Zoological Studies **46**(4): 521-528 (2007)

Zoological Studies

Effect of Biofilm Age and Type on Settlement of Cyprids of the Barnacle, *Fistulobalanus albicostatus* Pilsbry (Thoracica: Balanidae)

Ping-Hung Chen[1], Yung-Hui Chen[2], and I-Ming Chen[1,*]

[1]*Institute of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung 804, Taiwan*

[2]*Science Education Department, National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan*

(Accepted October 24, 2006)

Ping-Hung Chen, Yung-Hui Chen, and I-Ming Chen (2007) Effect of biofilm age and type on settlement of cyprids of the barnacle, *Fistulobalanus albicostatus* Pilsbry. *Zoological Studies* **46**(4): 521-528. We studied the effects of biofilms formed in seawater under different filtration treatments on the settlement of cyprids of the barnacle, *Fistulobalanus albicostatus*. Rubber panels (10 x 10 cm) were used to culture biofilms in different tanks where the seawater was either unfiltered or filtered twice a day through different mesh sizes of 1, 20, and 80 μm, respectively. Panels were cultured in each tank together with 350 cyprids, which were allowed to settle for 24 h. Alcohol-sterilized panels were used as controls. The numbers of settled cyprids significantly differed among the 5 treatments ($p < 0.05$) regardless of culture time. Cyprids settled better on panels with biofilms cultured in filtered seawater than in non-filtered water and on alcohol-sterilized panels during 5 d of culture, but settlement decreased on panels cultured for 12 d. The number of cyprids which settled on panels whose biofilms had been cultured for 15 d in non-filtered seawater and on which the films had fallen off from more than 75% of the total area of the panel did not significantly differ from the number that settled on panels cultured in the seawater filtered with the 1 μm mesh. This shows that biofilms cultured under different conditions affect the settlement of cyprids over time. http://zoolstud.sinica.edu.tw/Journals/46.4/521.pdf

Key words: Barnacle, *Fistulobalanus albicostatus*, Biofilm.

Barnacles are major sessile animals on hard coastal substrata. Recently, the effects of biofilms on both the inhibition (Raghukumar et al. 2000, Khandeparker et al. 2003) and stimulation (Maki et al. 1989 1990, Neal and Yule 1994a b) of the settlement of barnacle cyprids have been studied. Most studies focused on the effects on cyprid settlement of a single strain of bacteria cultured from biofilms for a short time in the laboratory. We still do not understand how naturally occurring biofilms and processes of succession affect the settlement of barnacle cyprids.

A biofilm is a complex agglomeration of organisms that includes bacteria, protozoa, algae, and invertebrates, in which the natural microbial population constitutes more than 90% of the biofilm (Costerton et al. 1995). Efforts have been devoted to understanding the effects of microbial films on larval settlement. Extracellular polymeric substances (EPSs) are reported to be major constituents of the biofilm matrix (Cooksey 1992, Costerton et al. 1994). More recently, Khandeparker et al. (2003) indicated that barnacle larvae are induced to settle by specific chemicals in the microbial film. However, most studies concentrated on the bacterial population alone and did not evaluate the potential influence of other sessile species within the biofilm on the settlement of barnacle larvae.

Fistulobalanus albicostatus Pilsbry is a common barnacle in East Asia, including Korea (Lee and Kim 1991), Japan (Nakamura 1997), Hong Kong (Huang et al. 1992), and the South China Sea (Dong et al. 1980, Hung 1994). In Taiwan, this species is abundant and often forms colonies around the docks of Kaohsiung Harbor (Hiro 1939,

*To whom correspondence and reprint requests should be addressed. Tel: 886-7-5252000 ext. 5055.
E-mail:iming@mail.nsysu.edu.tw

Utinomi 1967). Preliminary observations showed that cyprids of *F. albicostatus* quickly settle on artificial substrata within 1 d when the biofilm is just beginning to form. It has been shown that the composition of the biofilm gradually changes with time, and that its attractiveness to larvae of *F. amphitrite* accordingly declines (Faimali et al. 2004). In this study, we used biofilms cultured under 4 different seawater filtering conditions to assess the effects of the composition of the biofilm and its ageing/succession on the settlement of cyprids of *F. albicostatus*.

MATERIALS AND METHODS

Preparation of the biofilms

Seawater was pumped directly from Kaohsiung Harbor, southwestern Taiwan. Three types of seawaters were created by filtering the water through different mesh sizes. Fouling organisms larger than bacteria, cyprid antennule discs, and nauplii were respectively filtered out using mesh sizes of 1, 40, and 80 μm (Lee and Kim 1991, Berntsson et al. 2000). The biofilm panels were made of hard black rubber with dimensions of 12 x 12 cm. Biofilms were formed by immersing panels into 3 different 100 L tanks containing the different seawaters filtered as above and into unfiltered seawater. The fauna occurring in the biofilms were categorized into 4 major groups: bacteria, diatoms, protozoa, and other algae, including blue-green algae and algae with chlorophyll.

Culture of cyprids in the laboratory

Adult barnacles were collected from Kaohsiung Harbor. Egg masses containing embryos with eyes or developed appendages were induced to hatch in 6 well cell culture panels filled with 30 ppt sterilized seawater, 50 μg/ml of streptomycin sulfate, and 10 μg/ml of penicillin (Landau and D'Agostino 1977). In total, 100 eggs were introduced into each well of a 6-well culture plate. The plate was cultured at 30°C in a 14: 10 h L: D cycle. After hatching, 20-25 nauplii from stages I to VI were transferred into 24 well cell culture panels and cultured under the same conditions as above. An algal culture at a concentration of 2 x 10^4 cells/ml of *Skeletonema costatum* was added as food into each well. One-third of the seawater in each well was replaced daily, and bottom precipitates were removed before food was added. After the nauplii had metamorphosed to cyprids, they were transferred into new wells for settlement tests without feeding.

Experimental protocol

Three panels from 4 different culture tanks were selected and individually dried at 105°C for 24 h to estimate the biomass of the biofilm (mg). After being selected, the outer 1 cm of each experimental panel was cut away to form 10 x 10 cm squares. A panel from each of the 4 different filtered culture tanks and 1 alcohol-sterilized panel (as a control treatment) were used to form a box with the biofilm-coated sides to the inside. In total, 350 cyprids were introduced into each box for settlement on the panels for 24 h. The box was immersed in a tank containing 30 ppt sterilized seawater at 30°C with a 14: 10 h L:D cycle. Eight replicates were used every 2 d for the first 10 d and for each day for 5 d afterward. The cyprid settlement rate and dry weight were analyzed by linear regression and one-way ANOVA followed by Duncan's test to compare differences in the biomass of the biofilm and the number of cyprid larvae which had settled on the panels among the various treatments.

RESULTS

Growth of biofilms

Bacteria, diatoms, and other algae occurred in the biofilms formed in the unfiltered seawater treatments from the beginning of the experiment to the 15th d, but protozoa began to appear on the 3rd d. The biomass of the biofilms cultured from 4 treatments of seawater varied with time (Fig. 1). In the unfiltered seawater treatment, the biofilm began to increase around the 4th d(Table 1), reached its maximum at an average of 221.2 ± 26.8 mg on the 9th d, and then became stationary until the 13th d. After 75% of the total area of the biofilm had flaked off the panel, its biomass dropped to 18.3 ± 4.9 mg.

Bacteria and diatoms appeared in the biofilm cultured in 20 μm filtered seawater, while bacteria and other algae appeared in the biofilm in 80 μm filtered seawater from the 1st to 5th d of the experiment. Starting from the 6th d, 4 major groups could be found in the biofilms of both treatments. In both the 20 and 80 μm filtered treatments, the biomass remained low until the 10th d. After that, the biomasses of both groups significantly increased, and between the 13th and 15th d reached a maximum with respective averages of 95.0 ± 16.7 and 160.0 ± 24.1 mg.

In the biofilm cultured in the 1 μm filtered treatment, the biofilm formed contained only bacteria until the 6th d when protozoa and other algae began to appear. The biomass of the biofilm cultured in 1 μm filtered seawater was relatively lower than those of the other treatments with an average of 17.0 ± 5.9 mg at the end of the test. The growth rate of the biomass in the unfiltered treatments significantly differed from the rest of the treatments, but those of the different filtered treatments were similar to each other (Table 2).

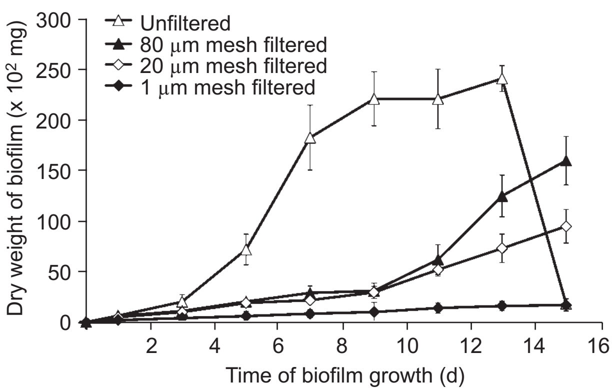

Fig. 1. Growth of biomass on 4 different types of panels cultured in seawater which was either filtered through 80, 20, and 1 μm filters or was unfiltered for 15 d. Part of the unfiltered biofilm had flaked off after 13 d. Values of biomass are given as the mean ± std. dev.

Cyprid larval settlement on the panels

Cyprid larvae rarely settled on the alcohol-sterilized panels throughout the test (Fig. 2). Similarly, fewer cyprid larvae settled on the biofilms cultured in unfiltered seawater until the film began to flake off on the 13th d (Table 3). After that, the number of cyprid larvae that settled on the clear surface quickly increased and reached a maximum with an average of 21.38 ± 2.33 around the 14th d. The number of cyprid larvae which settled on the 1 μm filtered panels was frequently higher than those in the other treatments, and reached its maximum with an average number of 48.88 ± 12.79 on the 5th d, but gradually decreased until the end of the test. In the 1 μm filtered treatments, the highest number of larvae settled on the panel during the first few days but gradually decreased to the lowest on the 11th d when

Table 1. Duncan's comparison of the biomass of biofilms formed in seawater filtered through different mesh sizes (unfiltered, and 80, 20, and 1 μm) with time. Means underlined with the same line do not differ significantly at the α = 0.5 level

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Unfiltered	—	—	—	—										—	—
		—	—	—	—									—	—
				—	—										
							—	—							
								—	—	—	—	—	—		
80 μm	—	—	—	—	—	—	—	—	—						
		—	—	—	—	—	—	—	—	—					
							—	—	—	—	—				
										—	—				
													—	—	
														—	—
20 μm	—	—	—	—	—										
		—	—	—	—	—									
			—	—	—	—	—	—							
				—	—	—	—	—	—						
					—	—	—	—	—	—					
						—	—	—	—	—	—				
											—	—			
												—	—		
														—	—
1 μm	—	—	—	—	—	—	—	—	—	—	—				
		—	—	—	—	—	—	—	—	—	—	—			
		—	—	—	—	—	—	—	—	—	—	—	—		
				—	—	—	—	—	—	—	—	—	—	—	
						—	—	—	—	—	—	—	—	—	—

the biomass of the biofilm significantly increased (Fig. 1). Both the 20 and 80 μm filtered treatments showed similar decreasing tendencies in the number of larvae settling on the panels with time and reached minimum values around the 11th d (Fig. 2). Covariance analysis showed that the decreasing tendency in the number of settled cyprid larvae on panels cultured in 1 μm filtered seawater significantly differed from those of the other 2 treatments, while there was little difference between the 20 and 80 μm filtered treatments (Table 4).

The total settlement rate on the experimental

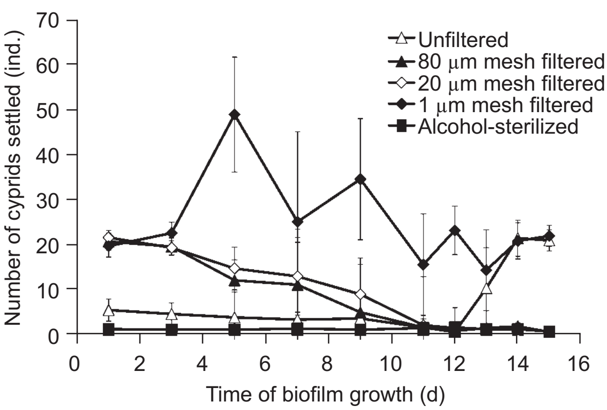

Fig. 2. Number of cyprids settling on 4 panels cultured in unfiltered, or 80, 20, or 1 μm filtered seawater, respectively, and on a panel sterilized with 95% alcohol as the control. The data are given as the mean ± std. dev.

boxes during the 1st day was 19.5% ± 1.1% (Fig. 3). The highest cyprid larval settlement rates were on panels from the 80- 20-, and 1 μm filtered treatments (Fig. 4). A smaller amount settled on the panel from the unfiltered seawater, and the lowest amount was the control which had been wiped with alcohol. On the 5th (Fig. 4), more cyprid larvae had settled on panels from 1 μm filtered seawater, while there were fewer on the panels from the 80- and 20-μm-filtered waters, and the lowest amount occurred on the alcohol-sterilized panels. The total rate of settlement on the experimental boxes on the 5th d was 22.9% ± 3.5% (Fig. 3). On the 12th d (Fig. 4), still more cyprid larvae had settled on the biofilm from the 1 μm filtered water. But it was just 48% of the amount on the 5th d. Larval settlement rates on the 4 other panels showed no significant differences ($p > 0.05$). The total rate of settlement on the experimental boxes was 7.7% ± 2.2% (Fig. 3). On the 15th d (Fig. 4), the panels with flaked-off biofilms from unfiltered seawater were used in the experiment. Cyprid larval settlement levels on the biofilms from unfiltered and 1 μm filtered seawater showed no significant difference ($p > 0.05$). There were similar low amounts of cyprid larval settlement on the 3 other panels without a statistically significant difference ($p > 0.05$). The total rate of settlement on the experimental boxes was 12.7% ± 1.2% (Fig. 3).

Table 2. Growth tendency of the biomass of biofilms cultured in different seawaters filtered through different mesh sizes. (a) Regression equation and (b) probability table from the covariance analysis on the growth of biomass of biofilms cultured in different panels in the time period indicated in (a)

(a)

Treatment	a	b	r^2	N	F	Time (d)
Unfiltered	3.0	- 5.2	0.9	30	308.5**	1-10
80 μm	2.3	-18.0	0.9	21	165.4**	9-15
20 μm	1.2	7.7	0.9	21	124.8**	9-15
1 μm	0.1	0.1	0.5	45	47.2**	1-15

** Significant at the $\alpha < 0.01$ level.

(b)

Treatment	Unfiltered	80 μm	20 μm
80 μm	0.10**		
20 μm	0.11**	0.01	
1 μm	0.13**	0.03	0.02

** Significant at the $\alpha < 0.01$ level.

DISCUSSION

Many factors have been reported to influence the settlement of barnacle cyprids (Berk 2001, Head et al. 2003). There is little doubt that biofilms on the substratum play an important role in inducing settlement of cyprids in many species, including *F. albicostatus* in our study. Recently, much effort has focused on bacterial strains cultured from biofilms and related chemicals that mediate settling processes (Qian et al. 2003). Qian et al. (2003) showed, however, that there are potential risks in applying laboratory data to explain field observations, since many factors are either strictly controlled for or excluded in laboratory experiments. For example, the number of larvae settling on the panels cultured in 1 μm filtered seawater suddenly increased on the 5th d, fluctuated, and gradually decreased until the end of the test.

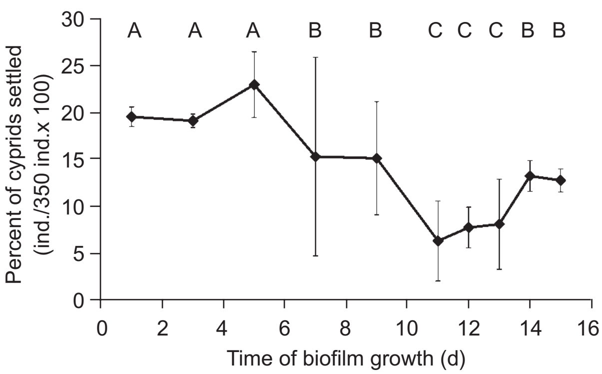

Fig. 3. Percent of cyprids settling on each experimental box plotted against time of biofilm growth. The data are presented as the mean ± std. dev. Different letters (A, B, C) indicate significant differences ($p < 0.05$) according to the ANOVA test followed by Duncan's multiple-range test.

Larval settlement on the alcohol-sterilized panels was low. Larvae showed different preferences for settling on various biofilms and alcohol-treated panels. After 1 d of biofilm formation, the amounts of larval settlement on the other biofilms were statistically higher than that on the alcohol-sterilized panels. We concluded that a relationship exists between biofilms and larval settlement. There were slight differences in the dry weight but significant differences in larval settlement rates among the biofilms. The effects of different types of the biofilms from the various filtered seawaters were apparently the reason. There was extensive larval settlement on the biofilm from 1 μm filtered seawater on the 5th d.

In the early development stage, the biofilm is mainly composed of bacteria that enhance settle-

Table 3. Duncan's comparisons of the number of cyprids settling on different types of panels cultured in seawaters undergoing different filtration treatments. Means underlined with the same line do not differ significantly at the $\alpha = 0.5$ level

Day	1	3	5	7	9	11	12	13	14	15
Unfiltered	—	—	—	—	—	—	—			
									—	—
80 μm					—	—	—	—	—	—
			—	—						
	—	—								
20 μm						—	—	—	—	—
			—	—						
	—	—								
1 μm	—	—				—	—	—	—	—
				—	—					

ment (O'Connor and Richardson 1998, Maki et al. 2000). Previous studies showed that during the settling process, barnacle larvae only respond to chemicals excreted from specific bacterial strains (Raghukumar et al. 2000). Even though only organisms of a size smaller than most bacteria could have been able to form the biofilm in the experiments with the 1 μm filtered seawater, the composition within the biofilm might have changed during the succession process, resulting in a weakening of the settlement-inducing factors. Our results showed that the early development stage of the biofilm is a critical period for settlement of barnacle cyprids, as afterwards, the inducement for larvae to settle weakens with aging of the biofilm. Organisms of a size smaller than 20 or 80 μm

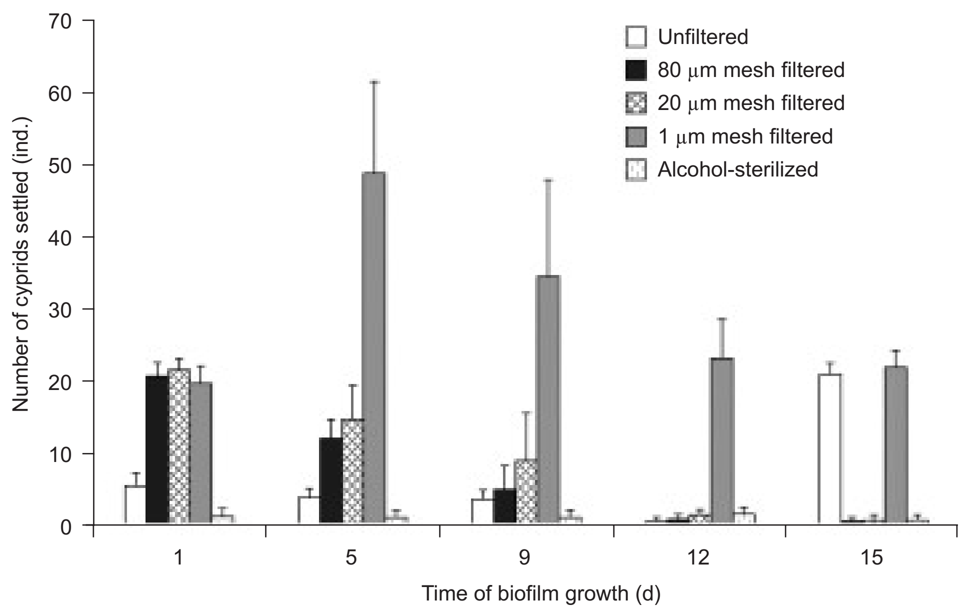

Fig. 4. Numbers of cyprids settling on panels that had undergone 5 different processes (unfiltered; 80, 20, and 1 μm mesh filtration; and alcohol sterilization). Cyprid counts of the biofilms are from days 1, 5, 9, 12, and 15.

Table 4. Number of larvae settling on different types of panels with time. (a) Regression equation, and (b) probability table from the covariance analysis on the number of larvae settling on different panels in the time period indicated in (a)

(a)

Treatment	a	b	r^2	N	F	Time (d)
Unfiltered	6.0	-66.6	0.7	40	105.0**	11-15
80 μm	-2.0	23.6	0.7	48	116.4**	1-11
20 μm	-1.9	24.4	0.6	48	80.6**	1-11
1 μm	-3.5	60.5	0.3	40	14.9**	5-12

** Significant at the $\alpha < 0.01$ level.

(b)

Treatment	Unfiltered	80 μm	20 μm
80 μm	6.84		
20 μm	8.30*	1.46	
1 μm	23.82**	16.98*	15.52**

* Significant at the $\alpha < 0.05$ level. ** Significant at the $\alpha < 0.01$ level.

gradually replaced the microbes during the succession processes. Previous studies showed that many benthic organisms are able to secrete chemicals that prevent settlement of larvae of other benthic species (Noda 1998, Lindquist 2002). Some of these might have indirectly affected the settling processes by pre-occupying the space of the biofilm needed for barnacle larval settlement. For example, the presence of barnacle shells does not enhance the settlement of cyprids (Jeffery 2002, Hansson et al. 2003). In our experiment, bacteria was the major group occurring in all treatments in the early development stage of the biofilms. The number of barnacle larvae settled was also higher in the early development stage of the biofilms than that in the later period when protozoa and other algae had begun to appear. However, after protozoa began to appear in the biofilm on the 6th d, settlement of barnacle larvae began to decrease in the 1, 20, and 80 μm filtered treatments. In the unfiltered treatment, it was not until the 13th d when part of the biofilm had flaked off the panel that settlement of larvae significantly increased. This shows that protozoa might prey on bacteria, which results in a weakening of the effects of induction of settlement of barnacles by bacteria.

In conclusion, our study indicated that biofilm types, defined by both size categories and compositions, influence larval settlement. Even when the biofilm was mainly composed of microbes smaller than 1 μm, aging or succession of the biofilm which produces changes in the microbial composition gradually reduced larval settlement. Organisms between 1 and 20 μm seemed to be critical for enhancing the degree of settlement, since the number of settled larvae differed significantly between the 2 treatments. It would be worthwhile analyzing other filtering conditions between these 2 treatments and the effect of the protozoa appearing in the biofilm to more-exactly identify the biofilm types.

Acknowledgments: This research was supported by the National Science Council (NSC93-2313-B-110-004) of the R.O.C. We thank the Office of Kaohsiung Harbor for providing space for this research.

REFERENCES

Berk SG, R Mitchell, RJ Bobbie, JS Nickels, DC White. 2001. Microfouling on metal surfaces exposed to seawater. Int. Biodeter. Biodegr. **48:** 167-175.

Berntsson KM, PR Jonsson, M Lejhall, P Gatenholm. 2000. Analysis of behavioural rejection of microtextured surfaces and implications for recruitment of the barnacle *Balanus improvisus*. J. Exp. Mar. Biol. Ecol. **251:** 59-83.

Cooksey KE. 1992. Extracellular polymers in biofilms. *In* LF Melo, TR Bott, M Fletcher, B Capdeville, eds. Biofilms: science and technology. The Netherlands: Kluwer Academic Publishing, pp. 137-147.

Costerton JW, Z Lewandowski, DE Caldwell, DR Korber, HM Lappin-Scott. 1995. Microbial biofilms. Annu. Rev. Microbiol. **49:** 711-745.

Costerton JW, Z Lewandowski, D DeBeer, D Caldwell, D Korber, G James. 1994. Minireview: biofilms, the customized microniche. J. Bacteriol. **176:** 2137-2142.

Dong Y, Y Chen, R Cai. 1980. Preliminary study on the Chinese cirripedian fauna. Acta Oceanol. Sin. **2:** 124-131. (in Chinese with English abstract)

Faimali M, F Garaventa, A Terlizzi, M Chiantore, R Cattaneo-Vietti. 2004. The interplay of substrate nature and biofilm formation in regulation of *Balanus amphitrite* Darwin, 1854 larval settlement. J. Exp. Mar. Biol. Ecol. **306:** 37-50.

Hansson LJ, IR Hudson, RJ Seddon. 2003. Massive recruitment of the barnacle *Semibalanus balanoides* in the Clyde Sea (Scotland, UK) in the spring of 2000. J. Mar. Biol. Assoc. UK **83:** 923-924.

Head RM, K Overbeke, J Klijnstra. 2003. The effect of gregariousness in cyprid settlement assays. Biofouling. **19:** 269-278.

Hiro F. 1939. Studies on the cirripedian fauna of Japan. IV. Cirripeds of Formosa (Taiwan), with some geographical and ecological remarks on the littoral forms. Memoirs of the College of Science, Series B, Vol. 15, No. 2. Kyoto, Japan: Kyoto Imperial Univ. Press, pp. 245-284.

Huang XM, SK Yan, S Lin, DQ Zheng. 1992. Biofouling communities on piers and pilings in Mirs Bay. *In* Morton, B. S., ed. Proceedings of the Fourth International Marine Biological Workshop: the marine flora and fauna of Hong Kong and southern China. Hong Kong: Hong Kong Univ. Press, pp. 529-543.

Hung ZG. 1994. Marine species and their distribution in China Sea. Beijing: China Ocean Press, pp. 516-523.

Jeffery CJ. 2002. New settlers and recruits do not enhance settlement of a gregarious intertidal barnacle in New South Wales. J. Exp. Mar. Biol. Ecol. **275:** 131-146.

Khandeparker L, AC Anil, S Raghukumar. 2003. Barnacle larval destination: piloting possibilities by bacteria and lectin interaction. J. Exp. Mar. Biol. Ecol. **289:** 1-13.

Landau M, A D'Agostino. 1977. Enhancement of laboratory cultures of the barnacle *Balanus eburneus* using antibiotics. Crustaceana **33:** 223-225.

Lee C, CH Kim. 1991. Larval development of *Balanus albicostatus* Pilsbry (Cirripedia, Thoracica) reared in the laboratory. J. Exp. Mar. Biol. Ecol. **157:** 231-244.

Lindquist N. 2002. Chemical defense of early life stages of benthic marine invertebrates. J. Chem. Ecol. **28:** 1987-2000.

Maki JS, L Ding, J Stokes. 2000. Substratum bacterial interactions and larval attachment: films and exopolysaccharides of *Halomonas marina* and their effect on barnacle cyprid larvae, *Balanus amphitrite* Darwin. Biofouling **16:** 159-170.

Maki JS, D Rittschof, MO Samuelsson, U Szewzyk, AB Yule, S Kjelleberg, JD Costlow, R Mitchell. 1990. Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. Bull. Mar. Sci. **46:** 499-511.

Maki JS, D Rittschof, AR Schmidt, AG Snyder, R Mitchell.

1989. Factors controlling attachment of bryozoan larvae: a comparison of bacterial films and unfilmed surfaces. Biol. Bull. **177:** 295-302.

Nakamura K. 1997. Growth and shell strain of a barnacle *Balanus albicostatus* population in an aquarium. Fisheries Sci. **63:** 22-28.

Neal AL, AB Yule. 1994a. The tenacity of *Elminius modestus* and *Balanus perforatus* cyprids to bacterial films grown under different shear regimes. J. Mar. Biol. Assoc. UK **74:** 251-257.

Neal AL, AB Yule. 1994b. The interaction between *Elminius modestus* Darwin cyprids and biofilms of *Deleya marina* NCMB 1877. J. Exp. Mar. Biol. Ecol. **176:** 127-139.

Noda T, K Fukushima, T Mori. 1998. Daily settlement variability of the barnacle *Semibalanus cariosus*: importance of physical factors and density-dependent processes. Mar. Ecol. Prog. Ser. **169:** 289-293.

O'Connor NJ, DL Richardson. 1998. Attachment of barnacle (*Balanus amphitrite* Darwin) larvae: responses to bacterial films and extracellular materials. J. Exp. Mar. Biol. Ecol. **226:** 115-129.

Qian PY, T Thiyagarajan, SCK Lau, SCK Cheung. 2003. Relationship between bacterial community profile in biofilm and attachment of the acorn barnacle *Balanus amphitrite*. Aquat. Microb. Ecol. **33:** 225-237.

Raghukumar S, AC Anil, L Khandeparker, JS Patil. 2000. Thraustochytrid protists as a component of marine microbial films. Mar. Biol. **136:** 603-609.

Utinomi H. 1967. Comments on some new and already known cirripeds with emended taxa, with special reference to the parietal structure. Publ. Seto. Mar. Biol. Lab. **15:** 199-237.