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Field Endocrinology and Conservation Biology[1]

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SYNOPSIS. Field endocrinology techniques allow the collection of samples (*i.e.*, blood, urine, feces, tissues) from free-living animals for analysis of hormones, receptors, enzymes, etc. These data reveal mechanisms by which individuals respond to environmental challenges, breed, migrate and regulate all aspects of their life cycles. Field endocrinology techniques can also be used to address many issues in conservation biology. We briefly review past and current ways in which endocrine methods are used to monitor threatened species, identify potential stressors and record responses to environmental disturbance. We then focus on one important aspect of conservation: how free-living populations respond to human disturbance, particularly in relation to ecotourism. Breeding adult Magellanic penguins, *Spheniscus magellanicus*, appear to habituate well to tourists, and breed in an area where about 70,000 people visit during the season. Baseline levels of corticosterone return to normal after exposure of naïve birds to humans. However, penguin chicks appear to show a heightened adrenocortical response to handling stress in nests exposed to tourists, compared to chicks living in areas isolated from human intrusions. Given that developmental exposure to stress can have profound influences on how individuals cope with stress as adults, this potential effect of tourists on chicks could have long-term consequences. This field endocrine approach identified a stressor not observed through monitoring behavior alone.

INTRODUCTION

As human population numbers continue to expand at near logarithmic rates, how wildlife and wild places respond to anthropogenic disturbances is of great critical concern. Indeed, whole journals are devoted to the conservation of species (*i.e., Conservation Biology; Biological Conservation*) and the environmental consequences of human disturbance (*i.e., Environmental Conservation; Environmental Health*).

There is no question that human disturbance now pervades all aspects of the environment and threatens habitats at all times of the year. Indeed, it is now probably true that there is no pristine place left on earth. Even the most remote oceanic trenches or polar and mountain regions with the organisms therein, are accumulating pollutants brought in by air or ocean currents (Macdonald and Bewers, 1996; Wania, 2003). As a result, disturbances caused by humans may force permanent changes in life history characteristics of organisms so they can survive such perturbations. How anthropogenic disturbances will be dealt with in conservation practice requires integration of scientific information into protocols for managers at the field level, and enormous political efforts (including cultural and economic change). However, much research remains to be done at the basic biological level. We still know relatively little about how animals respond to environmental change to adjust their life cycles accordingly and thus maximize fitness (Wingfield, 2004*a*, *b*).

Individuals in their natural habitat are exposed to two major types of environmental change: *predictable* cycles of seasons, tides etc. that an organism can anticipate, and *unpredictable* events (storms, predators, change in social status) that require responses on a facultative basis and are disruptive to the predictable life cycle (McEwen and Wingfield, 2003; Wingfield, 2004*a*, *b*). To do this, organisms must perceive signals from their habitat, use them to predict future changes, and integrate this information with social cues so that morphology, physiology and behavior are regulated appropriately (Jacobs and Wingfield, 2000). Moreover, an individual may not experience its environment in exactly the same way as another so that physiological and behavioral responses must be ''customized'' to the experience (Wingfield, 2004*a*, *b*). To achieve this, suites of environmental signals are transduced into neural, neuroendocrine and endocrine cascades that orchestrate all aspects of the life cycle.

We may never understand the impacts of human disturbance unless we have a clear understanding of how organisms deal with a changing environment. Although it is obvious that many aspects of human disturbance fall into the realm of unpredictable perturbations, the specter of global warming and climate change may result in dramatic alteration of predictable components of the life cycle as well. Examples are earlier springs and later autumns, and changed rainy and dry seasons. Whether or not organisms will be able to respond to all these dramatic changes in the predictable life cycle as well as cope with unpredictable perturbations is unknown, but could have catastrophic consequences. Here we provide an heuristic framework for classifying different aspects of human disturbance, and then give examples to show how field endocrine approaches help identify whether a population is coping or not and, more importantly, if it is not coping, what the specific problem may be.

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HUMAN DISTURBANCE IN THE PREDICTABLE AND UNPREDICTABLE ENVIRONMENT

Characteristics of direct human disturbance

Nisbet (2000, p. 313) defines human disturbance as ''. . . any human activity that changes the contemporaneous behavior or physiology of one or more individuals within a breeding colony of waterbirds.'' This definition can be extended to other vertebrates as well—*i.e.*, it may have universal relevance for human disturbance on animals in general. Direct human activity that has the potential to disturb natural populations can be classified in different types (after Nisbet, 2000) as follows:

1. ''Research Procedures.'' Activities of investigators applied to individuals of the species under study.
2. ''Investigator Intrusions.'' Activities of investigators on other local species that may be disturbed unintentionally.
3. ''Visitor Intrusions.'' Activities of humans other than researchers who may disturb animals deliberately to photograph individuals, or casual intrusions and disturbances by humans pursuing recreational activities etc.
4. ''Visitor Approaches.'' Activities of humans as they approach animals—*i.e.*, they may not cause disturbance in the sense of movements away from the intruder, but they may cause alarm and vigilance distracting the animal away from its normal activity.
5. ''Vehicle Activities.'' Movements of motor vehicles, aircraft and water craft in or close to animal populations.
6. ''Positive Management.'' Activities of managers to benefit natural populations of animals. These include erecting fences, eliminating predators, patrolling the protected area etc.
7. ''Negative Management.'' Activities of managers specifically designed to reduce numbers and control activity of natural populations of animals. This could include killing (hunting) animals, modifying the habitat etc. Disturbance to other species in the same area may be considerable.
8. ''Persecution, Harassment and Vandalism.'' Human activity intended to disturb and harm natural populations by indiscriminate hunting and killing, destruction of nests/dens, pursuing animals in vehicles or with dogs etc.

Examples of studies showing how these and other human activities affect wildlife include those on forest fragmentation (Danielson *et al.*, 1997; Sousa *et al.*, 2003), urbanization and road building (Develey and Stouffer, 2001; Forman and Alexander, 1998), tourist visitation (Culik and Wilson, 1995; Duchense *et al.*, 2000) and scientific investigator activities (Frederick and Collopy, 1989). All have been documented negative impacts on wildlife and this list only touches the surface.

Before going on to consider the hormonal correlates (and consequences) of these types of disturbance, it is important to consider a classification of the types of effects (Nisbet, 2000). Such effects of human disturbance (*i.e.*, direct human activity) that may be useful especially in conjunction with field endocrine techniques are as follows (based on Nisbet, 2000 but also supplemented by our own interpretations):

1. Physiological effects such as increases in heart rate without overt changes in behavior—except perhaps increased vigilance. Nisbet (2000) points out that this response should not be considered as adverse unless it decreases survival or reproductive success.
2. Moving away from a nest, territory, feeding site etc. and then returning after the disturbance ends. Again, Nisbet (2000) points out that this should not be considered adverse unless the movement results in loss of nest, status, food resources etc.
3. Permanent movement away from a nest, territory, feeding site or other resource (*i.e.*, abandonment or ''desertion''). This may likely have adverse effects.
4. In colonial/group living species, abandonment of a colony, home range or even larger scale distribution. Unless the population is able to relocate successfully, then adverse effects will result. Even if relocation is possible, the new site may not be ideal and reduced survival and reproductive success may result.
5. Disturbance results in direct reduction of reproductive effort either by loss of eggs and/or young, failure to reach reproductive maturity, attract a mate or find a suitable nest site.
6. Disturbance results in an increase in adult mortality in local, regional or total populations.

This kind of framework can be applied broadly to all vertebrates, and also possibly many invertebrates. Nisbet (2000) points out that cause and effect of disturbance on animal populations should be assumed only after rigorous comparisons with control experiments or situations. Field endocrinology may provide additional tools to assess this. It will also be important to determine whether some populations are able to habituate to at least low level disturbance or whether individuals and local populations differ in the degree of tolerance of disturbance.

Characteristics of indirect human disturbance

In addition to the direct effects of human activities discussed above, a separate suite of indirect disturbances exist, where the consequences of human activities to wildlife are separated either geographically or temporally from the actual disturbance activity. For these factors it is less straightforward to document how change is occurring because we often lack before and after comparisons. Likewise, effects can be gradual, and may not be manifest or observable in animal systems until it is too late. However, despite the difficulty in knowing *a priori* how or what indirect disturbances may affect wildlife, many studies are now focusing on documenting or predicting the potential consequences of indirect disturbances. Examples include factors such as pollution effects from agriculture and power plants (Guillette and Iguchi, 2003; Milnes *et al.*, 2004), introduction of diseases and non-indigenous species into new habitats (Phillips *et al.*, 2003; Vredenburg, 2004), and, on the broadest scale, global climate change (Scheel *et al.*, 1996; Burns *et al.*, 2003).

FIELD ENDOCRINOLOGY APPROACHES

Physiological consequences of disturbances are very complex—and may not be manifest at the same time frame as the particular disturbance. Thus, we stress that quantification of physiological consequences of disturbances is important. Such quantification will also be important to determine whether some populations are able to habituate to at least low level disturbance or whether individuals and local populations differ in the degree of tolerance of disturbance. Nisbet (2000, p. 315) points out that it is important to distinguish between these. He defines tolerance as ‘‘the intensity of disturbance that an individual tolerates without responding in a specified way.’’ It is different from habituation in which an individual learns not to respond to a stimulus when no reinforcement follows. Field endocrinology may provide additional tools to assess these consequences of effects of human disturbances on wildlife.

Historically, the majority of studies that examine the effects of anthropogenic disturbances have focused on behavioral consequences to individuals. These include factors as simple as whether an animal modifies its current activities when approached by a human (Fernández-Juricic *et al.*, 2001; Lord *et al.*, 2001). More commonly, how reproductive behaviors are modified by human disturbances is often measured (Fowler *et al.*, 1995; Frederick and Collopy, 1989; Giese, 1996). This includes such factors as: do animals abandon breeding/nest sites and nesting attempts, or do the offspring receive less food or care. While behavioral activities are important to monitor, it has become increasingly obvious that physiological characteristics and responses of animals to human disturbances are also important characteristics to track. Indeed, animals have been known to show no obvious behavioral changes in response to a human caused effect, while physiological characteristics such as heart rate may have increased significantly (Culik *et al.*, 1990; Weimerskirch *et al.*, 2002). Note, however, that many of these studies may be confounded by implanting a device, or handling, so that the organism is then more sensitive to human presence. This suggests that even when appearing outwardly ‘‘calm,’’ animals may in fact be experiencing physiological costs of anthropogenic disturbances. Furthermore, there is increasing evidence that stressors or perturbations during development can have significant future consequences at time points in the life cycle far removed from the initial stressor (Champagne and Meaney, 2001; Kitaysky *et al.*, 2003). This has been shown in cases where young have been subjected to stressors, either directly or through physiological signals passed to them from the mother, resulting in significant later physiological consequences such as decreased memory and learning capacity, increased aggression, and a suite of other negative characteristics that in general cause older individuals to be more susceptible to stressors (Liu *et al.*, 2000; Shanks, 2002; Teicher *et al.*, 2003). Clearly, monitoring physiological and behavioral responses to anthropogenic disturbances is critical to obtain the full spectrum of possible outcomes. Indeed, the physiology of an animal may not only be useful in monitoring *a posteriori* effects of disturbances, but may be equally useful as *a priori* predictors of which individuals, populations or species could be more susceptible to anthropogenic disturbances (Wingfield *et al.*, 1997).

Many aspects of physiology can be influenced by human disturbances. Increasingly, measures of endocrine function are being used as indicators of physiological status such as susceptibility to environmental stress and reproductive potential (Wingfield *et al.*, 1997). Of particular concern are endocrine disrupters, compounds that, when taken into the body, act to mimic (agonist) or interfere (antagonist) with the activities of naturally occurring hormones (for recent review see Lintlemann *et al.*, 2003). Note that endocrine disrupters may also act by being toxic in addition to potentially interfering with the reproductive system. More research is needed to determine the impact of endocrine disrupters on organisms in their natural habitat. For example, what are the effects of pollutants on reproductive success or survival rates? Are some species coping better than others, and do toxic effects reduce the ability of an individual to cope with other aspects of its environment?

Our laboratory focuses on the mechanisms by which vertebrate organisms regulate their life cycles. Two major foci are hormonal regulations and behavioral changes with environmental perturbations. The first includes environmental and hormonal regulation of the temporal sequence of life history stages that constitute the predictable life cycle of morphology, physiology and behavior. This includes such phenomena as migration, breeding, winter strategies, etc. (Jacobs and Wingfield, 2000). Human disturbance may have profound influences at many levels in this predictable component of the life cycle (*i.e.*, the seasons). Thus, vertebrates use cues from the environment (*e.g.*, day length, rainfall, temperature) to regulate the endocrine system and anticipate future events such as spring or winter by changing morphology, physiology and behavior so that an individual is ready to respond. An example is the development of the reproductive system in anticipation of spring. In recent years it is thought that global climate change is resulting in earlier and earlier springs, at least in the northern hemisphere. This in turn causes changes in spring migration times and earlier onset of breeding in many songbird species (Visser *et al.*, 2003). How organisms in general respond to change in the predictable life cycles, and whether some can adjust and others not, has undetermined implications for fitness parameters such as reproductive success. Field endocrinology techniques may help in monitoring such changes.

The second focus of our laboratory is on how organisms deal with the unpredictable components of their environment. Individuals cope with many natural perturbations such as storms, predators, change in social status etc., and do so on a facultative basis (McEwen and Wingfield, 2003; Wingfield *et al.*, 1998). Human disturbance falls into this category because populations of organisms in the field have no clear way to predict onset of logging, urbanization, etc.

Most vertebrates show an increase in circulating levels of glucocorticosteroids when exposed to perturbations in their environment. Glucocorticosteroids and other hormones of the hypothalamic-pituitary-adrenal (HPA) stress response facilitate changes in behavior and physiology to allow the organism to escape from or endure the disturbance (Sapolsky *et al.*, 2000; Wingfield and Ramenofsky, 1999). While baseline levels of glucocorticosteroids are necessary for all animals to maintain homeostatic energetic balance (Dallman *et al.*, 1993), chronically elevated levels of glucocorticosteroids are detrimental, causing muscle wasting, impaired immune function, and reduced growth rates (Sapolsky *et al.*, 2000; Wingfield and Romero, 2000). In nature, however, chronically elevated glucocorticosteroids are probably rare, because animals either cope with the stressors or die (Wingfield and Romero, 2000). However, laboratory studies have shown that the adrenocortical response to stress response can be gradually reduced (*i.e.,* habituated) as animals grow accustomed to either confinement or some form of low-intensity stress (Konarska *et al.*, 1989; Marti and Armario, 1998).

The use of stress hormone data for assessing an animal's response to disturbances is not without it difficulties. Indeed, it is important to have a frame of reference for which to compare the levels of hormone that are being assessed. As glucocorticosteroids have multiple functions in the body (see above), there is the potential for naturally occurring diel or seasonal changes in circulating baseline or constituent plasma hormone levels. Thus, some baseline information on resting or ''normal'' hormonal profiles of undisturbed animals is required, prior to making comparisons or claims as to the trends observed in sampled individuals.

Historically, hormone titers of glucocorticosteroids from animals are collected from blood samples. This technique is useful, for it allows the assessment of instantaneous stress hormone levels. However, the collection of the sample (taking blood) is itself an intrusion. Thus, when designing experiments to assess stress hormone profiles, the effects of current research activities must be considered so they do not affect the hormonal response of the animals examined. Increasingly, studies wishing to use non-invasive techniques for assessing hormonal profiles in animals are using scat analysis (Creel *et al.*, 2002; Wasser *et al.*, 1997). Using scat allows sample collection without disturbing the focal animal. However, there are a number of drawbacks of this technique. First, hormone titers in scat represent a summation of the stress profile over an undetermined amount of time prior to collection. Thus, it is impossible to assess finer scale disturbance effects (*i.e.,* acute stressors). In addition, many hormones are metabolized extensively prior to excretion making it difficult, without extensive standardization or validation processes, to know how hormone profiles in scat correlate with blood plasma levels. Regardless, a number of researchers have successfully used hormone analysis in scat to document the effects of anthropogenic disturbances on wildlife. These include the effects of snowmobiles on wolves, *Canis lupus*, and elk, *Cervus canadensis* (Creel *et al.*, 2002), clear cutting and the spotted owl, *Strix occidentalis* (Wasser *et al.*, 1997), and ecological stressors on African elephants, *Loxodonta africana* (Foley *et al.*, 2001).

Direct assessment of glucocorticosteroid levels in plasma has the benefit of representing the condition of the animal at the time measured. The capture stress protocol, where animals are captured and restrained while serial blood samples are collected (typically 3–5 samples over the course of an hour), allows for the assessment of baseline or constituent plasma hormone levels (if collected within three minutes) as well as provides a comparative measure of how severely an animal responds to an acute stressor. It is assumed that an animal showing a larger expression of corticosterone or cortisol in the plasma, in response to restraint, perceives the stressor in a more negative way than for a animal with a lower glucocorticosteroid secretion profile.

FIELD ENDOCRINOLOGY: AN EXAMPLE OF PENGUINS AND PEOPLE IN PATAGONIA

We have used the capture stress protocol to examine how tourist visitation affects the physiological stress response in Magellanic penguins (*Spheniscus magellanicus*). Quantifying how tourist disturbance affects wildlife is becoming increasingly popular and important. Ecotourism is marketed as an ecologically friendly and viable system which provides for the conservation of resources, while also benefiting the economic structure of local communities (Goodwin, 1996). Several studies have examined how ecotourism is affecting specific animals. Galapagos marine iguanas, *Amblyrhynchus cristatus*, seemed to habituate to human disturbances (Romero and Wikelski, 2001), while woodland caribou, *Rangifer tarandus caribou* (Duchense *et al.*, 2000), chimpanzees, *Pan troglodytes schweinfurthi* (Johns, 1996), African rhinoceros, *Rhinoceros unicornis* (Lott and McCoy, 1995), and the chicks of hoatzins, *Opisthocomus hoatzin* (Müllner *et al.*, 2004) all showed negative consequences of tourist visitation.

Magellanic penguins breed in the temperate zone with a wide geographic distribution, extending from

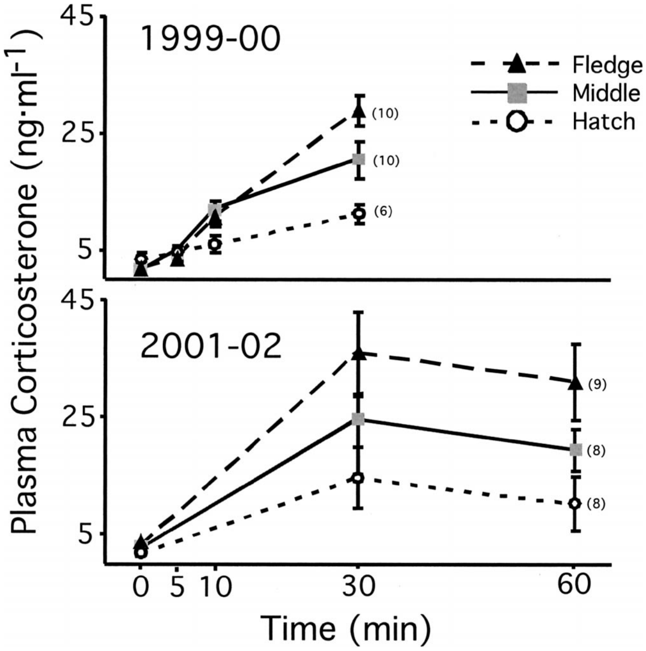

FIG. 1. Patterns of corticosterone increase for Magellanic penguin chicks subjected to the capture stress protocol. Chicks were sampled at three ages—near hatch, middle-aged and near fledge—during two breeding seasons (1999–2000 and 2001–2002). Sample sizes are included in parentheses with each group.

42°S on the eastern coast of South America, around Cape Horn, and north to 29°S on the Pacific coast, and including the Falkland (Malvinas) Islands (Williams, 1995). We worked at Punta Tombo, Argentina (44°02′S, 65°11′W), where the largest colony of Magellanic penguins is found (over 200,000 breeding pairs; Boersma *et al.*, 1990; Gandini *et al.*, 1996). More that 70,000 tourists visit Punta Tombo annually (Yorio *et al.*, 2001). Tourists are restricted by fencing to a small portion of the colony, but within this area there are several hundred nests so that tourists can walk directly among the birds (Yorio and Boersma, 1992; Fowler, 1999).

Previous work at Punta Tombo showed that adult Magellanic penguins in areas visited by tourists have habituated to having humans in and around their nesting sites (Yorio and Boersma, 1992; Fowler, 1999; Walker, 2003). Penguins in tourist areas show reduced defensive behavioral responses as well as lower glucocorticosteroid stress responses to capture and restraint. Visitors find the penguins to be calm and accepting of humans with penguins often walking directly among the tourists.

We investigated the effects of tourist visitation on Magellanic penguins by examining how chicks living in tourist areas develop and/or modify their stress responses as compared to chicks in non-visited (‘‘naïve’’) areas (Walker *et al.*, 2005*a*). Magellanic penguin chicks are semi-altricial (Starck, 1993). At hatching, chicks are covered in down, cannot feed or defend themselves, are unable to thermoregulate well, and are nest-bound. Hatchling Magellanic penguin chicks normally have an underdeveloped glucocorticosteroid stress response, which gradually matures or intensifies as they grow toward fledging (Fig. 1; Walker *et al.*, 2005*b*). This process of gradual development of the stress response has also been observed in other altricial or semi-altricial species such as the northern mockingbird, *Mimus polyglottus* (Sims and Holberton, 2000) and American kestrel, *Falco sparverius* (Love *et al.*, 2003), and is thought to have evolved as a mechanism for defenseless chicks to avoid the negative consequences of elevated glucocorticosteroids.

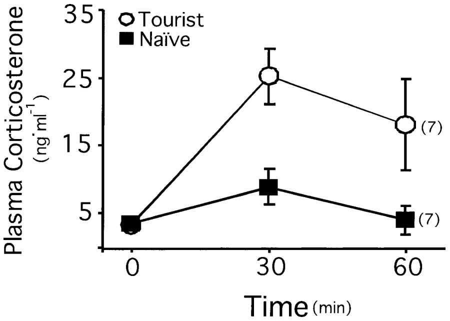

FIG. 2. Plasma corticosterone levels in newly hatched Magellanic penguin chicks living either in tourist-visited areas or unvisited (‘‘naïve’’) areas of the Punta Tombo penguin colony. Penguin chicks were subjected to a capture stress protocol; captured and held outside their nest, with sequential blood samples taken over a 60 minute period.

Interestingly, within days after hatching, Magellanic penguin chicks in tourist-visited areas exhibit significantly elevated levels of corticosterone (the glucocorticosteroid in birds) as compared to unvisited chicks, in response to capture and restraint (Fig. 2). As yet, we are uncertain of the mechanism(s) for this rapid expression of the stress response, but may be likely due to the increased incidence of the parents standing up and exposing chicks as tourists approach (Boersma, personal observations). In contrast, chicks in non-visited areas are rarely exposed or un-brooded, as adults do not experience close encounters that make them shift or stand up. In addition, maternal effects may be playing a role in the expression of an increased corticosterone response in chicks in the tourist area. However, as Magellanic penguin adults living in tourists areas have a reduced capability to secrete corticosterone in response to stressors (Walker, 2003), how the female would signal the opposite effect in her chicks is unknown.

Regardless of the mechanism causing the rapid and early onset of corticosterone stress response expression, chicks in tourist areas have elevated corticosterone. Elevated corticosterone at an early age can have significant negative physiological consequences for individuals later in life (see reviews: Bertram and Hanson, 2002; Maccari *et al.*, 2003; Welberg and Seckl, 2001). Post-fledging captive black-legged kittiwake chicks (*Rissa tridactyla*) subjected to elevated corticosterone early in life were less successful in remembering how to find food in a choice-reward experiment

(Kitaysky *et al.*, 2003). Whether high levels of corticosterone early in life affects Magellanic penguins as adults is unknown. Such studies of changes in long-term life history characteristics on a free-living, long-lived species may prove difficult. However, the effects could be manifest as differences in foraging capability, physiological condition later in life, or ability to recruit to the breeding population.

Our data show the benefit of using endocrine techniques to examine the consequences of human disturbances to wildlife. Our findings showing the increased expression of the stress response in young chicks would be difficult using the non-invasive techniques of scat analysis and/or behavioral observations. Even in studies that suggest anthropogenic disturbances have little negative impact—as in the adult Magellanic penguins breeding in tourist areas—finer scale and multi-level analyses of disturbances effects may provide a more complete understanding of the actual costs to the animal.

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