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Establishment and Succession of an Epibiotic Community on Chromated Copper Arsenate-Treated Wood in Mediterranean Waters

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Abstract Colonization and succession of an epibiotic animal community on chromated copper arsenate (CCA)-treated wood were studied for 18 months in the eastern Mediterranean (Saronikos Gulf, Aegean Sea). Pine wood panels, 200 × 100 × 25 mm, impregnated with CCA at retentions of 0, 12, 24, and 48 kg m^{-3} were used. The abundance or surface coverage of the most characteristic taxa (polychaetes, mollusca, crustacea bryozoa, sponges, ascidians) was measured in situ, while 12 months after submersion two panels of each retention were removed and examined in the laboratory. A total of 26 taxa were identified, among which polychaetes of the family *Serpulidae* dominated. The controls carried the largest number of species (17) but the lowest number of individuals. On panels with CCA retentions of 12 and 24 kg m^{-3}, 14 and 16 species were observed, respectively, while at 48 kg m^{-3}, only 9 species were found. Only the controls were affected by boring bivalves of the family Teredinidae and started to break up within 3 months of submersion. Statistically significant differences in barnacle and polychaete abundance were found between treated and untreated panels. There were no significant differences among panels treated at the three CCA loadings. Ordination by nonmetric multidimensional scaling showed a seasonal effect on the colonization of the treated panels, with the highest recruitment during the warmer months of the study.

Wooden constructions in the sea, such as pilings, bulkheads, and docks, are exposed to marine fungi, bacteria, and wood-boring animals. To prevent decay, wood is treated with preservatives which inhibit the settlement and the growth of fouling organisms. Copper is the preferable biocide today (Srinivasan and Swain 2007); in particular, chromated copper arsenate (CCA) is the most common antifouling paint currently in use (Hingston et al. 2001). The three metals copper, chromium, and arsenic—in the form of oxides—are pressure-impregnated in aqueous solution into the wood, then chemically bound to the lignocellulose complex of the wood (Anderson et al. 1991). It has been demonstrated, however, that they leach from the treated wood when it is submerged in estuaries (Weis and Weis 1992b) or in marine (Breslin and Adler-Ivanbrook 1998) and freshwater (Warner and Solomon 1990) ecosystems and thus enter the environment.

The effects of CCA on the biota may register at various levels of biological organization. For example, an elevated occurrence of metaplasia in the digestive diverticula and of micronuclei in gill cells was found in oysters (*Crassostrea virginica*) residing on CCA-treated wood inside a canal (Weis et al. 1993). CCA leachate reduced the fertilization success of the sea urchin *Arbacia punctulata* sperm by 90% and totally inhibited the larval development of fertilized eggs (Weis et al. 1992). Snails, *Nassarius obsoletus*, fed on seaweed growing on CCA-treated wood died or became inactive after 4 weeks (Weis and Weis 1992a). Growth of

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the barnacle *Balanus eburneus* was reduced on recently installed CCA-treated wood, as was the size of bryozoan colonies (Weis and Weis 1992b). However, attention should be drawn to the high concentrations of leachate likely to occur under the conditions in which all the above experiments/observations were conducted. The laboratory work was done under static conditions, while the fieldwork was conducted in areas of restricted water flow. In laboratory experiments in a flow-through system, Adler-Ivanbrook and Breslin (1999) found less evidence for effects of CCA leachate on bivalves. Wendt et al. (1996) looked at the effects of water flow and concluded that the deleterious effects were only likely under conditions of very restricted water movement. Overall, it should be considered that different organisms have different sensitivities to different toxicants and that sensitivities differ within the same organisms depending on their life stage (McPherson and Chapman 2000).

Populations may be affected by impacts on settlement, survival, growth, and competitive ability. Such effects will be integrated in the process of community development. Studies of the effects of CCA at the community level have shown a decrease in the diversity of epibionts growing directly on treated wood (e.g., Weis and Weis 1992b, 1996) or of the benthic communities of the adjacent sediments (e.g., Neary et al. 1989, Weis and Weis 1994, Weis et al. 1998). However, similar or higher numbers of taxa on CCA-treated compared to untreated wood have been reported (Brown et al. 2003). Different experimental protocols (e.g., exposure time) and conditions which affect leaching rates of CCA (see Hingston et al. 2001) may be responsible for discrepancies among the above studies. Epibiota studies offer a particularly good insight into the impact of wood preservatives on immediately adjacent fauna, but to date, such work has tended to focus on numbers of recruits of particular species after a period of exposure (Cookson et al. 1996, Brown and Eaton 1997, Cragg and Eaton 1997). Separating effects of settlement from confounding factors such as survival and competitive ability requires either data collection immediately after settlement (Brown et al. 2001) or data collection at regular intervals as in the short-term study by Weis and Weis (1992b). In this study, we followed a regular observation regime of CCA-treated and untreated panels submerged in Saronikos Gulf (Aegean Sea) over a period of 18 months. As the preservative loading is likely to affect the leaching rate (Archer and Preston 1994, Hayes et al. 1994), experiments were carried out using three levels of preservative loading (12, 24, and 48 kg m^{-3}) and also untreated wood. A previous study by Brown et al. (2003) in seven European exposure sites (including Saronikos Gulf) investigated the effect on nontarget epibiota by comparing the fouling communities developed after determined exposure periods. This study aims to provide additional information on the effects of CCA since the continuity of our data permitted effects on settlement to be investigated and enabled the impact of CCA-treated wood on colonization and succession of epibiotic communities to be assessed for the first time in Mediterranean waters. The study was conducted within the framework of the PINTO research program (Preservative Impact on Non-Target Organisms; MAS2-CT94-0100).

Materials and Methods

Experimental panels, measuring 25 × 100 × 200 mm, were machined from Scots pine (*Pinus silvestris*) sapwood. They were prepared and conditioned at 20°C and 65% relative humidity for a minimum of 4 weeks, then end-sealed with epoxy resin (Schering Europox 710). They were treated in accordance with the Bethel full cell process according to BS 5589 (1989) to obtain retentions of 12, 24, and 48 kg m^{-3} CCA (Celcure AO). Panels were attached to racks constructed from 32-mm uPVC pipe and ABS fittings. Each rack carried eight randomly arranged panels: two of each retention and two nontreated control blocks. Twelve racks were submerged vertically at a depth of approximately 1 m, hung from a concrete jetty, in a semisheltered site in Saronikos Gulf, Aegean Sea. The results presented here were obtained during the 18-month exposure period from August 1995 to January 1997. During the experiment, the water temperature ranged from 12.9°C in February to 25.9°C in July, while the salinity remained close to 39 psu. The maximum chlorophyll *a* concentration (1.39 μg L^{-1}) appeared in February, corresponding to the winter phytoplankton bloom, and the minimum (0.07 μg L^{-1}) in the summer (V.A. Katsiki, HCMR, Greece; personal communication).

The panels of six racks were examined in situ, weekly for the first 6 months and twice a month for the rest of the experimental period. However, monthly data are presented here for the purpose of clarity. Individuals of large solitary animals, such as barnacles and mussels, were enumerated, while for the rest the percentage cover of the panel was estimated using an 18-mm^2 grid. To present our data in a comparative way, an arbitrary scale was used, as follows: 1 = presence, 2 ≤ 10% cover, 3 = 11%–20% cover, 4 = 21–30% cover,… 7 > 50% cover for colonial species and serpulids and 1 = 1–10 individuals, 2 = 11–20 individuals, 3 = 21–30 individuals, 4 = 31–40 individuals, and 5 = 41–50 for solitary species. Arbitrary scaling is a semiquantitative method for estimating the density or surface coverage of epibiotic organisms (Crisp and Southward 1958). A semilogarithmic scale is suitable for large-scale surveys (Hawkins and Jones 1992). In this study we chose to use a proportional scale since the relatively small surface of panels as well as the low abundance of epibiotic organisms, observed for most of the experimental period, required a finer scale in order to get better resolution.

Twelve months after the beginning of the experiment, the panels from one randomly selected frame were carefully removed and brought to the laboratory. Fouling organisms were identified to the species level or higher taxa and enumerated using a stereo light microscope. Thus, a quantitative description of the established community was possible. All statistical analyses were carried out using the software package STATISTICA. For the ordination of monthly data from the six frames exposed for 18 months, multidimensional scaling (MDS) was applied using the software package PRIMER (Clarke and Warwick 1994).

Results

Settlement and Succession

Based on field observations, the first settlement of animal groups on the experimental panels occurred 2 weeks after their installation in August 1995. The first organisms to settle on both treated and untreated panels were the polychaetes of the family Serpulidae. Their tubes were very thin and short and covered only a very small part of the wood surface (<<10%). The abundance of serpulids increased slowly until July 1996, when a new mass settlement occurred in the three treatments (12, 24, and 48 kg m^{-3} CCA) and the surface coverage reached $\sim$ 40% (Fig. 1). Then their abundance increased more rapidly, reaching a maximum in September–October 1996 (100% cover), after which they declined slightly. Higher abundance was observed on the 48 kg m^{-3} CCA-treated panels (Fig. 1). On the control panels serpulid abundance remained relatively low and practically unchanged.

The second animal group to settle was the bryozoa in September 1995. They never formed large colonies on either the control or the treated wood. Sponges were first recorded on a control and a 24 kg m^{-3} CCA-treated panel in November 1995. Subsequently, they appeared sporadically and always at low densities (one individual per panel) on different panels until autumn 1996, when a mass settlement occurred mainly on control panels. A total of 21 sponges were encountered during the course of the experiment. However, in most cases, they failed to remain attached on the panels for more than 5 months, and in only one case did they survive and grow to the end of the experiment (24 kg m^{-3} treated wood, 14 months).

Initial settlement of barnacles, *Balanus amphitrite*, occurred on two CCA-treated panels (12 and 48 kg m^{-3} CCA) in October 1995. However, they did not develop further until March 1996, when their numbers begun to increase in all treatments, reaching maximal values (>30 individuals per panel) in June 1996 (Fig. 1). Then their densities dropped on all retentions. Untreated panels had lower numbers of barnacles compared to CCA-treated panels but followed the same pattern of seasonal evolution (Fig. 1).

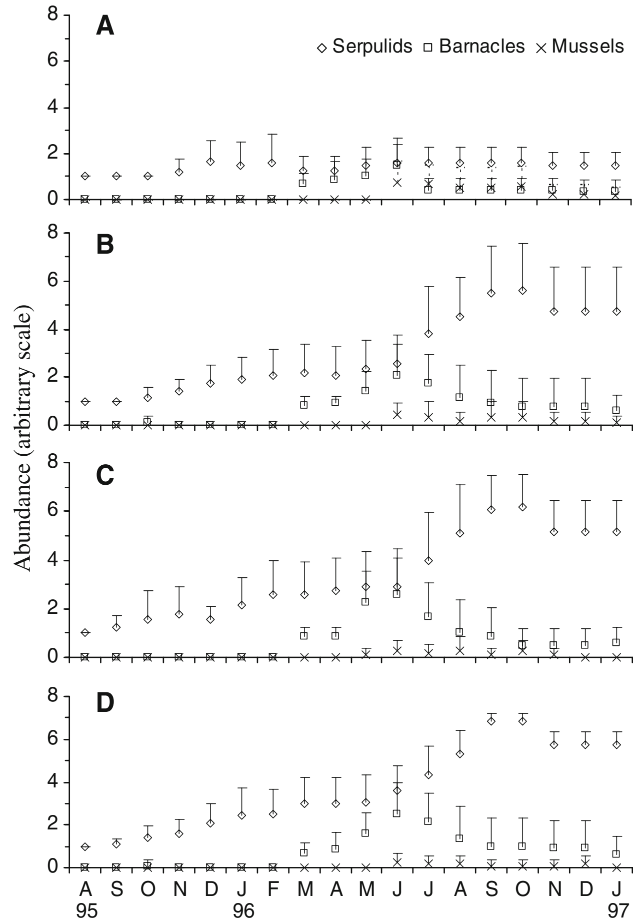

Fig. 1 Population changes in serpulids, barnacles and mussels between August 1995 (A95) and January 1997 (J97) on the control panel (**a**) and the 12 kg m^{-3} (**b**), 24 kg m^{-3}, and (**c**) and 48 kg m^{-3} retentions. Mean values of abundances on the following arbitrary scales. Serpulids: 1 = presence, 2 ≤ 10% cover, 3 = 11–20% cover, 4 = 21–30% cover,…, 7 > 50% cover. Barnacles and mussels: 1 = 1–10 individuals, 2 = 11–20 individuals, 3 = 21–30 individuals, 4 = 31–40 individuals, and 5 = 41–50 individuals. Error bars: standard deviation of measurements (n = 12). For clarity only "plus" error bars are presented

The settlement of mussels, *Mytilus galloprovincialis,* was first observed on a 24 kg m^{-3} CCA-treated panel in May 1996. However, mass settlement of these organisms was observed a month later, in June 1996. Abundance on control panels (21–30 individuals per panel) was higher than on CCA-treated panels until November 1996, when it declined and equaled that of treated panels (≤10 individuals per panel; Fig. 1). In January 1997 mussels disappeared completely from the panels with the highest retentions (24 and 48 kg m^{-3}).

A single occurrence of ascidians was noticed from March to April 1996. Their main settlement occurred in September 1996 but they always covered a low percentage of the panel surface (<<10%).

The settlement of the boring Teredinidae was manifested in October 1995 by the appearance, on the surface of the untreated wood, of small holes (1–2 mm in diameter) through which the siphons of the bivalve protrude and through which it maintains contact with the external seawater environment. Although there was no other sign of Teredinidae on the surface, the bivalves formed tunnels in the wood until it started to fragment and break up. Towards the end of October 1995 one of the untreated panels had already lost one of its corners, while by February 1996 another panel was broken up and had fallen from the frame.

Figure 3 displays the results of ordination by MDS based on the data obtained from panels of six frames removed in January 1997 (18-month exposure). Panels form three distinct groups in the two-dimensional plot. The presence of Teredinidae strongly influences the ordination, with control panels forming a group at the top-left part of the graph. CCA-treated panels form two distinct groups, each with 60% similarity. Data representing the initial colonization of the panels, from August to February 1996, form a group at the bottom-left part of the graph. Data obtained from spring 1996 to the end of the experiment form a different group, representing the second settlement phase of the fouling organisms.

Community Characteristics

Species identification was carried out on the panels of one frame randomly selected at the end of the first year and the results are presented in Table 1. On the whole, 26 species were identified. The most abundant were the polychaetes (Fig. 2), particularly the serpulids. Serpulid abundance ranged between <400 and >1500 individuals per panel (on control and 12 kg m^{-3} CCA-treated wood, respectively). The polychaetes were also the group with the highest number of species (15). They were followed by crustacea, with eight species. The abundance of different crustacean species was generally low (<10 individuals per panel) except that of the species belonging to the Tanaidacea (~70 individuals per panel on 12 kg m^{-3} CCA-treated wood) and the barnacle *B. amphitritae* (20–40 individuals per panel on CCA-treated panels of different retentions). Mollusca were represented by two species (Table 1). Species of the family Teredinidae were completely absent from treated panels, while *M. galloprovincialis* was found only on the control and a 24 kg m^{-3} CCA-treated panel. Mussel abundance on the control was higher by a factor of 4. Overall, the numbers of species and individuals varied among the four treatments (Table 1, Fig. 2). The control had the lowest number of individuals, although it possessed the highest number of species, i.e., 17. Retentions of 12 and 24 kg m^{-3} had slightly lower numbers of species (14 and 16 species, respectively), while at a retention of 48 kg m^{-3}, only 9 species were found.

The mean abundance of the most numerous groups – serpulids, spirorbids, barnacles, and total polychaetes—on treated and untreated panels removed 1 year after submergence were compared using one-way ANOVA. Statistically significant differences ($p < 0.05$; $n = 6$) were observed between treated and untreated panels for barnacles, serpulids, and total polychaetes (serpulids, hydroides, spirorbids). Mussels were not tested since their abundance, mainly on treated panels, was very low. However, no statistical significant differences were found between different CCA loadings. Similarly, one-way ANOVA did not show any statistically significant difference among the abundances of epibiotic organisms encountered on different racks ($p > 0.05$; $n = 8$).

Discussion

The establishment and succession of epibiotic communities depends on several biotic (e.g., larval selectivity, species interactions, adult–larval interactions, slime films) and abiotic (e.g., availability of substrates, water depth, temperature, salinity, and current strength) factors (Qiu et al. 2003, and references therein). CCA retention is an additional, anthropogenic factor to be considered here. Two distinct phases of settlement can be recognized in the MDS ordination based on the abundance of different taxa (polychaetes, bivalves, bryozoa, barnacles, sponges, ascidians) (Fig. 3). The first is the initial phase of recruitment on the panels from their submersion in midsummer to the middle of autumn, and the second occurs in the spring of the second year. Our findings reflect the effect of temperature on fouling, since the area of submergence of panels presents a marked seasonal variation of temperature (12.9–25.9°C). Recruitment was higher during the warmer months compared to the cooler months, as has been reported in the past (Braithwaite et al. 2007, Greene and Grizzle 2007). Since we did not find any statistically significant differences between racks, it seems that replicates were not blocked by racks, which would indicate a spatial variation of the abundance of epibiotic organisms.

The numbers of species and individuals are affected by CCA, the first being lower on treated panels and the second

Table 1 Species encountered and total number of taxa in different CCA retentions after 1 year of submergence

	CCA concentration			
	0 kg m^{-3}	12 kg m^{-3}	24 kg m^{-3}	48 Kg m^{-3}
Polychaeta				
Amphitrite johnstoni	+	–	–	–
Capitella capitata	+	–	–	–
Eulalia viridis	–	+	+	–
Hydroides sp.	+	+	+	+
Lepidontous sp.	–	+	–	–
Nereis zonata	–	–	+	–
Nereis sp.	–	–	+	–
Phyllodoce laminosa	–	–	–	+
Polydora giardi	–	+	+	–
Potamila reniformis	+	–	–	–
Serpulidae	+	+	+	+
Spirorbidae	+	+	+	+
Syllis gracilis	+	+	+	+
Syllis sp.	–	+	–	–
Trypanosyllis sp.	–	–	+	–
Mollusca				
Mytilus galloprovincialis	+	–	+	–
Teredinidae	+	–	–	–
Crustacea				
Balanus amphitrite	+	+	+	+
Corophium acherusicum	+	–	+	–
Corophium acutum	+	+	+	–
Corophium sextonea	+	–	+	–
Corophium sp.	+	+	–	–
Anthura gracilis	–	+	–	+
Limnoria sp.	+	–	–	–
Tanaidacea	+	+	+	+
Bryozoa				
Schizoporella errata	+	+	+	+
Total no. of taxa (*S*)	17	14	16	9

Note: (+) Presence and (–) absence of species

on untreated. Decreased species diversity, although associated with lower numbers of macroinvertebrates, was found by Neary et al. (1989) in pond sediments affected by CCA. Fewer species and lower diversity were also found on CCA-treated wood by Weis and Weis (1992b). It is believed that the lower numbers of specimens in the present study may be due to the drastic reduction of available space on the control panels since large parts of the wood were tunneled by Teredinidae. This problem could probably be treated in future studies by using natural habitats at similar depths as controls. Unfortunately no such data exist in the present study. The reduced number of individuals on the control panels was largely due to the lower numbers of serpulids and, to a lesser extent, of the barnacles *B. amphitrite*.

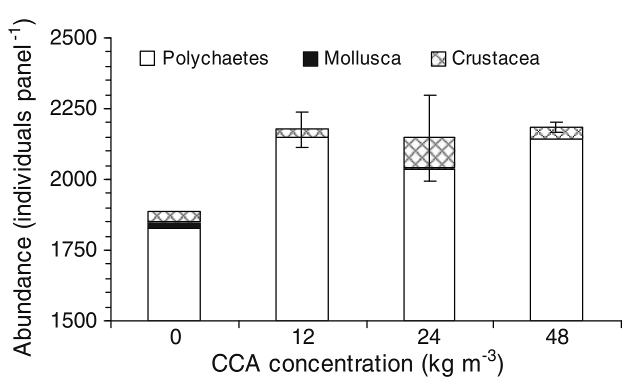

Fig. 2 Mean abundance of different taxonomical groups (polychaetes, mollusca, and crustacea) identified on a randomly selected rack 1 year after installation. Error bars: deviation from the mean of each replicate. Note that data from one control panel are lacking

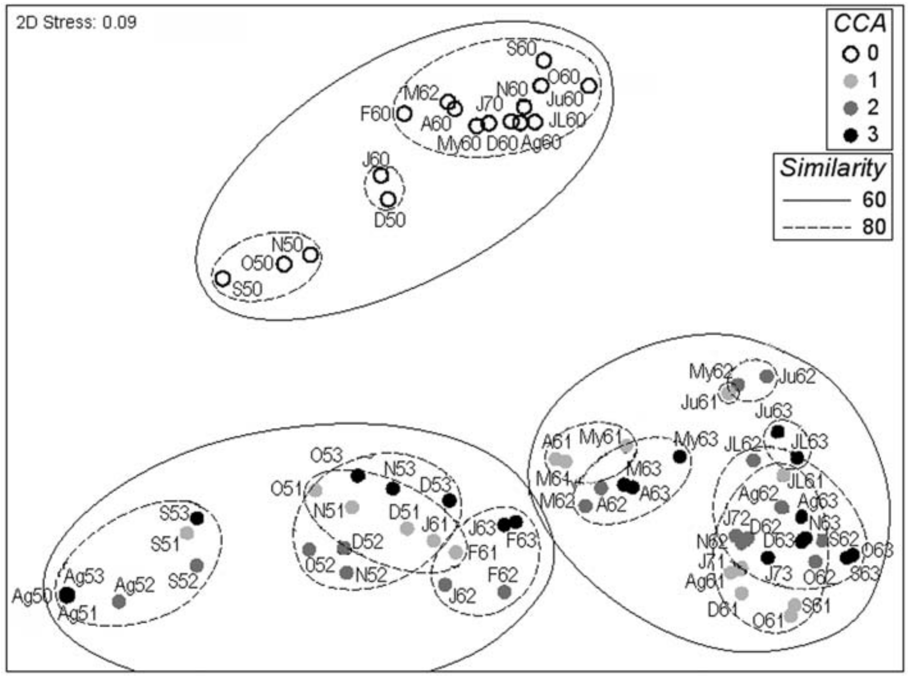

Fig. 3 MDS ordination based on the arbitrary scale of macrofaunal abundance on individual panels after 18 months of exposure (August 1995–January 1996). Letters represent the sampling month. The first number of each label indicates the sampling year (5 = 1995, 6 = 1996, 7 = 1997), and the second the CCA loading (0 = control, 1 = 12 kg m^{-3}, 2 = 24 kg m^{-3}, 3 = 48 kg m^{-3}). Similarity corresponds to the Bray-Curtis index

CCA loading did not seem to have a direct relation with the species present, although the absence of all amphipods from panels with loading of 48 kg m^{-3} should be noted. Similarly, Brown and Eaton (2001) found no dose–response relationship between species abundance and CCA loading in a similar long-term exposure trial conducted in Langstone Harbour (Portsmouth, UK).

Biofouling of serpulids was noticed from the first weeks and increased—mainly on treated panels—during the course of the experiment, in contrast to other exposure sites of CCA-treated and untreated woods in the North Sea, Bay of Biscay, and North Atlantic (Brown et al. 2003). It seems that high temperature and salinity, as well as eutrophication, enhance the fouling by serpulids, as was also found by Khatoon et al. (2007) in experiments conducted on different substrates in tropical marine shrimp ponds.

The higher densities of barnacles on the treated panels contrasts with the report by Weis and Weis (1992b), who found fewer specimens of a related species, *Balanus eburneus,* on treated wood. Such differences may be due to different behavior of substratum selection of the two species. Difference in substratum selection behavior was also found by Rittschof et al. (1984) between *B. amphitrite* and *Semibalanus balanoides* as described by Crisp and Meadows (1963). According to M. J. Anderson and Underwood (1994) barnacle larvae settle in greater numbers on dark than on light surfaces. Probably, in our experiments barnacles were also attracted by the dark-greenish color of the CCA-treated panels. Finally, the low abundance of barnacles as well as of serpulids on the controls may be related to the presence of Teredinidae. Teredinidae were the only very abundant organisms on those panels, and were absent from treated woods. Weis and Weis (1992b), who found higher numbers of barnacles on untreated wood compared to CCA-treated wood, did not mention any borers. They may have been absent due to the shorter duration of their study.

The mussels *M. galloprovincialis* showed a preference for untreated wood. The related species *M. edulis* was found to prefer to settle on rough substrata covered by microfilm (Bayne 1964, Dobretsov and Railkin 1996, Satuito et al. 1997). Dobretsov and Railkin (1996) suggested that roughness influences larvae by increasing water turbulence. All these substratum characteristics apply to the control wood, which is made rough by tunneling by Teredinidae and where the formation of microbial biofilm is not inhibited by the preservative. Similarly, Hunt and Scheibling (1998) observed colonies of *Mytilus trossulus* and *M. edulis* on a variety of substrata on the shore of Nova Scotia, but rarely on smooth otherwise unoccupied surfaces. Menge (1991) found that there is competition for space between mussels and barnacles in New England. Mussels settled preferentially on rugose rock surfaces, particularly substrata occupied by *Semibalanus balanoides,* either eliminating them by competitive exclusion or being eliminated themselves by predators. In the present study, mussels settled 4 months later (June 1996) and reached a lower maximum abundance than barnacles. However, mussels and barnacles co-occurred on the control panels from July 1996 to the end of the experiment (January 1997), presenting similar densities (Fig. 1). On the contrary, on CCA-treated panels barnacles always prevailed over mussels. Moreover, mussel abundance on the controls was always higher than on CCA-treated wood. These findings indicate a higher degree of impact of CCA on mussel than on barnacle attachment and growth. Similarly, Mayer-Pinto and Junqueira (2003) found that *Balanus* spp. are tolerant to toxic conditions, while according to Jelic-Mrcelic et al. (2006), and references therein) mussels are copper-sensitive organisms. The difference in abundance between retentions might have been more obvious had the mussel settlement occurred closer to the time of submergence when leaching was more intense. Wendt et al. (1996) reported field studies with bioassays indicating that, in nonstagnant environments, effects of leachate are likely to be short-lived and localized. Such information could be obtained in the future if new panels are submerged every month, or at least near the time of known larval settlement.

The importance of predation in shaping patterns of succession was mentioned by Menge et al. (1983) and Hurlbut (1991). During the present study shrimp, crabs, and small fish were seen on the panels, and on one occasion scraping was obvious. It is believed that the local consumer regime may have caused small-scale patchiness and variability between panels, which, however, was minimized by the use of a large number of replicates in our field data.

In conclusion, the overall epibiotic community which developed on the wooden panels was similar, with quantitative differences induced by the presence of CCA. The presence of CCA appeared to promote settlement in some species but reduce it in others. The temporal changes observed were basically a result of the larval availability in the water. It is not clear whether differences observed between treated wood and controls are direct effects of CCA or of the modification of the substratum by the boring bivalve Teredinidae, established only on the control panels. More refined experiments are required to assess the subtle interspecific relationships.

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