Ultrasonography as a non-invasive technique for monitoring the gonadal development in European sea bass (*Dicentrarchus labrax*) females

*Ús de l'ecografia com a tècnica no-invasiva per al monitoratge del desenvolupament gonadal en femelles de llobarro (*Dicentrarchus labrax*)*

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Abstract: The reproductive dynamics of fishes and their variability between individuals determine the fecundity at population level and are therefore very relevant to fisheries management, yet they are also difficult to characterize by conventional methods. Bioenergetic models, such as Dynamic Energy Budget (DEB) could be an alternative to estimate population fecundity. These models aim to understand the dynamics of the main life processes, including reproduction. However, to properly account for individual variability, these models must be fed with repeated measurements of the same fish. The aim of this work is to develop a methodology capable to quantify the volume of the gonads of fish non-invasively throughout their lifespan. To achieve this goal, the gonad volume of 32 female European sea bass was measured with ultrasounds throughout an entire reproductive cycle. The volume estimated with ultrasounds has proven to be a great predictor of gonads weight. The relationship between weight and volume is given by W = 1.028*V - 5.263, where W is the estimated weight of one gonad lobe and V is the volume of the lobe estimated with ultrasounds.
Key words: bioenergetic models, reproductive dynamics, gonads, ultrasound.

Resum: *La dinàmica reproductiva dels peixos i la seva variabilitat entre els individus determinen la fecunditat de cada població i per tant són molt rellevants per a una correcta gestió pesquera, però són difícils de caracteritzar amb els mètodes convencionals. Els models bioenergètics, com el* Dynamic Energy Budget *(DEB) podrien ser una alternativa per a estimar la fecunditat poblacional. Aquests models pretenen entendre la dinàmica dels principals processos vitals, entre ells la reproducció. Però, per tenir en compte correctament la variabilitat individual, aquests models s'han d'alimentar amb mesures repetides dels mateixos peixos. L'objectiu d'aquest treball és desenvolupar una metodologia que permeti quantificar el volum de les gònades dels peixos al llarg de la seva vida de forma no invasiva. Per assolir aquest objectiu, s'han mesurat amb tècniques ecogràfiques el volum de la gònada de 32 femelles de llobarro al llarg de tot un cicle reproductiu. El volum estimat amb les ecografies ha demostrat ser un molt bon predictor del pes de la gònada. La relació entre pes i volum ve donada per W = 1.028*V - 5.263, on W és el pes estimat d'un lòbul de la gònada i V és el volum del lòbul estimat per ecografia.*
Paraules clau: *models bioenergètics, dinàmica reproductiva, gònades, ecografia.*

INTRODUCTION

Information on temporal variations of the reproductive potential of fish populations (in other words, which factors affect it and how it can be predicted) is essential for developing fishery management plans that maximize the stocks' yield and sustainability (Serrat *et al.*, 2019). Although this relevance, fish fecundity is difficult to estimate at population level due to the complexity of the egg development process. The number of eggs that can be counted in a gonad on a given moment is not usually a reliable predictor of the fish fecundity, and even less reliable when accounting for the fecundity at population level. Thus, the conventional approach to estimate fecundity implies a continuous monitoring of the population, starting before the beginning of the spawning season, using specialized histological methods. But even in these cases, is not always possible to obtain reliable fecundity estimates at population level due to the between-fish variability, both for seasonal patterns and individual fecundity (Serrat *et al.*, 2019).

On the other hand, bioenergetic models allow to explain by mechanistic ways all the biological processes of an organism from energetic dynamics. For example, in the case of the Dynamic Energy Budget theory (DEB), the energy assimilated by an organism is stored as reserve energy, and this energy is mobilized to feed growth and reproduction processes. Thus, according to this theory, the reproductive capability of one fish depends on the quantity of energy that it has available at each moment, for each process (Koojman, 2010). In this way, the reproductive potential of a fish

could be indirectly estimated from the quantity of energy that it invests in reproductive processes. Actually, DEB models including explicitly the spawning dynamics have already been proposed (Muller *et al.*, 2019; Pecquerie *et al.*, 2009), but this indirect approach to estimate the reproductive potential has not been applied to fisheries management yet.

The main goal within which this communication is framed is to develop a DEB model able to explain mechanistically the main reproductive processes in order to obtain estimates of the reproductive potential robust enough to be useful for improving fisheries management and/or aquaculture production.

Anyways, the parameter estimates in the DEB model at individual level needs repeated measurements from different observable variables throughout the lifespan of the same individual (i.e., a temporal sequence of observations that will allow to identify the system dynamics). The provided information by conventional histological methods is essential too, but it corresponds to a "single frame" of the whole reproductive process, since it is a very invasive survey that implies the fish sacrifice. Thus, it can be useful to be provided of non-invasive methodologies that allow to generate sequential information of the reproductive dynamics. A potentially useful variable for that matter is the gonads weight. Ultrasonography is a highly promising non-invasive technique since it allows to estimate the size of the internal organs, especially fish gonads (Jennings *et al.*, 2005; Næve *et al.*, 2018).

Therefore, the specific aims of this work are 1) to develop an ultrasound-based measuring protocol for the fish gonads and 2) to deploy a statistical model that allows to generate estimates for gonads weight as precise and accurate as possible from observations by ultrasonography.

MATERIALS AND METHODS

This study takes the European sea bass (*D. labrax*) as a model species. A dioic species of high commercial interest in the Mediterranean, both for fisheries and aquaculture (FEAP, 2016). For the experiments, a total of 32 4 sea bass females, with and average weight of 2503 g and 4 years of age have been used. They were supplied from aquaculture breeding (Aquicultura Balear S.A.U.) and maintained in the LIMIA facilities in Port d'Andratx. Between November 2021 and April 2022, following the species spawning season (Pawson *et al.*, 2007), each female was sampled biweekly by echography. Prior to echographies, the specimens were tranquilized with a phenoxyethanol dose of 1000 ppm. Echography samplings were performed using an ultrasonic probe with a linear array with a frequency of 7.5-10 MHz, a focal point of 25-35 mm and a signal amplification (gain) of 85% (Fig. 1A). In each echography, 11 measurements from the left lobe of the gonad were taken: total length and the height and width in 5 equidistant points of the longitudinal axis (Fig. 1B).

Specimens were sacrificed sequentially throughout the experimentation timeframe, always after being surveyed by echography for last time the day before. Once sacrificed, gonads were extracted, weight and volume (by volumetry) were measured and the same 11 measurements as in echography surveys were taken using a calliper, in both gonad lobules. The obtained measurements with echography were compared (by lineal regression) with the same measurements obtained with the calliper.

For gonad weight estimates from ultrasound measurements, 3 geometric models were compared in order to reconstruct the volume of the lobule from echography measurements: 1) a method based in the combination of geometric figures (trunked cones of ellipsoid bases) and two polygonal models implemented in two libraries of the R software: 2) *geometry* and 3) *α-shape 3D* (Fig. 1C). Lastly, estimated volumes of each lobule were compared (by lineal regression) with their real weight.

RESULTS

The measurements of the gonad lobes taken by ultrasonography and measurements taken with the calliper once gonads were extracted (after the fish sacrifice) show a good correlation. For the total length of the gonad and for the central measurements of height and width, the correlation coefficients were $R^2 = 0.86$, $R^2 = 0.90$ and $R^2 = 0.88$, respectively.

Once reconstructed the volumes of each gonad lobule with the tree methods above-mentioned, their respective analyses of lineal regression (estimated volume vs real weight) were performed. The three lineal models show a p-value inferior to 0.001 for the slope of the pattern line, and very similar R^2 values (0.951, 0.946 and 0.949, respectively) and AIC values (275.06, 277.87 and 276.23, respectively). Despite this, the method 1 (volume reconstruction by trunked ellipsoid cones) was selected, as it presents the pattern line slope closer (1.028 ± 0.043 g/cm^3) to the lineal model that links the lobes weight with their volume (measured by volumetry after the sacrifice), which shows a slope of 1.067 ±

0.043 g/cm^3. The other two methods showed slopes slightly higher (1.529 ± 0.067 g/cm^3 and 1.593 ± 0.068 g/cm^3, respectively).

Consequently, the proposed formula to estimate the weight of gonad lobules from echography measurements is defined as W = 1.028*V - 5.263, where W is the estimated weight of one gonad lobe and V is the volume of the lobe estimated with ultrasounds (Fig. 2A)

DISCUSSION

With the obtained results, the comparison between the gonad measurements by ultrasonography and the same measurements obtained with a calliper (once the fish is sacrificed) shows that ultrasonography is a reliable technique for measuring *in vivo* and non-invasively the size of internal organs as the gonads of adult specimens of *D. labrax*. The handling protocol has proven to be safe and does not pose any risk to the fish welfare, which have exhibited a normal gonadal development throughout the whole reproductive cycle.

Any of the three methods allows to precisely assess the reconstruction of the gonad lobules volume. Furthermore, the estimated volumes with the three methods are good predictors of the gonad weight. Predictions of gonad weights obtained by the three methods are precise and accurate enough, and there is no evidence of biases along the observed range of sizes. However, the volume estimates with the method 1 (trunked ellipsoid cones) show a slope closer to the slope of the lineal relationship between the lobule weight and its volume measured by volumetry. The other two volume reconstruction methods show a similar precision but seem to be less accurate, as they slightly underestimate the volume measured by volumetry. This underestimation might be related to the fact that the surface of the reconstructed volume with the methods 2 and 3 is defined by geometric planes (Fig. 1C) and hence the transversal sections of the reconstructed volume are rhomboids. In contrast, transversal sections of volumes reconstructed with the method 1 are ellipsoids and hence are closer to the spindle shape of the gonad lobes.

The obtention of the straight pattern to relate ultrasonography measurements of one gonad with its weight is the first step to fulfil in order to achieve more ambitious objectives. First of all, as echographies are almost non-invasive, the gonad weight can be estimated in a same fish throughout a full spawning season. As an example, we show the variation pattern of the gonad weight of two individuals throughout the reproductive cycle (Fig. 2B). Despite the two fishes had a very similar total weight, there is an apparent individual variability, both for the gonad volume and the peak moment of the maximum volume and, therefore, the length of the reproductive period.

Being able to estimate the gonad wight of fishes throughout their lifespan, individually and using non-invasive techniques with a relatively simple methodology, can allow to describe the reproductive dynamics for *D. labrax* as well as other fish species. The obtained data through this methodology can be useful to enhance bioenergetic models such as the derived from the DEB theory. Specifically, our goal is to combine the data of the gonad weight with other measurements of observable variables, such as blood hormone levels of several sexual hormones and yolk precursors of the eggs, and quantitative data about the histological composition of the gonads. This information will allow to achieve the main goal within which this communication is framed: to develop a model DEB able to explain mechanistically the main reproductive processes in order to generate estimates of the reproductive potential robust enough to be useful for improving fisheries management.

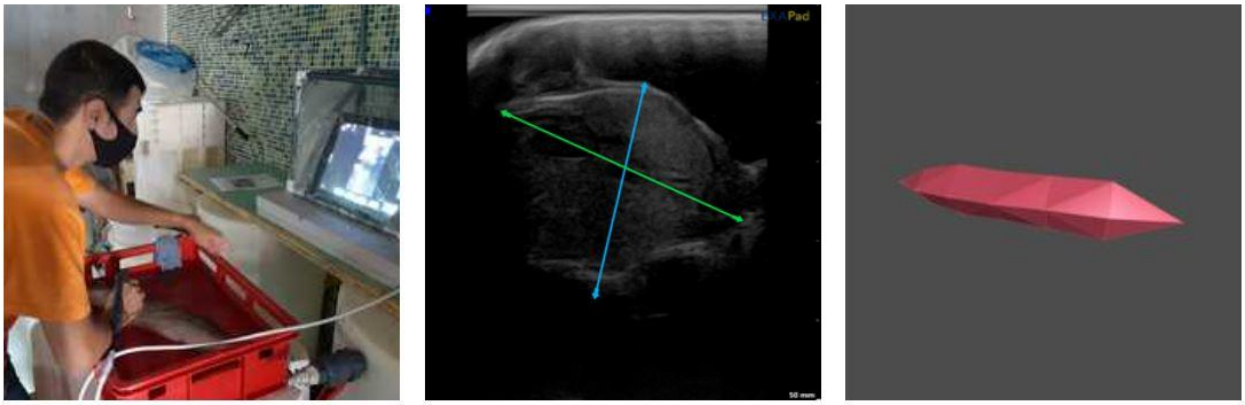

FIGURE 1: *A) Echography procedure of a specimen of* D. labrax. *B) Ultrasonography image of a transversal section of a gonad lobule of* D. labrax, *where there can be observed the measurements of its height (green) and width (blue). C) Volumetric reconstruction of a gonad lobule from ultrasonography measurements, using the method α-shape 3D.*

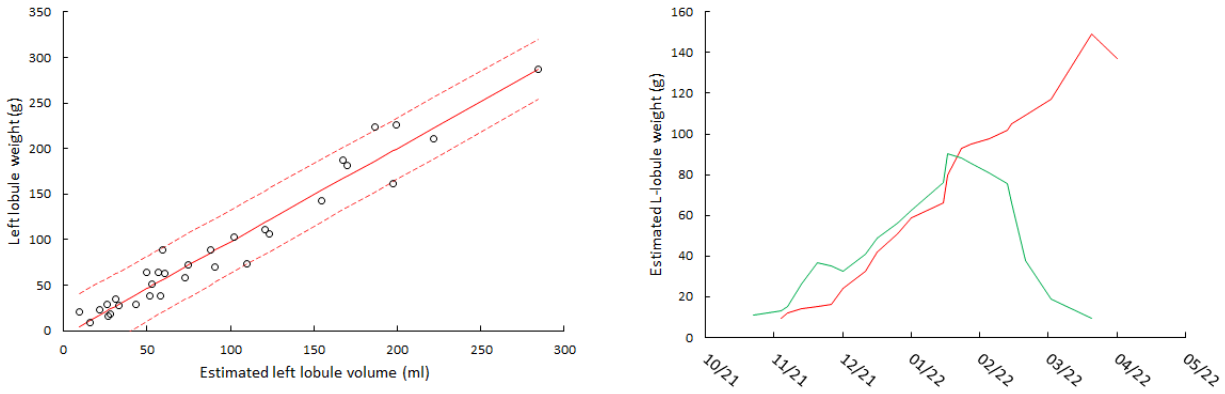

FIGURE 2: *A) Straight-line pattern obtained for the weight estimates of gonad lobules from the volume estimates using the method 1 (trunked ellipsoid cones), with the CI of 95%. B) Comparison of the variation in the estimated volume of the left lobule of the gonad of two different fish (red and green) of similar total weight, where there can be observed differences in the maximum weight and the moment where the peak weight is reached.*

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