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Measuring Acoustic Activity as a Method to Evaluate Welfare in Captive Beluga Whales (*Delphinapterus leucas*)

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Abstract

Animal welfare evaluation is a difficult task. Behavioural and physiological parameters are commonly used, but their interpretation is not always robust. The study of vocal behaviour as an indicator of animal welfare has proven to be effective in some terrestrial captive mammals, but little is known about its application in marine mammals. The acoustic activity of two beluga whales (*Delphinapterus leucas*) was monitored during two procedures: (1) before and after air transportation to new facilities and (2) before and after the introduction of four harbour seals (*Phoca vitulina*) to the same facilities. After transportation, the underwater vocalization rate dropped dramatically, remained very low during the next 4 wks, and did not reach the same level as before the transport until the 5th wk. Similarly, the vocalization rate decreased just after the introduction of the harbour seals, and it remained low for 2 wks. The observed decrease in the acoustic activity of beluga whales in both situations and the persistence of this change through time suggest that the acoustic behaviour in this species is very sensitive to environmental stressors. We propose that observation of underwater acoustic activity in captive beluga whales is a potentially effective method to monitor stress level and adaptations to environmental changes in their facilities. This technique must be explored further since it could be valuable in cetacean management in oceanaria and rehabilitation centres.

Key Words: beluga whale, *Delphinapterus leucas*, welfare, environmental enrichment, transport, stress, acoustics

Introduction

Animal welfare is a main concern for captive animal keepers. Managing the captive environment to minimize stress and optimize well-being for the animals requires not only an understanding of the environmental factors that are stressful, but also the development of appropriate measures for regular monitoring (Owen et al., 2004).

Physical well-being is difficult to measure in many species. Confidence has long been placed on indirect measures such as good physical health, the absence of evident behavioural and physiological symptoms of stress, and the exhibition of species' typical behaviour patterns (Fowler, 1978; Thomson & Geraci, 1986; Novak & Suomi, 1988; Dierauf, 1990; Baker & Aureli, 1997; Wielebnowski et al., 2002; Owen et al., 2004). In recent years, however, studies have simultaneously examined both physiological and behavioural parameters in an effort to quantitatively evaluate physical well-being, with relative blood cortisol levels being the most common physiological parameter (e.g., Clarke & Boinski, 1995; Flow & Jacques, 1997; Waples & Gales, 2002). When the behavioural and cortisol measures are mutually consistent, the results are usually interpreted as a more valid indicator of physical well-being than behavioural measures alone (Broom & Johnson, 1993; Stoskopf & Gibbons, 1994; Boinski et al., 1999). The relationship between stress, behaviour, hormones, and well-being is admittedly complex and controversial, however. Therefore, there is not yet a precise and effective method to measure the physical well-being in captive animals.

Acoustic activity is rarely included among those behaviours monitored to evaluate the suitability of housing or handling in zoos and oceanaria. Vocalizations are only considered relevant to animal welfare as indicators of pain in laboratory research (Morton & Griffiths, 1985; Cooper & Vierck, 1986). There is an extensive body of literature documenting that vocalization rate, types of vocalization, and acoustic structure within a call type may vary with stressful contexts such as aggression, panic, social separation, and levels of stress hormones among rodents (Goldstein et al., 1996; Blanchard et al., 1997), primates (Bayart et al., 1990; Champoux & Suomi, 1994; Friedman et al., 1995; Laudenslager et al., 1995; Boinski et al., 1999), and domestic pigs (*Sus scrofa domesticus*) (Von Borell & Ladewig, 1992; Désautés et al., 1997; Weary et al., 1997). Furthermore, acoustic behaviour already is commonly exploited in food animal research to evaluate welfare and minimize distress during slaughter (Warris et al., 1994; Grandin, 1998), castration (White et al., 1993; Weary et al., 1998; Taylor & Weary, 2000), branding (SchwartzkopfGenswein et al., 1997), and weaning (Cockram et al., 1993). Few studies, and only on primates (Crowell Comuzzie, 1993, with olive baboons [*Papio hamadryas anubis*]; Krishnamurthy, 1995, with François langurs [*Trachypithecus francoisi*]; Mulligan et al., 1994, with Rhesus macaques [*Macaca mulatta*]) and on cheetahs [*Acinonyx jubatus*] (Ruiz-Miranda et al., 1998), suggest that acoustic behaviour could be exploited as a useful monitor of psychological well-being in captive colonies because of associations with stereotypical behaviours or aversive social situations.

The study of vocal behaviour as an indicator of animal welfare has proven to be effective in some terrestrial captive mammals, but little is known about its application on marine mammals. Early studies suggest the use of distress calls by odontocete species (Reiss & Castellote, in prep.), but the potential use of these vocalizations as a measure of well-being in captivity remains unstudied.

It is widely accepted that transport, even within a facility, can cause changes in an animal's behaviour and physiology that can be related to stress; however, much of the work on this has been restricted to laboratory rodents (Tuli et al., 1995), laboratory primates (Honess et al., 2004), and livestock species such as pigs (*Sus scrofa*) (Bradshaw et al., 1996), cattle (*Bos taurus*) (Knowles et al., 1994, 1996; Fisher et al., 1999, 2002, 2004; Palme et al., 2000), and goats (*Capra hircus*) (Sanhouri et al., 1989). Transport is a critical situation, and often crucial during rescue, of the overall handling procedures in marine mammals (Antrim & McBain, 2001). Despite being a common activity among aquaria and wildlife rehabilitation centres, little is known of the effects of transportation on marine mammals.

Two different experiments are presented in this report: (1) transport effect and (2) environmental disturbance effect on the acoustic behaviour of two beluga whales (*Delphinapterus leucas*). These two captive beluga whales were transported in July 2003 from Mar del Plata Aquarium in Argentina to L'Oceanogràfic, an oceanarium newly opened in Valencia, Spain. Their underwater acoustic activity was monitored before and after the transport and introduction to the new facilities, as well as before and after the introduction of four harbour seals (*Phoca vitulina*) in the same facilities, which was done for environmental enrichment purposes. In this report, underwater acoustic monitoring is presented as a potential new method to evaluate animal welfare.

Materials and Methods

Facilities

The study began in Mar del Plata Aquarium in Argentina. The beluga whale exhibit has a configuration of three interconnected pools. Depth is 7 m, total water volume is 2,900,000 l, and the water surface is 414 m^2.

After transportation, the study continued at L'Oceanogràfic in Valencia, Spain. The beluga whale exhibit has a configuration of four interconnected pools. Depth is 5 m, total water volume is 3,582,000 l, and the water surface is 800 m^2.

Study Subjects

Two captive beluga whales, an adult male and a sub-adult female, have been studied for 2 y (March 2003 to March 2005) on a continuous basis, starting before transportation by air from Mar del Plata Aquarium to L'Oceanogràfic facilities in July 2003. The male, approximately 12-y old at the date of transport, has been in captivity for 7 y. The female, approximately 6-y old at the date of transport, has been in captivity for 4 y. Their medical records do not show any particular injury or past illness. Both whales have experienced at least two transports in the past: from capture site to facilities in Russia by truck and from Russia to Argentina by airplane. Both whales have been kept together since 2000 before their arrival at L'Oceanogràfic. No specific training was carried out for any of the experienced transports.

As part of the marine mammal environmental enrichment program at L'Oceanogràfic, four captive harbour seals, one juvenile male and three adult females, were introduced in the beluga whale facilities in November 2003. The seals were introduced in one of the pools, with connecting gates closed and covered by foam PVC panels to avoid visual contact with the beluga whales. Their presence was gradually exposed to beluga whales by eliminating the PVC panels, but keeping the bar-gates closed for a period of 3 mo prior to the gate opening. Harbour seals were kept in the beluga whale facilities for a total of 7 mo. A study timeline is presented in Figure 1.

Recording Protocol and Digitization

Recording sessions of 50 min started 5 mo before the transport from the Argentinean facilities and continued in L'Oceanogràfic starting the day of arrival for a period of 2 y and 8 mo. In 2003, sessions were recorded three times per day before feedings: morning sessions (0700-1145 h), midday sessions (1145-1615 h), and afternoon sessions (1615-2100 h). In 2004 and 2005, sessions were recorded three times per week at random times using a Brüel&Kjaer hydrophone model 8103 (sensitivity: -211 dB re 1

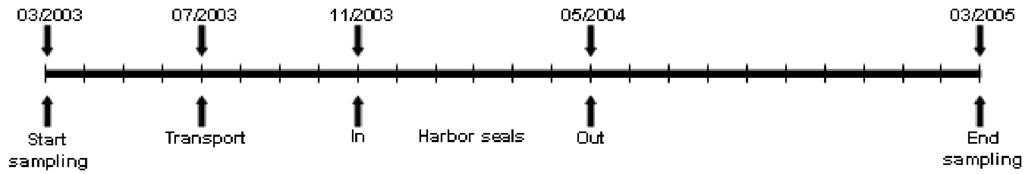

Figure 1. Timeline of the study

V/μPa ± 2 dB; frequency response: 1.0 Hz to 100.0 kHz +1.0/-6.0 dB) and a Brüel&Kjaer Nexus conditioner amplifier model 2690 (10.0 Hz – 80.0 kHz pass filters; gain: 0 to 80 dB). Hydrophone depth was constant at 4 m in a fixed position in the pool system for all recordings.

Conditioned and amplified signals were digitized in real-time at 24 bit/192 kHz sampling rate (80.0 kHz Nyquist filter) with a Roland Edirol FA-101 sound acquisition board. Data of each recording session was temporarily stored in a laptop (2.5 GHz Intel Pentium IV processor, 1GB RAM) hard drive using *Adobe Audition*, *Version 1.5*, audio editing software and later archived in TDK DVD-Rs.

Spectral Analysis and Underwater Vocalization Rate

Audio files were displayed as spectrograms (Hanning window, fast Fourier transforms size: 512 samples, overlap 50%) using *Adobe Audition*, *Version 1.5*, audio editing software and were visually and aurally inspected for detection of vocalizations. Beluga whale underwater vocalizations were identified by two experienced observers, according to defined acoustic parameters and variations in vocalization spectral contours. All the vocalizations observed were used in the analysis. When pulsed vocalizations occurred in train series (i.e., echolocation clicks), the number of trains was used for the total count of vocalizations. Acoustic activity was measured as the total number of vocalizations observed in each 50-min session (NVs). The number of vocalizations per hour (60 min) (NVh) was calculated to obtain a standardized vocalization rate:

$$NVh = \frac{NVs \times 60}{50}$$

Weekly vocalization rate (WVR) was calculated as the mean value per hour of all recording sessions within the week as a measure of the acoustic activity of both beluga whales:

$$WVR = \frac{\Sigma_{i=1}^{n} NVh_i}{n}$$

n = total number of recording sessions within a week. There were five sessions per week for 2003 and three sessions per week for 2004 and 2005.

Results

Recording Schedule

During 2003, the NVs recorded did not differ between time of the day (morning, midday, and afternoon) (Figure 2; Kruskal-Wallis test: KW-H[2, 859] = 0.42, $p > 0.05$). Based on these results, we designed a random sampling schedule for 2004-2005.

Weekly Vocalization Rate

During the 2-y period, 43,354 vocalizations were identified. Underwater acoustic activity included social vocalizations, echolocation clicks, and jaw claps. WVR was calculated from the beginning of the study period in March 2003 until March 2005. WVRs of the last week in the Argentinean facilities and the following nine weeks in L'Oceanogràfic are presented in Table 1 as results from the transport effect on their acoustic behaviour.

The acoustic behaviour also was monitored during the period of the introduction of the harbour seals (November 2003 until May 2004) and the WVR during the 2 wks prior to the introduction and 4 wks after is presented in Table 2 as results from the environmental disturbance effect on their acoustic behaviour.

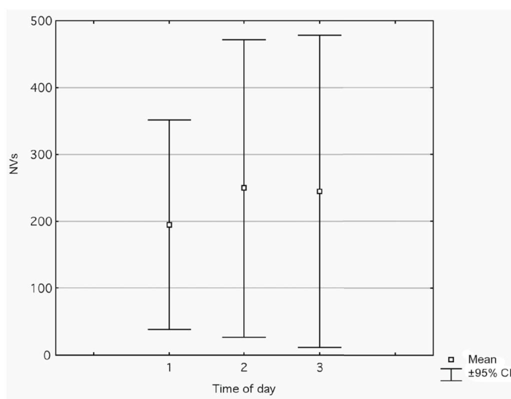

Figure 2. Box and whisker plot (mean ± 95% CI) of the total number of vocalizations per session (NVs) of belugas during morning sessions 1 (0700-1145 h), midday sessions 2 (1145-1615 h), and afternoon sessions 3 (1615-2100 h)

Table 1. Weekly vocalization rate (WVR) of belugas (calculated as the mean sounds per hour of all recording sessions within the week) during the period before and after the air transport; Week -1 = last week before transport, week 1 = first week after transport; n = 137 recording sessions.

Week	WVR
-1	606.8
1	65.7
2	61.3
3	72.8
4	148.0
5	498.3
6	407.9
7	558.3
8	295.2
9	304.7

Table 2. Weekly vocalization rate (WVR) of belugas (calculated as the mean sounds per hour of all recording sessions within the week) during the period before and after the introduction of harbour seals; weeks –2 and -1 = last two weeks before introduction of seals; week 1 = first week after introduction; *n* = 30 recording sessions.

Week	WVR
-2	492.6
-1	401.2
1	224.8
2	303.1
3	406.4
4	594.3

Statistical Analysis

Statistical analysis focused on data from two experiment periods: (1) the beluga whale transport and (2) the introduction of the harbour seals to the beluga whale facilities. When necessary, data were log-transformed to meet the requirements of parametric tests. All tests were at $\alpha = 0.05$ level.

Experiment 1: Beluga Whale Transport Effect—This dataset consisted of WVR from 1 wk before and 9 wks after transport. One-way analysis of variance (ANOVA) was used to determine if there was any difference in the WVR before and after the transport to L'Oceanogràfic facilities. Results showed highly significant differences (Figure 3; $F_{9,\,127} = 16.12$, $p < 0.001$) between vocalization rates by different weeks analyzed for the transport period. Tukey HSD post-hoc analysis was used to measure the level of significance of these differences, and results are presented in Table 3. The

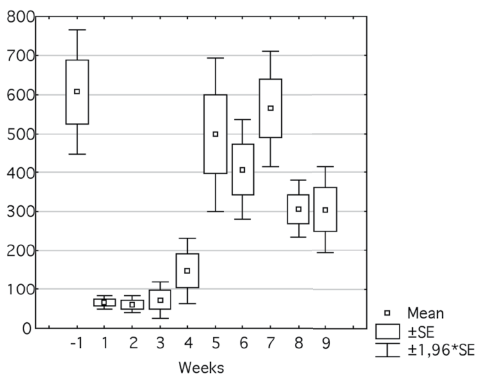

Figure 3. Box and whisker plot (mean ± SE and 95% CI) of the weekly vocalization rate (WVR) of belugas during the week before (-1) transport and nine weeks after (1 to 9) arrival to L'Oceanogràfic facilities

Table 3. Post-hoc Tukey HSD values from ANOVA; week -1 = lg vocalization rate (lgWVR) from the week before transport from Mar del Plata Aquarium, and week 1= lg-vocalization rate (lgWVR) from first week after transport to L'Oceanogràfic. Bold numbers indicate significant differences.

	lg Weekly Vocalization Rate (lgWVR)								
	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}
Week -1									
Week 1	**0.002**								
Week 2	**0.001**	1.000							
Week 3	**0.000**	0.995	1.000						
Week 4	**0.007**	1.000	0.988	0.890					
Week 5	0.986	**0.000**	**0.000**	**0.000**	**0.000**				
Week 6	0.991	**0.000**	**0.000**	**0.000**	**0.000**	1.000			
Week 7	1.000	**0.000**	**0.000**	**0.000**	**0.000**	0.949	0.972		
Week 8	0.898	**0.001**	**0.000**	**0.000**	**0.006**	1.000	0.999	0.607	
Week 9	0.821	**0.001**	**0.000**	**0.000**	**0.011**	0.993	0.988	0.408	1.000

analysis grouped week -1 with weeks 5 to 9 as a high vocalization rate cluster, and weeks 1 to 4 as a low vocalization rate cluster.

Experiment 2: Environmental Disturbance Effect—This dataset consisted of WVRs from 2 wks before and 4 wks after the introduction of four harbour seals in the beluga whale facilities. A one-way ANOVA was used to determine if there was any difference in the WVR before and after the presence of harbour seals. Results show significant differences (Figure 4; $F_{5, 24} = 2.8$, $p = 0.038$) between vocalization rates of the different weeks analyzed for the introduction of the harbour seals. LSD post-hoc analysis was used to measure the level of significance of these differences, and results are presented in Table 4. Weeks 1 and 2 were grouped as a low vocalization rate cluster.

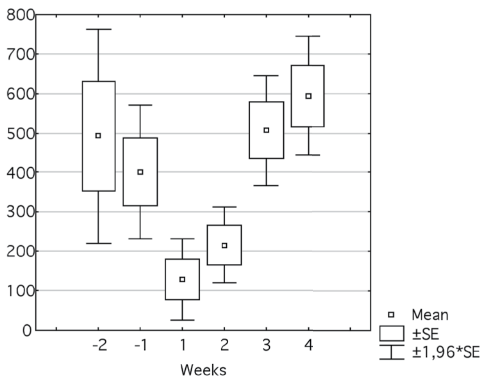

Figure 4. Box and whisker plot (mean ± SE and 95% CI) of the weekly vocalization rate (WVR) of belugas two weeks before (-2 and -1) and four weeks after (1 to 4) the introduction of harbour seals in the beluga whale facilities

Table 4. Post-hoc LSD values from ANOVA; Weeks –2 and -1 = lg-weekly vocalization rate (lg-WVR) from two weeks before introduction of harbour seals to the beluga whale facilities; week 1 = lg-weekly vocalization rate (lg-WVR) from first week after introduction of harbour seals. Bold numbers indicate significant differences.

	lg-weekly vocalization rate (lgWVR)				
	{1}	{2}	{3}	{4}	{5}
Week -2					
Week -1	0.730				
Week 1	**0.008**	**0.009**			
Week 2	0.507	0.665	**0.092**		
Week 3	0.726	0.520	**0.011**	0.382	
Week 4	0.561	0.356	**0.004**	0.273	0.866

Discussion

Stress and associated health problems are a recognized concern in captive cetaceans (Caldwell & Caldwell, 1968; Dierauf, 1990; Sweeney, 1990), therefore minimizing stress for captive individuals is an important strategy of management. It is widely admitted that environmental changes, such as transportation or introduction of novel stimuli, induce stress to marine mammals in captivity. In this study, both the air transport and the environmental disturbance of introducing harbour seals into the facilities drastically reduced the acoustic activity of beluga whales. After transportation, the vocalization rate dropped dramatically, remained very low during the next 4 wks, and did not reach the same level as before the transport until the 5th wk. Even if the exposure of the seal group to beluga whales was gradual, with increasing visual contact over several weeks, the vocalization rate drastically decreased just after the introduction of the cohabitants, and the effect lasted for 2 wks. Our results suggest that acoustic recordings are a valuable method to monitor stress levels since we showed that changes in the environment are strongly reflected in the acoustic behaviour of both beluga whales.

Animals may display a variety of responses to a stressor, including behavioural and physiological responses (Hofer & East, 1998). These symptoms in cetaceans are typically disguised, and stressors, or even diseases, often are not detected until they are advanced. In many cases, a behavioural response may be the first indicator and a clue to underlying causes, and there is good reason to believe that behavioural observations can be used to assess animal well-being (Carlstead et al., 1993; Baker & Aureli, 1997; Waples & Gales, 2002; Owen et al., 2004). The systematic collection of quantitative behavioural data allows investigators to elucidate both immediate and long-term manifestations of stress. Daily trainer reports at L'Oceanogràfic include behavioural observations and food intake of both beluga whales as part of the veterinary control procedure. The trainers noticed an adaptation period of 8 d after the arrival to the new facilities, in which the animals showed disruptive behaviour, inattentiveness during feeding sessions, and presented lower interest and motivation to trainer interaction. Inappetence is a standard early sign of disease in cetaceans (Sweeney & Ridgway, 1975) and one that may also be a symptom of stress (Selye, 1973; Fowler, 1978; Sweeney, 1990; Antrim & McBain, 2001); however, food intake was successful for both beluga whales since the arrival to the new facilities, and the negative behaviours observed were gradually reduced during the 8-d period. Therefore, observations of the behavioural effect of transportation and introduction to new facilities disappeared after 8 d. Acoustic behaviour remained affected for up to 4 wks after arrival, however, and the vocalization rate did not reach the same level as before the transport until the 5th wk. This strong difference in the duration of behavioural versus acoustic responses to stressors suggests that the acoustic monitoring technique may be an efficient method to evaluate stress in captive beluga whales.

There is evidence of a decrease or even a ceasing in the acoustic activity of beluga whales in the presence of natural predators (i.e., killer whales) or engine noise disturbance. This acoustic response has been observed in both captive and free-ranging beluga whales (Morgan, 1979; Lésage et al., 1999; Karlsen, 2002; Van Parijs et al., 2003) and free-ranging narwhals (*Monodon monoceros*) (Finley et al., 1990) and has been associated with threat, startle, fright, alarm, or stress contexts and interpreted as a survival strategy to avoid detection by predators (Schevill, 1964; Fish & Vania, 1971; Morgan, 1979; Finley et al., 1990; Lésage et al., 1999). These observations further support our proposed viability of using the acoustic activity as a measure of animal welfare in captive beluga whales.

Acoustic activity is incorporated in animal welfare research in few terrestrial mammals, and to our knowledge, this is the first report of its application in marine mammals. This lack of acoustic behaviour in welfare evaluations is to a certain extent justified: calibrated acoustic equipment is usually expensive and not always easy to operate and maintain. Mammals produce numerous and diverse categories and subcategories of vocalizations. Robust interpretations of the communicative significance of vocal signals may depend on detailed understandings of analysis techniques (Owren & Bernacki, 1998) and natural history (Kroodsma, 1996; Blumstein & Armitage, 1997). Furthermore, efforts must be made to understand the natural history and evolution of a specific vocal behaviour before invoking it as a useful measure of animal well-being (Weary & Fraser, 1995).

The technique presented in this report refers to the total acoustic activity independently of the behaviour and communicative significance, in which the useful information for evaluation is the overall production of vocalizations as a measure of well-being, rather than detecting specific vocalizations associated to stressful contexts. Therefore, detailed acoustic and behavioural analysis is not needed for our purpose, making the analysis simple and time affordable for welfare evaluation.

If the measurement of the acoustic activity in captive beluga whales proves to be an effective method for evaluating animal welfare, it is bound to be a useful tool in captive management. It would allow the monitoring of stress levels during the adaptation period after the transportation and introduction to new facilities. In addition, common contexts in captivity, such as nearby construction or facility modifications, also would be potential situations for monitoring stress levels or adaptation periods. Similarly, this technique could potentially be used in rehabilitation centres, in which stress level during both transportation and rehabilitation procedures of odontocetes is crucial. Acoustic activity measurements would also benefit environmental enrichment protocols. To date, there is little empirical data on the effectiveness of environmental enrichment techniques. There is no clear methodology on how to evaluate enrichment protocols (Shepherdson et al., 1998; Mellen & Sevenich-Macphee, 2001). Monitoring acoustic activity in captive beluga whales allows measuring the effect of the new environmental elements.

This technique has several benefits over traditional methods such as behavioural observations or measurement of hormonal levels. Audio recordings conducted for this purpose do not require expensive calibrated equipment and can be reliable when based on compressed digital audio formats. Recorders based in such formats are commercially available and affordable. Identification of vocalizations does not require extensive training or experience. Animals do not need to be trained, and this method is not invasive since it is merely observational. Furthermore, acoustic activity seems to be a more accurate method than behavioural observations to evaluate the effect of environmental stressors, as shown in the daily trainer reports.

Our proposed hypothesis is based on observations of two beluga whales, and our results must thus be interpreted as suggestive, rather than conclusive. More research is clearly needed before obtaining final conclusions. Monitoring other beluga whale transports or environmental changes in facilities could help validate this proposed hypothesis. Moreover, parallel studies of blood corticoid levels could lead to robust deductions about quantitative relationships between acoustic activity and stress level.

Hopefully, our efforts will be a source for future studies on captive animal welfare. We recommend that zoos and oceanaria consider incorporating acoustic activity monitoring into their standards for animal welfare and psychological well-being. This can be implemented without expensive acoustic equipment and little investment in training, data collection, and analysis.

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