Linking physiological approaches to marine vertebrate conservation: using sex steroid hormone determinations in demographic assessments

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Sex, age and sexual maturation are key biological parameters for aspects of life history and are fundamental information for assessing demographic changes and the reproductive viability and performance of natural populations under exploitation pressures or in response to environmental influences. Much of the information available on the reproductive condition, length at sexual maturity and sex determinations of endangered species has been derived from direct examination of the gonads in dead animals, either intentionally or incidentally caught, or from stranded individuals. However, morphological data, when used alone, do not provide accurate demographic information in sexually monomorphic marine vertebrate species (e.g. sharks, sea turtles, seabirds and cetaceans). Hormone determination is an accurate and non-destructive method that provides indirect information about sex, reproductive condition and sexual maturity of free-ranging individuals. Correlations between sex steroid concentrations and biochemical parameters, gonadal development and state, reproductive behaviour and secondary external features have been already demonstrated in many species. Different non-lethal approaches (e.g. surgical and mark–recapture procedures), with intrinsic advantages and disadvantages when applied on free-ranging organisms, have been proposed to assess sex, growth and reproductive condition. Hormone determination from blood samples will generate valuable additional demographic information needed for stock assessment and biological conservation.

Key words: Conservation, life history, marine vertebrates, sex steroids, stock assessment

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Introduction

Biological parameters describing key aspects of life history, such as sex, age structure, the dynamics of growth and sexual maturation (recruitment for breeding), are generally considered fundamental information for assessing demographic changes, reproductive viability and the performance of natural populations under exploitation pressures or in response to...
environmental influences, for establishing the accurate conservation status of free-ranging populations and for developing conservation and sustainable fisheries management strategies (DeMaster, 1984; Perrin and Reilly, 1984; Eberhardt, 1983; Balazs, 1993; Crouse and Frazer, 1995; Haddon, 2001; Heppell et al., 2003; de Mitcheson, 2009; alternative approaches are described by Kirkwood, 1991; Cook, 1995; Punt and Donovan, 2007). However, certain basic information needed to predict the population dynamics of threatened species is particularly difficult to obtain solely by visual determination. This is especially true for sexually monomorphic marine vertebrate wildlife (e.g. sharks, seabirds, cetaceans and sea turtles; Caldwell, 1962; Bercovitz et al., 1978; Owens et al., 1978; Merchant-Larios, 1999; Wibbels et al., 2000; Bertellotti et al., 2002; Casale et al., 2005; Dubiec and Zagalska-Neubauer, 2006). Consequently, much of this information has been derived from dead animals, either directly or incidentally caught, or from stranded individuals (Hohn et al., 1985; Read, 1990; Valencia and Leyton, 1992; Merchant-Larios, 1999; Kellar et al., 2009; Casale et al., 2011). Even when data can be obtained directly from free-ranging animals by photo-identification or tagging studies, the population estimates for wild marine fauna are complicated by the difficulties of capturing and tagging, the effects of tagging, the recovery rate and the requirement for long-term monitoring series (Gibbons, 1987; Clapham, 1992; Limpus, 1992; Balazs, 1995, 1999; Balazs and Chaloupka, 2006; Dugger et al., 2006).

Measurement of hormone concentrations provides an alternative methodology that can be used for understanding physiological cycles, functional aspects of reproductive endocrinology and the relationship between the hormone dynamics and the reproductive and nesting behaviour (e.g. Licht et al., 1979, 1980, 1985; Owens, 1980; Licht, 1982; Owens and Morris, 1985; Wibbels et al., 1990, 1992; Williams, 1992a; Al-Habsi et al., 2006; Awruch et al., 2008b). Recent publications have demonstrated the utility of hormone measurement as a non-destructive method to obtain information on the reproductive condition of endangered species without conspicuous sexual dimorphism (Rolland et al., 2005; Daoquen et al., 2006; Awruch et al., 2008a; Kellar et al., 2009; Valente et al., 2011). Currently, hormone biomarkers are sufficiently accurate to provide information about overall health (e.g. biochemical parameters and mould) in response to environmental (e.g. season and El Niño event) and regional conditions (e.g. tourism and chemical contaminants; Romero and Wikelski, 2001, 2002a,b; Mashburn and Atkinson, 2004; Oki and Atkinson, 2004; Labrada-Martagón et al., 2013). Such biomarkers thus contribute valuable information on the physiological responses of wildlife to xenobiotics and environmental change (Guillette et al., 1994, 1999; Guillette and Gunderson, 2001; Hall et al., 2003).

Vertebrates have evolved neuroendocrine signals, patterns of sexual development and complex behavioural responses (social and sexual behaviours mediated by the nervous system) that are, in general, highly conserved across taxa, including elasmobranchs (Callard et al., 1989; Crews et al., 1994; Romero, 2004; Pratt and Carrier, 2005); two examples are the stress response (to potentially dangerous stimuli) and the sex steroid and thyroid hormone control systems (Randall et al., 1997; Wingfield et al., 1997; Romero, 2002; Willmer et al., 2005; Rastogi, 2007). There are, of course, exceptions found in amphibians and reptiles (Dickhoff and Darling, 1983; Willmer et al., 2005), such as temperature-dependent sex determination (Crews et al., 1994). Due to the need to match research with conservation measures and the need for accurate data and understanding, a main current goal for ecologists is the development and application of less invasive tools for physiological studies of threatened wildlife (Bercovitz et al., 1978; Hunt et al., 2004; Mashburn and Atkinson, 2004; Millsbaugh and Washburn, 2004; Sanvito et al., 2004; Bush et al., 2005; Palme, 2005). The interpretation of the health and physiological condition of vertebrates could be seriously affected by the acute physiological stress generated by the capture, restraint and handling conditions. For example, variation in haematocrit, haemoglobin concentration, serum sex steroid concentrations and other biochemical variables (e.g. aspartate aminotransferase activity and levels of glucose, triglycerides and lactate) have been correlated with the handling/sampling time (the time elapsed between capture of the animal and blood sampling) and with cortisol and corticosterone concentrations (Jessop et al., 1999, 2002, 2004b,c; Franklin et al., 2003; Labrada-Martagón et al., 2010) derived from the hypothalamic–pituitary–adrenal axis response (Romero, 2004).

In this review, we reinforce the contribution and relevance of physiological studies as a complement to the more standard demographic data of many threatened and endangered populations of marine vertebrate species that are needed for accurate management and conservational strategies. We give particular attention to those long-lived, monomorphic marine species in which sex and maturity are impossible to assess by non-lethal, visual/external examinations. We discuss the questions that have already been addressed and the demographic knowledge derived from analytical determination of hormone concentrations in marine megafauna. We briefly review the more invasive techniques and procedures that have generated demographic data and population parameters in marine vertebrates, specifically those focused on establishing sex and reproductive status. We then summarize the information derived from each method, as well as the advantages and disadvantages of these techniques, with concluding remarks focusing on the need for wide implementation of hormone determination as a valuable, precise and non-lethal tool for those marine species for which demographic data are lacking.

The journey from lethal take to non-invasive ecological data

Steroid hormone determinations

At present, the value (informational, educational, economical and ecological) that a live free-living animal has in the wild is generally accepted (Stonehouse, 1990; Ávila and Saad, 1998; Carwardine and Watterson, 2002; Bjorndal and Jackson,
2003); however, it has not always been so. As researchers began to understand the negative effects that some techniques (e.g. branding, mutilation, banding and surgery) could have on the wellbeing, health, survival and fitness of wildlife under study (Hammond, 1990; Dugger et al., 2006; Mellish et al., 2007; Petrouskas et al., 2008), they moved towards evaluation and application of less invasive methods (Hammond, 1990; Fossi et al., 1992; Jensen et al., 2003; Hunt et al., 2004; Mashburn and Atkinson, 2004; Millsbaugh and Washburn, 2004; Sanvito et al., 2004; Bush et al., 2005; Al-Habsi et al., 2006; Sulikowski et al., 2006, 2012; Awruch et al., 2008a; Kellar et al., 2009). One of the breakthroughs occurred in the 1970s, when sea turtle researchers confirmed that serum hormone determination by radioimmunoassay provided equivalent information on the reproductive endocrinology of these organisms without the need to kill the animals, as had been done until then (Owens et al., 1978; Licht et al., 1979, 1980, 1985; Licht, 1980, 1982).

Considerable effort in the study of reproductive endocrinology (the relationship between hormone concentrations and reproductive behaviour) has been focused on adult seabirds (Groscolas et al., 1986; Hector et al., 1986a,b; Williams, 1992a; Williams and Sharp, 1993; Cherel et al., 1994; Maugat et al., 1994; Lormée et al., 2000), mature sharks (Manire et al., 1995; Manire and Rasmussen, 1997; Heupel et al., 1999) and nesting sea turtles (see reviews by Owens, 1997; Hamann et al., 2003).

There is still a lack of understanding about some aspects of endocrine dynamics and their relationship with reproductive ecology, which limits the conclusions that can be drawn about reproductive status when steroid hormone concentrations are used alone. An example is provided by deferred-breeding seabirds, in which physiologically mature individuals with functional gonads and adult hormone levels do not manifest observable breeding events (Williams, 1992b). Variations in the concentrations of gonadotrophins have been evaluated together with steroid hormones in an attempt to understand deferred breeding in penguins (Williams, 1992a, 1992b). Other physiological biomarkers, such as vitellogenin and lipoproteins, which are precursors of egg yolk, could be explored in the future to evaluate reproductive status in seabirds, as has been done in sea turtles (Herbst et al., 2003; Valverde et al., 2008) and birds (Vanderkist et al., 2000). Estrone has also been evaluated and is suggested to be the primary natural estrogen circulating in plasma of adult female sea turtles (Coufal et al. 2003; Coufal and Whittier, 2003), given the minimal or undetectable estradiol-17β (E) concentrations reported throughout the nesting period in some female green sea turtle populations (Licht et al., 1980; Wibbels et al., 1992; Al-Habsi et al., 2006).

Hormone measurements can provide indirect clues about the reproductive ecology or behavioural status of marine organisms without the necessity for killing the organisms under study and without the support of additional information that is difficult to obtain by other direct means (e.g. gonad biopsies; Bercovitz et al., 1978; Owens et al., 1978; Wibbels et al., 1987a,b; Gross et al., 1995; Schwarzenberger et al., 1996; Owens, 1997, 1999; Mansour et al., 2002; Rolland et al., 2003; Awruch et al., 2008a; Kellar et al., 2009; Xia et al., 2011). Thus, despite some limitations, steroid hormones [particularly testosterone (T), progesterone (P) and E], measured in non-lethal samples (e.g. blood, amniotic fluid, eggs, blubber, faeces and saliva), have been proposed as an alternative tool for the assessment of sex identification, the reproductive status (gonadal activity and pregnancy), stage of maturity and size at maturity of wild threatened and endangered species. However, until the analytical process is well established and the natural variations and functional differences (e.g. season and sex) of hormone concentrations are well understood for the population of interest, some validating technique (histology, laparoscopy, ultrasound or photo-identification) has to be applied to a small subset of the sampled organisms of known sex or reproductive condition (Wibbels et al., 1987a, 2000; Gross et al., 1995; Wibbels, 1999, 2003; Rolland et al., 2005; Braun-McNeill et al., 2007; Blanvillain et al., 2008; Kellar et al., 2009; Sulikowski et al., 2012). This guarantees accurate interpretation of the information derived from the first and subsequent hormone determinations.

**Sharks: puberty and sexual maturity**

Sharks are the model species in which the hormonal changes (serum T, E and P) have been evaluated during growth (differences between pre-maturity stages) and related to the process of sexual maturity (elevated E and T levels), providing information about the sex steroid changes related to puberty and sexual maturity. These findings complement the well-known relationship of the sex steroids with other reproductive conditions, such as the pre-ovulatory stage, mating process and spawning cycle (Koob et al., 1986; Rasmussen and Gruber, 1993; Manire et al., 1995; Gelsleichter et al., 2002; Sulikowski et al., 2005, 2006; Awruch et al., 2008a,b). Even so, the information about reproductive endocrinology in sharks remains incomplete (Rasmussen and Gruber, 1993; Gelsleichter et al., 2002).

The studies on skates and sharks [e.g. white shark (Carcharodon carcharias), draughtboard shark (Cephaloscyllium laticeps) and winter (Leucoraja ocellata) and thorny (Amblyraja radiate) skates] have emphasized that understanding the endocrine dynamics (plasma E, T and P levels) involved during puberty and maturation would provide the opportunity to create an accurate, validated, non-lethal tool for reproductive information, such as the determination of size at sexual maturity, in free-living organisms (Sulikowski et al., 2005, 2006, 2012; Awruch et al., 2008a,b). During growth of the individual, sharks and skates show increased levels of serum T and E that have been associated with some anatomical changes of the gonads, such as annual testicular development, increased size/weight of the testes, ovarian mass, follicle size and shell gland mass (Callard et al., 1989; Manire and Rasmussen, 1997;
Sulikowski et al., 2005, 2006). In bonnethead sharks (Sphyrna tiburo), differences in serum androgen levels are evident (P < 0.05) between stages of maturity in males (early pubertal vs. full gonadal maturity); nevertheless, quantitative correlations between the increase in hormone concentrations and gonadal maturity observed (testis size) were not evaluated (Gelsleichter et al., 2002). Likewise, T concentrations measured in plasma of draughtboard shark males, as well as the gonadosomatic index, have shown a significant increase from the juvenile to the adult stage; however, the correlation between the androgen levels and the histological index was not reported (Awruch et al., 2008b). In winter and thorny skates, in which morphological, histological and hormone concentration data were combined to establish age and size at sexual maturity, the concentration of T in plasma of males is directly correlated to clasper length (r > 0.79), the percentage of mature spermatocytes (r > 0.74) and testis mass (r > 0.79) throughout the process of sexual maturity, while female skates show a significant correlation between the E concentration and ovarian mass (r > 0.82), shell gland mass (r > 0.80) and average follicle size (r > 0.80; Sulikowski et al., 2005, 2006).

Awruch et al. (2008a) demonstrated that plasma sex steroid determination is a non-lethal technique that provides accurate information about the stage of maturity (juveniles or adults) and size at maturity of chondrichthians. They reported minor differences (≤2%) between estimates of size at maturity based on lethal (gonadal condition) vs. non-lethal approaches (models based on hormone concentrations). The concentrations of T and E, together with total length, allowed them to discriminate the state of maturity of female draught-board sharks, while T and clasper length together enable discrimination between juvenile and adult male sharks. The T and E provided 90% accuracy when used to classify the reproductive state of female sharks (juveniles and adults), and the T combined with clasper length showed an accuracy of 97% for adult males when validated by gonad examination (Awruch et al., 2008a).

Sea turtles: sex identification and reproductive status

For sea turtles, the advantage of using hormone determination, measured in non-lethal biological samples, has been sex identification during stages without evident morphological differences (Owens et al., 1978; Owens, 1997). The concentration of circulating T (immature individuals) and T measured in amniotic fluid (hatchlings) has been broadly used to determine sex in loggerhead sea turtles (Caretta caretta), green sea turtles (Chelonia mydas), Kemp’s ridley sea turtles (Lepidochelys kempii) and hawksbill sea turtles (Eretmochelys imbricata), due to the lack of morphological differences between sexes at these age stages. The T and E concentrations showed significant differences between sexes in these species, facilitating the use of the E:T ratio for sex determination of the immature sea turtles (Owens et al., 1978; Wibbels et al., 1987a,b, 2000; Gross et al., 1995; Gregory and Schmid, 2001; Braun-McNeill et al., 2007; Xia et al., 2011). The E:T ratio, measured in either plasma or amniotic fluid, provided an overall accuracy of 96% when validated by histological examinations, resulting in a more accurate sexing method than T or E concentrations when used alone (71–86.7% overall accuracy) in Caretta and Chelonia (Gross et al., 1995; Xia et al., 2011). Immature green sea turtles, however, do not always show clear differentiation patterns in T concentrations between sexes. Exceptions appear to be 4-year-olds individuals from a Cayman turtle farm (Owens et al., 1978), artificially incubated hatchlings from China (Xia et al., 2011) and free-ranging Hawaiian immature turtles (not validated by a direct approach; Wibbels et al., 1993). The overlap in ranges of circulating T between sexes means that the use of T for sex identification and sex ratio in green sea turtle juveniles is problematic; other complementary visual methods have been recommended (Bolten et al., 1992; Wibbels et al., 2000; Labrado-Martagón, 2011).

In addition to physiological cycles, reproductive endocrinology and the relationship between the hormone dynamics and reproductive and nesting behaviours in female adults (e.g. Licht et al., 1979, 1980, 1985; Owens, 1980; Licht, 1982; Owens and Morris, 1985; Wibbels et al., 1990, 1992; Williams, 1992a; Al-Habsi et al., 2006), the evaluation of T concentration in plasma has been used to determine the reproductive status (active breeders vs. inactive ones) of adult male loggerhead sea turtles in order to gain information about their reproductive biology. Differences in plasma T concentrations have been reported between males with different reproductive behaviour and testis condition, with males classified as active breeders showing higher levels than non-breeders (Jessop et al., 2004a; Blanvillain et al., 2008). The T concentration measured in plasma provided 100% accuracy in discriminating the reproductive status of adult male loggerheads when validated with laparoscopy; here, a level of T ≤5 ng ml⁻¹ is considered to indicate a reproductively inactive male (Blanvillain et al., 2008). Increased levels of circulating T have been observed before the mating season in loggerhead and green sea turtle males, suggesting testicular development and a peak in spermatogenesis (Licht et al., 1985; Wibbels et al., 1990; Blanvillain et al., 2008). The concentration of T in plasma has been directly correlated with an increase in the seminiferous tubule diameter in the loggerhead turtles (Pearson’s r = 0.65, P < 0.0005; Wibbels et al., 1990; Blanvillain et al., 2008). Seasonal changes in circulating levels of T in sea turtles, related to the testicular recrudescence and spermatogenesis in adult males (Licht et al., 1985; Wibbels et al., 1987a, 1990; Jessop et al., 2004a), could be a confounding factor during the determination of sex ratios of adults. The accuracy of T levels in plasma, when used as a sexing method in immature loggerheads, decreased by 67% from summer to winter due to the overlapping values between females and males observed during the non-breeding period (Braun-McNeill et al., 2007). For that reason, for sexual determination it is recommended to use those blood samples taken during the summer, when the elevated values of T are found in loggerheads (Braun-McNeill et al., 2007; Blanvillain et al., 2008).
Sea turtles and seabirds: resource allocation and deferred sexual maturity

The evaluation of circulating hormone concentrations has helped in the identification of the environmental (e.g. photoperiod) and energetic cues (e.g. body condition and lipid reserves) that stimulate gonadal development, migration, nesting behaviour and reproduction in seabirds (both sexes) and sea turtles (females). The E concentration is closely related to the maturation of the ovarian follicles and vitellogenesis (Groscolas et al., 1986; Hector et al., 1986b; Hamann et al., 2003). The elevated levels of plasma steroids (E and T) and triglycerides and the increase in follicular and testicular size observed before migration, courtship and the pre-laying period in sea turtles and seabirds all suggest that resource allocation (metabolic preparation), follicular development and elevated activity of the gonads occur before arrival at the breeding grounds (Groscolas et al., 1986; Hector et al., 1986b; Hamann et al., 2002a, 2002b, 2003).

In Adélie (Pygoscelis adeliae) and Emperor penguins (Aptenodytes forsteri), the increase in plasma T concentration observed in males at the time of copulation has been related to the period of testicular development and spermatogenesis (Groscolas et al., 1986). In male and female black-browed (Diomedea melanophris), grey-headed (D. coryphaenoides) and wandering albatross (D. exulans), the highest levels of sex steroids in plasma can be found during the pre-laying period. In these species of seabirds, sexual differences in the dynamics of circulating P concentration can be found during the breeding cycle (Hector et al., 1986a,b).

Williams (1992b) explained deferred sexual maturity in Macaroni (Eudyptes chrysolophus) and Gentoo penguins (Pygoscelis papua) by variation of the plasma steroids, and concluded that delays in breeding should be related to behavioural or ecological factors instead of an immature physiological state, because late breeder male and female penguins were physiologically mature at 5 years and 1 year, respectively, prior to their first breeding event. Immature wandering albatross, which also exhibit deferred sexual maturity, have shown a significant increase in testicular size and plasma T concentration with age; even when no differences in the size of the gonads were found, immature individuals that had not yet bred, older than 5 years, showed lower concentrations of T than breeders. Albatross 10 years old were the group of immature birds (not breeders) that did not show differences in the concentration of T with respect to adults (Hector et al., 1986a).

Cetaceans: maturity and reproductive condition

In marine mammals, the study of sex steroids has mainly involved cetaceans [e.g. right whales (Eubalaena glacialis), common dolphins (Delphinus delphis) and finless porpoise (Neophocaena phocaenoides)] to assess reproductive stage (juveniles), status (pregnancy and lactation), seasonality, sex and maturity (Mansour et al., 2002; Rolland et al., 2005; Daoquan et al., 2006; Greig et al., 2007; Hao et al., 2007; Kellar et al., 2009). The concentration of T in serum has been used to describe the maturity condition (immature, pubescent and fully mature) and age at maturity of a captive finless porpoise male, in which the pattern of growth in body length coincided with the pattern of increase of the concentration of serum T measured in the individual (Daoquan et al., 2006). Blubber concentrations of T have been compared between stages of maturity in common dolphins; the highest T concentrations occur in mature dolphins, compared with pubertal and immature individuals. The concentration of T measured in adipose tissue biopsies taken from free-swimming common dolphins have not shown significant differences when compared with values measured in stranded and incidentally captured individuals of known stages of maturity. This suggests the utility of blubber T concentration to assess maturity condition in this species (Kellar et al., 2009).

In the North Atlantic right whale (E. glacialis), the faecal ratio of androgen to estrogen concentration is an accurate approach for sex determination; this has been validated with photo-identified individuals (Rolland et al., 2005). Faecal hormone concentrations have been suggested as an additional tool for assessing sexual maturity and reproductive state in free-ranging right whales. The concentration of faecal T showed significant differences between males when grouped according to maturity status (immature vs. adult). Pregnant and lactating right whale females were also identifiable from other female maturity subgroups (juvenile or resting adult) according to specific faecal hormone profile characteristics (concentrations of progestins, estrogens and androgens; Rolland et al., 2005). The lack of correlation between the weight of the faecal sample taken and the hormone concentrations measured (Rolland et al., 2005) guarantees the accuracy when using this approach in cetaceans. We lack additional information on sex hormone concentrations in marine mammals because of the difficulties inherent in obtaining blood or faecal samples in free-living cetaceans (Daoquan et al., 2006; Rolland et al., 2006; Kellar et al., 2009).

Additional advantages of steroid hormone determinations

Circulating hormone determination in endangered wildlife has additional benefits. In the field, the capture and handling protocol is limited to blood sampling (Wibbels, 1999), which can be completed in <15 min (Jessop et al., 1999, 2002, 2004; Blavillain et al., 2008), thus facilitating the collection of a large number of samples with minimal stress to the individuals. Sex steroids are fairly stable metabolites, so serum samples can be stored frozen (−20°C) for long periods (Wibbels, 1999) without using a large amount of laboratory space. The analytical procedures used to determine the hormone concentrations (radioimmunoassay and enzyme-linked immunosorbent assay) are relatively inexpensive, large numbers of samples can be analysed simultaneously in a short period of time, and the analytical protocols have been extensively described for many marine species. Thus, hormone determination is a practical and inexpensive means for evaluating the demographic data of large numbers of individuals.
The type of sample should be chosen according to the species of interest, thus facilitating the field work (e.g. blubber or faecal samples in cetaceans; amniotic fluid in hatchlings). This technique could be assessed for younger and immature individuals, such as calves, chicks and hatchlings; in the case of immature sea turtles, it could be used in individuals as short as 25 cm of straight carapace length (Wibbels, 1999).

**Comparison with classical approaches for assessment of population parameters**

The most common procedures used to determine sex, sexual maturation and reproductive condition of marine megafauna have been the following: (i) direct examination of gonads and hard body parts; (ii) mark–recapture monitoring and photo-identification; and (iii) visual examination of external morphology (Table 1). The analysis of growth marks in hard body parts generates estimates of age, growth rates and some insights about the age–length and age at maturity relationships (Table 1). Such data may also be helpful in understanding the relationship between skeletal development (growth) and physiological maturity in wildlife (e.g. Campana et al., 2002; Natanson et al., 2002; Zug et al., 2002; Luque et al., 2007; Avens et al., 2009; Goshe et al., 2010). The main limitation is that these techniques are only accurate when recovered carcasses are validated with known-age data (e.g. Bowen et al., 1983; Kusher et al., 1992; Bjorndal et al., 1998; Natanson et al., 2002; Snover et al., 2011).

Mark–recapture and sequential measurements of the same individuals at time intervals (Haddon, 2001; de Mitcheson, 2009) and photo-identification, based on a photographic catalogue and recognition of variations of the dorsal fin and fluke patterns (Karczmarski and Cockcroft, 1998), are the most commonly used methods for the determination of many important life history and demographic data, in both captive and live free-ranging organisms (Table 1). Photo-identification has been used as a gold standard approach to validate the conclusions based on hormone concentrations measured in cetaceans (Rolland et al., 2005). Studying the relationship between the reproductive endocrinology and the reproductive breeding ecology has been favoured by tagging methods in seabirds such as penquins, sea turtles and marine mammals (e.g. Strasburg, 1958; Stevens, 1983; Perrin and Reilly, 1984; Hohn et al., 1985; Natanson and Cailliet, 1986; Read, 1990; Kusher et al., 1992; Gross et al., 1995; Hazin et al., 2001; Blanvillain et al., 2008; Xia et al., 2011). These findings lead to a variety of integrative and comparative reviews about the reproductive biology and endocrinology (hormone dynamics) of some species (e.g. Licht, 1979, 1982; Owens, 1980, 1997; Owens and Morris, 1985; Callard et al., 1989; Valencia and Leyton, 1992; Hamann et al., 2003). Nevertheless, some aspects of the reproductive endocrinology and ecology, such as stage duration (e.g. juveniles) and size or age at maturity, are still inconclusive for groups such as sea turtles and sharks (Rasmussen and Gruber, 1993; Musick and Limpus, 1997; Gelsleichter et al., 2002; Seminoff et al., 2002; Heppell et al., 2003).

Laparoscopic (sea turtles and avian species) and cloacoscopic examinations (penguins) are sexing techniques used in live and free-ranging immature sea turtles, chicks and adult penguins without reproductive behaviour (e.g. copulation), and have also been used to determine the reproductive condition and maturity status of sea turtles (Ainley et al., 1983; Owens, 1999; Wibbels, 1999; Jessop et al., 2004a; Blanvillain et al., 2008). Laparoscopy is considered the most accurate method of the sexing techniques owing to the capacity to visualize the gonads directly (Wibbels et al., 2000; Blanvillain et al., 2008) and, together with histological examinations, has been broadly used as the gold-standard method of validation of other, less invasive sexing approaches. Cloacoscop, on contrast, requires structures well differentiated in size for successful sexing, and the characteristics of cloaca could differ between bird species, making the applicability of this tool difficult in the field (Samour et al., 1983; Boersma and Davies, 1987; Costantini et al., 2008). Ultrasonography is a promising external, non-invasive technique used to evaluate reproductive status based on ultrasound images of the internal organs. This tool is suggested as an alternative for the determination of the reproductive condition of sea turtles and marine mammals (dolphins); however, further studies are needed to evaluate its applicability, particularly to immature...
| Species | Megafauna group | Method | Demographic data | Disadvantages |
|---------|-----------------|--------|------------------|--------------|
| Blue shark (Prionace glauca) | Pelagic sharks | Histological examination of gonads | Age at maturity | Lethal approach requires carcass recovery by fisheries nets. |
| Whitetip shark (Carcharhinus longimanus) | Pelagic sharks | Histological examination of gonads | Age at maturity | Lethal approach requires carcass recovery by fisheries nets. |
| Blacktip shark (Carcharhinus limbatus) | Pelagic sharks | Histological examination of gonads | Age at maturity | Lethal approach requires carcass recovery by fisheries nets. |
| Leopard shark (Triakis semifasciata) | Pelagic sharks | Histological examination of gonads | Age at maturity | Lethal approach requires carcass recovery by fisheries nets. |
| Porbeagle shark (Lamna nasus) | Pelagic sharks | Histological examination of gonads | Age at maturity | Lethal approach requires carcass recovery by fisheries nets. |
| Shortfin mako (Isurus paucus) | Pelagic sharks | Histological examination of gonads | Age at maturity | Lethal approach requires carcass recovery by fisheries nets. |
| Short-finned pilot whale (Globicephala macrorhynchus) | Cetaceans | Skeletochronology | Age and growth | Requires previous clinical and physical examination of the health state of the individuals. |
| Bottlenose dolphin (Tursiops truncatus) | Cetaceans | Skeletochronology | Age and growth | Requires previous clinical and physical examination of the health state of the individuals. |
| Green sea turtle (Chelonia mydas) | Sea turtles | Reproductive biology/anatomy | Reproductive condition | Requires carcass recovery or bone biopsies. |
| Loggerhead sea turtle (Caretta caretta) | Sea turtles | Reproductive biology/anatomy | Reproductive condition | Requires carcass recovery or bone biopsies. |
| Olive Ridley sea turtle (Lepidochelys olivacea) | Sea turtles | Reproductive biology/anatomy | Reproductive condition | Requires carcass recovery or bone biopsies. |
| Spotted dolphin (Stenella attenuata) | Cetaceans | Reproductive biology/anatomy | Reproductive condition | Requires carcass recovery or bone biopsies. |
| Harbour porpoise (Phocoena phocoena) | Cetaceans | Reproductive biology/anatomy | Reproductive condition | Requires carcass recovery or bone biopsies. |
| Pygoscelids penguins | Earless seals | Reproductive biology/anatomy | Reproductive condition | Requires carcass recovery or bone biopsies. |
| Harp seal (Phoca greonlandica) | Earless seals | Reproductive biology/anatomy | Reproductive condition | Requires carcass recovery or bone biopsies. |
| Beluga (Delphinapterus leucas) | Earless seals | Reproductive biology/anatomy | Reproductive condition | Requires carcass recovery or bone biopsies. |

Table 1: Classical, direct approaches for population parameter assessment employed for marine megafauna.
| Method                        | Megafauna group | Species examples                                                                 | Demographic data                                                                 | Disadvantages                                                                                          | References                                                                                   |
|------------------------------|-----------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Cloacascopy                  | Penguins        | Adélie penguin (*Pygoscelis adeliae*), Chinstrap penguin (*Pygoscelis antarctica*), Humboldt penguin (*Spheniscus humboldti*) | Sex determination                                                                | Requires structures well differentiated in size, Not applicable to chicks, Applicable only in individuals older than 11 months, Accuracy is compromised towards the end of the breeding season, Specific variations in cloacae characteristics are difficult to assess in the field, Applicability is dependent on field conditions, Requires specialized equipment and trained experts | Ainley *et al.* (1983); Samour *et al.* (1983); Boersma and Davies (1987); Kerry *et al.* (1992); Zavalaga and Paredes (1997); Costantini *et al.* (2008) |
| Ultrasonography              | Sea turtles     | Kemp's Ridley sea turtle (*Lepidochelys kempi*), Loggerhead sea turtle (*Caretta caretta*), Bottlenose dolphin (*Tursiops truncatus*) | Reproductive condition                                                             | External, non-invasive, promising tool, Requires further validation studies, Requires complete gonadal matura- tion (follicle size), Not useful for sex determination in immature individuals (sea turtles), Requires specialized equipment, Requires anatomical knowledge for accurate interpretation, Time consuming per individual examination | Rostal *et al.* (1990, 1997); Brook *et al.* (2000); Brook (2001); Valente *et al.* (2007); Blanvillain *et al.* (2008) |
| Mark-recapture monitoring:   | Penguins        | Adélie penguin (*P. adeliae*), Emperor penguin (*Aptenodytes forsteri*), Magellanic penguin (*Spheniscus magellanicus*), Blue penguin (*Eudyptula minor*), Yellow-eyed penguin (*Megadyptes antipodes*), Humboldt penguin (*S. humboldti*), African penguin (*S. demersus*), Rockhopper penguin (*Eudyptes chrysolophus*), Green sea turtle (*Chelonia mydas*), Logge's head sea turtle (*C. caretta*), Hawksbill turtle (*Eretmochelys imbricata*), Bottlenose dolphin (*T. truncatus*), Spotted dolphin (*Stenella frontalis*), Pilot whale (*Globicephala melas*) | Reproductive/breeding cycles                                                           | Long-term monitoring series (years), High economic cost, Tagging, Absence of suitable tags for specific age classes, Tagging is dependent on field conditions, Accessibility to habitats (feeding grounds), Tagging confined to specific: Age classes, Sex, Life stages/size distribution, Location of recaptures, Tag loss, Photo-identification: Changes in marks over time | Ainley and DeMaster (1980); Ainley *et al.* (1983); Bjorndal *et al.* (1983); Carr (1984); Groscolas *et al.* (1986); Boersma *et al.* (1990); Dann and Cullen (1990); Darby and Seddon (1990); Hammond (1990); Clapham (1992); Limpus (1992); Green (1993); Balazs (1995, 1999); Crouse and Frazer (1995); Meylan (1995); Chaloupka and Musick (1997); Herzing (1997); Zavalaga and Paredes (1997); Karczmarski and Cockcroft (1998); Crawford *et al.* (1999); Bjorndal *et al.* (2000); Seminoff *et al.* (2002); Grellier *et al.* (2003); |
| Discriminant functions based on biometric data (seabirds): Bill, foot, wing and vent measurements | Visual examinations of phenotypical characteristics: Clasper morphology | Mean nesting size | Presence of secondary sexual characteristics | Plastron softness |
|---|---|---|---|---|
| Sharks | Seabirds | Sea Turtles | |
| Lemon shark *(Negaprion brevirostris)* | Bonnethead sharks *(Sphyrna tiburo)* | Whale shark *(Rhincodon typus)* | |
| Sandbar shark *(Carcharhinus plumbeus)* | Bonnethead sharks *(Sphyrna tiburo)* | Royal penguin *(Eudyptes schlegeli)* | |
| Spinner shark *(C. brevipinna)* | Winter skate *(Leucoraja ocellata)* | Rockhopper penguin *(Eudyptes chrysocephale)* | |
| Thorny skate *(Amblyraja radiata)* | Porbeagle shark *(L. nasus)* | Magellanic penguin *(S. magellanicus)* | |
| Shortfin mako *(I. oxyrinchus)* | Blueprint shark *(Cephaloscyllium laticeps)* | Blue penguin *(E. magellanicus)* | |
|draughtboard shark *(Cephaloscyllium chrysocome)* | Whale shark *(Rhincodon typus)* | Storm petrel *(Oceanodroma furcata)* | |
| Winter skate *(Leucoraja ocellata)* | Royal penguin *(Eudyptes schlegeli)* | Southern giant petrel *(Macronectes giganteus)* | |
| Great black-backed gull *(Larus marinus)* | Bonnethead sharks *(Sphyrna tiburo)* | Grey pelican *(Pelecanus occidentalis)* | |
| American coot *(Fulica americana)* | Winter skate *(Leucoraja ocellata)* | Great white pelican *(Pelecanus onocrotalus)* | |
| Green sea turtle *(Chelonia mydas)* | Greasy skate *(Raja clavata)* | Green turtle *(Chelonia mydas)* | |
| Loggerhead sea turtle *(Caretta caretta)* | Greasy skate *(Raja clavata)* | Loggerhead turtle *(Caretta caretta)* | |
or non-reproductive sea turtles (Rostal et al., 1990; Brook et al., 2000; Brook, 2001; Blanvillain et al., 2008). This technique has not been useful to identify the sex of immature loggerhead sea turtles, owing to the small size of the undeveloped gonads (Valente et al., 2007). As a result of the similarity between the general appearance of the reproductive structures described by ultrasound in active male dolphins and sea turtles (for which comparative data exist) and some correlations found between the measurements taken of the reproductive organs of adult male loggerhead sea turtles with laparoscopy and ultrasonography, such as the epididymal duct diameter (Pearson’s r = 0.91, P < 0.001), it is possible that ultrasonography may ultimately replace the laparoscopic procedure (Blanvillain et al., 2008).

Biometric data (e.g. bill, foot, wing and vent measurements) and comparison of phenotypic characteristics (e.g. clasper morphology, tail size, mean nesting size and plastron softness) are non-invasive, discriminant methods to assess demographic parameters, such as reproductive stage and age/sex determination (Table 1). In sharks, external secondary sexual characteristics of clasper (length, rotation and calcified condition), visual signs of mating and courtship (scars, sperm in clasper and haematoma around the cloaca) and length, when the size at maturity is known for the species, have been used, together with other histological and endocrine approaches, as a sexual discriminant criterion and to assess the maturity of males (e.g. Rasmussen and Gruber, 1993; Joung and Chen, 1995; Gelsleichter et al., 2002; Francis and Duffy, 2005; Joung et al., 2005; Sulikowski et al., 2005, 2006; Norman and Stevens, 2007; Awruch et al., 2008a). In the threatened whale shark (Rhincodon typus), the sex of 85% of the individuals observed off the coast of Australia (n = 325) was identified, while diving with the animal, by clasper presence, and sexual maturity was assessed in the total of males found (100%), confirming that clasper condition criterion is a non-invasive approach to assess demographic parameters for this species (Norman and Stevens, 2007). Nevertheless, an external visual criterion to assess female sexual maturity does not exist in sharks (Francis and Duffy, 2005; Norman and Stevens, 2007; Awruch et al., 2008a). Softness of the plastron has been suggested as a visual criterion to identify active male breeders from immature sea turtles; nevertheless, the T concentration measured in plasma has not shown a significant correlation with the percentage relative plastron softened area, and additional studies are needed (Blanvillain et al., 2008). The mean nesting size and the tail size are parameters widely used to define the adult stage, for both female and male sea turtles (Musick and Limpus, 1997; Seminoff et al., 2002, 2003; Casale et al., 2005; Koch et al., 2007; Lopez-Castro et al., 2010); there is no external visual criterion for sexing juvenile stages. However, size and the absence of secondary sexual characteristics (e.g. nails and tail development in male sea turtles) are not good indicators of the sex and reproductive stage when used alone as classification criteria in feeding grounds, where large immature and male adult sea turtles are found mixed together (Caldwell, 1962; Owens et al., 1978). Growth models suggest that sea turtles reach sexual maturity at a size larger than the estimated mean nesting size (Seminoff et al., 2002). Even when sea turtle species exhibit a common delayed maturity (Heppell et al., 2003), the size of these organisms is not a reliable indicator of maturity, because populations of the same species (e.g. green turtle; Zug et al., 1986; Green, 1993; Balazs, 1995; Seminoff et al., 2002) do not start to breed at the same age or minimal size (Zug et al., 1986; Miller, 1997). Laparoscopic and steroid-based studies suggest that the mean nesting size could mislead estimates of population structure, with potential underestimation of the number of males (Limpus et al., 1994; Miller, 1997; Labrada-Martagon et al., 2013).

Despite the great sex classification power of the discriminant analysis obtained from biometric data on seabirds (84–100%; Boersma and Davies, 1987; Kerry et al., 1992; Hull, 1996; Mawhinney and Diamond, 1999; Hocken and Russell, 2002; Copello et al., 2006), a variety of canonical discriminant functions (biometric classification parameters) has been suggested, between reproductive stages (e.g. flipper length in immature Magellanic penguins (Spheniscus magellanicus) vs. bill length in adults; Bertellotti et al., 2002) and between seabird species, making this approach applicable for all age classes (immature), time of birth (chicks) and conditions (wild vs. captive) difficult without previous validation (Table 1). However, this has to be interpreted with caution when used with data from other geographical populations (Hull, 1996; Zavalaga and Paredes, 1997; Bertellotti et al., 2002; Setiawan et al., 2004; Copello et al., 2006; Poisbleau et al., 2010). Currently, molecular techniques using non-invasive (eggs and feathers) or non-lethal samples (blood) give the most reliable and accurate method for sexing avian species (Bertellotti et al., 2002; Bush et al., 2005; Cerit and Avanus, 2007; for additional reviews see Seutin et al., 1991; Jensen et al., 2003; Bush et al., 2005; Dubiec and Zagalaska-Neubauer, 2006).

Conclusions

The limitations in pooled analyses include heterogeneity between studies and the different methods of recruiting the controls used (Raimondi et al., 2006). Thus, it is difficult to compare the accuracy and classification power of different approaches used to obtain life history data in wildlife (e.g. biometric data and hormone concentrations), owing to the heterogeneity of the information reported between studies (e.g. means, ranges, percentage of accuracy and correlation coefficients) and the classification criteria used (e.g. sex, age class and reproductive stages), in addition to the specific differences in this review (e.g. sharks, seabirds and sea turtles). Another limitation during the sensitivity analysis, performed on those published reports focusing on validation of the use of hormone concentrations, was the variety of samples (serum, plasma, amniotic fluid and faeces) and validation approaches or controls (e.g. histology, laparoscopy, cloacascopy and photo-identification) used between studies.
Histology (sharks, sea turtles and marine mammals), laparoscopy (sea turtles), laparotomy and cloacascopy (seabirds) are the most accurate methods for determining the sex and reproductive condition of the animals, and they have been considered as the reference method (gold standard) to evaluate less-invasive approaches, such as the hormone concentrations. The main disadvantages of the classical direct approaches are logistical complications (e.g. cost, specialized equipment required and dependence on field conditions) and their invasive or lethal characteristics when applied to threatened or endangered species. Photo-identification (marine mammals) and biometric (seabirds), phenotypic (sharks) and behavioural data (seabirds) are non-invasive, external validation methods of sex and reproductive stages, useful only when the information is available (e.g. known size at maturity and clasper condition); they require monitoring studies of the population through the breeding season (e.g. penguins and albatrosses) and expensive, long time series (e.g. 25 years of life history data for cetaceans). Using phenotypic characteristics of sea turtles is difficult for immature individuals and when adults are together with sub-adults. The large discriminant accuracy of biometric data when used for sexing seabirds is limited by considerable intra-specific (e.g. age class, reproductive condition and geographical population) and inter-specific variation.

The measurement of steroid hormone concentrations has shown great accuracy as a discriminant (71–100% accuracy) of sex and reproductive status (when data could be compared), with consistent accuracy between samples (e.g. amniotic fluid and plasma) and studies (Gross et al., 1995; Xia et al., 2011), thus demonstrating the reproducibility and diagnostic validity of this method. The advantages of hormone determination as a non-lethal and less-invasive tool for the generation of valuable demographic information needed for stock assessment and biological conservation include low cost, the facility for sample preservation, and little time and effort employed in both field and laboratory conditions. Validation of such data is needed in specific populations in order to guarantee the accurate interpretation of subsequent hormone determinations (Wibbels, 1999). There are many descriptive studies on the reproductive endocrinology (the relationship between hormone concentrations and reproductive behaviour) of marine species. A large number of associations have already been demonstrated between sex steroid concentrations and biochemical parameters, gonadal state, reproductive behaviour and secondary external features (e.g. clasper in sharks). However, there is a lack of information about direct quantitative correlations between the gonadal changes observed using lethal methods and the hormone concentrations measured in the individuals (e.g. Wibbels et al., 1990; Sulikowski et al., 2005, 2006; Blanvillain et al., 2008), even when the information was available, and there are even fewer studies that have proposed some quantitative approach to discriminate demographic parameters based on hormone concentrations (Awruch et al., 2008a).

In addition to the use of hormone concentrations as a sexing method, the most recent studies on sharks (Sulikowski et al., 2005, 2006, 2012; Awruch et al., 2008a) have suggested that measurements of circulating sex steroids can be used as an indirect approximation to assess sexual maturity and reproductive condition in those species without evident secondary sexual characteristics and in the absence of morphological data (e.g. gonadal condition). Clearly, the relationship between plasma hormone changes during puberty and the sexual maturation process could be studied in other marine vertebrate groups, by comparing sex steroid concentrations by age/size classes at pre-mature stages and between groups with or without evident secondary sexual characteristics. This would allow validation of the size classes and pre-mature stages and/or conditions at which hormone determination provides accurate demographic information. The link between deferred sexual maturity and circulating steroid concentrations needs further clarification.

Understanding the dynamics of the hormone concentrations during puberty and sexual maturity allows for identification of sex, stage duration, size range at maturation and reproductive condition, expanding the information derived from external characteristics and morphological measurements when used alone. For example, in skates the simultaneous evaluation of multiple techniques has demonstrated that hormone determinations provide more precise estimates of sexual maturity stage (expressing a lag period between gonadal development and functional maturity) than the morphological measurements alone (Sulikowski et al., 2006). In the East Pacific population of green sea turtles, hormone determination for immature turtles suggests a bias in the estimation of the population structure when considering the mean nesting size as a unique classification criterion (overestimation of adult females due to the absent secondary sexual characteristics expected in males; Labrada-Martagón et al., 2013).

We lack information about demographic data in many marine species and populations that are the focus of conservation interest. The current information available about reproductive endocrinology, stage duration (growth) and age/size at sexual maturity is centred on a few populations (e.g. green sea turtles from Hawaii, Florida and Australia), habitats (nesting grounds) and reproductive stages (adults) in some species. Consequently, key demographic parameters used to establish policies and conservation strategies remain unknown. The information derived from sex steroid concentrations in different stages and size classes (Rasmussen and Gruber, 1993; Awruch et al., 2008a; Sulikowski et al., 2012), together with other phenotypic (e.g. total length and clasper length; Awruch et al., 2008a) and biochemical parameters that have explained the steroid concentrations measured in immature sea turtles (e.g. glucose, cholesterol and thyroxine levels; Labrada-Martagón et al., 2013) and energy allocation patterns in adult females (triglycerides; Hamann et al., 2002a,b) form the basis for future determinations on demographic data (e.g. sex and reproductive stage), population structure and seasonal variations in the development and reproductive condition of the populations as well.
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References

Ainley DG, DeMaster DP (1980) Survival and mortality in a population of Adélie penguins. Ecology 61: 522–530.

Ainley DG, LeResche RE, Sladen WJL (1983) Breeding Biology of the Adélie Penguin. University of California Press, Berkeley, 240 pp.

Al-Habsi AA, Alkindi AYA, Mahmoud IY, Owens DWM, Khan T, Al-Abri A (2006) Plasma hormone levels in the green turtle Chelonia mydas during peak period of nesting at Ras Al-Hadd-Oman. J Endocrinol 191: 9–14.

Avens L, Taylor JC, Goshe LR, Jones TT, Hasting M (2009) Use of skeleto-chronological analysis to estimate the age of leatherback sea turtles Dermochelys coriacea in the western North Atlantic. Endang Species Res 8: 165–177.

Ávila S, Saad L (1998) Valuación de la ballena gris (Eschrichtius robustus) y la ballena jorobada (Megaptera novaeangliae) en México. In DH Benitez, E Vega, A Peña, S Ávila, eds, Aspectos Económicos sobre la Biodiversidad de México. CONABIO-IN, Ciudad de México, pp 123–143.

Awruch CA, Frusher SD, Pankhurst NW, Stevens JD (2008a) Non-lethal assessment of reproductive characteristics for management and conservation of sharks. Mar Ecol Prog Ser 355: 277–285.

Awruch CA, Pankhurst NW, Frusher SD, Stevens JD (2008b) Endocrine and morphological correlates of reproduction in the draughtboard shark Cephaloscyllium laticeps (Elasmobranchii: Scyliorhinidae). J Exp Zool A Ecol Genet Physiol 309: 184–197.

Balazs GH (1995) Growth rates of immature green turtles in the Hawaiian Archipelago. In KA Bjorndal, ed, Biology and Conservation of Sea Turtles. Smithsonian Institution Press, Washington, DC, pp 117–125.

Balazs GH (1999) Factors to consider in the tagging of sea turtles. In KL Eckert, KA Bjorndal, FA Abreu-Grobois, M Donnelly, eds, Research and Management Techniques for the Conservation of Sea Turtles, IUCN/SSC Marine Turtle Specialist Group, No. 4, Pennsylvania, pp 101–109.

Balazs GH, Chaloupka M (2004) Spatial and temporal variability in somatic growth of green sea turtles (Chelonia mydas) resident in the Hawaiian Archipelago. Mar Biol 145: 1043–1059.

Balazs GH, Chaloupka M (2006) Recovery trend over 32 years at the Hawaiian green turtle rookery of French Frigate Shools. Atoll Res Bull 543: 147–158.

Bercovitz AB, Czekala NM, Lasley BL (1978) A new method of sex determination in monomorphic birds. J Zoo Anim Med 9: 114–124.

Bertellotti M, Tella JL, Godoy JA, Blanco G, Forero MG, Donázar JA, Ceballos O (2002) Determining sex of Magellanic Penguins using molecular procedures and discriminant functions. Waterbirds 25: 479–484.

Bjorndal KA, Jackson JBC (2003) Roles of sea turtles in marine ecosystems: reconstructing the past. In PL Lutz, JA Musick, J Wynen, eds, The Biology of Sea Turtles, Vol II. CRC Press, Boca Raton, pp 259–270.

Bjorndal KA, Meylan AB, Turner BJ (1983) Sea turtles nesting at Melbourne Beach, Florida, I. Size, growth and reproductive biology. Biol Conserv 26: 65–77.

Bjorndal KA, Bolten AB, Bennett RA, Jacobson ER, Wronskey TJ, Valeski JJ, Eliazer PJ (1998) Age and growth in sea turtles: limitations of skeleto-chronology for demographic studies. Copeia 1998: 23–30.

Bjorndal KA, Bolten AB, Chaloupka M (2000) Green turtle somatic growth model: evidence for density dependence. Ecol Appl 10: 269–282.

Blanvillain G, Pease AP, Segars AJL, Rostal DC, Richards AJ, Owens DWM (2008) Comparing methods for the assessment of reproductive activity in adult male loggerhead sea turtles Caretta caretta at Cape Canaveral, Florida. Endang Species Res 6: 75–85.

Boersma PD, Davies EM (1987) Sexing monomorphic birds by vent measurements. Auk 104: 779–783.

Boersma PD, Stokes DL, Yorio PM (1990) Reproductive variability and historical change of Magellanic penguins (Spheniscus magellanicus) at Punta Tombo, Argentina. In LS Davis, JT Darby, eds, Penguin Biology. Academic Press, Inc., San Diego, pp 15–43.

Bolten AB, Bjorndal KA, Grumbles JS, Owens DWM (1992) Sex ratio and sex specific growth rates of immature green turtles, Chelonia mydas, in the southern Bahamas. Copeia 1992: 1098–1103.

Bowen WD, Sergeant DE, Ørtsland T (1983) Validation of age estimation in the harp seal, Phoca groenlandica, using dentinal annuli. Can J Fish Aquat Sci 40: 1430–1441.

Branstetter S (1987) Age and growth estimates for blacktip, Carcharhinus limbatus, and spinner, C. brevipinna, sharks from the northwestern Gulf of Mexico. Copeia 1987: 964–974.

Braun-McNeill J, Epperly SP, Owens DWM, Avens L, Williams E, Harms C (2007) Seasonal reliability of testosterone radioimmunoassay (RIA) for predicting sex ratios of juvenile Loggerhead (Caretta caretta) turtles. Herpetologica 63: 275–284.

Brook FM (2001) Ultrasonographic imaging of the reproductive organs of the female bottlenose dolphin, Tursiops truncatus aduncas. Reproduction 121: 419–428.

Brook FM, Kinoshita R, Brown B, Metreweli C (2000) Ultrasonographic imaging of the testis and epididymis of the bottlenose dolphin, Tursiops truncatus aduncas. J Reprod Fertil 119: 233–240.
Bush KL, Vinsky MD, Aldridge CL, Paszkowski CA (2005) A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater Sage-Grouse (Centrocercus urophasianus). Conserv Genet 6: 867–870.

Caldwell DK (1962) Carapace length—body weight relationship and size and sex ratio of the northeastern Pacific green sea turtle, Chelonia mydas carrinegra. Contributions In Science Los Angeles County Museum 62: 3–10.

Callard IP, Klosterman LL, Sorbera LA, Fileti LA, Reese J (1989) Endocrine regulation of reproduction in elasmobranchs: archetype for terrestrial vertebrates. J Exp Zool 252(Suppl 52): 12–22.

Campana SE, Natanson LJ, Mykleboll S (2002) Bomb dating and age determination of large pelagic sharks. Can J Fish Aquat Sci 59: 450–455.

Carr A (1984) The Sea Turtle. So Excellent a Fishe, Ed. 2. University of Texas Press, Austin, 280 pp.

Carwardine M, Watterson K (2002) The Shark Watcher's Handbook. A Guide to Sharks and where to see them. Princeton University Press, Princeton, 287 pp.

Casale P, Freggi D, Basso R, Argano R (2005) Size at male maturity, sexing methods and adult sex ratio in Loggerhead turtles (Caretta caretta) from Italian waters investigated through tail measurements. Herpetolog J 15: 145–148.

Casale P, Mazaris AD, Freggi D (2011) Estimation of age at maturity of loggerhead sea turtles caretta caretta in the Mediterranean using length-frequency data. Endang Species Res 13: 123–129.

Cerit H, Avanus K (2007) Sex identification in avian species using DNA typing methods. Worlds Poult Sci / 63: 91–100.

Chaloupka M, Limpus C, Miller J (2004) Green turtle somatic growth dynamics in a spatially disjunct Great Barrier Reef metapopulation. Coral Reefs 23: 325–335.

Chaloupka MY, Musick JA (1997) Age, growth, and population dynamics. In PL Lutz, JA Musick, eds, The Biology of Sea Turtles. CRC Press, Boca Raton, pp 233–276.

Cherel Y, Maquet R, Lacroix A, Gilles J (1994) Seasonal and fasting-related changes in circulating gonadal steroids and prolactin in king penguins, Aptenodytes patagonica. Physiol Zool 67: 1154–1173.

Clapham PJ (1992) Age at attainment of sexual maturity in humpback whales, Megaptera novaeangliae. Can J Zool 70: 1470–1472.

Cook JG (1995) The International Whaling Commission's revised management procedure as an example of a new approach to fishery management. In AS Blix, L Walløe, Ø Ulltang, eds, Developments in Marine Biology, Vol 4, Elsevier Science, Tromsø, pp 647–657.

Copello S, Quintana F, Somoz G (2006) Sex determination and sexual size-dimorphism in Southern Giant-Petrels (Macronectes giganteus) from Patagonia, Argentina. Emu 106: 141–146.

Costantini V, Guaricci AC, Laricchiuta P, Rausa F, Lacalandra GM (2008) DNA sexing in Humboldt Penguins (Spheniscus humboldti) from feather samples. Anim Reprod Sci 106: 162–167.

Coufal KA, Whittier JM (2003) Identification of estrone as the major circulating estrogenic steroid in marine turtle plasma. In JA Seminoff, ed, Proceedings of the 22nd Annual Symposium on Sea Turtle Biology and Conservation, NOAA Technical Memorandum NMFS-SEFSC-503, Miami, pp 200–201.

Coufal KA, Whittier JM, Limpus CJ (2003) Quantification of estrone in plasma of female Chelonia mydas, Caretta caretta, Eretmochelys imbricata, and Natator depressus populations in Eastern Australia. In JA Seminoff, ed, Proceedings of the 22nd Annual Symposium on Sea Turtle Biology and Conservation, NOAA Technical Memorandum NMFS-SEFSC-503, Miami, p 186.

Crawford RJ, Shannon LJ, Whittington PA (1999) Population dynamics of the African Penguin Spheniscus demersus at Robben Island, South Africa. Mar Ornithol 27: 139–147.

Crewe D, Bergeron JM, Bull JJ, Flores D, Tousignant A, Skipper JK, Wibbels T (1994) Temperature-dependent sex determination in reptiles: proximate mechanisms, ultimate outcomes, and practical applications. Dev Genet 15: 297–312.

Crouse DT, Frazer NB (1995) Population models and structure. In KA Bjornsdal, ed, Biology and Conservation of Sea Turtles. Smithsonian Institution Press, Washington, DC, pp 601–603.

Dann P, Cullen JM (1990) Survival, patterns of reproduction, and lifetime reproductive output in Little blue penguins (Eudyptula minor) on Phillip Island, Victoria, Australia. In LS Davis, JT Darby, eds, Penguin Biology. Academic Press, Inc., San Diego, pp 63–84.

Daoquan C, Hao YJ, Zhao QZ, Wang D (2006) Reproductive seasonality and maturity of male Neochorana phocaenoides asiaseorientalis in captivity: a case study based on the hormone evidence. Mar Freshw Behav Physiol 39: 163–173.

Darby JT, Seddon PJ (1990) Breeding biology of Yellow-eyed penguins (Megadyptes antipodes). In LS Davis, JT Darby, eds, Penguin Biology. Academic Press, Inc., San Diego, pp 45–62.

DeMaster DP (1984) Review of techniques used to estimate the average age at attainment of sexual maturity in marine mammals. Reports of the International Whaling Commission Special 6: 175–179.

de Mitcheson YS (2009) Biology and ecology considerations for the fishery manager. In KL Cochrane, SM Garcia, eds, A Fishery Manager’s Guidebook. FAO, Iowa, pp 21–51.

Dickhoff WW, Darling DS (1983) Evolution of thyroid function and its control in lower vertebrates. Am Zool 23: 697–707.

Dubiec A, Zagalska-Neubauer M (2006) Molecular techniques for sex identification in birds. Biol Lett 43: 3–12.

Dugger KM, Ballard D, Ainley DG, Barton KJ (2006) Effects of flipper bands on foraging behavior and survival of Adélie penguins (Pygoscelis adeliae). Auk 123: 858–869.

Eberhardt LL (1985) Assessing the dynamics of wild populations. J Wildl Manag 9: 997–1012.

Fossi CM, Marsill L, Leoncio C, Notarbartolo Di Sciara G, Zanardelli M, Focardi S (1992) The use of non-destructive biomarker in
Mediterranean cetaceans: preliminary data on MFO activity in skin biopsy. *Mar Pollut Bull* 24: 459–461.

Francis MP, Duffy C (2005) Length at maturity in three pelagic sharks (*Lamna nasus, Isurus oxyrinchus, and Prionace glauca*) from New Zealand. *Fish Bull* 103: 489–500.

Franklin CE, Davis BM, Peuccker SKJ, Stephenson H, Mayer R, Whittier JM, Lever J, Grigg GC (2003) Comparison of stress induced by manual restraint and immobilisation in the estuarine crocodile *Crocodylus porosus*. *J Exp Zool* 298A: 86–92.

Gelsleichter J, Rasmussen LEL, Manire CA, Tyminsik J, Chang B, Lombardi-Carlson L (2002) Serum steroid concentrations and development of reproductive organs during puberty in male bottlenose sharks, *Sphyrna tiburo*. *Fish Physiol Biochem* 26: 389–401.

Gibbons JW (1987) Why do turtles live so long? *BioScience* 37: 262–269.

Goshe LR, Avens L, Scharf FS, Southwood AL (2010) Estimation of age at maturation and growth of Atlantic green turtles (*Chelonia mydas*) using skeletochronology. *Mar Biol* 157: 1725–1740.

Green D (1993) Growth rates of wild immature green turtles in the Galapagos Islands, Ecuador. *J Herpetol* 27: 338–341.

Gregory LF, Schmid JR (2001) Stress responses and sexing of wild Kemp’s Ridley sea turtles (*Lepidochelys kepii*) in the Northeastern Gulf of Mexico. *Gen Comp Endocrinol* 124: 66–74.

Greig DJ, Mashburn KL, Rutishauser M, Gulland FM, Williams TM, Atkinson S (2007) Seasonal changes in circulating progesterone and estrogen concentrations in the California sea lion (*Zalophus californianus*). *J Mammal* 88: 67–72.

Grellier K, Hammond PS, Wilson B, Sanders-Reed CA, Thompson PM (2003) Use of photo-identification data to quantify mother–calf association patterns in bottlenose dolphins. *Can J Zool* 81: 1421–1427.

Groscolas R, Jallageas M, Goldsmith A, Assenmacher I (1986) The endocrine control of reproduction and molt in male and female emperor (*Aptenodytes forsteri*) and adelie (*Pygoscelis adeliae*) penguins: I. Annual changes in plasma levels of gonadal steroids and LH. *Gen Comp Endocrinol* 62: 43–53.

Gross TS, Crain DA, Bjorndal KA, Bolten AB, Carthy RR (1995) Identification of sex in hatching loggerhead turtle (*Caretta caretta*) by analysis of steroid concentrations in chorioallantoic/amniotic fluid. *Gen Comp Endocrinol* 99: 204–210.

Guillette LJ, Gunderson MP (2001) Alterations in development of reproductive and endocrine systems in wildlife populations exposed to endocrine-disrupting contaminants. *Reproduction* 122: 857–864.

Guillette LJ, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR (1994) Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ Health Perspect* 102: 680–688.

Guillette LJ, Brock JW, Rooney AA, Woodward AR (1999) Serum concentrations of various environmental contaminants and their relationship to sex steroid concentrations and phallic size in juvenile American alligators. *Arch Environ Contam Toxicol* 36: 447–455.

Haddad M (2001) Modelling and Quantitative Methods in Fisheries. Chapman and Hall/CRC, Boca Raton, 406 pp.

Haii AJ, Kalantzi Ol, Thomas GO (2003) Polybrominated diphenyl ethers (PBDEs) in grey seals during their first year of life—are they thyroid hormone endocrine disrupters? *Environ Pollut* 126: 29–37.

Hamann M, Jessop TS, Limpus CJ, Whittier JM (2002a) Interactions among endocrinology, seasonal reproductive cycles and the nesting biology of the female green sea turtle. *Mar Biol* 140: 823–830.

Hamann M, Limpus CJ, Whittier JM (2002b) Patterns of lipid storage and mobilisation in the female green sea turtle (*Chelonia mydas*). *J Comp Physiol B* 172: 485–493.

Hamann M, Limpus CJ, Owens DWM (2003) Reproductive cycles of males and females. In PL Lutz, JA Musick, J Wynenek, eds, The Biology of Sea Turtles, Vol II. CRC Press, Boca Raton, pp 135–161.

Hammond PS (1990) Capturing whales on film—estimating cetacean population parameters from individual recognition data. *Mamm Rev* 20: 17–22.

Hao YJ, Chen DQ, Zhao QZ, Wang D (2007) Serum concentrations of gonadotropins and steroid hormones of *Neophocaena phocaenoides asioorientalis* in middle and lower regions of the Yangtze River. *Theriogenology* 67: 673–680.

Hazin F, Fischer A, Broadhurst M (2001) Aspects of reproductive biology of the scalloped hammerhead shark, *Sphyrna lewini*, off northeastern Brazil. *Environ Biol Fish* 61: 151–159.

Hector JAL, Croxall JP, Follett BK (1986a) Reproductive endocrinology of the wandering albatross *Diomedea exulans* in relation to biennial breeding and deferred sexual maturity. *Ibis* 128: 9–22.

Hector JAL, Follett BK, Prince PA (1986b) Reproductive endocrinology of the Black-browed albatross *Diomedea melanophris* and the Grey-headed albatross *D. chrysostoma*. *J Zool* 208: 237–253.

Heppell SS, Snover ML, Crowder LB (2003) Sea turtle population ecology. In PL Lutz, JA Musick, J Wynenek, eds, The Biology of Sea Turtles, Vol II. CRC Press, Boca Raton, pp 275–306.

Herbst LH, Siconolfi-Baez LL, Torelli JH, Klein PA, Kerben MJ, Schumacher IM (2003) Induction of vitellogenesis by estradiol-17 and development of enzyme-linked immunosorbant assays to quantify plasma vitellogenin levels in green turtles (*Chelonia mydas*). *Comp Biochem Physiol B* 135: 551–563.

Herzing DL (1997) The life history of free-ranging Atlantic spotted dolphins (*Stenella frontalis*): age classes, color phases, and female reproduction. *Mar Mamm Sci* 13: 576–595.

Heupel MR, Whittier JM, Bennett MB (1999) Plasma steroid hormone profiles and reproductive biology of the epaulette shark, *Hemiscyllium ocellatum*. *J Exp Zool* 284: 586–594.

Hickerson E (2000) Assessing and tracking resident, immature loggerheads (*Caretta caretta*) in and around the Flower Garden Banks, northwest Gulf of Mexico. MSc thesis, Texas A&M University, College Station, TX.
Hocken AG, Russell JJ (2002) A method for determination of gender from bill measurements in Otago blue penguins (Eudyptula minor). N Z J Zool 29: 63–69.

Hohn AA, Chivers S, Barlow J (1985) Reproductive maturity and seasonality of male spotted dolphins, Stenella attenuata, in the eastern tropical Pacific. Mar Mamm Sci 1: 273–293.

Hull CL (1996) Morphometric indices for sexing adult Royal Eudyptes schlegeli and Rockhopper E. chrysocome Penguins at Macquarie Island. Mar Ornithol 24: 23–27.

Hunt KE, Trites AW, Wasser SK (2004) Validation of a fecal glucocorticoids assay for Steller sea lions (Eumetopias jubatus). Physiol Behav 80: 595–601.

Jensen T, Pernasetti FM, Durrant B (2003) Conditions for rapid sex determination in 47 avian species by PCR of genomic DNA from blood, shell membrane blood vessels, and feathers. Zoo Biol 22: 561–571.

Jessop TS, Limpus CJ, Whittier JM (1999) Plasma steroid interactions during high-density green turtle nesting and associated disturbance. Gen Comp Endocrinol 115: 90–100.

Jessop TS, Knapp R, Whittier JM, Limpus CJ (2002) Dynamic endocrine responses to stress: evidence for energetic constraints status dependence in breeding male green turtles. Gen Comp Endocrinol 126: 59–67.

Jessop TS, Hamann M, Limpus CJ (2004a) Body condition and physiological changes in male green turtles during breeding. Mar Ecol Prog Ser 276: 281–288.

Jessop T, Sumner J, Lance V, Limpus C (2004b) Reproduction in shark-attacked sea turtles is supported by stress-reduction mechanisms. Proc Biol Sci 271 (Suppl 3): 591–594.

Jessop TS, Summer JM, Limpus CJ, Whittier JM (2004c) Interplay between plasma hormone profiles, sex and body condition in immature hawksbill turtles (Eretmochelys imbricata) subjected to a capture stress protocol. Comp Biochem Physiol A Mol Integr Physiol 137: 197–204.

Joung SJ, Chen CT (1995) Reproduction in the sandbar shark, Carcharhinus plumbeus, in the waters off northeastern Taiwan. Copeia 1995: 659–665.

Joung SJ, Liao YY, Liu KM, Chen CT, Leu LC (2005) Age, growth, and reproduction of the spinner shark, Carcharhinus brevipinna, in the northeastern waters of Taiwan. Zool Stud 44: 102–110.

Karczmarski L, Cockcroft VG (1998) Matrix photo-identification technique applied in studies of free-ranging bottlenose and humpback dolphins. Aquat Mamm 24: 143–147.

Kellar NM, Trego ML, Marks CI, Chivers SJ, Danil K, Archer FI (2009) Blubber testosterone: a potential marker of male reproductive status in short beaked common dolphins. Mar Mamm Sci 25: 507–522.

Kendall WL, Langtimm CA, Beck CA, Runge MC (2004) Capture-recapture analysis for estimating manatee reproductive rates. Mar Mamm Sci 20: 424–437.

Kerry KR, Agnew DJ, Clarke JR, Else GD (1992) Use of morphometric parameters for the determination of sex of Adelie penguins. Wildl Res 19: 657–663.

Kirkwood GP (1991) Annex R Comprehensive assessment of whale stocks progress report on development of revised management procedures. Report of the International Whaling Commission 41: 213.

Koch V, Brooks L, Nichols WJ (2007) Population ecology of the green/black turtle (Chelonia mydas) in Bahia Magdalena, Mexico. Mar Biol 153: 35–46.

Koob TJ, Tsang P, Callard IP (1986) Plasma estradiol, testosterone, and progesterone levels during the ovulatory cycle of the skate (Raja erinacea). Biol Reprod 33: 267–275.

Kusher DJ, Smith SE, Calliet GM (1992) Validated age and growth of the leopard shark, Triakis semifasciata, with comments on reproduction. Environ Biol Fish 35: 187–203.

Labrada-Martagón V (2011) Evaluación del estado de salud de la tortuga verde del Pacífico Oriental (Chelonia mydas) que habita en la costa de Baja California Sur, a través de biomarcadores fisiológicos. PhD thesis dissertation. CIBNOR, La Paz, 207 pp.

Labrada-Martagón V, Méndez L, Gardner SC, Lopez-Castro MC, Zenteno-Savín T (2010) Health Indices of the green turtle (Chelonia mydas) along the Pacific coast of Baja California Sur, Mexico. I. Blood biochemistry values. Chelonia Conserv Biol 9: 162–172.

Labrada-Martagón V, Méndez L, Mangel M, Zenteno-Savín T (2013) Applying generalized linear models as an explanatory tool of sex steroids, thyroid hormones and their relationships with environmental and physiologic factors in immature East Pacific green sea turtles (Chelonia mydas). Comp Biochem Physiol A Mol Integr Physiol 166: 91–100.

Langtimm CA, Beck CA, Edwards HH, Fick-Child KJ, Ackerman BB, Barton SL, Hartley WC (2004) Survival estimates for Florida manatees from the photo-identification of individuals. Mar Mamm Sci 20: 438–463.

Licht P (1979) Reproductive endocrinology of reptiles and amphibians: gonadotropins. Annu Rev Physiol 41: 337–351.

Licht P (1980) Evolutionary and functional aspects of pituitary gonadotropins, in the green turtle, Chelonia. Am Zool 20: 565–574.

Licht P (1982) Endocrine patterns in the reproductive cycle of turtles. Herpetologica 38: 51–61.

Licht P, Wood J, Owens DWM, Wood FE (1979) Serum gonadotropins and steroids associated with breeding activities in the green sea turtles Chelonia mydas. I. Captive animals. Gen Comp Endocrinol 39: 274–289.

Licht P, Rainey W, Clifton K (1980) Serum gonadotropin and steroids associated with breeding activities in the green sea turtle, Chelonia mydas. II Mating and nesting in natural populations. Gen Comp Endocrinol 40: 116–122.

Licht P, Wood J, Wood FE (1985) Annual and diurnal cycles in plasma testosterone and thyroxine in the male green sea turtle Chelonia mydas. Gen Comp Endocrinol 57: 335–344.

Limpus CJ (1992) Estimation of tag loss in marine turtle research. Wildl Res 19: 457–469.
Limpus CJ, Couper PJ, Read MA (1994) The green turtle, *Chelonia mydas*, in Queensland: population structure in a warm temperate feeding area. *Mem Qld Mus* 35: 139.

Lopez-Castro MC, Koch V, Mariscal-Loza A, Nichols WJ (2010) Long-term monitoring of black turtles *Chelonia mydas* at coastal foraging areas off the Baja California Peninsula. *Endang Species Res* 11: 35–45.

Lormée H, Jouventin P, Lallemand J, Chastel O (2000) Reproductive endocrinology of tropical seabirds: sex-specific patterns in LH, steroids, and prolactin secretion in relation to parental care. *Gen Comp Endocrinol* 117: 413–426.

Luque SP, Higdon JW, Ferguson SH (2007) Dentine deposition rates in belugas (*Delphinapterus leucas*): an analysis of the evidence. *Aquat Mamm* 33: 241–245.

McDonald SE (1996) Endoscopy. In WJ Rosskopf, RW Woerpel, eds, *Endoscopy*. E. Licht, Inc., Norwalk, pp 699–717.

Manire CA, Rasmussen LEL (1997) Serum concentrations of steroid hormones in the male bonnethead shark, *Sphyrna tiburo*. *Gen Comp Endocrinol* 107: 414–420.

Mansour AA, Mkay DW, Lien J, Orr JC, Banoub JH, Ølen N, Stenson G (2002) Determination of pregnancy status from blubber samples in minke whale (*Balaenoptera acutorostrata*). *Mar Mamm Sci* 18: 112–120.

Marshburn KL, Atkinson S (2004) Evaluation of adrenal function in serum and feces of Steller sea lion (*Eumetopias jubatus*): influences of molt, gender, sample, storage and age on glucocorticoid metabolism. *Gen Comp Endocrinol* 136: 371–381.

Matta NE, Ramirez N, Zúñiga BC, Vera V (2008) Sex determination in birds by molecular tools. *Acta Biol Colomb* 14: 27–40.

Mauger R, Jouventin P, Lacroix A, Ishii S (1994) Plasma LH and steroid hormones in King penguin (*Aptenodytes patagonicus*) during the onset of the breeding cycle. *Gen Comp Endocrinol* 93: 36–43.

Mawhinney K, Diamond T (1999) Sex determination of great black-backed gulls using morphometric characters. *J Field Ornithol* 70: 206–210.

Mellish JA, Hennen D, Thomson J, Petrauskas L, Atkinson S, Calkins D (2007) Permanent marking in an endangered species: physiological response to hot branding in Steller sea lions (*Eumetopias jubatus*). *Wildl Res* 34: 43–47.

Merchant-Larios H (1999) Determining hatching sex. In KL Eckert, KA Bjorndal, FA Abreu-Grobois, M Donnelly, eds, *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication, No. 4, Pennsylvania, pp 130–135.

Meylan A (1995) Sea turtle migration—evidence from tag returns. In KA Bjorndal, ed, *Biology and Conservation of Sea Turtles*. Smithsonian Institution Press, Washington, DC, pp 91–100.

Miller JD (1997) Reproduction in sea turtles. In PL Lutz, JA Musick, eds, *The Biology of Sea Turtles*. CRC Press, Boca Raton, pp 51–81.

Millspaugh JJ, Washburn BE (2004) Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. *Gen Comp Endocrinol* 138: 189–199.

Musick JA, Limpus CJ (1997) Habitat utilization and migration in juvenile sea turtles. In PL Lutz, JA Musick, eds, *The Biology of Sea Turtles*. CRC Press, Boca Raton, pp 137–163.

Natanson LJ, Cailliet GM (1986) Reproduction and development of the Pacific angel shark, *Squatina californica*, off Santa Barbara, California. *Copeia* 1986: 987–994.

Natanson LJ, Mello JJ, Campana SE (2002) Validated age and growth of the porbeagle shark (*Lamna nasus*) in the western North Atlantic Ocean. *Fishery Bulletin National Oceanic and Atmospheric Administration* 100: 266–278.

Norman BM, Stevens JD (2007) Size and maturity status of the whale shark (*Rhincodon typus*) at Ningaloo Reef in Western Australia. *Fish Res* 84: 81–86.

Ogden JA, Conlogue GJ, Rhodin AGJ (1981) Roentgenographic indicators of skeletal maturity in marine mammals (Cetacea). *Skeletal Radiol* 7: 119–123.

Oki C, Atkinson S (2004) Diurnal patterns of cortisol and thyroid hormones in the Harbor seal *Phoca vitulina* during summer and winter seasons. *Gen Comp Endocrinol* 136: 289–297.

Owens DW (1997) Hormones in the life history of sea turtles. In PL Lutz, JA Musick, eds, *The Biology of Sea Turtles*. CRC, Boca Raton, pp 315–341.

Owens DWM (1980) The comparative reproductive physiology of sea turtles. *Am Zool* 20: 549–563.

Owens DWM (1999) Reproductive cycles and endocrinology. In KL Eckert, KA Bjorndal, FA Abreu-Grobois, M Donnelly, eds, *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication, No. 4, Pennsylvania, pp 119–123.

Owens DWM, Morris YA (1985) The comparative endocrinology of sea turtles. *Copeia* 1985: 723–735.

Owens DWM, Hendrickson JR, Lance VA, Callard IP (1978) A technique for determining sex of immature *Chelonia mydas* using a radioimmunoassay. *Herpetologica* 34: 270–273.

Palme R (2005) Measuring fecal steroids: guidelines for practical application. *Ann N Y Acad Sci* 1046: 75–80.

Parham JF, Zug GR (1997) Age and growth of loggerhead sea turtles (*Caretta caretta*) of coastal Georgia: an assessment of skeletochronological age-estimates. *Bull Mar Sci* 61: 287–304.

Peddemors V (1989) Minimum age at sexual maturation of a female South-East Atlantic bottlenose dolphin *Tursiops truncatus*. *S Afr J Mar Sci* 8: 345–347.

Perrin WF, Reilly SB (1984) Reproductive parameters of dolphins and small whales of the family Delphinidae. Report of the International Whaling Commission (Special Issue 6): 97–134.
Petrauskas L, Atkinson S, Gulland F, Mellish JA, Horning M (2008) Monitoring glucocorticoid response to rehabilitation and research procedures in California and Steller sea lions. J Exp Zool A Ecol Genet Physiol 309: 73–82.

Poisbleau M, Demongin L, Noordwijk HJV, Strange IJ, Quillfeldt P (2010) Sexual dimorphism and use of morphological measurements to sex adults, immatures and chicks of rockhopper penguins. Ardea 98: 217–224.

Pratt HL, Carrier JC (2005) Elasmobranch courtship and mating behavior. In WC Hamlett, ed, Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Batoids and Chimaeras. Science Publishers, Enfield, pp 129–169.

Punt AE, Donovan GP (2007) Developing management procedures that are robust to uncertainty: lessons from the International Whaling Commission. ICES J Mar Sci 64: 603–612.

Raimondi S, Paracchini V, Atruip H, Barros-Dios JM, Benhamou S, Boffetta P, Cote ML, Dialyna IA, Dolzan V, Filiberti R et al (2006) Meta- and pooled analysis of GSTTI and lung cancer: a HuGE-GSEC review. Am J Epidemiol 164: 1027–1042.

Randall D, Burggren W French K (1997) Eckert Animal Physiology: Mechanisms and Adaptations, Ed 14. Freeman and Company, New York, 728 pp.

Rasmussen LE, Gruber SH (1993) Serum concentrations of reproductively-related circulating steroid hormones in the free-ranging lemon shark, Negaprion brevirostris. Environ Biol Fish 38: 167–174.

Rastogi SC (2007) Essentials of Animal Physiology, Ed 4. New Age International Limited Publishers, New Delhi, 578 pp.

Read AJ (1990) Age at sexual maturity and pregnancy rates of harbour porpoises Phocoena phocoena from the Bay of Fundy. Can J Fish Aquat Sci 47: 561–565.

Richardson JI, Hall DB, Mason PA, Andrews KM, Bjorkland R, Cai Y, Bell R (2006) Eighteen years of saturation tagging data reveal a significant increase in nesting hawksbill sea turtles (Eretmochelys imbricata) on Long Island, Antigua. Anim Conser 9: 302–307.

Rolland RM, Hunt KE, Kraus SD, Wasser SK (2005) Assessing reproductive status of right whales (Eubalaena glacialis) using fecal hormone metabolites. Gen Comp Endocrinol 142: 308–317.

Rolland RM, Hamilton PK, Kraus SD, Davenport B, Gillett RM, Wasser SK (2006) Faecal sampling using detection dogs to study reproduction and health in North Atlantic right whales (Eubalaena glacialis). J Cetacean Res Manag 8: 121–125.

Romero LM (2002) Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen Comp Endocrinol 128: 1–24.

Romero LM (2004) Physiological stress in ecology: lessons from biomedical research. Trends Ecol Evol 19: 249–255.

Romero LM, Wikelski M (2001) Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Niño events. Proc Natl Acad Sci U S A 98: 7366–7370.

Romero LM, Wikelski M (2002a) Exposure to tourism reduces stress-induced corticosterone levels in Galápagos marine iguanas. Biol Conserv 108: 371–374.

Romero LM, Wikelski M (2002b) Severe effects of low-level oil contamination on wildlife predicted by the corticosterone-stress response: preliminary data and research agenda. Spill Sci Technol Bull 7: 309–313.

Rostal DC, Grumbles JS, Byles R, Marquez R, Owens DWM (1997) Nesting physiology of Kemp’s Ridley sea turtles, Lepidochelys kempi, at Rancho Nuevo, Tamaulipas, Mexico, with observations on population estimates. Chelonian Conserv Biol 2: 538–547.

Rostal DC, Robeck TR, Owens DWM, Kraemer DC (1990) Ultrasound imaging of ovaries and eggs in Kemp’s Ridley sea turtles (Lepidochelys kempi). J Zoo Wildl Med 21: 27–35.

Samour HJ, Stevenson M, Knight JA, Lawrie AJ (1983) Sexing penguins by cloacal examination. Vet Rec 113: 84–85.

Sanvito S, Galimberti F, Delahanty KM, McKay DW (2004) Blood spots in Pinnipedia hormone studies: measure of cortisol levels in southern elephant seal (Mirounga leonina). Aquat Mamm 30: 251–256.

Schwarzenberger F, Möstl E, Palme R, Bamberg E (1996) Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim Reprod Sci 42: 515–526.

Seminoff JA, Resendiz A, Nichols WJ, Jones TT (2002) Growth rates of wild green turtles (Chelonia mydas) at a temperate foraging area in the Gulf of California, México. Copeia 2002: 610–617.

Seminoff JA, Jones TT, Resendiz A, Nichols WJ, Chaloupka M (2003) Monitoring green turtles Chelonia mydas at a coastal foraging area in Baja California, Mexico: multiple indices describe population status. J Mar Biol Ass 83: 1355–1362.

Setiawan AN, Darby JT, Lambert DM (2004) The use of morphometric measurements to sex yellow-eyed penguins. Waterbirds 27: 96–101.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Can J Zool 69: 82–90.

Snover ML, Hohn AA, Goshe LR, Balazs GH (2011) Validation of annual section estimates. J Exp Zool A Ecol Genet Comp Endocrinol 317: 9–20.

Smith DC, Steel PH (1961) Age and growth, sexual maturity and reproductive cycle of the Pacific mackerel, Scomber japonicus. Can J Zool 39: 553–564.
Sulikowski JA, Kneebone J, Elzey S, Jurek J, Howel WH, Tsang PCW (2006) Using the composite variables of reproductive morphology, histology and steroid hormones to determine age and size at sexual maturity for the thorny skate Amblyraja radiata in the western Gulf of Maine. J Fish Biol 69: 1449–1465.

Sulikowski JA, Williams LJ, Domeier ML (2012) The use of a nonlethal technique to assess the reproductive biology of the white shark, Carcharodon carcharias. In ML Domeier, ed, Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 467–476.

Valencia J, Leyton V (1992) Gonadal cycles of Pygoscelis penguins of the South Shetland Islands. In WC Hamlett, ed, Reproductive Biology of South American Vertebrates. Springer, New York, pp 198–207.

Valente AL, Parga ML, Espada Y, Lavin S, Alegre F, Marco I, Cuenca R (2007) Ultrasonicographic imaging of loggerhead sea turtles (Caretta caretta). Vet Rec 161: 226–232.

Valente AL, Velarde R, Farga ML, Marco I, Lavin S, Alegre F, Cuenca R (2011) Reproductive status of captive loggerhead sea turtles based on serum levels of gonadal steroid hormones, corticosterone and thyroxine. Vet J 187: 255–259.

Valverde RA, Selcer KW, Lara LR, Sibaja-Cordero JA (2008) Lack of xenosterrogen-induced vitellogenin in male olive ridley sea turtles (Lepidochelys olivacea) from the Pacific coast of Costa Rica. Rev Biol Trap 56: 49–57.

Vanderkist BA, Williams TD, Bertram DF, Lougheed LW, Ryder JL (2000) Indirect, physiological assessment of reproductive state and breeding chronology in free-living birds: an example in the Marbled Murrelet (Brachyramphus marmoratus). Funct Ecol 14: 758–765.

Wibbels T (1999) Diagnosing the sex of sea turtles in foraging habitats. In KL Eckert, KA Bjorndal, FA Abreu-Grobois, M Donnelley, eds, Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4, Pennsylvania, pp 139–143.

Wibbels T (2003) Critical approaches to sex determination in sea turtles. In PL Lutz, JA Musick, J Wynen, eds, The Biology of Sea Turtles, Vol II. CRC Press, Boca Raton, pp 103–126.

Wibbels T, Owens DWM, Amoss MS Jr (1987a) Seasonal changes in the serum testosterone titers of Loggerhead sea turtles captured along the Atlantic Coast of the United States. In WN Witzell, ed, Proceedings of the Cape Canaveral, Florida Sea Turtle Workshop. NOAA Technical Report NMFS 53, Miami, pp 59–64.

Wibbels T, Owens DWM, Morris YA, Amoss MS Jr (1987b) Sexing techniques and sex ratio for immature Loggerhead sea turtles captured along the Atlantic coast of the United States. In WN Witzell, ed, Proceedings of the Cape Canaveral, Florida Sea Turtle Workshop. NOAA Technical Report NMFS 53, Miami, pp 65–74.

Wibbels T, Owens DWM, Limpus CJ, Reed PC, Amoss MS Jr (1990) Seasonal changes in serum gonadal steroids associated with migration, mating and nesting in the Loggerhead sea turtle (Caretta caretta), Gen Comp Endocrinol 79: 154–164.

Wibbels T, Owens DWM, Licht P, Limpus CJ, Reed PC, Amoss MS Jr (1992) Serum gonadotropins and gonadal steroids associated with ovulation and egg production in sea turtles. Gen Comp Endocrinol 87: 71–78.

Wibbels T, Balazs GH, Owens DWM, Amoss MS Jr (1993) Sex ratio of immature green turtles inhabiting the Hawaiian Archipelago. J Herpetol 27: 327–329.

Wibbels T, Owens DWM, Limpus CJ (2000) Sexing juveniles sea turtles: is there an accurate and practical method? Chelonian Conserv Biol 3: 756–761.

Williams TD (1999) Diagnosing the sex of sea turtles in foraging habitats. In KL Eckert, KA Bjorndal, FA Abreu-Grobois, M Donnelley, eds, Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4, Pennsylvania, pp 139–143.

Williams TD (2003) Critical approaches to sex determination in sea turtles. In PL Lutz, JA Musick, J Wynen, eds, The Biology of Sea Turtles, Vol II. CRC Press, Boca Raton, pp 103–126.

Williams TD, Owens DWM, Amoss MS Jr (1987a) Seasonal changes in plasma levels of gonadal steroids and LH in breeding adults. Gen Comp Endocrinol 85: 230–240.

Williams TD (1992b) Reproductive endocrinology of macaroni (Eudyptes chrysophalus) and gentoo (Pygoscelis papua) penguins: II. Plasma levels of gonadal steroids and LH in immature birds in relation to deferred sexual maturity. Gen Comp Endocrinol 85: 241–247.

Williams TD, Sharp PJ (1993) Plasma prolactin during the breeding season in adult and immature macaroni (Eudyptes chrysophalus) and gentoo (Pygoscelis papua) penguins. Gen Comp Endocrinol 92: 339–346.

Willmer P, Stone G, Johnston IA (2005) Environmental Physiology of Animals. Blackwell Publishing, Oxford, 754 pp.

Wingfield JC, Hunt KE, Breuner C, Dunlap K, Fowler GS, Freed LR, Lepton J (1997) Environmental stress, field endocrinology, and conservation biology. In JR Clemmons, R Buchholz, eds, Behavioral Approaches to Conservation in the Wild. Cambridge University Press, Cambridge, pp 95–131.

Wood JR, Wood FE, Critchley K (1983) Hybridization of Chelonia mydas and Eretmochelys imbricata. Copeia 1983: 839–842.

Xia ZR, Li PP, Gu HX, Fong JJ, Zhao EM (2011) Evaluating noninvasive methods in sex identification in green sea turtle (Chelonia mydas) hatchlings. Chelonian Conserv Biol 10: 117–123.

Zavala CB, Paredes R (1997) Sex determination of adult Humboldt penguins, Spheniscus humboldti, using morphometric characters. J Field Ornithol 68: 102–112.

Zug GR (1990) Age determination of long-lived reptiles: some techniques for seaturtles. Annales des Sciences Naturelles Zoologie et Biologie Animale 11: 219–222.

Zug GR (1998) Estimates of age and growth in a population of green sea turtles (Chelonia mydas) from the Indian River lagoon system, Florida: a skeletochronological analysis. Can J Zool 76: 1497–1506.

Zug GR, Wynn AH, Ruckdeschel C (1986) Age Determination of Loggerhead Sea Turtles, Caretta caretta, by Incremental Growth Marks in the Skeleton. Smithsonian Institution Press, Washington, 34 pp.

Zug GR, Balazs GH, Wetherall JA, Parker DM, Shavnurkav K (2002) Age and growth of Hawaiian green seaturtles (Chelonia mydas): an analysis based on skeletochronology. Fish Bull 100: 117–127.