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Seasonal, ontogenetic and sexual changes in lipid metabolism of the small-spotted catshark (*Scyliorhinus canicula*) in deep-sea free-living conditions

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ABSTRACT

Marine predators, such as elasmobranchs, exhibit variations in nutritional conditions related to both reproductive traits and food availability in the marine environment throughout the year. The main objective of this study was to examine changes in several blood physiological parameters in a demersal shark, the small-spotted catshark (*Scyliorhinus canicula*), in the wild in relation to season, sex and maturity stage. For this purpose, 108 individuals at different developmental stages were captured and released alive in the western Mediterranean. Blood was obtained from caudal vessels and plasma lipid fractions (total cholesterol, triglycerides and phospholipids) and a ketone body (3-β-hydroxybutyrate) were measured. During summer, plasma triglyceride and phospholipid levels were lower in adults than in juveniles (mainly in females, probably related to breeding season and laying eggs). Plasma cholesterol levels also showed higher values in summer, indicating higher physical activities during summer and revealing that lipid fractions are more related to reproduction than to nutrition. Plasma 3-β-hydroxybutyrate variations showed a different pattern. No differences were found between sex or maturity stage during summer, although the highest values in adult and juvenile males during winter indicates higher physical activity of males. This study, uses an innovative methodology to establish a correlation between lipid fractions and ketone bodies from the blood of wild individuals and changes in sexual and nutritional status. This method was conducted without damage to the target species and provides new information on the physiology of this abundant elasmobranch in the Mediterranean Sea.

1. Introduction

Sharks are considered key species in marine ecosystems due to their role in maintaining the structure and functioning of food webs (Baum and Worm 2009). They are highly sensitive to ecosystem changes including human impacts (Myers et al. 2007; Dulvy et al. 2014). In comparison with other predators such as seabirds or marine mammals, few ecophysiological studies have been conducted in free-living elasmobranchs (García-Garrido et al., 1990; Speers-Roesch and Treberg 2010; Carrier et al. 2012; Gallagher et al. 2014). Such studies would be a huge step toward understanding how environmental seasonal changes or ontogeny/sexual variations in particular nutritional requirements affect the physiology of these marine predators. For example, variations in the quality or quantity of prey in the environment during the year can affect the physiological status of elasmobranchs (Pethybridge et al. 2014). Similarly, due to differences in the reproductive requirements or the morphology and behaviour between males and females, some variations in particular physiological parameters associated with reproduction may be present (Wearmouth and Sims 2008).

The physiology of elasmobranchs can be studied by measuring particular blood metabolites related to nutritional stores (García-Garrido et al., 1990; Ballantyne 2014; Gallagher et al. 2014). Thus, plasma levels of total cholesterol or triglycerides are useful indicators of the use of lipid reserves and of animal condition (García-Garrido et al., 1990). Although elasmobranches usually present low levels of blood cholesterol or triglycerides (Crabtree et al. 1972), increments in these metabolites indicate the mobilisation of lipid stores as a consequence of variations in the energy requirements associated with reproduction or body condition (García-Garrido et al., 1990). Similarly, an increase in ketone bodies in the blood, such as 3-β-hydroxybutyrate, indicates higher

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muscular activity or the mobilisation of lipid reserves during starvation situations (Ballantyne 1997; Speers-Roesch 2006). Plasma levels of the ketone body 3-β-hydroxybutyrate in elasmobranches are as high as those seen in fasted mammals, indicating their preference for ketone body oxidation rather than fatty acid oxidation in muscle under routine conditions (Speers-Roesch and Treberg 2010).

In the present study, the physiological state (plasma biochemistry) of the small-spotted catshark (*Scyliorhinus canicula*) was examined in free-living conditions. The small-spotted catshark is a widespread and abundant elasmobranch particularly appropriate as an experimental model due its strong resistance to handling and the relative ease of capture. Despite these facilities, there is a surprising scarcity of studies on the ecophysiology in free-living conditions of this shark species. This is an abundant demersal shark present throughout the Mediterranean Sea and in some areas of the Atlantic Ocean (Navarro et al. 2016). Similar to several sharks, the small-spotted catshark is oviparous, laying eggs enveloped in a shielding sleeve and anchored in algae and other solid structures (Ellis and Shackley 1997). Males and females are sexually active throughout the year (Capapé et al. 2014). In relation to food habits, the small-spotted catshark is a nocturnal opportunistic predator that exploits a wide range of benthic crustaceans and demersal fish (Valls et al. 2011). The main aims of the present study were to examine the effect of season (winter and summer), sex (males and females) and maturity stage (juveniles and adults) on three plasma lipid parameters (cholesterol, triglyceride, phospholipids) and one ketone body (3-β-hydroxybutyrate).

2. Material and methods

2.1. Sampling procedures

The study was conducted at the mouth of the Ebro River, in the western Mediterranean Sea (Fig. 1). This is a relatively highly productive area in the Mediterranean Sea due to the combination of the contributions of organic matter by the Ebro River in the wider area of the continental shelf, and the effect of the Liguro-Provençal-Catalan current along the slope of the continental platform (Estrada 1996; Salat 1996). During winter (February) and summer (July) of 2013, a total of 108 small-spotted catshark individuals were caught (Table 1). All individuals were captured in depths ranging between 250 and 350 m during two experimental fishing cruises (ECOTRANS project; Institute of Marine Sciences ICM-CSIC, Spain). To reduce the effect of circadian rhythms on the physiological parameters, all individuals were captured between 10:00 and 12:00 a.m. Once caught, they were kept in tanks with a continuous flow of seawater and were sampled within 1 h after capture. The body length (±0.1 cm), body mass (±0.1 g) and sex (visually external reproductive organs) were recorded for each individual. The maturity state of each small-spotted catshark was determined based on body length (body length > 35 cm were considered adults; body length < 30 cm were considered juveniles; ECOTRANS Project, unpublished data). For each individual, 0.3 mL of blood from the caudal vessels was extracted using fine 1 mL syringes. Immediately after the extraction, blood was stored in heparinised tubes and centrifuged for 10 min at 5500 rpm to separate the plasma fraction. Plasma samples were frozen at −70 °C until analysis. After blood sampling and body size measurements, each individual was returned to the water tank for 30 min and then released alive in the area of capture.

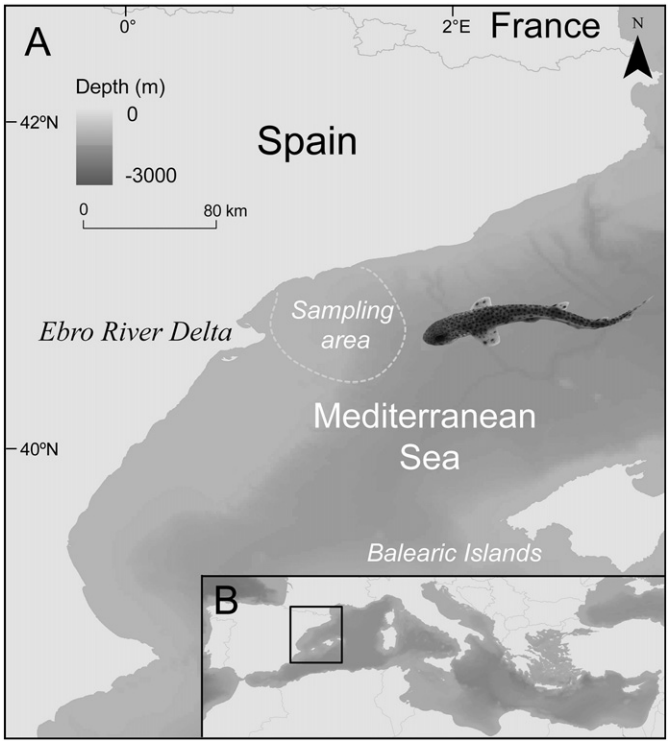

Fig. 1. Map of the study area in the NW Mediterranean Sea (A) and the position within the Mediterranean basin (B). The sampling area where small-spotted catshark (*Scyliorhinus canicula*) individuals were collected is indicated in the map.

2.2. Biochemical determination and statistical analysis

Three lipid fractions: triglycerides, cholesterol and phospholipids, and one ketone body, 3-hydroxybutyrate, were measured in plasma samples using commercial kits adapting the corresponding colorimetric methods to microsamples using a spectrophotometer TECAN Infinite 200 with plates of 96 wells (Triglycerides –LQ, Cholesterol –LQ and Phospholipids from Spinreact, Sant Esteve de Bas, Spain; and 3-Hydroxybutyrate from Ranbut, Randox Lab., Crumlin, UK).

The differences in plasma levels of total cholesterol, triglycerides, phospholipids and 3-β-hydroxybutyrate between sexes (male, female), seasons (winter, summer) and maturity stage (juvenile, adult) were examined by ANOVA tests. Pair-relationships between physiological parameters were tested using Pearson's correlation test. Before statistical analyses, we log-transformed all physiological variables to ensure a normal distribution. Significance levels for all tests were adopted at $p < 0.05$.

3. Results

Total body weight and length, and plasma levels of the plasma indicators of lipid metabolism of small-spotted catshark (*Scyliorhinus canicula*) are shown in Table 1. All the plasma lipid fractions analysed showed significant differences according to the season (Table 2), being on average higher in the summer. Nevertheless, the state of maturity presented interactions with sex in the case of total cholesterol and with season for triglycerides and phospholipids (Table 2). Cholesterol showed higher values during summer than during winter. In summer, adult females presented lower concentrations of cholesterol than juvenile females (and also males), while in winter both groups of females showed lower values in this parameter than males (Fig. 2A, Tables 1 and 2). These seasonal differences were also observed in the case of triglycerides, but the increase in levels during summer was in juvenile males and females. In the case of phospholipids, both groups of males and juvenile females showed higher values in summer, whereas the highest values of adult females during winter decreased in the summer (Fig. 2B-C, Tables 1 and 2).

In the case of the ketone body, 3-β-hydroxybutyrate, the pattern observed was clearly different from those of the other physiological parameters (Fig. 2D, Tables 1 and 2). In winter, males showed higher values than females, especially adult males that showed values more than double those presented by all the other groups (Fig. 2D, Tables 1 and 2). During summer we did not find differences in plasma 3-β-