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In situ measurements of the functional response of benthic suspension feeders exposed to cadmium and anti-fouling paint

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Abstract

In situ measurements of the functional response (feeding, respiration and excretion) of *Mytilus edulis* and *Ciona intestinalis* showed that the effects of 2.5 µg l^{-1} tributyl tin and 100 µg l^{-1} cadmium on an assemblage of the two species was lower than what could be predicted from the response of the two species separately. This is explained by biological interactions between the species and by the fact that the two species may react in different ways to the same disturbance. Thus, results from single species tests seem inadequate for making predictions of pollution effects in marine environments, and tests should instead be carried out at the community or ecosystem level.

Introduction

Concern about changes in the marine environment and their effects on the biota have evoked an extensive research. Many investigations have focussed on bivalve molluscs, where lethal toxicity levels, accumulation rates, and to a limited extent, sublethal effects have been studied (review in AKBERALI and TRUMAN 1985). The need of parallel tests on higher levels of biological organization e.g. ecosystems, instead of solely single species tests in hazard assessments has been emphasized (GIDDINGS and EDDLEMON 1978, CAIRNS 1983) as well as the importance of detecting sublethal effects, which are visible before structural changes such as shifts in species composition will occur (MATTEWS et al. 1982). To evaluate the possible danger arising from contamination with pollutants it is thus necessary to understand the mechanisms of their effects on the physiology of the species (THEEDE 1980, 1984), and to take into account effects on interactions between species. (O'NEILL and REICHLE 1980, CAIRNS 1983).

The extrapolation of the results of physiological measurements made in the laboratory for the interpretation of physiological response in natural populations is often fraught with difficulties, both conceptual and technical. We have approached this problem by using a portable computer-operated *in situ* continuous flow-through system (LINDBLAD et al. 1986, 1988).

The toxic effects of the heavy metal cadmium on marine organisms, and its modification by abiotic factors such as salinity are well documented (MØHLENBERG and JENSEN 1980, THEEDE 1980, 1984). Organic tin compounds enter the marine environment as a result of industrial activities, and from anti-fouling paint used on boats and fish farm net cages. Tin compounds have been shown to cause injuries in especially molluscs (GOLDBERG 1986, HIS and ROBERT 1987).

We report here the acute effects of cadmium and anti-fouling paint based on tributyl tin, on the functional responce of two common benthic suspension feeders, *Mytilus edulis* and

Ciona intestinalis. Measurements of filtration rate, respiration, and excretion of NH_4 and PO_4 were made *in situ* on natural populations, both on the two species separately and on mixed populations.

Material and methods

Mytilus edulis and *Ciona intestinalis* were allowed to recruit naturally on to ceramic tiles, submerged at 9 m depth near the Tjärnö marine biological laboratory (59°N, 11°E) on the Swedish west coast. Salinity and temperature annually range between 15 and 29 ‰ S, and 1 and 20° C, respectively. During the experimental period salinity was about 25 ‰ S, and temperature 17° C. Tiles with 2 year old fouling communities were retrieved by SCUBA and immediately brought to a raft outside a boat-house. All species except *Mytilus* and/or *Ciona* where then removed from the substrate. The mussels were about 6 cm in length and the ascidians 5 to 8 cm. The experiments were carried out in an *in situ* continuous flow-through system, with enclosures submerged to 1 m depth, decribed by KAUTSKY (1984) and LINDBLAD et al. (1986, 1988). Natural sea-water and *in situ* incubations were used to minimize experimental artifacts (cf. JØRGENSEN 1975, BAYNE et al. 1976). The equipment allowed the use of several parallel 15 l transparent acrylic enclosures. A tile with 30 to 40 specimen of the test animals was placed in each enclosure. Surface sea-water was pumped into an overflow tank 2 m above sea level, and flowed steadily through each enclosure at around 1.0 l min^{-1}. Oxygen concentration, temperature and flow were recorded continuously. Water samples for particle and nutrient analysis were taken after an initial 2–4 hours of acclimation. A second sample was taken two hours later. In the cadmium experiment the water flow was then turned off and a sea water solution of $CdCl_2.6H_2O$, giving a nominal concentration of 100 µg l^{-1}, was added to the enclosures. The enclosures were aerated during 10 h of cadmium exposure. After exposure to cadmium, the water flow was turned on again and two more particle and nutrient samples were collected at 2-hourly intervals. In the experiment with anti-fouling paint, "Interracing" containing tributyl tin was painted inside PVC tubes, which were connected to the inlet of water to the enclosures. The concentration of TBT calculated from a leakage rate of approximately 22.5 µg cm^{-2} d^{-1} (LAUGHLIN et al. 1984), gave a nominal concentration of 2.5 µg l^{-1} in the enclosures, which corresponds well with actually measured concentrations (GOLDBERG 1986).

In Baltic and North Sea coastal waters concentrations of more than 2 µg l^{-1} cadmium have been reported (JANKOVSKY et al. 1977, MEELHUS et al. 1978, BRÜGMANN 1981). Significantly higher concentrations, of more than 10 µg l^{-1} cadmium, have also been found in other coastal waters and estuaries (review in BRYAN 1984). The concentration of 100 µg l^{-1} cadmium for 10 hours used in this experiment was thus considered a concentration high enough to give detectable effects, but not high enough to cause severe injuries in the animals. In the TBT experiment we used a considerably lower concentration, 2.5 µg l^{-1}, which is similar to concentration reported in the water around small boat marinas (LAUGHLIN et al. 1984, GOLDBERG 1986).

In each experiment there were three experimental set-ups: *Mytilus* and *Ciona* separately, and *Mytilus* + *Ciona* combined. There were two replicates for each species combination except at 100 µg l^{-1} Cd, where, unfortunately, the second replicate of *Ciona* had to be excluded, since the enclosure broke. For each treatment there was one control. An empty enclosure was used to correct for the time lag between in- and outflowing water, and the metabolism of the plankton passing through the containers. Each replicate enclosure also served as its own control, since there were at least two measurements before and two after exposure.

Filtration rate

Particles in the 2 – 30 μm size range were counted with an Elzone 80 XY particle counter (Particle Data Inc. Elmhurst, Illinois, USA), using 48 and 190 μm orifice tubes. Three replicate counts were made on each sample. The dominating size fraction of the seston in the sea-water was in the range 4–8 μm. The filtration rate, FR, defined as the volume of water cleared of suspended particulates, in the 4–8 μm range, per unit time and biomass, was calculated as follows:

$$FR = (N_0 - N_1)/N_0/B$$

where N_0 = The concentration of particles in the outflowing water from an empty enclosure
(= inflowing water to the test enclosures)
N_1 = The concentration of particles in the outflowing water from the enclosures containing test organisms
F = Flow rate in $l \cdot min^{-1}$
B = Biomass, as ash free dry weight, of the experimental organisms

Respiration

Oxygen consumption was calculated as the different in oxygen content between the in- and outflowing water multiplied by the flow rate. Respiration was then expressed as oxygen consumed per unit time and biomass.

Excretion

Samples for the analysis of NH_4 and PO_4 were taken the same way as for particle analysis, two times before and two times after exposure, and analysed according to the New Baltic Manual (CARLBERG 1972). Nutrient release was then calculated as the difference in concentration between in- and outflowing water per unit time and biomass.

O:N and O:P-ratios

The atomic ratios between respiration and excretion were also calculated. Each oxygen value was divided by the interpolated value of the corresponding excretion value, since nutrients were measured only on four occasions.

O:N is an index of the catabolic balance between carbohydrate, lipid an protein substrates in the metabolism, and used to describe the physiological status of the organisms (cf. BAYNE et al. 1985).

Perturbation index

In order to standardize measurements and eliminate differences in biomass and condition between organisms in different enclosures the results are also given as a ratio of the measured parameter after and before treatment. This is then related to the equivalent ratio for the control enclosures, thus:

$$\text{Perturbation index (PI)} = \frac{(\text{X enclosure after / X enclosure before})}{(\text{X control after / X control before})}$$

where X denotes the parameter measured, e.g. filtration rate, respiration, excretion etc. (LINDBLAD et al. 1986).

To get an overall measure of disturbance, an Absolute Disturbance Index (ADI) was calculated accordingly to LINDBLAD et al. (1988). ADI is the absolute distance between the metabolic activities of an undisturbed system and a disturbed point in a multidimen-

Table 1

Cadmium 100 µg l^{-1}. Mean values of physiological measurements in *Mytilus*, *Ciona* and *Mytilus* + *Ciona* ± s.e., before and after exposure to 100 µg l^{-1} cadmium. * only one measurement

	Mytilus 1	*Mytilus* 2	*Mytilus* control	*Mytilus* + *Ciona* 1	*Mytilus* + *Ciona* 2	*Mytilus* + *Ciona* control	*Ciona* 1	*Ciona* control
Filtration rate (l/min/g)								
Before exposure	0.016±0.001	0.014±0.001	0.015±0.0006	0.017±0.0004	0.016±0.00006	0.017±0.001	0.040±0.002	0.020*
After exposure	0.018±0.00001	0.015±0.0001	0.016±0.00005	0.016±0.0005	0.018±0.0003	0.019±0.0002	0.050±0.0005	0.013±0.0002
Respiration (μgO_2/g/h)								
Before exposure	863.8±17.2	541.2±10.8	806.3±16.3	1039.8±19.8	530.2±10.6	999.3±20.5	2387.2±41.7	1439.8±25.2
After exposure	935.5±18.7	665.4±13.3	897.3±17.9	1078.9±21.6	685.1±13.7	1079.7±21.6	2557.7±42.3	1364±27.3
NH_4-excretion (µg/g/h)								
Before exposure	17.65±0.87	21.70±1.10	22.51±1.09	45.71±1.83	49.20±2.01	40.87±2.11	98.27±4.81	68.40±3.12
After exposure	20.52±0.90	22.14±0.98	12.11±0.63	22.21±0.98	25.09±1.65	23.12±1.32	74.89±2.98	42.87±2.29
PO_4-excretion (µg/g/h)								
Before exposure	10.29±0.53	5.25±0.44	6.20±0.61	8.72±0.98	7.86±0.76	5.89±0.83	35.12±1.98	20.08±1.10
After exposure	11.60±0.64	11.22±0.98	8.18±0.67	7.00±0.55	5.89±0.54	5.69±0.39	36.58±1.77	14.80±0.95
O:N-atomic ratio								
Before exposure	42.8±1.08	21.8±0.64	31.3±0.61	19.9±0.33	9.4±0.11	21.4±0.52	21.3±0.22	18.4±0.29
After exposure	39.9±0.87	26.3±0.62	64.9±1.59	42.4±0.82	23.1±0.28	40.9±0.64	29.9±0.37	27.9±0.24
O:P-atomic ratio								
Before exposure	162.5±4.23	199.7±3.92	252.1±4.92	231.2±3.57	130.6±2.25	328.6±5.24	131.6±2.01	138.9±3.01
After exposure	156.2±3.69	114.8±2.88	212.5±4.86	297.9±4.01	161.1±2.86	367.6±5.36	135.4±2.72	178.6±3.03

Table 2

TBT 2.5 µg l^{-1}. Mean values of physiological measurements in *Mytilus, Ciona* and *Mytilus* + *Ciona* ± s.e., before and after exposure to 2.5 µg l^{-1} TBT. * only one measurement

	Mytilus 1	*Mytilus* 2	*Mytilus* control	*Mytilus* + *Ciona* 1	*Mytilus* + *Ciona* 2	*Mytilus* + *Ciona* control	*Ciona* 1	*Ciona* 2	*Ciona* control
Filtration rate (l/min/g)									
Before exposure	0.0145±0.0035	0.009*	0.0135±0.0005	0.0225±0.0005	0.0235±0.0005	0.0138±0.0003	0.069±0.011	0.012*	0.069±0.011
After exposure	0.0038±0.0073	0.0020±0.0040	0.0118±0.0018	0.0042±0.0043	0.0049±0.0072	0.0140±0.0010	-0.0095±0.031	0.0005±0.011	0.080±0.0005
Respiration ($\mu gO_2/g/h$)									
Before exposure	940±20	860±17	1130±23	1470±30	1880±38	980±20	4006±81	6640±130	2930±60
After exposure	580±12	810±16	840±17	1370±28	1520±30	820±16	3810±76	5170±100	2940±59
NH_4-excretion (µg/g/h)									
Before exposure	19.06±1.2	25.95±1.9	26.24±1.9	54.14±5.0	52.29±1.8	31.56±1.0	119.70±9.5	185.73±6.6	110.98±8.9
After exposure	27.41±0.5	30.68±1.0	15.45±0.7	32.77±3.8	31.81±1.7	19.06±1.5	119.77±4.8	186.15±2.0	95.00±7.3
PO_4-excretion (µg/g/h)									
Before exposure	11.58±1.1	5.10±0.3	8.12±1.2	7 .54±0.8	6.44±0.2	5.28±0.5	33.29±3.7	28.11±5.9	23.68±8.9
After exposure	12.21±1.6	11.89±1.3	7.55±1.1	10.17±1.8	11.82±0.1	5.83±0.5	93.96±6.2	99.75±7.5	38.55±3.6
O:N-atomic ratio									
Before exposure	31.7±0.68	39.6±0.84	37.7±1.01	23.8±0.53	31.4±0.77	27.2±0.45	29.6±0.33	31.3±0.77	23.1±0.48
After exposure	16.5±0.22	25.7±0.52	47.7±0.86	36.6±0.84	41.8±0.68	37.4±0.77	27.8±0.48	24.3±0.54	27.1±0.61
O:P-atomic ratio									
Before exposure	157±3.11	193±4.01	257±5.11	279±6.07	297±6.13	277±6.13	235±10.81	457±14.3	240±7.14
After exposure	72±1.91	131±2.71	182±3.77	235±5.14	247±5.65	188±4.99	79±2.12	100±3.07	148±3.15

sional space where each dimension represents the PI of a measured parameter (X). It is proportional to the disturbance; larger values indicate a more disturbed situation. ADI is expressed as: $ADI = [(\Sigma (PI (X) - 1)^2)/n]^{0.5}$

Results

All physiological measurements are summarized in table 1 and 2. As shown by the PI's (Figs. 1a, b) the filtration rate and respiration in *Mytilus, Ciona* and *Mytilus+Ciona* were almost unaffected when exposed to 100 μg l^{-1} cadmium (Table 1, Figs. 1a, b). While excretion of ammonium was highly stimulated in *Mytilus* and slightly stimulated in *Ciona*, it was somewhat lowered in the treatments with mixed populations of *Mytilus* and *Ciona*, compared to the controls (Fig. 1c). Excretion of phosphate was affected the same way as the ammonium excretion except for the first replicate of *Mytilus* where excretion decreased instead of increased (Fig. 1d).

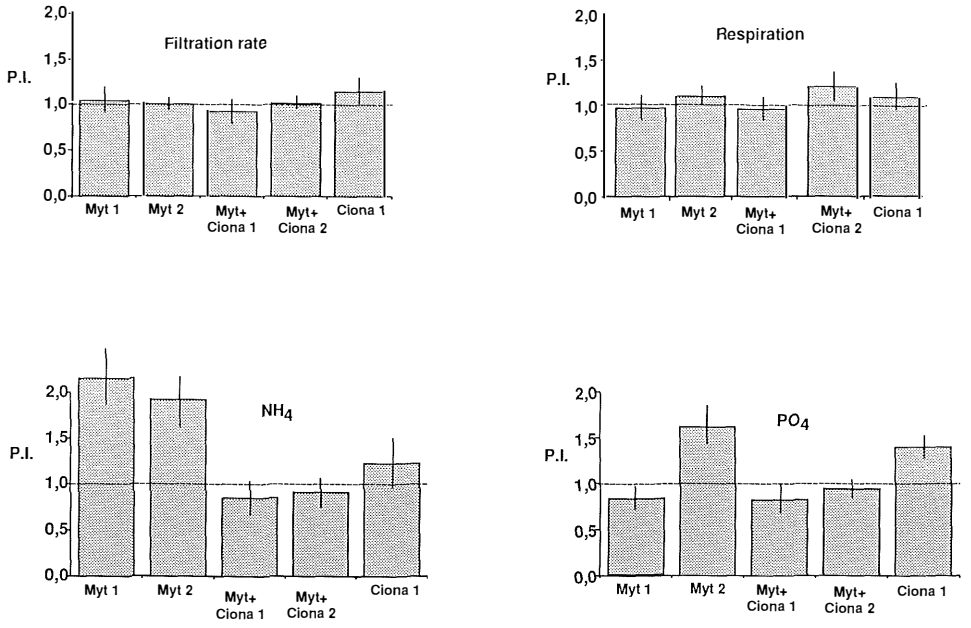

Figure 1

Perturbation indices ± s.e. for *Mytilus, Ciona* and *Mytilus* + *Ciona* exposed to 100 μg l^{-1} of cadmium for 10 h. Controls = 1 (dashed line). a. Filtration rate. b. Respiration. c. Excretion of ammonium. d. Excretion of phosphate

O:N ratios were lowered in *Mytilus* and *Ciona*, which indicates that these organisms had a higher protein catabolism, whereas for the combinations of *Mytilus+Ciona* O:N slightly increased (Fig. 2a). As in the case with nutrient release O:P ratios was affected the same way as the O:N ratios except for one replicate of *Mytilus* (Fig. 2b).

When exposed to 2.5 μg l^{-1} TBT both *Mytilus* and *Ciona* completely stopped feeding (Fig. 3a). A control experiment with no test animals was also performed to check that an actual retention of seston was not masked by a leakage of particles from the tubes with anti-fouling paint. In respiration, on the other hand, there was little difference between the tin-treatments and the controls (Fig. 3b). Ammonium excretion was highly stimulated in

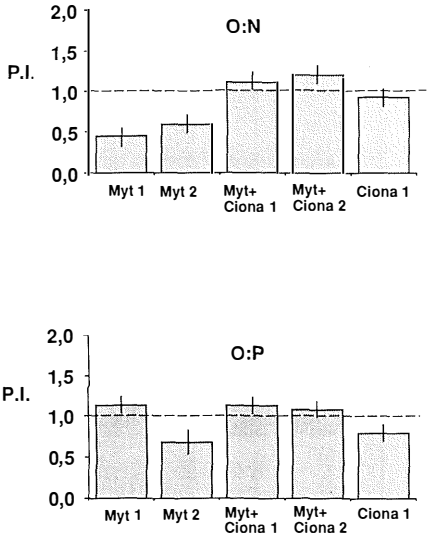

Figure 2

Perturbation indices ± s.e. for *Mytilus, Ciona* and *Mytilus* + *Ciona* exposed to 100 µg l^{-1} of cadmium for 10 h. Controls = 1 (dashed line). a. O:N ratios. b. O:P ratios

Mytilus but unaffected in *Ciona* and *Mytilus+Ciona* (Fig. 3c), whereas the phosphate excretion increased in all species (Fig. 3d). The O:N and O:P ratios decreased for *Mytilus* and *Ciona,* but were unchanged or increased in the mixed species (Figs. 4a, b).

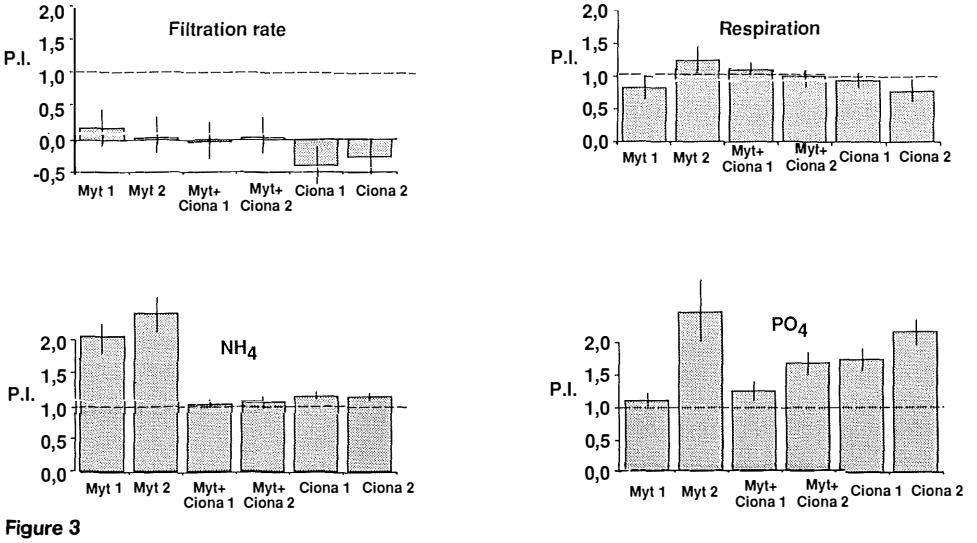

Figure 3

Perturbation indices ± s.e. for *Mytilus, Ciona* and *Mytilus* + *Ciona* exposed to 2.5 µg l^{-1} of TBT. Controls = 1 (dashed line). a. Filtration rate. b. Respiration. c. Excretion of ammonium. d. Excretion of phosphate

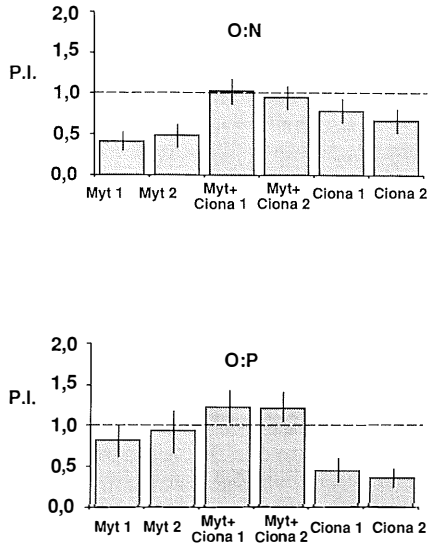

Figure 4

Perturbation indices ± s.e. for *Mytilus, Ciona* and *Mytilus* + *Ciona* exposed to 2.5 µg l^{-1} TBT. Controls = 1 (dashed line). a. O:N ratios. b. O:P ratios

Discussion

The overall response of the treated animals, expressed as the Absolute Disturbance Index is shown in Figs. 5a, b. It is clearly indicated that the mixed populations displayed a different reaction to the added toxicants, cadmium and organic tin, than did the single

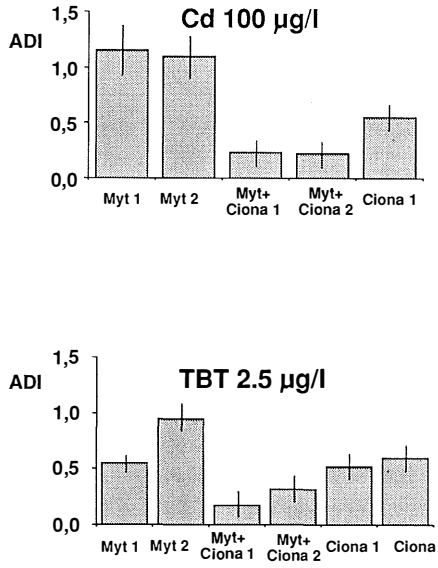

Figure 5

Absolute disturbance indices ± s.e. for *Mytilus, Ciona* and *Mytilus* + *Ciona* exposed to a. 100 µg l^{-1} cadmium for 10 h and b. 2.5 µg l^{-1} TBT

species. In terms of overall physiological response the two-species assemblages were less disturbed than the single species ones. Partly, this was due to the opposite physiological responses in *Mytilus edulis* and *Ciona intestinalis*, which realized the net response of the „community". Both cadmium and TBT caused a high ammonium and phosphate release in *Mytilus edulis* which resulted in the high ADI-values observed for that species, while in *Ciona intestinalis* the release was much lower. The results suggests some sort of interaction between the species. A possible mechanism is that some limiting resource, such as food, was released. ANDRÉ and KAUTSKY (unpubl.) showed that exposure to 1000 μg l^{-1} cadmium caused decreased retention of food-particles < 7 μm in *Mytilus* but not in *Ciona*. Small seston and/or suspended feces may thus have increased as a food resource for *Ciona* in the mixed populations. Different morphological and physiological properties in different species may also increase the possibility that at least some species survive and can maintain the suspension feeder function.

Our results show that the effects of two different pollutants on an assemblage of two species were quite different from what could be predicted from the response of the two species separately, indicating that results from single species tests alone is insufficient for making predictions about responses on higher levels of biological organization. In order to give appropriate predictions about the effects of pollutants in the marine environment it is thus necessary to perform tests also on higher levels of organization, preferably on the ecosystems level.

Acknowledgement

The work was carried out at the Tjärnö marine biological laboratory, with financial support from the Swedish National Research Council and the National Swedish Environmental Protection board.

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