Mar Biotechnol (2009) 11:153–160
DOI 10.1007/s10126-008-9130-9

ORIGINAL ARTICLE

Pyrethroids as Promising Marine Antifoulants: Laboratory and Field Studies

Danqing Feng · Caihuan Ke · Shaojing Li · Changyi Lu · Feng Guo

Received: 5 January 2008 / Accepted: 20 June 2008 / Published online: 25 July 2008

Abstract Due to the regulations and bans regarding the use of traditional toxic chemicals against marine fouling organisms and the practical impediments to the commercialization of natural product antifoulants, there is an urgent need for compounds that are antifouling-active, environmentally friendly, and have a potential for commercial application. In this study, a series of common, commercially available pyrethroid products, which are generally used as environmentally safe insecticides, was evaluated for antifouling activity in the laboratory using an anti-settlement test with cyprids of the barnacle *Balanus albicostatus* and also in a field experiment. Laboratory assay showed that all eleven pyrethroids (namely, rich *d-trans*-allethrin, Es-biothrin, rich *d*-prallethrin, S-prallethrin, tetramethrin, rich *d*-tetramethrin, phenothrin, cyphenothrin, permethrin, cypermethrin, and high active cypermethrin) were able to inhibit barnacle settlement (EC_{50} range of 0.0316 to 87.00 μg/ml) without significant toxicity. Analysis of structure–activity relationships suggested that the cyano group at the α-carbon position had a significant influence on the expression of antifouling activity in pyrethroids. In the field, the antifouling activity of pyrethroids was further confirmed, with the most potent pyrethroids being cypermethrin and high active cypermethrin, which displayed efficiency comparable with that of tributyltin. In summary, our investigation indicated that these pyrethroids have a great and practical commercial potential as antifouling agents.

Keywords Pyrethroids · Marine antifoulants · Commercialization · Antifouling activity · Barnacle · Structure–activity relationships

D. Feng · C. Ke · S. Li · C. Lu
Key State Laboratory of Marine Environmental Science,
College of Oceanography and Environmental Science,
Xiamen University,
Xiamen, People's Republic of China

C. Ke (✉) · S. Li · F. Guo
Department of Oceanography,
College of Oceanography and Environmental Science,
Xiamen University,
Xiamen, People's Republic of China
e-mail: chke@xmu.edu.cn

Introduction

It is well known that marine fouling organisms, settling on ship hulls and other man-made constructions immersed in the sea, constitute a worldwide technical and economical problem (Yebra et al. 2004; Townsin 2003; Rittschof 2000). The most widespread solution to fouling is based upon coatings containing toxic chemicals such as organotin and copper (Alberte et al. 1992; Yebra et al. 2004). However, these compounds have been found to be highly toxic to non-target species, to persist in the environment, and to pollute the marine ecosystem (Ellis 1991; Cardwell et al. 1999; Clare et al. 1992). This has resulted in regulations and bans on their use in many countries (Rittschof 2001; van Wezel and van Wlaardingen 2004). In the wake of this, a great deal of research has been focused on finding new antifouling agents which are environmentally friendly.

In many studies, it is suggested that marine natural products with antifouling activity are promising and eco-friendly alternatives for classical antifoulants, since these natural products already exist in the marine environment and are biodegradable (e.g., Abarzua and Jakubowski 1995; Clare 1996, 1998; Burgess et al. 2003; Fusetani 2004; Hellio et al. 2005). So far, more than 100 marine natural product antifoulants have been isolated and identified, including mainly terpenoids, steroids, fatty acids, amino acids, heterocyclics, acetogenins, alkaloids, and polyphenolics (Targett 1997; Rittschof 1999, 2001; Yebra et al.

2004; Fusetani 2004; Fusetani and Clare 2006; Paul et al. 2006). However, many obstacles need to be overcome before commercialization of marine natural product antifoulants is possible (Clare 1996; Rittschof 1999, 2000, 2001; Yebra et al. 2004). One challenge is the production of these natural products on a large scale, since most of them are not available in sufficient quantities to be harvested from exotic and rare marine organisms. On the other hand, many natural product antifoulants are too complex in chemical structure to be synthesized for commercial use. Costly and time-consuming government registration of novel antifoulants is another stumbling block. At the present time, there are no coatings based upon marine natural antifoulants available in the market, and the development of this technology still seems to be far in the future (Rittschof 1999; Yebra et al. 2004).

With the urgency to find novel antifoulants and the hurdles to the industrial application of natural product antifoulants, efforts are necessary to solve this predicament. One desirable strategy is to search for compounds that have a good antifouling performance in an environmentally benign way and, at the same time, have practical commercial potential.

Pyrethroids are synthetic analogs of pyrethrins, the natural insecticidal compounds isolated from the flowers of the plant *Chrysanthemum cinerariaefolium* (Ensley 2007). Many pyrethroids are widely used as highly active insecticides not only in agriculture but also in indoor pest control and have many public health applications because they are low in toxicity to mammals, do not accumulate in organisms, and do not persist for a long time in the environment (Ensley 2007; Lutnicka et al. 1999). These favorable properties of pyrethroids also meet some of the criteria for an ideal antifoulant. If pyrethroids were to be used for marine antifouling, the production of a number of pyrethroids on an industrial scale and their reasonable prices would not only make these antifoulants available in sufficient amounts but also make them commercially competitive because of their comparatively low cost. Much is known about pyrethroids and there is an extensive literature dealing with their chemical nature (e.g., Fernández-Álvarez et al. 2007; Leahey 1985; Zhou et al. 1995), their environmental fate and effects (e.g., Lutnicka et al. 1999; Friberg-Jensen et al. 2003; Helliwell and Stevens 2000), and their possible impacts on human health (e.g., Hadnagy et al. 2003; Sumida et al. 2001; Surrallés et al. 1995). This would all facilitate the studies necessary for governmental registration for the use of pyrethroids as antifoulants (Rittschof et al. 2003), and so there are many advantages in the application of pyrethroids as antifoulants. Synthetic pyrethroids have been investigated for their efficacy as wood preservatives in the sea, and they are active against the marine borer *Limnoria* (Eaton and Cragg 1996; Cragg and Eaton 1997). However, information is still scarce concerning the effect of pyrethroids on biofouling.

The aim of this study was to describe and evaluate the antifouling activity of a series of common, commercially available pyrethroids. These were tested in the laboratory for their anti-settlement activity against the cyprid larvae of the barnacle *Balanus albicostatus*. The data were discussed also in terms of structure–activity relationships. Most importantly, the chosen pyrethroids were subjected to conventional submerged assay (Takasawa et al. 1990; Etoh et al. 2002) to explore their antifouling activity in the field.

Materials and Methods

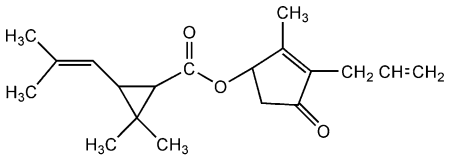
Test Pyrethroids

Eleven pyrethroids, namely rich *d-trans*-allethrin, Es-biothrin, rich *d*-prallethrin, S-prallethrin, tetramethrin, rich *d*-tetramethrin, phenothrin, cyphenothrin, permethrin, cypermethrin, and high active cypermethrin, were used in our initial study. There are four pairs among the 11 pyrethroid products, the two components of each pair differing in the type and the ratio of isomeric configurations of one pyrethroid compound, namely rich *d-trans*-allethrin/Es-biothrin, rich *d*-prallethrin/S-prallethrin, tetramethrin/rich *d*-tetramethrin, and cypermethrin/high active cypermethrin. All these pyrethroids were purchased from the Changzhou Kangmei Chemical Industry Ltd., China, all with a purity above 90%. Their chemical structures are shown in Fig. 1. The reasons for choosing these pyrethroids were that they were all very easy to obtain from the market in large amounts at low prices, and some of them are analogous in chemical structure, which may enable us to obtain some knowledge of their structure–activity relationships in the present work.

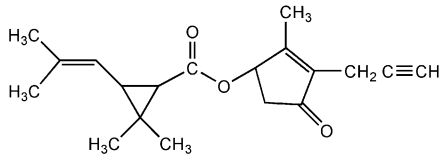
Larval Culture of *B. albicostatus*

Adults of *B. albicostatus* were collected from intertidal rocks in Xiamen, China. Adults were left to dry overnight after which they released the nauplius I and nauplius II stages upon immersion in seawater. Naupliar larvae actively swimming towards the light were collected using a pipette and cultured in glass beakers with filtered seawater (FSW, 0.22 μm, salinity 30‰, and temperature 25°C) at an initial density of one larva per milliliter. They were fed with the diatom *Chaetoceros muelleri* at a concentration of 2.5×10^5 cells/ml. Each day, nauplii were sieved from the cultures by mesh and transferred to glass beakers with fresh FSW and phytoplankton. After 5–6 days, most of the nauplii had metamorphosed to cyprids, and the cyprids were collected by filtration.

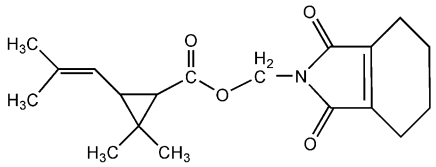
Fig. 1 Chemical structures of tested pyrethroids



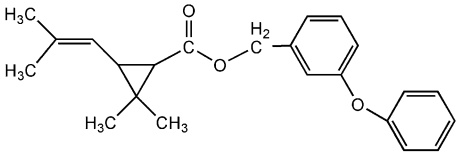
Rich *d-trans*-allethrin **(1)** / Es-biothrin **(2)**



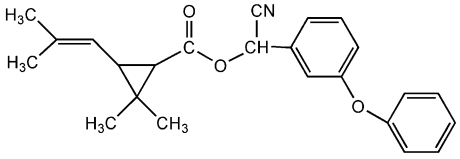
Rich *d*-prallethrin **(3)** / S-prallethrin **(4)**



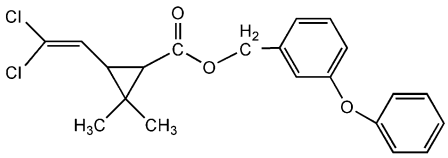
Tetramethrin **(5)** / Rich *d*-tetramethrin **(6)**



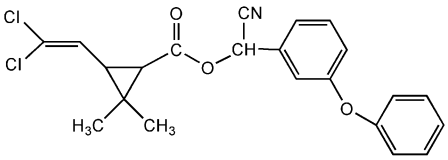
Phenothrin **(7)**



Cyphenothrin **(8)**



Permethrin **(9)**



Cypermethrin **(10)** / High active cypermethrin **(11)**

Anti-Attachment Test with *B. albicostatus* Cyprids

The anti-attachment test was carried out according to the methods described by Hellio et al. (2005) and Kitano et al. (2004). Test samples of the pyrethroids were dissolved in ethanol, and aliquots of the solution were applied to glass Petri dishes. After complete evaporation of the ethanol at room temperature, 10 ml FSW and 30 cyprids were added to each Petri dish. FSW was used as a control because previous pilot studies had shown that there was no significant difference in cyprid settlement between non-treated and ethanol-treated dishes. Three replicates were set up for the FSW control and for each of the treatment groups. The Petri dishes were incubated in the dark at 25°C for 48 h and, after this time, the numbers of larvae that settled or died were enumerated with the aid of a stereomicroscope. Cyprids that did not move, had extended appendages, and did not respond to a touch with a metal probe were counted as dead (Rittschof et al. 1992). Cyprids that permanently attached and metamorphosed were scored as settled (Rittschof et al. 2003; Hellio et al. 2005). The assay was repeated two to four times with different batches of larvae, but for each tested pyrethroid at each time, all treatments were run with a single batch of larvae. The percentages of settled or dead larvae were analyzed by one-way analysis of variance followed by a Dunnett's test for multiple comparisons of treatment means with a control. The significance level was defined as $P<0.05$. The antifouling activities of pyrethroids were expressed as EC_{50} values (the concentration that reduced the settlement rate by 50% relative to the control) and the toxicities of pyrethroids expressed as LC_{50} values (the concentration that resulted in 50% mortality), estimated using the Spearman–Karber method (Hamilton et al. 1977, 1978; Reichelt-Brushett and Michalek-Wagner 2005).

Field Experiment

The pyrethroids were also subjected to conventional submerged assay based on Takasawa et al. (1990) and Etoh et al. (2002). Briefly, each pyrethroid was mixed with 15% polyvinyl butylated resin in methanol, and all

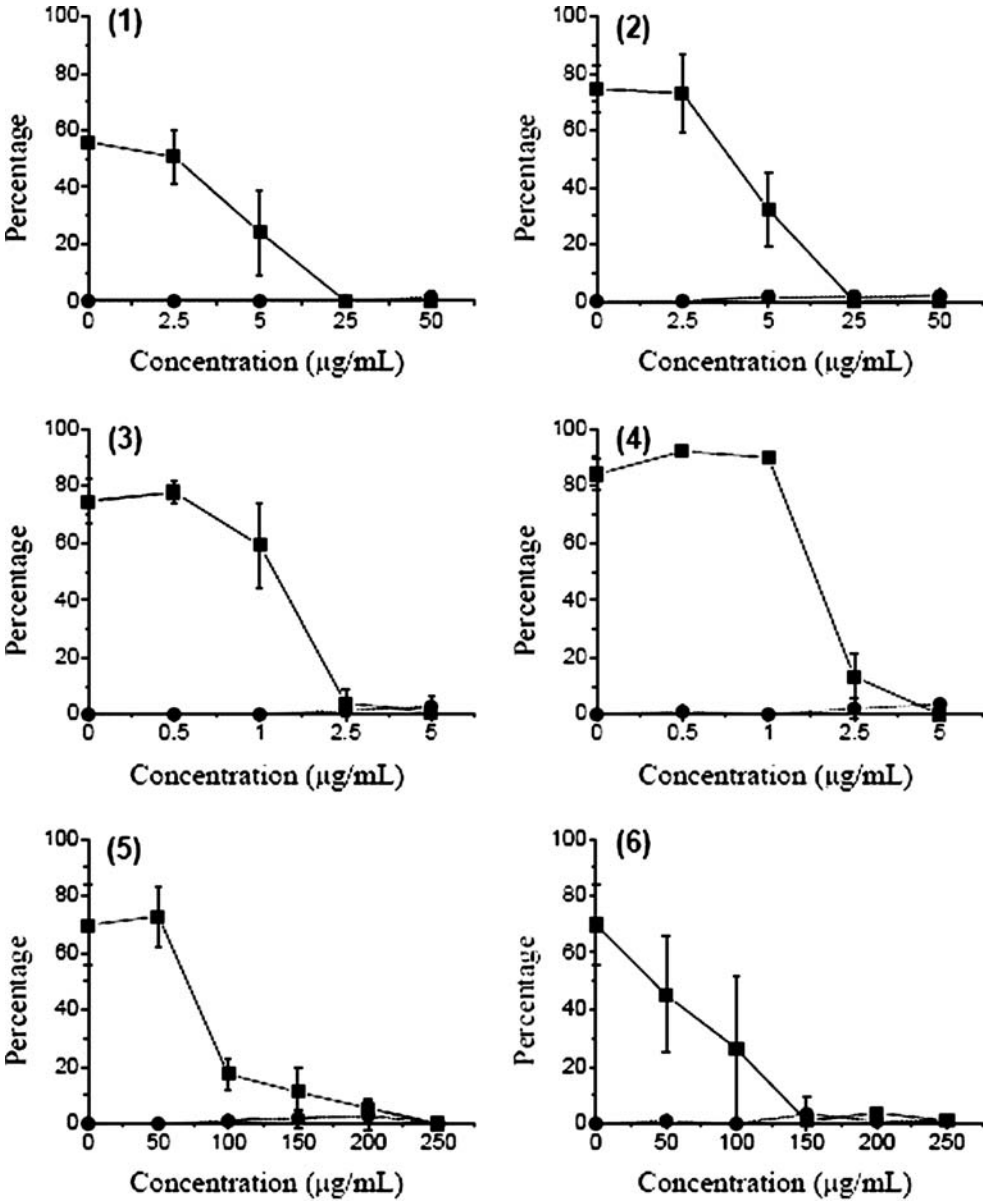


Fig. 2 Effects of pyrethroids on settlement and mortality of *B. albicostatus* cyprids. *1*: Rich *d-trans*-allethrin; *2*: Es-biothrin; *3*: Rich *d*-prallethrin; *4*: S-prallethrin; *5*: Tetramethrin; *6*: Rich *d*-tetramethrin; *7*: Phenothrin; *8*: Cyphenothrin; *9*: Permethrin; *10*: Cypermethrin; *11*: High active cypermethrin. Rates of settlement and mortality at different concentrations are plotted. *Square*, percentage settlement; *circle*, percentage mortality. Data plotted are means±SD of three replicates

prepared samples were applied individually to sample zones (5 cm in diameter) on a polyvinyl chloride plate (35×60×0.3 cm). In the field experiment, each pyrethroid was tested at 150 and 300 mg/zone. Fifteen percent polyvinyl butylated resin in methanol was used as the positive control. TBTCl (Hongding International Chemical Industry, Ltd.) mixed with 15% polyvinyl butylated resin in methanol and applied to sample zones at 150 and 300 mg/zone was used as the negative control. Three replicates were set up for the controls and for each treatment. After the solvent had evaporated, the three replicate plates were exposed to biofouling at a depth of 1 m at a fish farm in Tongan Bay, Xiamen. The field experiment was carried out for 6 months from January 2005 to July 2005. The difference in wet biomass of fouling organisms between each pyrethroid sample zone and the positive and negative control zones was statistically analyzed using independent samples *t* test. During the period of the experiment, two important seawater parameters, temperature and salinity, were measured each month, since Yebra et al. (2004) suggested that the performance of antifouling paints might change under different sea water conditions and it is useful to characterize the marine environment faced by antifouling paints.

Results and Discussion

The effects of 11 pyrethroids on settlement and mortality of *B. albicostatus* cyprids are shown in Fig. 2. Their EC_{50} and LC_{50} values are summarized in Table 1. As shown in Fig. 2 and Table 1, larval settlement was inhibited significantly by

Fig. 2 (continued)

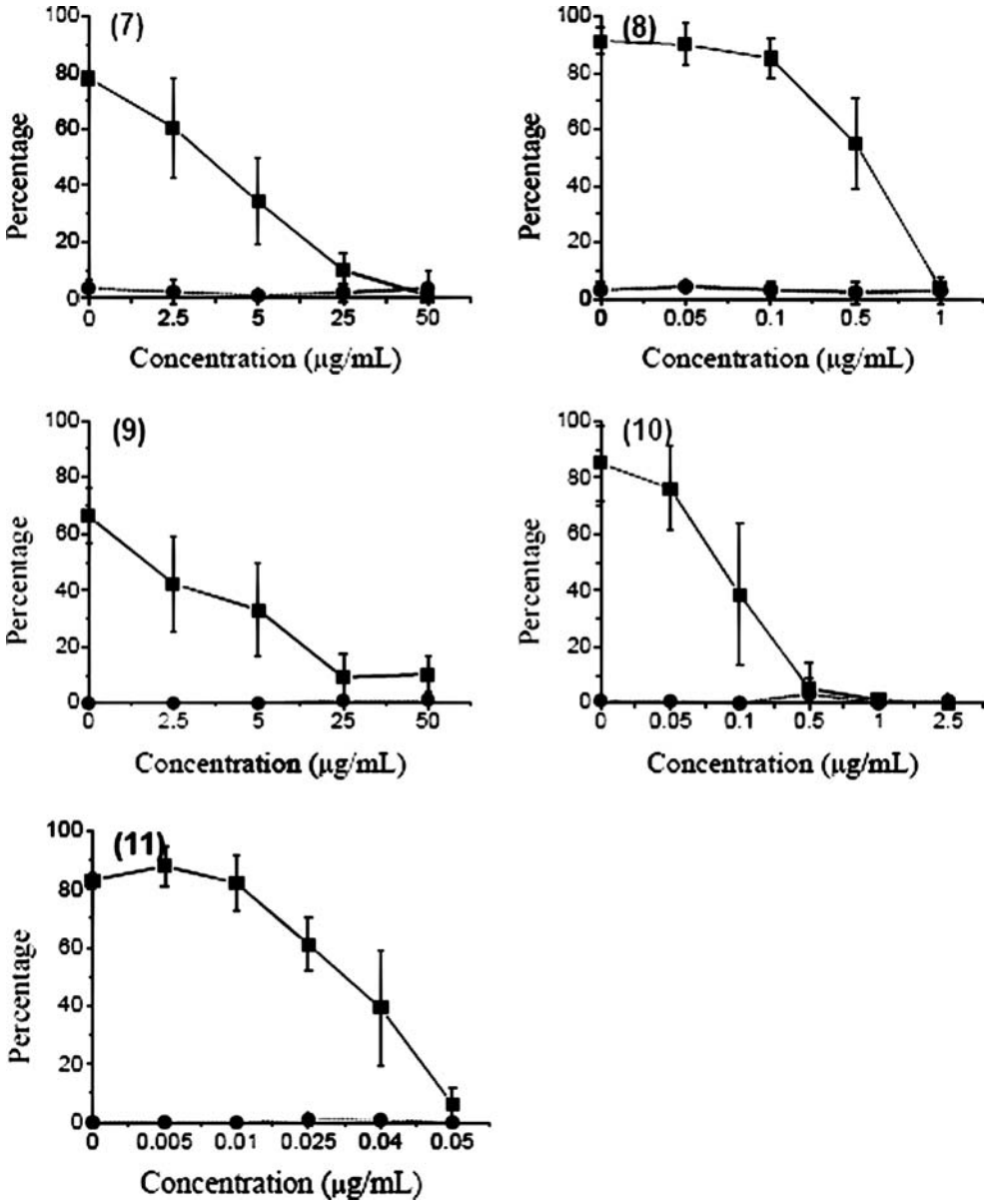


Table 1 Antifouling activity of pyrethroids against *B. albicostatus* cyprid larvae

Pyrethroid	EC_{50} (μg/ml)	LC_{50} (μg/ml)
Rich *d-trans*-allethrin **(1)**	5.33	>250
Es-biothrin **(2)**	5.71	>250
Rich *d*-prallethrin **(3)**	1.38	>20
S-prallethrin **(4)**	1.78	>20
Tetramethrin **(5)**	87.00	>250
Rich *d*-tetramethrin **(6)**	72.83	>250
Phenothrin **(7)**	5.03	>250
Cyphenothrin **(8)**	0.44	>250
Permethrin **(9)**	4.91	>250
Cypermethrin **(10)**	0.11	>250
High active cypermethrin **(11)**	0.0316	>250

each pyrethroid in a dose-dependent manner ($P<0.0001$), without significant toxicity ($P>0.05$). Rittschof (1999) suggested that non-toxic compounds are those that do not directly kill fouling propagules at or near the levels at which they deter fouling. In the present work, each of the 11 pyrethroids yielded an LC_{50} value much greater than its EC_{50} value, indicating that all these compounds had the ability to inhibit larval settlement by a non-toxic mechanism.

The antifouling activities exhibited by the pyrethroids varied, with EC_{50} values ranging from 0.0316 to 87.00 μg/ml (Table 1). Rich *d-trans*-allethrin and Es-biothrin, the two products of allethrin differing in the type and ratio of isomeric configurations of allethrin, showed similar antifouling activity. A similar phenomenon was also observed in the rich *d*-prallethrin and S-prallethrin pair (the two products of prallethrin) and the tetramethrin and rich *d*-tetramethrin pair

(the two products of tetramethrin). However, there was an obvious difference in the antifouling activity between cypermethrin and high active cypermethrin (the two products of cypermethrin). Therefore, based on the results obtained from the antifouling assay, it was suggested that isomeric composition may or may not have an impact on the antifouling activity of pyrethroids. The antifouling activities of rich *d-trans*-allethrin and Es-biothrin against cyprids were both clearly lower than those of rich *d*-prallethrin and S-prallethrin, which is likely to be due to the difference in chemical structure (Fig. 1), suggesting that the side chain of the cyclopentenolone ring in prallethrin (the propyne group) might be more potent in its antifouling activity than the propene group. In addition, by comparing the EC_{50} values of phenothrin and cyphenothrin and considering that the only difference between the structures of these two compounds is the lack of a cyano group in phenothrin, it is evident that the presence of a cyano group at the α-carbon position of pyrethroids enhanced antifouling activity. This was further confirmed by similar results obtained from structure–activity relationship studies of permethrin and cypermethrin. Based on the above analysis, it is suggested that in pyrethroids containing a cyano group at the α-carbon position, the cyano group is an important functional group in the expression of potent antifouling activity. This proposal is also supported by the result that cyphenothrin, cypermethrin, and high active cypermethrin all exhibited much greater antifouling activity against cyprids than did the other pyrethroids tested in the present work. The antifouling activity exhibited by cypermethrin (in which the gem-dimethyl group at isobutyl in cyphenothrin is substituted by two chlorine atoms) was observed to be stronger than that of cyphenothrin. However, when the gem-dimethyl group at the isobutyl in phenothrin is substituted by two chlorine atoms, the resulting permethrin showed almost the same antifouling activity as cyphenothrin. This inconsistency means that substituting the methyl group for chlorine atoms would not always increase the antifouling activity in pyrethroids and also indicates that the cyano group might have a significant influence on the increase of antifouling activity when substituting methyl by chlorine groups in pyrethroids and that the chlorine atom might be another component expressing potent antifouling activity in cypermethrin.

How can pyrethroids decrease larval settlement of barnacles in a non-toxic way? Knowledge of their mechanism of impact on insects may provide some useful information, since barnacles and insects both belong to the phylum Arthropoda. A series of studies (Narahashi 1992, 1996, 2002; Narahashi et al. 1998) suggest that pyrethroids cause knockdown or death of insects by acting on the nervous system, with the voltage-gated sodium channel of the neuronal membrane as the target site. This channel would be kept open by pyrethroids, causing membrane depolarization, repetitive discharges, and synaptic disturbances, leading to hyperexcitation in the insects. This information may be useful as a starting point for understanding the mechanism of the inhibitory effect of pyrethroids on barnacle settlement.

After the laboratory experiment with *B. albicostatus* cyprids, the 11 pyrethroids were subjected to conventional submerged assay to check their antifouling activity in the field. During the period of the field assay, seawater temperature varied between 10.5°C and 29.5°C and salinity between 29.0‰ and 35.0‰, indicating that the experimental plates were subjected to a wide range of temperature but a comparatively narrow range of salinity variation. The results of the conventional submerged assay are shown in Table 2. After 6-month exposure in the sea, each pyrethroid sample zone had a much lower wet mass of fouling organisms compared to the positive control, indicating that all 11 pyrethroids were strongly resistant to marine biofouling. Thus, the antifouling activity based on the experimental anti-settlement tests with *B. albicostatus* cyprids was also observed in the conventional submerged assay. Furthermore, among these pyrethroids, cypermethrin and high active cypermethrin exhibited the highest antifouling activity and were as effective against fouling as TBTCl, which is recognized as one of the most effective antifouling agents. In each of the tested pyrethroids, the 300-mg/zone treatment resulted in a lower wet mass of fouling organisms than the 150-mg/zone treatment, suggesting that increased concentration would result in an increase in the antifouling efficiency of pyrethroids.

Table 2 Wet mass (g/zone, means±SD) of fouling organisms in each sample zone after 6 months in the sea

Pyrethroid	Wet mass biomass (g/zone)	
	150 mg/zone	300 mg/zone
Rich *d-trans*-allethrin (1)	8.31 ± 0.74^{a}	$5.16\pm0.32^{a,c}$
Es-biothrin (2)	$8.90\pm0.49^{a,b}$	$6.82\pm0.17^{a,c}$
Rich *d*-prallethrin (3)	$8.43\pm0.26^{a,b}$	$6.94\pm0.83^{a,c}$
S-prallethrin (4)	4.08 ± 0.44^{a}	$3.46\pm0.39^{a,c}$
Tetramethrin (5)	4.63 ± 0.58^{a}	$2.90\pm0.23^{a,c}$
Rich *d*-tetramethrin (6)	4.77 ± 0.61^{a}	$3.67\pm0.51^{a,c}$
Phenothrin (7)	$6.98\pm0.97^{a,b}$	$3.48\pm0.22^{a,c}$
Cyphenothrin (8)	$7.73\pm0.55^{a,b}$	2.30 ± 0.12^{a}
Permethrin (9)	$9.06\pm0.24^{a,b}$	$4.79\pm0.37^{a,c}$
Cypermethrin (10)	2.88 ± 0.44^{a}	2.43 ± 0.27^{a}
High active cypermethrin (11)	4.40 ± 0.39^{a}	2.37 ± 0.29^{a}
TBTCl	3.60±0.46	2.24±0.26
Positive control	18.63±1.50	

Each zone was 5 cm in diameter. For each pyrethroid sample zone, (a) indicates wet biomass significantly different from the positive control, (b) indicates wet biomass significantly different from the negative control (TBTCl) at a sample amount of 150 mg/zone, and (c) indicates wet biomass significantly different from the negative control (TBTCl) at a sample amount of 300 mg/zone

With the imminent total ban of tributyltin (TBT) antifoulants initiated by the International Maritime Organization under societal and business pressure, considerable effort has been devoted to the search for new antifouling alternatives. Herbicides, fungicides, and bactericides have been used as antifouling agents in marine paints, e.g., Irgarol 1051, diuron, zinc pyrithione, copper pyrithione, and Sea-Nine 211 (Thomas 2001; Okamura et al. 2002). However, these new antifouling compounds, like TBT, act through toxic mechanisms (Konstantinou and Albanis 2004) and have been reported to be highly toxic to many non-target marine organisms (Okamura et al. 2000a, b, 2002; Kobayashi and Okamura 2002; Goka 1999). Thus, there is growing concern regarding the environmental fate and potential risks of their use (Thomas et al. 2002, 2003; Shade et al. 1993; Turley et al. 2000). In the UK, restrictions have been imposed on the use of Irgarol 1051 and Sea-Nine 211, and diuron is no longer approved for use as an antifoulant (Thomas et al. 2002). Therefore, other antifouling alternatives need to be developed urgently. Based on the results of the present work, all the pyrethroids tested inhibited barnacle settlement in a non-toxic way and maintained their antifouling activity in the sea for quite a long time (≥6 months). As stated in the introduction, there are many advantages for the commercial application of pyrethroids as antifoulants, and therefore, these compounds have excellent potential for the development of environmentally friendly antifouling coatings. Further work is in progress to focus effort towards a practical goal, and so important issues such as the compatibility of the proposed pyrethroids with existing coating technology, the effective release rates of these pyrethroids from the paint, and the durability of the paint are being studied.

Acknowledgments We express our sincere thanks to Dr. Dan Rittschof for his constructive suggestions. Thanks are also due to Professor John Hodgkiss for his help in the preparation of the manuscript. This research was supported by Xiamen Sci-Tech Bureau under contract No. 3502Z20073014 and the National Natural Science Foundation of China (NNSFC) under contract No. 40276041 and contract No. 40676081.

References

Abarzua S, Jakubowski S (1995) Biotechnological investigation for the prevention of biofouling. I. Biological and biochemical principles for the prevention of biofouling. Mar Ecol Prog Ser 123:301–312

Alberte RS, Snyder S, Zahuranec B (1992) Biofouling research needs for the United States Navy: program history and goals. Biofouling 6:91–95

Burgess JG, Boyd KG, Armstrong E, Jiang Z, Yan L, Berggren M, May U, Pisacane T, Granmo A, Adams DR (2003) The development of a marine natural product-based antifouling paint. Biofouling 19S:197–205

Cardwell RD, Brancato MS, Toll J, DeForest D, Tear L (1999) Aquatic ecological risks posed by tributyltin in United States surface waters: pre-1989 to 1996 data. Environ Toxicol Chem 18:567–577

Clare AS (1996) Marine natural product antifoulants: status and potential. Biofouling 9:211–229

Clare AS (1998) Towards non-toxic antifouling. J Mar Biotechnol 6:3–6

Clare AS, Rittschof D, Gerhart DJ, Maki JS (1992) Molecular approaches to nontoxic antifouling. Invert Reprod Dev 22: 67–76

Cragg SM, Eaton RA (1997) Evaluation of creosote fortified with synthetic pyrethroids as wood preservatives for use in the sea. II Effects on wood-degrading micro-organisms and fouling invertebrates. Mater Org 31:197–216

Eaton RA, Cragg SM (1996) Evaluation of creosote fortified with synthetic pyrethroids as wood preservatives for use in the sea Part 1: efficacy against marine wood-boring molluscs and crustaceans. Mater Org 29:211–229

Ellis DV (1991) New dangerous chemicals in the environment: lessons from TBT. Mar Pollut Bull 22:8–10

Ensley S (2007) Pyrethrins and pyrethroids. In: Gupta RC (ed) Veterinary toxicology: basic and clinical principles. Elsevier, New York, pp 494–498

Etoh H, Kondoh T, Noda R, Singh IP, Sekiwa Y, Morimitsu K, Kubota K (2002) Shogaols from *Zingiber officinale* as promising antifouling agents. Biosci Biotech Biochem 66:1748–1750

Fernández-Álvarez M, Sánchez-Prado L, Lores M, Llompart M, García-Jares C, Cela R (2007) Alternative sample preparation method for photochemical studies based on solid phase microextraction: synthetic pyrethroid photochemistry. J Chromatogr A 1152:156–167

Friberg-Jensen U, Wendt-Rasch L, Woin P, Christoffersen K (2003) Effects of the pyrethroid insecticide, cypermethrin, on a freshwater community studied under field conditions. I. Direct and indirect effects on abundance measures of organisms at different trophic levels. Aquat Toxicol 63:357–371

Fusetani N (2004) Biofouling and antifouling. Nat Prod Rep 21: 94–104

Fusetani N, Clare A (2006) Antifouling compounds. Springer, Berlin

Goka K (1999) Embryotoxicity of zinc pyrithione, an antidandruff chemical, in fish. Environ Res Sect A 81:81–83

Hadnagy W, Leng G, Sugiri D, Ranft U, Idel H (2003) Pyrethroids used indoors-Immune status of humans exposed to pyrethroids following a pest control operation—a one year follow-up study. Int J Hyg Environ Health 206:93–102

Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714–719

Hamilton MA, Russo RC, Thurston RV (1978) Correction to: Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714–719 (Environ Sci Technol 12, 417)

Hellio C, Tsoukatou M, Maréchal JP, Aldred N, Beaupoil C, Clare AS, Vagias C, Roussis V (2005) Inhibitory effects of Mediterranean sponge extracts and metabolites on larval settlement of the barnacle *Balanus amphitrite*. Mar Biotechnol 7:297–305

Helliwell S, Stevens MM (2000) Efficacy and environmental fate of alphacypermethrin applied to rice fields for the control of chironomid midge larvae (Diptera: Chironomidae). Field Crops Res 67:263–272

Kitano Y, Nogata Y, Shinshima K, Yoshimura E, Chiba K, Tada M, Sakaguchi I (2004) Synthesis and anti-barnacle activities of novel isocyanocyclohexane compounds containing an ester or an ether functional group. Biofouling 20:93–100

Kobayashi N, Okamura H (2002) Effects of new antifouling compounds on the development of sea urchin. Mar Pollut Bull 44:748–751

Konstantinou IK, Albanis TA (2004) Worldwide occurrence and effect of antifouling paint booster biocides in the aquatic environment: a review. Environ Int 30:235–248

Leahey JP (1985) The pyrethroid insecticides. Taylor & Francis, London

Lutnicka H, Bogacka T, Wolska L (1999) Degradation of pyrethroids in an aquatic ecosystem model. Wat Res 33:3441–3446

Narahashi T (1992) Nerve membrane Na^+ channels as targets of insecticides. Trends Pharmacol Sci 13:236–241

Narahashi T (1996) Neuronal ion channels as the target sites of insecticides. Pharmacol Toxicol 79:1–14

Narahashi T (2002) Nerve membrane ion channels as the target site of insecticides. Mini Rev Med Chem 2:419–432

Narahashi T, Ginsburg K, Nagata K, Song J, Tatebayashi H (1998) Ion channels as targets for insecticides. Neurotoxicology 19:581–590

Okamura H, Aoyama I, Liu D, Maguire RJ, Pacepavicius GJ, Lau YL (2000a) Fate and ecotoxicity of the new antifouling compound Irgarol 1051 in the aquatic environment. Water Res 34:3523–3530

Okamura H, Aoyama I, Takami T, Maruyama T, Suzuki Y, Matumoto M, Katsuyama I, Hamada J, Beppu T, Tanaka O, Maguire RJ, Liu D, Lau YL, Pacepavicius GJ (2000b) Phytotoxicity of the new antifouling compound Irgarol 1051 and a major degradation product. Mar Pollut Bull 40:754–763

Okamura H, Watanabe T, Aoyama I, Hasobe M (2002) Toxicity evaluation of new antifouling compounds using suspension-cultured fish cells. Chemosphere 46:945–951

Paul VJ, Puglisi MP, Ritson-Williams R (2006) Marine chemical ecology. Nat Prod Rep 23:153–180

Reichelt-Brushett AJ, Michalek-Wagner K (2005) Effects of copper on the fertilization success of the soft coral *Lobophytum compactum*. Aquat Toxicol 74:280–284

Rittschof D (1999) Fouling and natural products as antifoulants. In: Fingerman M, Nagabhushanam R, Thompson MF (eds) Recent advances in marine biotechnology. Oxford & IBH Publishing Co, Dew Delhi, pp 245–257

Rittschof D (2000) Natural product antifoulants: one perspective on the challenges related to coatings developments. Biofouling 15:119–127

Rittschof D (2001) Natural product antifoulants and coatings developments. In: McClintock JB, Baker BJ (eds) Marine chemical Ecology. CRC, Boca Raton, pp 543–566

Rittschof D, Clare AS, Gerhart DJ, Mary SA, Bonaventura J (1992) Barnacle in vitro assays for biologically active substances: toxicity and settlement inhibition assays using mass cultured *Balanus amphitrite amphitrite* Darwin. Biofouling 6:115–122

Rittschof D, Lai CH, Kok LM, Teo SLM (2003) Pharmaceuticals as antifoulants: concept and principles. Biofouling 19S:207–212

Shade WD, Hurt SS, Jacobson AH, Reinert KH (1993) Ecological risk assessment of a novel marine antifoulant. In: Gorsuch JW, Dwyer FJ, Ingersoll CG, La Point TW (eds) Environmental Toxicology and risk assessment, ASTM STP 1173, Vol. 2. American Society for Testing and Materials, Philadelphia, pp 381–408

Sumida K, Saito K, Ooe N, Isobe N, Kaneko H, Nakatsuka I (2001) Evaluation of in vitro methods for detecting the effects of various chemicals on the human progesterone receptor, with a focus on pyrethroid insecticides. Toxicol Lett 118:147–155

Surrallés J, Xamena N, Creus A, Catalán J, Norppa H, Marcos R (1995) Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures. Mutation Res 341:169–184

Takasawa R, Etoh H, Yagi A, Sakata K, Ina K (1990) Nonylphenols as promising antifouling agents found by a simple bioassay method using the blue mussel, *Mytilus edulis*. Agric Biol Chem 54:1607–1610

Targett NM (1997) Natural antifouling compounds from marine organisms: A review. In: Nagabhushanam R, Thompson MF (eds) Fouling organisms of the Indian Ocean: biology and control technology. Oxford and IBH Publishing Co, New Delhi, pp 85–103

Thomas KV (2001) The environmental fate and behaviour of antifouling paint booster biocides: a review. Biofouling 17:73–86

Thomas KV, McHugh M, Hilton M, Waldock M (2002) Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate. Sci Total Environ 293:117–127

Thomas KV, McHugh M, Hilton M, Waldock M (2003) Increased persistence of antifouling paint biocides when associated with paint particles. Environ Pollut 123:153–161

Townsin RL (2003) The ship hull fouling penalty. Biofouling 19S: 9–15

Turley PA, Fenn RJ, Ritter JC (2000) Pyrithiones as antifoulants: environmental chemistry and preliminary risk assessment. Biofouling 15:175–182

van Wezel AP, van Wlaardingen P (2004) Environmental risk limits for antifouling substances. Aquat Toxicol 66:427–444

Yebra DM, Kiil S, Dam-Johansen K (2004) Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings. Prog Org Coat 50:75–104

Zhou JL, Rowland S, Mantoura RFC (1995) Partition of synthetic pyrethroid insecticides between dissolved and particulate phases. Water Res 29:1023–1031