The community-level response to sewage impact in intertidal mytilid beds of the Southwestern Atlantic, and the use of the Shannon index to assess pollution

La respuesta al impacto cloacal a nivel comunitario en los bancos intermareales de mitílidos del Atlántico sudoccidental, y el uso del índice de Shannon para la determinación de contaminación

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Abstract. - A study of an intertidal mytilid community in the Southwestern Atlantic shore was carried out in order to characterize the mytilid bed's response to an intertidal sewage discharge. Four Stations were sampled around the outfall, with a Control site. Total Weight and Organic Carbon content of sediments among mussels, as well as water quality variables were measured. All macrofauna were counted, and species number, Shannon's diversity index and evenness were calculated. The mytilid *Brachidontes rodriguezi* increases its density inversely to distance to the effluent. The polychaetes *Capitella capitata* and *Boccardia polybranchia* characterized the most impacted Stations, while crustaceans (*Corophium insidiosum*, *Jassa falcata* and *Caprella* sp.) were dominant at medial distances. Both environmental and biological data show significative differences between Stations. The most impacted Station has the lowest species number, but the highest values of both diversity and evenness; while control Station has the lowest values of diversity and evenness. The study rejects the use of univariate indices to assess organic pollution in this community, being the presence of indicator species with mytilid proportion the best tool for sewage impact assessment.

Keywords: mytilid beds, community structure, univariate index, intertidal sewage, Southwestern Atlantic.

Resumen. - Se realizó un estudio de la comunidad intermareal de mitílidos del Atlántico sudoccidental con el objetivo de caracterizar su respuesta a la descarga de un efluente cloacal intermareal. Cuatro estaciones fueron muestreadas en el área impactada, más un sitio Control. Peso total y contenido orgánico fueron medidos del sedimento retenido entre los bivalvos, al igual que variables de calidad del agua. Toda la macrofauna fue contada, y el número de especies, diversidad y equitatividad fueron calculadas. El mitílido *Brachidontes rodriguezi* incrementa su densidad en forma inversa a la distancia al efluente. Los poliquetos *Capitella capitata* y *Boccardia polybranchia* caracterizan las estaciones más impactadas, mientras que los crustáceos (*Corophium insidiosum*, *Jassa falcata* y *Caprella* sp.) fueron dominantes a distancias intermedias. Tanto los datos ambientales como los biológicos muestran diferencias significativas entre estaciones. La estación más impactada tiene el más bajo número de especies, aunque los más altos valores de diversidad y equitatividad, mientras que el sitio Control tiene los más bajos. En este estudio se rechaza el uso de índices univariantes para la determinación de contaminación orgánica por efluentes cloacales en esta comunidad. La mejor herramienta de análisis resultó ser la presencia de especies indicadoras y la proporción de mitílidos. Palabras clave: bancos de mitílidos, estructura comunitaria, índices univariados, cloaca intermareal, Atlántico sudoccidental.

Introduction

Historically men have to disposal sewage. Coastal cities have solved this situation by dumping in the coastal line, being organic enrichment the oldest form of marine pollution (Pearson & Rosenberg 1978). However, large cities settled on the shore have become the eutrophication in a common hazard in marine coastal

areas in many parts of the world, with consequent potential damaging effects on both inshore fisheries and recreational facilities (Rosenberg 1985).

In the Southwestern Atlantic shore most (if not all) sewage discharge is still produced in shallow waters without any treatment or, like in the argentinian Mar del Plata City (38° S – 57° W), on the intertidal zone. The

City has a permanent population of about 550,000 people, but during summer time, it receives more than 2,000,000 tourists. The domestic sewage has a pretreatment plant that discharges a continuous flow with a mean rate of $2,5 \text{ m}^3 \cdot \text{seg}^{-1}$, but in summer season reaches more than $4 \text{ m}^3 \cdot \text{seg}^{-1}$. The City needs a way to evaluate the extents and degree of man-caused impact in the marine environment.

The search for a cost-effective method for assessing environmental impact has a long history. From indicator species, or group of species, to community structure, men have attempted to found an unequivocal way to evaluate the extents and degree of the antropogenic effects. A complete framework was developed for the relationship between benthic communities and organic pollution, base on the response of soft-bottom macrobenthic communities from USA and Europe (Pearson & Rosenberg 1978). This model predicts low values of species number in the vicinity of an organically enriched area, with great amount of individuals (opportunistic species). So, we can assess this kind of impact through indices that reflect this uneven individuals by species-distribution. Several measures have been proposed, being the Shannon diversity index H' (Shannon & Weaver 1963), probably the most widespread employed in pollution ecology (Warwick & Clarke 1993). The Shannon index has been incorporated in some programmes (like PRIMER, Plymouth Routines in Multivariate Ecological Research)(Plymouth Marine Laboratory 1994), and in Norway is part of the environmental legislation, and it is used to classified sediment quality (but see Gray 2000).

In contrast with soft-bottom models, only a few hard-bottom related studies have been carried out (i.e. Borowitzka 1972, Littler & Murray 1978), most if not all in intertidal substrates dominated by algae. However, considering intertidal mussel beds affected by sewage, at last in one known case (using relative percent cover, and not all the species), the response of the intertidal community was opposite to the described model. Intertidal mussel beds developed as epilithic communities in the Southwestern Atlantic shore have shown an unsuspected behavior in community structure, being the lowest value of diversity and evenness (but no the species number) in not impacted areas, rather than in organically impacted ones (López Gappa *et al.* 1990, 1993).

So, is this behavior a common feature in intertidal mussel beds or is an exceptional case among a set of "classical" benthic response? In order to test this hypothesis, and to find a methodology easy and cheap to assess organic pollution in the Southwestern Atlantic shore, a spatial design with quantitative data along a

predicted organically enrichment gradient was performed on a epilithic intertidal mytilid community developed in hard substrates exposed to domestic sewage.

The Study area

The sampling area is an open coast subjected to a longshore current (south to north) and to autumn-winter storms, with extense medium-sand beaches only interrupted by quartzitic outcrops and abrasion platforms of hard substrate. Around the effluent an abrasion platform is exposed to low tides, being azoics to the north (except for a few opportunistic algae), while to the south (to the City) they are cover by an unsuspected well developed mytilid community, where the study was carried out. These platforms are hard substrates of "caliche" (consolidated loess), characterized by an irregular and slowly slope (almost horizontal). Grooves lying perpendicular to the shoreline are common. Semidiurnal tides varies between 0.60 and 0.90 m (see Isla & Ferrante 1997, Isla & Aliotta 1999). Seawater have a range of temperature between 8 to 21° C, and salinity between 33.5 to 33.8 psu, being considered as residual waters of the continental shelf (Guerrero & Piola 1997).

Material and methods

During November of 1997, four Stations (1 to 4) were sampled south to the effluent (Fig. 1). In each Station two tidal levels were randomly sampled with a 78 cm^2 corer into the mussel beds, 4 sampling units in the upper fringe (exposed in neap tides), and other 4 in the lower fringe (exposed in spring tides). The low level was absent from the Station 1. A Control Station (C) has also sampled 9 km north, where the intertidal community was developed in a similar intertidal hard substrate (Santa Elena Formation).

Environmental variables (Dissolved Oxygen, Turbidity, Temperature, Salinity and pH) were measured *in situ* by a U-10 Horiba in each Station, and also in two wind conditions (North and East). Sediment samplings were obtained replicated in each level for determination of Total Organic Carbon (TOC, Walkley & Black 1965). The material retained in a 1 mm screen mesh was separated and identified under a stereomicroscope, but sediment accumulated between mussels were retained during sorting for total weight determination. Species richness, the Shannon diversity index (Shannon & Weaver 1963) and evenness (Pielou 1969) were calculated for each sampling unit.

Figure 1

Sampling design in an abrasion platform affected by sewage inhabited by a mytilid community in the Southwestern Atlantic.

The Station Control (C) is placed 9 km north to the effluent.

Diseño de muestreo en una plataforma de abrasión afectada por efluentes cloacales habitada por una comunidad de mitílidos en el Atlántico sudoccidental. La estación Control (C) está ubicada 9 km al norte del efluente.

Water quality data were analyzed using a two-way Anova, considering Stations and wind conditions, and differences in TOC and sediment weight were tested by a one-way Anova. Differences in densities between tidal levels and Species, and between Stations and Species were performed also with a one-way Anova. Biological analyses were performed on a reduced matrix with the 14 species of dominance up to 0.05 %. Both environmental and biological data (abundances) were

double root transformed to achieve homogeneity of variances (verified by the Cochran's C test). Whenever a difference was established in the Anova, a Post-hoc SNK test was performed to the appropriated alpha level. A Redundancy Analysis (RDA) was performed in order to determine the relationship between biological and environmental variables. For diversity analysis, the statistic Jack-Knife was utilized to test differences between all possible pair of Stations.

Figure 2

Mean values ($\pm$ sd) of environmental variables (Salinity, Temperature, Dissolved Oxygen, Turbidity, and pH) in impacted Stations (1 to 4) and Control Station (C) of the SW Atlantic shore in the two wind conditions.

Valores medios ($\pm$ ds) de variables ambientales (Salinidad, Temperatura, Oxígeno disuelto, Turbidez, y pH) en Estaciones impactadas (1 a 4) y Estación Control (C) de la costa SO del Atlántico en dos condiciones de viento.

Results

Environmental variables

The data were analyzed by a two-way Anova, considering Stations (1, 2, 3, 4 and C) and wind condition. The results shows a gradient according to distance to the effluent, but also a strong influence of the wind (Fig. 2). Differences due to Stations, Wind and Interactions were significant ($p < 0.05$). Planned comparison shows that Control Station (C) was different from Stations 1 to 4, and Station 1 differs from Stations 2, 3 and 4.

Sedimentary pattern

Total weight of sediments accumulated between mussels show a decreasing gradient from the control area (C) to the most impacted Station (1)(Fig. 3a), while TOC shows the opposite tendency, revealing the existence of an organically enrichment gradient (Fig. 3b). Post-hoc comparison shows that both parameters have highly significant differences ($p < 0.01$) between Stations 1-2 and the rest.

Figure 3

Mean ($\pm$ sd) of a: sediments accumulated between mussels (Kg.m⁻²); b: Total Organic Carbon (%) in sediments. Station 1 is the closest to the intertidal sewage discharge and 4 the most far to the south; Station C (Control) is placed 9 km north.

Media ($\pm$ ds) de a: sedimentos acumulados entre bivalvos (Kg.m⁻²); b: Carbono orgánico total (%) en sedimentos. La Estación 1 es la más cercana al efluente y la 4 la más lejana hacia el sur; la Estación C (Control) está 9 km al norte.

Community pattern

About 43 species (36,667 individuals) were identified from the intertidal community. Mean dominance in the area corresponds to the mytilid *Brachidontes rodriguezi* (42.86 %), the amphipods *Jassa falcata* (23 %) and *Corophium insidiosum* (12.9 %), the polychaetes *Syllis prolixa* (7.66 %) and *Syllis gracilis* (2.64 %), and the caprellid *Caprella* sp. (4.15 %). The presence of the algae *Enteromorpha* sp., *Bryopsis* sp. and *Cladophora* sp. was constant, and in minor degree also for *Ulva lactuca*, but always in low frequency and abundance. *Mytilus edulis platensis*, the other mytilid in the area, varies between 0.35 to 3.6 %. However, spatial distribution is not even according to proportion and density, being the molluscs characteristic of the control Station (C), and polychaetes in Station 1, while crustaceans are dominant at intermediate Stations (Fig. 4).

A first analysis of differences in the community was conducted on tidal levels, being differences not significant; so all the sampling units were pooled for a one-way Analysis of Variance, being differences due to Station and Species significant, as well as interactions (Table 1). Due to significant interactions, the benthic response was analyzed Station by Station.

In Station 1, community structure is characterized by the presence of *Boccardia polybranchia* (12.4 %), *Capitella capitata* (11.4 %) and *Corophium insidiosum* (18 %), while the proportion of *Brachidontes rodriguezi* were low (38 %). Species number is low, but diversity and evenness were high (Fig. 5). Stations 2 and 3 (at 230 and 450 m from the effluent, respectively) present a variable proportion of the mytilid *B. rodriguezi* (between 15.5 to 33.5 %), and the associated fauna reach high abundance, mainly constituted by *Jassa falcata* (26 to 35 %) and *C. insidiosum* (14.6 a 39.9 %). In Station 4 (about 800 m from the effluent), *C. insidiosum* decreases in its dominance (6.7 to 9.7 %), while *J. falcata* is still dominant (30.8 to 47.5 %). The proportion of *B. rodriguezi* remains low (18.2 to 23.9 %), due to the abundance of *Caprella* sp. (7.6 to 14.5 %) and the polychaete *Syllis prolixa* (10.8 to 16.7 %). Species number increases but diversity and evenness remains relatively constant in these three Stations (Fig. 5). Differences in diversity were also significant (Table 2).

In the control Station (C) *B. rodriguezi* is the dominant species (85 %) and *Mytilus edulis platensis* only reaches 1 to 1.3 %. The associated fauna were *Syllis prolixa* (3.8 to 4.8 %), *Syllis gracilis* (1.2 to 1.9 %) and *Jassa falcata* (0.1 to 3.7 %). Diversity reaches their minimum value, as well as evenness, but species number was not different from other Stations.

Figure 4

Mean density (ind.m⁻²) of the intertidal mussel community in the SW Atlantic shore affected by an organic gradient due to sewage.

Densidad media (ind.m⁻²) de la comunidad intermareal de bivalvos en la costa SO del Atlántico afectada por un gradiente orgánico debido a un efluente cloacal.

Figure 5

Species number (S), diversity (H' Shannon Index) and evenness (J') in an intertidal mytilid bed affected by sewage in the Southwestern Atlantic shore. Organic gradient decreases from Stations 1 to 4. Station C is the Control site.

Número de especies (S), diversidad (índice H' de Shannon) y equitatividad (J') en un banco de mitílidos intermareales afectados por un efluente cloacal en la costa SO del Atlántico. El gradiente orgánico disminuye desde la Estación 1 a la 4. La Estación C es el sitio Control.

Table 1

Summary of all Effects in the two-way Anova (Stations and Species as Factors). * $p < 0.05$
 Resumen de todos los efectos del Anova de dos vías (Especies y Estaciones como factores). * $p < 0.05$

	Df Effect	MS Effect	Df Error	MS Error	F	p-level
Station	4	2.90772	31	0.135830	21.4071	0.000000*
Species	11	18.81903	341	0.060869	309.1751	0.000000*
Interaction	44	2.04683	341	0.060869	33.6270	0.000000*

Table 2

Values of t (Jack-Knife test) for H' diversity measurements in intertidal Stations affected by an organic enrichment gradient in the SW Atlantic shore. In parenthesis the degree of freedom. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Valores de t (prueba de Jack-Knife) para mediciones de diversidad de H' en estaciones intermareales afectadas por un gradiente de enriquecimiento orgánico en el Atlántico SO. En paréntesis los grados de libertad. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	1	2	3	4
2	54.05*** (34)			
3	39.09*** (21)	34.78*** (37)		
4	2.63* (34)	1.96ns (40)	0.55ns (37)	
C	2.96** (31)	2.91** (37)	2.83** (34)	2.98** (37)

Redundancy Analysis

The relationship of environmental and biological variables (Fig. 6) shows sampling units of impacted stations 1 and 2 related to TOC and Turbidity, associated to the polychaetes *Capitella capitata* and *Boccardia polybranchia*. In the opposite side of Axis I (that explain 54 % of the total variance) the intermediate stations 3 and 4 were associated mainly to crustaceans and the polychaetes *Syllis gracilis* and *Caulleriella alata*, while the Control station were associated to *Brachidontes rodriguezi*, and the polychaetes *Syllis gracilis* and *Protoariciella uncinata*, highly related to pH, dissolved oxygen, and sediment accumulated between mussels. Axis II (explaining a 39 % of the variance) discriminate diverse stations (1, 2, 3 and 4) from poorly diverse station (Control site). However, considering only TOC and sediment between mussels (because the other environmental variables were highly correlated), explained variance of Axis I and II were 82 and 18 %, respectively.

Figure 6

Redundancy analysis (RDA). Relationship of sampling units of Stations, Species, and environmental variables in the intertidal *Brachidontes rodriguezi* community exposed to sewage discharge. 1 to 4: Impacted Stations; C: Control site. B.poly: *Boccardia polybranchia*; C.cap: *Capitella capitata*; J.fal: *Jassa falcata*; C.insid: *Corophium insidiosum*; Capre: *Caprella* sp.; C.alata: *Caulleriella alata*; S.pro: *Syllis prolixa*; S.gra: *Syllis gracilis*; P.unci: *Protoariciella uncinata*; B.rodri: *Brachidontes rodriguezi*. TOC: Total Organic Carbon; Turb: Turbidity; Sed: Sediment accumulated between mussels; Oxy: Dissolved oxygen and pH.

Análisis de redundancia. Relación entre unidades de muestreo de las Estaciones, Especies y variables ambientales en la comunidad intermareal de *Brachidontes rodriguezi* expuesta a la descarga cloacal. 1 a 4: Estaciones impactadas; C: sitio Control; B.poly: *Boccardia polybranchia*; C.cap: *Capitella capitata*; J.fal: *Jassa falcata*; C.insid: *Corophium insidiosum*; Capre: *Caprella* sp.; C.alata: *Caulleriella alata*; S.pro: *Syllis prolixa*; S.gra: *Syllis gracilis*; P.unci: *Protoariciella uncinata*; B.rodri: *Brachidontes rodriguezi*. TOC: Carbono orgánico total; Turb: Turbidez; Sed: Sedimento acumulado entre bivalvos; Oxy: Oxígeno disuelto; y pH.

Discussion

Environmental data on water quality show a typical gradient from the effluent, produced by the dilution of sewage discharge in the coastal waters. Parameters that reflect this pattern are pH, Dissolved Oxygen, Salinity, and Turbidity. However, wind direction affected the distribution of sewage loadings. The flume has a variable behavior according to wind and tidal movements, being the southern coast affected by north wind, while the northern coast is affected by south and east winds (see Isla *et al.* 1998). Due to wind and longshore current (both predominantly south to north), north coast is severely affected by sewage, while to the south coast, average water quality allows the existence of a relatively well developed mytilid community.

Sediment composition also reflects the organic enrichment. An organically gradient is present from Station 1 to the south. However, the minimum value is observed in the intermediate Station 3 (perhaps due to the enbayment position). Sediments accumulated between mussels show the reverse, decreasing from Control sites to impacted areas, being the lowest value in Station 1, near the effluent, and the maximum value was recorded in the control site, where mytilid density is the greatest. It is a well known fact that mussel matrix retain sediments (see Seed & Suchanek 1992), so the sediment retained is a denso-dependent variable, while density is a sewage-dependent factor. This could be seen in the RDA, in where the two factors (organic matter and sediments) and main species can explain the 100 % of the variance.

The original description of the epilithic community shows that dominance in quartzitic rocks were established by the mytilid *Brachidontes rodriguezi*, reaching a mean dominance of 94 % in the high intertidal to 59 % in the lower intertidal, while *Mytilus edulis platensis* increases to the lower intertidal, from 0.59 to about 38 % (Olivier *et al.* 1966, Penchaszadeh 1973). The diminution of the *Mytilus* dominance could not directly related to sewage effect, because other studies on this community also show the dominance (between 70 to 90 %) of *B. rodriguezi*, being *Mytilus* only occasional, and always in low abundance (Scelzo *et al.* 1996).

On the other hand, in the Mar del Plata port the displacement of the mytilid community from intertidal areas was attributed to the pollution effect (Bastida *et al.* 1980) and to competitive exclusion by barnacles (Vallarino & Elías 1997). In the abrasion platforms affected by sewage, the proportion of the mytilid

Brachidontes rodriguezi remains low till the control areas, due to the negative effect of organic enrichment, and also for the presence and abundance of other species, tolerant to organic pollution.

Several polychaetes species were also found associated to this mussel beds, being *Capitella capitata* the dominant polychaete in organically impacted areas (5,448 ind.m⁻²), as well as the spionid *Boccardia polybranchia* (5,833 ind.m⁻²). In enriched sediments associated to the blue mussel *Mytilus edulis* in Denmark, Svane & Setyobudiandi (1996) have also found *C. capitata* and the spionid *Polydora ciliata* related to local hydrodynamics that increases both sedimentation and/or faeces and pseudofaeces. In the Wadden Sea *C. capitata* and *P. ligni* reach their highest average abundance inside *Mytilus* beds (4,000 and 3,000 ind.m⁻², respectively), while in the adjacent areas of sand-flats did not exceed 750 ind.m⁻² (Günther 1996). *Capitella* spp. are known to increase in patches enriched with organic matter (see Ragnarsson & Rafaelli 1999), and in particular *C. capitata* is regarded as classical indicator of organic enrichment of sediments (see Pearson & Rosenberg 1978, Pocklington & Wells 1992). It is also known the relationship of spionids with organically enriched sediments (Pearson & Rosenberg 1978). In intertidal areas of California (Dorsey *et al.* 1983) and in an Australian sewage (Dorsey 1982) *Boccardia proboscidea* was found in high abundances. In other abrasion platform of the Southwestern Atlantic shore, López Gappa *et al.* (1990) have found high densities of *Boccardia* sp. (up to 500,000 ind. m⁻²) between mussels and in sandy substrates at lower intertidal and subtidal areas at 50 to 100 m from sewage.

Other effects related to organic pollution is the great crustacean richness and density. Several species of *Corophium* have been mentioned in or around pollution areas (Pearson & Rosenberg 1978). *C. insidiosum* was found in many works on the South Atlantic shore, but is the first time that their occurrence in high densities are registred in relation to a mussel beds affected by sewage. *Caprella* sp. and *Jassa falcata* are common members of this community, but never have been registered in densities of thousands. *J. falcata* was also subdominant in subtidal mytilids beds of the Mediterranean Sea at intermediate distances from pollution sources (Bitar 1982). Günther (1996) also observed an increasing abundance of mobile epifauna (mainly crustaceans) inside the *Mytilus* beds compared to adjacent flat.

This most impacted Station (1) has high diversity and evenness. Although species number are the lowest

of the sampled area, the numerical distribution of indicator species and *Brachidontes rodriguezi* are more even than in other areas far from the effluent. Diversity index, like Shannon's, would be useful in detection of pollution, because we expected that impacted areas had lower values than non-impacted ones. However, the example of the intertidal community in this part of South America shows an opposite effect, being the lowest values of H' and J' in the "normal" community due to the dominance of mytilids. López Gappa *et al.* (1990), studying the same epilithic mytilid community 120 km south to Mar del Plata City, found the same pattern in a similar abrasion platform (but tidal level corresponds mostly to a more high level, and using relative percent cover). These authors conclude, like Osman (1977), that there is an optimum degree of disturbance at which diversity reaches a maximum. Near the effluent, the low species number was due to the negative effects of organic pollution, while in the Control site the drop in both diversity and evenness (rather than in species number) was due to the space monopolization by *Brachidontes rodriguezi*. The optimum degree of disturbance by domestic sewage seems to be between 200 to 800 m far from the effluent, in where highly density of associated fauna (mainly crustaceans and polychaetes) and high diversity values were observed. Trends in epilithic intertidal community showed herein at the spatial scale can be observed also along a series of short-term studies (Vallarino, pers. com., 2001).

In the same way, Warwick & Clarke (1993) show that variability (standard deviation) in diversity (H') tends to increase with increasing levels of perturbation (only significative for macrobenthos). In the present work this tendency was rejected, because Station 1 (the most impacted) shows the lowest species number, but the highest diversity and evenness values (see Figure 5), and also shows a similar variability with the Control Station, however differences are highly significative in diversity values (see Table 2). In agreement with Del Valls & Conradi (2000) the uncertainty and high variability inherent in both ecosystems and methods of measurement require a range of values and a burden-of-evidence approach. Ultimately, judgements on environmental quality, assuming the persistence of habitat, can only be determined by the responses or condition of multiple (but never single or a large number of) measurements conducted as part of integrative assessments. Gray (2000) have demonstrated that Shannon index is insensitive, showing little variation along a transect survey carried out in the Norwegian Shelf, and that the use of multivariate statistics gives a much more precise way of detecting changes in benthic assemblages. However, the Shannon index is still successfully used in assessment of

pollution. In subtidal areas of this study area the index has revealed to be usefull for this kind of studies (Elías *et al.* 2001).

In this community, the classic response to organic pollution is the presence and abundance of indicator species (mainly polychaetes), while the new one is the utilization of the proportion of mytilids as the most powerful method for assessing organic pollution from sewage discharge in the Southwestern Atlantic shore.

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