Assessing reproductive status of right whales (Eubalaena glacialis) using fecal hormone metabolites

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Abstract

Long-term studies of the endangered North Atlantic right whale, Eubalaena glacialis, have revealed declining reproductive parameters over the past two decades, threatening recovery of this small population if current trends continue. Little is known about right whale reproductive physiology, and investigating this reproductive decline has been limited by a lack of non-lethal methods for assessing reproductive status (e.g., sexual maturation, ovarian activity, pregnancy, lactation, and reproductive senescence) in free-swimming whales. This paper describes validation of existing radioimmunoassay techniques to study reproduction in right whales by measuring estrogens, progestins, androgens, and their related metabolites in fecal samples. Over the past decade fecal steroid hormone assays have been used to assess reproductive status and function in a wide range of terrestrial wildlife species, but this is the first application of this methodology in wild cetaceans. Analysis of fecal hormone metabolite levels in combination with life history data from photographically identified whales shows that this non-invasive method can be used to determine gender, detect pregnancy and lactation, and to assess age at sexual maturity in right whales and potentially other endangered whale populations.

Keywords: Right whale; Eubalaena glacialis; Reproduction; Fecal hormones; Estrogen; Progesterone; Testosterone

1. Introduction

The western North Atlantic right whale, Eubalaena glacialis, was hunted to very low levels by the early 1900s and remains one of the most critically endangered of the large whales (Clapham et al., 1999; International Whaling Commission, 2001). Despite protection from commercial whaling since the 1930s, currently this population numbers just over 300 individuals (International Whaling Commission, 2001) and may be decreasing (Fujiiwara and Caswell, 2001) in contrast to healthy, growing populations of southern hemisphere right whales (Eubalaena australis; Best et al., 2001; Payne et al., 1990). Because the northern right whale migratory range includes densely human-populated coastal habitats from Florida to the Gulf of St. Lawrence, there has been significant anthropogenic mortality from collisions with large ships and fishing-gear entanglements (Knowlton and Kraus, 2001). More recently it has been recognized that population growth may also be limited by declining reproduction. Based on photographic identification of individual whales, long-term studies of this population have revealed a decrease in calves per mature female per year, an increase in inter-birth intervals, an increasing age at first calving, and highly variable calf production over the past two decades (Kraus et al., 2001). Recent demographic models have predicted extinction of the North Atlantic right whale within 200 years if current trends in mortality and reproduction continue (Caswell et al., 1999; Fujiiwara and Caswell, 2001).

Most of the existing information on the reproductive physiology of the mysticete (baleen) whales has been...
derived from examination of the reproductive tracts and gonads of dead whales either stranded or killed during whaling operations (Lockyer, 1984). Since right whales have been protected for over 60 years, there is almost no information on the reproductive physiology of this species, including the reproductive cycle of females, pregnancy rates, incidence of abortion or stillbirths, seasonality of reproduction, the age of sexual maturation or reproductive senescence (if it occurs). Because it is not possible to collect blood from free-swimming right whales, there have previously been no methods to measure reproductive hormones to investigate the physiological reasons for this declining reproductive success.

However, the recent application of radioimmunoassay methods to quantify reproductive hormone metabolites in feces from numerous terrestrial mammals (reviewed in Schwarzenberger et al., 1996) presented a possible strategy for determining the reproductive status of right whales. Over the last decade, measurement of the metabolites of 17β-estradiol and progesterone (estrogens and progestins) in fecal samples has been used to characterize reproductive status of females in a wide variety of captive and free-ranging terrestrial wildlife species (for example, Czekala et al., 1994; Garnier et al., 1998; Monfort et al., 1993; Stoops et al., 1999; Velloso et al., 1998; Wasser, 1996, Wasser et al., 1996, 1997). Similarly, assays for fecal androgens (testosterone and metabolites) have been validated to investigate relationships between sex, reproductive state, season, social rank, and behavior in an array of wild mammals (for example, Barrett et al., 2002; Cavigelli and Pereira, 2000; Creel et al., 1997; Diomiak et al., 2004; Moss et al., 2001). Because it had been previously demonstrated that floating right whale feces could be successfully collected (Kraus and Stone, 1985; see Section 2), this study was undertaken to evaluate whether it was feasible to adapt these assays to evaluate reproductive status in right whales.

In mammals, the major reproductive steroid hormones (i.e., estrogen, progesterone, and testosterone) are secreted into the bloodstream and metabolized in the liver by hydroxylation or conjugation with sulfate or glucuronide sidegroups. These hormones and their metabolites are excreted in bile (and/or urine) and are further metabolized into a wide variety of forms in feces (Adlercreutz and Martin, 1980). The fecal metabolites can have highly variable affinities to radioimmunoassay (RIA) antibodies (Wasser et al., 2004), but can be measured by using antibodies to the parent hormone that cross-react with some of the fecal metabolites (Adlercreutz and Järvenpää, 1982; Palme et al., 1996; Wasser et al., 2004). The concentration of fecal metabolites reflects the general pattern of serum hormone levels, with a lag time from hours to days that is determined by the metabolic clearance rates and intestinal transit time for the species (Palme et al., 1996; Wasser et al., 1994). Because hormone metabolism and routes of excretion vary considerably between different species (Schwarzenberger et al., 1996), these assays require validation for every new species studied.

While fecal hormone assays have been used extensively in terrestrial wildlife, they have not been widely applied to aquatic mammals (except for female sea otters, Enhydra lutris; Larson et al., 2003). The North Atlantic right whale presented a unique opportunity to test the usefulness of these assays in non-captive cetaceans because virtually the entire population has been photographically identified (Hamilton et al., 1998), and there are over two decades of demographic and life history data available on individual whales in the North Atlantic Right Whale Catalog (Hamilton and Martin, 1999).

The objectives of this study were to: (1) determine the feasibility of collecting fecal samples from right whales for these studies, (2) validate RIAs for estrogens, progestins, and androgens for right whale fecal samples, and (3) evaluate the relationship between fecal hormone metabolite levels and the gender, age, and reproductive status (e.g., pregnant, non-pregnant, and lactating) of individual whales with known life histories.

2. Methods

2.1. Sample collection and handling

Fecal samples were collected from right whales during shipboard photo-identification surveys in the Bay of Fundy, Canada, between July and September 1999–2002. During these months, North Atlantic right whales congregate in large numbers to feed on aggregations of their primary zooplankton prey, Calanus finmarchicus (Murison and Gaskin, 1989). Based on sighting data collected since 1980, right whales are known to feed in high densities within a relatively well-defined area in the Bay that is approximately 24 × 30 km (Brown et al., 1995), and other whale species are only occasionally sighted among feeding right whales.

In over 50% of sample collection events, right whales were observed defecating at the surface, either during courtship activity when large numbers of whales aggregate, or while fluking up to dive. In these instances, the individual whale was identified using photographs taken just before or after defecation (see below for whale identification methods). Alternately, feces were found floating on the water in the area of diving right whales that were not photographed (these data are not used in this study). Right whale feces were identifiable by size, shape, a typical brown to orange-red color, characteristic strong odor, and the presence of fine baleen hairs in the sample.

A 300 μm nylon mesh dipnet (Sea-Gear, Melbourne, FL) attached to a telescoping boathook was used to