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# Measurement and analysis of small pelagic fish condition: A suitable method for rapid evaluation in the field

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ABSTRACT

As condition is a key variable in population dynamics (especially for survival, growth and reproduction), the use of well-defined and accurate fish condition indices is capital. In particular, condition indices (morphometric, bioenergetic and biochemical) have never been compared and validated for the European anchovy *Engraulis encrasicolus*, the European pilchard *Sardina pilchardus* and the European sprat *Sprattus sprattus*. The accuracy of two indirect methods, the morphometric relative condition index $K_n$ and the bioenergetics index determined with the Distell Fish Fatmeter was investigated by comparing with a direct measure of relative lipid content carried out with a thin layer chromatography-flame ionization detector. Estimations from the fatmeter correlated quite well with the relative lipid contents of all species, regardless of the reproductive period ($R^2 = 0.69$ for anchovy, $R^2 = 0.75$ for sprat and $R^2 = 0.48$ for sardine). $K_n$ correlated more poorly with relative lipid content ($R^2 = 0.22$ for anchovy and $R^2 = 0.41$ for sardine, ns for sprat), especially during the reproductive period, pointing out the difficulty for such an index to precisely reflect changes in fat allocation. During the reproductive period, changes in $K_n$ could reflect other processes, such as changes in protein content. Therefore, these different types of commonly used indices do not reflect exactly the same type of energy stores. The high repeatability of the fatmeter was brought to light, so that only one measurement on each fish side may be necessary to evaluate the relative lipid content of a small pelagic fish. Finally, fatmeter measurements were not affected by freezing storage up to one month for anchovy ($R^2 = 0.66$) and sardine ($R^2 = 0.90$), making it possible to use frozen samples of both commercial and scientific survey. In contrast, the freezing storage for sprat should be avoided. Based on this study, the Fatmeter appears to be a suitable indirect method to assess condition and fat content of sardine and anchovy on a large number of individuals.



## 1. Introduction

Body condition is a key variable widely used in ecological studies particularly on fish, mammals and birds to define the nutritional or the physiological status of an individual (Bolger and Connolly, 1989; Stevenson and Woods, 2006). Commonly, body condition is defined as the quantity of nutrient reserves, which represents the quantity of metabolizable tissues exceeding those required for daily nutritional demands (Schamber et al., 2009; Schulte-Hostedde et al., 2001). Body condition indices thus inform on the quantity of energy extracted from the environment and can give for instance important insights on foraging behavior or prey distribution (Lloret et al., 2013). They are also used as indicators of an individual's well-being which can affect its future performances (Stevenson and Woods, 2006; Wilson and Nussey, 2010). For example, individuals with larger nutritional reserves may have a greater survival rate, a larger reproductive success and a higher growth rate (Millar and Hickling, 1990), ultimately resulting in a link between body condition and fitness for several species (Jakob et al., 1996). Measuring body condition is thus of the utmost importance for physiologists and ecologists to understand population dynamics (Schulte-Hostedde et al., 2005) and monitor the status of fish stock (Lambert and Dutil, 1997).

A large number of condition indices are available from literature. They can be sorted in three categories, namely, morphometric, bioenergetic and biochemical indices. While some studies demonstrated a correlation between bioenergetic (measure of the relative amount of fat in one fat depot, mainly in muscles) and biochemical indices (Nielsen et al., 2005; Trudel et al., 2005; Vogt et al., 2002) or between biochemical and morphometric indices (Brown and Murphy, 2004; Pangle and

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Sutton, 2005), the use of three types of indices differs in terms of both methodology and meaning of the term condition. Morphometric indices are indirect and based on the assumption that for a given size, heavier individuals are in a better condition (Green, 2001). These indices assume that the bulk of available energy reserves is located in somatic and germ tissues, and that the total weight is a suitable reflection of condition. They are extensively used because of their simplicity and have often been selected to monitor fish health (Lambert and Dutil, 1997), investigate the effect of marine pollution (Bervoets and Blust, 2003) or manage fisheries (Cone, 1989). Direct estimations of fish condition can also be determined through lipid content, protein or ash quantification (Stevenson and Woods, 2006). As lipids are the first component of energy reserves to be mobilized (McCue, 2010), the majority of studies using direct estimations relies on them, but because of the complexity and the diversity of energy stores, proteins might also be crucial, especially when lipid concentrations are very low. Biochemical lipid quantification methods are time-consuming and expensive, leading to a preferential use of morphometric indices over biochemical ones (Cone, 1989; Schamber et al., 2009). Finally, bioenergetic indices appear as a compromise between morphological and biochemical indices. They estimate lipid content through indirect measurements, e.g. water content can be measured as it is strongly and inversely related to fat content (Craig et al., 1978; Simat and Bogdanović, 2012), as is done by the fatmeter (Distell Fish Fatmeter; Kent, 1990). This electronic device provides some advantages because it is easy to use, portable, fast and non-destructive, allowing researchers to keep the individual alive or intact for further analyses. While several authors recommended validating indirect indices against direct measurements of condition, only few studies have investigated the accuracy of indirect indices for fish. When these validations were realized, significant relationships between biochemical and morphometric indices were sometimes missing (McPherson et al., 2011; Nielsen et al., 2005), pointing out that morphometric condition indices were not always indicative of energy reserves.

The aim of this study was firstly to compare three types of indices (morphometric, biochemical and bioenergetics) to understand the different information they provide. Comparative studies that involved three different types of condition indices are rare in literature and focused mostly on herring (Davidson and Marshall, 2010; McPherson et al., 2011). The second aim was to determine a reliable method, easy and quick enough to monitor body condition of small pelagic fish at the population scale (i.e. allowing rapid measurements of a large number of individuals).

In this study, for the first time, the use of an electronic instrument that makes non-invasive measurements of water content to determine the relative lipid content of fish (Distell Fish Fatmeter MFM-992 (Kent, 1990)) was investigated for three widespread small pelagic fish species namely the European pilchard *Sardina pilchardus* (Walbaum 1792), the European anchovy *Engraulis encrasicolus* (L. 1758) and the sprat *Sprattus sprattus* (L. 1758). They are the most significant species of the Gulf of Lions in terms of biomass and economic value. Up until recently, the estimation of relative lipid content by a fatmeter was done on medium and large species such as Atlantic herring *Clupea harengus* (L. 1758), eel *Anguilla anguilla* (L. 1758) and carp *Cyprinus carpio* (L. 1758) (Davidson and Marshall, 2010; Klefoth et al., 2013; McPherson et al., 2011). However, the apparition of a new model aiming at smaller fish opened new perspectives on species which could not be monitored before.

In order to know if indirect condition indices reflect lipid storage, the fatmeter and a common morphometric index, the relative condition were compared with biochemical analyzes of lipid content realized with a thin layer chromatography, a direct method considered here as a benchmark. Furthermore, only one study has ever investigated the effects of storage duration on fatmeter measurements (herring, Vogt et al., 2002). Here, various freezing durations were compared to determine precisely when differences were induced on fatmeter measurements. Moreover, the number of replications necessary to obtain reliable estimations of relative lipid content was explored for the first time using repeatability analyses.

## 2. Material and methods

### 2.1. Study area and fish sampling

In the Gulf of Lions (42°26′–43°12′N and 3°09′–5°27′E), a total of 499 anchovies, 488 sardines and 187 sprats were sampled from January 2013 to February 2014. June and July samples came from a standardized acoustic survey (PELMED) onboard the RV "L'Europe", while the other samples were collected by commercial pelagic trawlers. For each sampling event, the same protocol was used, i.e. fish were randomly selected and measured directly onboard (scientific surveys) or one day after, in which case they were stored in ice (commercial fishing). For each fish, the total length (TL, to the nearest 1 mm), total wet weight (W, to the nearest 1 g), sex, maturity stage (based on macroscopic observation of the gonads) and fat content (with Distell Fish Fatmeter) were recorded. The usual five maturity stages were also estimated (ICES, 2008): the first stage during which gonads are inactive, two intermediate stages during which they are active, the fourth stage corresponding to the spawning event and the fifth stage during which the gonads are inactive and recovering.

### 2.2. Morphometric index

As the three species exhibited an allometric growth pattern (Beveren et al., 2014), the relative condition index $K_n$ (Le Cren, 1951) was used as a proxy of individual fish condition (Green, 2001). Indeed, $K_n$ prevents from the assumption of isometric growth and avoid a potential length effect. The index $K_n$ was computed as

$$K_n = W/W_r \tag{1}$$

where $W$ is the mass of an individual and $W_r$ is the theoretical mass of an individual of a given total length (TL in mm) predicted by a length–weight relationships ($W_r = \alpha \cdot \mathrm{TL}^{\beta}$). Length–weight relationships were calculated based on a 30-year dataset including more than 42,000 individuals and were characterized by $\alpha = 0.0029 \pm 0.0001$ and $\beta = 3.302 \pm 0.007$ for anchovy, $\alpha = 0.0038 \pm 0.0001$ and $\beta = 3.241 \pm 0.011$ for sardine, and $\alpha = 0.0063 \pm 0.0005$ and $\beta = 3.022 \pm 0.003$ for sprat.

### 2.3. Bioenergetic index (Distell Fish Fatmeter)

Based on the strong inverse relationship between water and fat content in fish (Craig et al., 1978; Simat and Bogdanović, 2012), the microstrip sensor (microwave) of the fatmeter estimates the relative fat content (% lipids) of an individual from a permittivity calculation (Kent et al., 1992). The MFM-992 fatmeter used in this study was equipped with a small sensor head (3 centimeter wide), making it more adapted to analyze small fish. For each species the calibration provided by the manufacturer was used (sardine2, anchovy2 and sprat2). Relative lipid content was measured twice on the same location on both sides of the fish (along the lateral line as recommended in user manual) and the average of these four measurements was used as the final value. Even if temperature does not affect fatmeter measurements (Klefoth et al., 2013), all measurements were made following the same experimental conditions to avoid any potential bias.

### 2.4. Repeatability of fatmeter measurements

Repeatability was assessed by testing the similarity between different fatmeter measurements (reliability of measurement). In addition, the number of measurements required to obtain an accurate estimate was determined (Lessells and Boag, 1987). Among all fish available