CuSO_4$ > $KMnO_4$ > formalin > NaOCl > organic iodine in the chronic one.

$CuSO_4$ and $KMnO_4$ were the most effective disinfectants, NaOCl and organic iodine were the least. Formalin was toxic for Dinophyta cysts in chronic test, but not in the acute one.

DISCUSSION AND CONCLUSIONS

The present investigation is included in a broad research programme on resistance of aquatic resting stages to chemicals: effects of chemicals on the resting forms of Ciliophora, Rotifera, and Crustacea representatives have been already presented (Pati and Belmonte, 2003). The characteristics of both the cyst and the motile stage of the species used in the present paper allow us to suspect it could be a species of the genus *Alexandrium* (Dinophyta). Even if cysts tested in this study have not been identified at species level, we consider that the results obtained could be useful both in the comparison of the different cyst resistance to chemicals (Table 4), and/or in the management of aquaculture systems from disturbing species.

The low germination rate of tested cysts (about 12%) was probably affected by

the length of resting (about two years), and/or lab conditions of storage. This notwithstanding, the germination represents the possibility for the species to return to being active even after a long time of absence. This fact should be carefully considered, since the cyst is very abundant in sediments, and the species is possibly connected (responsible for?) to massive fish mortality occurred in the environment a few months before the cyst collection (SANNIO *et al.*, 2000).

Tab. 3 - Costs of disinfectant treatments reported in Euro (€).
Ta= acute test; Tc= chronic test. Lethal treatment: disinfectant dose where full cyst inactivation occurs.

Chemical	Exposure	Cost of chemical (€/mg)	Cost of o.d. treatment (€/m^3 of treatment solution)	Cost of lethal treatment (€/m^3 of treatment solution)
Formalin	Ta	0.000000411	0.1028	3.29
	Tc			0.82
NaOCl	Ta	0.000008	0.40	6.40
	Tc			3.20
Organic iodine	Ta	0.000004480	0.0448	0.52
	Tc			0.13
$CuSO_4$	Ta	0.000001382	0.0138	0.04
	Tc			0.01
$KMnO_4$	Ta	0.000003367	0.0067	0.08
	Tc			0.05

Tab. 4 - Comparison of lethal doses (disinfectant doses where full cysts inactivation occurs) for resting stages of different phyla. Bold indicates the greatest tolerance.

SUMMARY OF LETHAL TREATMENTS IN CHRONIC EXPOSURES						
	Formalin	NaOCl	Organic iodine	$CuSO_4$	$KMnO_4$	References
Dinophyta (*Alexandrium* sp)	200 ppm (8 o.d.)	40 ppm (8 o.d.)	2.88 ppm (16 o.d.)	1 ppm (1 o.d.)	1.6 ppm (8 o.d.)	present work
Ciliophora (*Fabrea salina*)	25 ppm (1 o.d.)	80 ppm (16 o.d.)	2.88 ppm (16 o.d.)	0.25 ppm (1/4 o.d.)	3.2 ppm (16 o.d.)	Pati and Belmonte, 2003
Rotifera (*Hexarthra fennica*)	6.25 ppm (1/4 o.d.)	20 ppm (4 o.d.)	46.08 ppm (256 o.d.)	**1024 ppm (1024 o.d.)**	**51.2 ppm (256 o.d.)**	Pati and Belmonte, 2003
Crustacea (*Artemia franciscana*)	**1600 ppm (64 o.d.)**	**1280 ppm (256 o.d.)**	**184.32 ppm (1024 o.d.)**	**1024 ppm (1024 o.d.)**	**51.2 ppm (256 o.d.)**	Pati and Belmonte, 2003

The acute treatment test simulated both short aquaculture treatments and natural situations where organisms may be abruptly exposed to chemicals (pollution events). Instead the chronic treatment simulated both industrial disinfestation with long exposures, and chronically polluted sites where the organisms are exposed to chemicals for long periods.

The tested cyst exhibits a remarkable tolerance to applied chemicals, with doses usually considered as effective (ordinary doses). In the acute treatment, in fact, no disinfectant lowered germination to zero, and in the chronic treatment (*i.e.*, long exposure) only $CuSO_4$ was effective.

Slight increase of cyst germination (even though not significant) at 1 o.d. in formalin acute test suggests a possible efficacy of this chemical against the load of micro-organisms which cover the cysts and are harmful for flagellate health, so ameliorating its germination rate. A similar trend in Ta was observed also in *Hexarthra fennica* (Pati and Belmonte, 2003). Also Sahul Hameed and Balasubramanian (2000) reported that resistance of resting forms towards chemicals can be greater than that of the bacterial cyst load, and this aspect is considered useful for the treatment of cysts which are used as live feed sources in aquaculture (Hagiwara *et al.*, 1995; Lavens and Sorgeloos, 1996)

The present Dinophyta cyst seems to be more resistant than cysts of *Fabrea salina* (Ciliophora) and resting eggs of *Hexarthra fennica* (Rotifera) against formalin (Table 4).

Chlorine in various forms has been widely used for water disinfection. Sodium hypochlorite is the most common chemical employed. It was not very effective against Dinophyta cysts: in the acute test, excystment occurred up to 16 o.d. Resistance of cysts against NaOCl varies depending on species: cysts of *Fabrea salina* (Ciliophora) and resting eggs of *Brachionus plicatilis, B. rotundiformis* (Rotifera) and *Artemia franciscana* (Crustacea) showed an increase of germination at low concentrations of sodium hypochlorite (Balompapueng *et al.*, 1997; Douillet, 1998; Pati and Belmonte, 2003). Cysts of *Hexarthra fennica* (Rotifera), instead, exhibit a high sensitivity towards NaOCl (Pati and Belmonte, 2003). Furthermore, the present Dinophyta cyst is more resistant to formalin and NaOCl than some viruses (Chang *et al.*, 1998), bacteria (Sahul Hameed *et al.*, 2003), algae, and planktonic animals (Junli *et al.*, 1997).

Our results confirmed that NaOCl needs a long time to perform a disinfectant action (see also Hallegraeff *et al.*, 1997, for cysts of *Gymnodinium catenatum*). Results of the lowest doses in acute treatments show that toxic effects of NaOCl are reversible after 2-hour exposures. NaOCl administrations, such as formalin ones, need additional treatments because chlorine must be removed either by chemical addition or extensive degassing.

Regarding organic iodine, at the lowest tested dose (that is, o.d. dose) the germination percentage increased (even though not significantly) in chronic tests more than in acute ones. This suggests that probably also this chemical, similarly to formalin, acts against micro-organisms aggressive to cysts in cultures, increas-

ing the germination success, but differently from the formalin, iodine must be continuously present.

Iodophores present several advantages: they are not easily deactivated by organic matter, and the brown colouration of the chemical fades as the activity decreases. This gives a good indication of whether the chemical is no longer active and needs replacing. Furthermore, iodine based disinfectants do not evaporate as fast as some chlorine based ones, so remaining active for longer periods.

The inhibition effect of $CuSO_4$, on the present Dinophyta cyst germination was higher than that observed on resting eggs of *A. franciscana* and *F. salina* (Table 4). Furthermore, it was the only chemical inducing aberrant germinations. Induction of hatching anomalies by $CuSO_4$ was observed also in *A. franciscana* and *F. salina* (Pati and Belmonte, 2003) and it confirms the high toxicity of this chemical, probably due to both the presence of heavy metal and the high oxidant capacity. Copper toxicity is widely reported in literature (Koivisto and Ketola, 1995; McPherson and Chapman, 2000). Luna-Andrade *et al.* (2002) reported that indiscriminate use of copper in aquaculture ponds has a negative impact on the production of rotifers which, in turn, affects the final yield.

Furthermore, copper residence time in the water is high (up to 2 months), probably caused by its complexation and adsorption on natural organic matter (Van Hullebusch *et al.*, 2002). Nutrient addition significantly influenced the metal uptake rate in phytoplankton species (Wang and Dei, 2001): this is very important because aquaculture farms are located in areas where organic content levels are very high. Metal uptake in marine phytoplankton generally involves an initial rapid surface sorption, followed by metal transport into the intracellular environment (Hudson, 1998). In the cell, copper elicits toxicity by overwhelming cellular defence mechanisms that typically control copper homeostasis (Schlenk *et al.*, 1999). Evidence is emerging for copper-induced mutagenesis via Reactive Oxygen Species (ROS) production: copper, in fact, undergo red-ox cycling resulting in the production of ROS; as a consequence, enhanced lipid peroxidation occurs with damage to both the antioxidant systems in animals and DNA (Guecheva *et al.*, 2001).

$KMnO_4$ was less toxic than $CuSO_4$ for the tested Dinophyta cysts. However these seem to be more sensitive to $KMnO_4$ than *Fabrea salina*, *Hexarthra fennica* and *Artemia franciscana* (Table 4). Peterson *et al.* (1995) reported, for cyanobacteria, that both $CuSO_4$ and $KMnO_4$ cause membrane damage and the release of Dissolved Organic Carbon (DOC). Both $KMnO_4$ and $CuSO_4$ treatments against present Dinophyta cysts are cheaper than others, but they might be very toxic for breeding fish species (De Boeck *et al.*, 1995).

Resistance of cysts to chemicals can be due primarily to cyst wall peculiarities. Cyst walls show remarkable species-specific features with regard to composition, morphology, size, shape and sculpturing. Cyst covering represents an effective barrier isolating the internal organism from a hostile environment (Gutierrèz *et al.*, 1990), toxic substances (Clegg, 1974), and micro-organisms (Stabili *et al.*, 1999). Cyst coverings of animal species are usually composed by layers of chitin,

acid mucopolysaccharides, and proteins or lipoproteins (GILBERT, 1974; BUSSERS, 1976). Dinoflagellate cysts can be enclosed by one, two, or three wall layers, that may be composed of organic compounds (biopolymers of carotenoids and/or carotenoid esters) which are resistant to both natural decay and laboratory acid treatments (DALE, 1983).

The cyst resistance towards hard environmental conditions, however, derives also from many biochemical, metabolic, and physiological adaptations of the internal encysted individual (FENCHEL, 1990; CLEGG and JACKSON, 1998; CLEGG *et al*., 2000).

Undesirable consequences to aquaculture management may result from tolerance of encysted stages against chemicals: disinfectant administrations, in fact, may cause a selection of the planktonic community towards species having resting stages in their life-cycle (a similar hypothesis was presented by NÆSS, 1991, regarding copepod populations).

Tolerance toward aquaculture disinfectants of the tested dinoflagellate seems to be close to that of *Hexarthra fennica* and greater than that of *Fabrea salina* (see Table 4). We can therefore argue that the chemical treatments usually applied in aquaculture farming to remove bacteria, viruses, and other diseases, are probably ineffective in the total elimination of these dinoflagellate cysts and, potentially, they could be ineffective even in the prevention of toxic dinoflagellate blooms which have also been reported in aquaculture farms where disinfectant treatments were regularly applied (ZINGONE and ENEVOLDSEN, 2000).

Furthermore, the similar chemical resistance of the present dinoflagellate cyst and that of resting stages of both Rotifera (a common live feed of breeding species) and Ciliophora (natural feed) may represent a serious problem for aquaculture treatment management: doses necessary to completely inactivate Dinophyta cysts, in fact, are effective also against Rotifera and Ciliophora, categories which could represent a natural food supply.

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