Marine Biology 90, 345–351 (1986)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Review of the Gonad Index (GI) and an introduction to the concept of its "critical value": application to the skipjack tuna *Katsuwonus pelamis* in the Atlantic Ocean

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Abstract

With special reference to tunas and using recent results obtained on the skipjack tuna *Katsuwonus pelamis*, the authors point out that Gonad Index (GI, defined as the ratio between the weight of the gonads and the size of the fish to the cube) is an inaccurate indicator of each of the different sexual maturity stages commonly used to describe the sexual maturation process of marine fishes. They also show that it would be of interest to determine a GI critical value in order to distinguish females in an advanced stage of maturation from the others. Using diameter measurements of skipjack tuna oocytes they adjust a non linear model between GI and the diameter of the most advanced group of oocytes. This permits the estimation of a GI critical value. This critical value is discussed.

Introduction

History and reliability of the Gonad Index

The sexual maturity of female *Katsuwonus pelamis* has long been determined by means of a simple optical examination of the macroscopic stage of the gonads. Even though macroscopic scales of maturity are still used in the field to make fast and gross determinations of the maturation stage, the inaccuracy of this method and its subjective components have often been pointed out (Bunag, 1956; Raju, 1964; Batts, 1972; Cayré, 1981). In order to dispose of objective criteria, certain authors have used a fine cytological description of the oocytes and (jointly or not) the measurements of their diameters (Brock, 1954; Bunag, 1956; Raju, 1964; Yoshida, 1966; Batts, 1972).

The long and laborious task of measuring the ova has been avoided by using different maturity indices calculated from the gonad weights in relation to either the body weight (i.e. Gonado-somatic Index, Yuen, 1955) or the size of fishes (Kikawa, 1953). Such indices are only usable when they show the sexual maturity stage independently of the size of the considered fish.

At present the most commonly used index is the gonad index (GI):

$$GI = \frac{G.W.}{L^3} \cdot 10^k,$$

where G.W. = Gonad weight; L = length of the fish; k = factor, which is chosen according to the units adopted for G.W. and L.

Moreover, if the GI truly reflects the precise stage of sexual maturity for the females, it should be related to a quantitative histological or cytological factor through a monotone function, which would reflect the maturity stage; the diameter of the most advanced mode of the oocytes is the cytological reference factor most frequently used. Schaefer and Orange (1956) noted that there is a linear relationship between GI and the diameter of the most advanced group of oocytes when this diameter is $< 300\,\mu$. Nevertheless several authors who have worked in particular with skipjack tuna (*Katsuwonus pelamis*) have shown that, for a given maturity stage (determined by visual examination of the gonads or by using the size of the biggest oocytes), the GI could vary significantly and thus not permit a clear distinction of successive maturity stages (Yoshida, 1966; Matsumoto *et al.*, 1984; Cayré and Farrugio, 1983). For this reason, the GI does not seem to be a reliable measure of the maturity stage for skipjack tuna.

Several authors (Yoshida, 1966; Cayré and Farrugio, 1983; Matsumoto *et al.*, 1984) have also pointed out that when the diameter of the most advanced group of oocytes is large ($> 300\,\mu$), there is no evident relationship between the GI and this diameter.

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Concept of critical value of GI for skipjack tuna

Skipjack tunas, which are oceanic pelagic migrators, seem to be able to accomplish their sexual maturation cycle in a very short period of time (within one week: Kaya *et al.*, 1982; Cayré and Farrugio, 1983); this facilitates their reproduction as soon as they find favourable environmental conditions. We can then talk about an "opportunistic" reproduction mode since it is done without being affected by spawning area or season, in the strict sense of these terms (Sharp, 1981; Cayré, 1981).

Under such reproduction conditions it is important to know from which average GI value it can be ensured that the females are at an advanced maturity stage near spawning. At this point it is of interest to mention that Cayré and Farrugio (1983) stated that within the maturing gonads of the skipjack females a maximum of four groups (or modes) of oocytes maturing toward the ovulating eggs can be observed.

These authors consider that a female has reached the prespawning stage when the last mode observed in the size frequency distributions of the oocytes is clearly separated from the others; this mode can be clearly distinguished from the other three when it reaches an average diameter of 300 μ. They have then calculated the percentage of females which show this advanced mode by GI[1] class; the "critical GI value" (which indicates spawning imminence) has then been defined by these authors as the value for which 100% of the females show this advanced mode. The critical GI value determined in this way is 35.

Purpose of the study

In the present study our purpose was to:

(1) verify the reliability of the GI, with respect to the sexual maturation of skipjack females, comparing it to an objective parameter which indicates the maturity stage; the parameter used is the modal size of the most advanced group of oocytes found in the gonads, and not any maturity stage determined macroscopically;

(2) describe the mathematical relationship between the GI and these oocytes, and thus the maturity stage; and

(3) ensure the pertinence of, and understand the significance of the critical GI value as defined and determined by Cayré and Farrugio (1983).

Material and methods

Biological material

The gonads of 443 female *Katsuwonus pelamis* caught in the East Tropical Atlantic (between Lat. 20°N and 10°S, east of Long. 30°W) were sampled and weighed to within 0.1 g. Samples of gonad tissue were taken from the median part of the gonads and put in Gilson liquid to free the oocytes.

The measurements of the diameter of approximately 200 oocytes per sample were made using an ocular micrometer mounted on a binocular lens. When maturing oocytes of any size greater than 100 microns were observed in a sample, these 200 measurements per sample do not include the measurements of the small (i.e. diameter $< 100\,\mu$), non advancing oocytes, which constitute the reserve stock of oocyte cells.

The GIs were calculated according to the formula previously mentioned.

Determination of the diameter of the largest oocyte group

Instead of trying to determine the modal size of the group of the most advanced oocytes, we preferred, like Schaefer and Orange (1956), to determine the diameter corresponding to the 95th percentile of the size frequency distribution for the totality of the oocytes in a sample; the size of the most advanced group of oocytes will thus be expressed by this diameter, d_{95}, which in fact is the lower size limit for the 5% group of the biggest oocytes in a sample.

At this point it is important to emphasize that there is a shrinkage of the oocytes due to the Gilson liquid; all oocyte sizes given in this study from here on do not take this fact into account.

In order to obtain the real size of an oocyte before having been placed in the Gilson liquid, the formula calculated by Cayré (1981) concerning skipjack should be used:

$$d = 0.776\, d_0 + 15.788,$$

where d = diameter (in microns) of the oocyte after 15 to 30 d in the Gilson liquid and d_0 = diameter of the "fresh" oocytes before being placed in the Gilson liquid.

Mathematical relationship between GI and diameter of the largest oocytes (d_{95})

We chose model (1), of which a detailed description can be found in Tomassone *et al.* (1982), because it takes into account the diverse aspects of the relation between GI and the diameter of the most advanced group of oocytes mentioned previously (i.e. two parts with different relationships, separated at the "GI critical value" point C).

$$\text{Model (1):}\quad \begin{array}{l} d_{95k} = b_1\,GI_k + a_1 + \varepsilon_k \text{ when } GI_k \leqq C \\ d_{95k} = b_2\,GI_k + a_2 + \varepsilon_k \text{ when } GI_k > C, \end{array}$$

where d_{95k} = diameter corresponding to the 95th percentile of the size frequency distribution of oocytes for the kth fish; b_1 and b_2 = slope values of the linear equations; a_1 and a_2 = origin intercepts of the linear equations; C = Gonad Index critical value; and k = residual deviation from the model for the kth fish.

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[1] $G.I. = \frac{G.W.}{L^3} \cdot 10^5$;

G.W. = Gonad weight (g)
L = Fork length (cm)

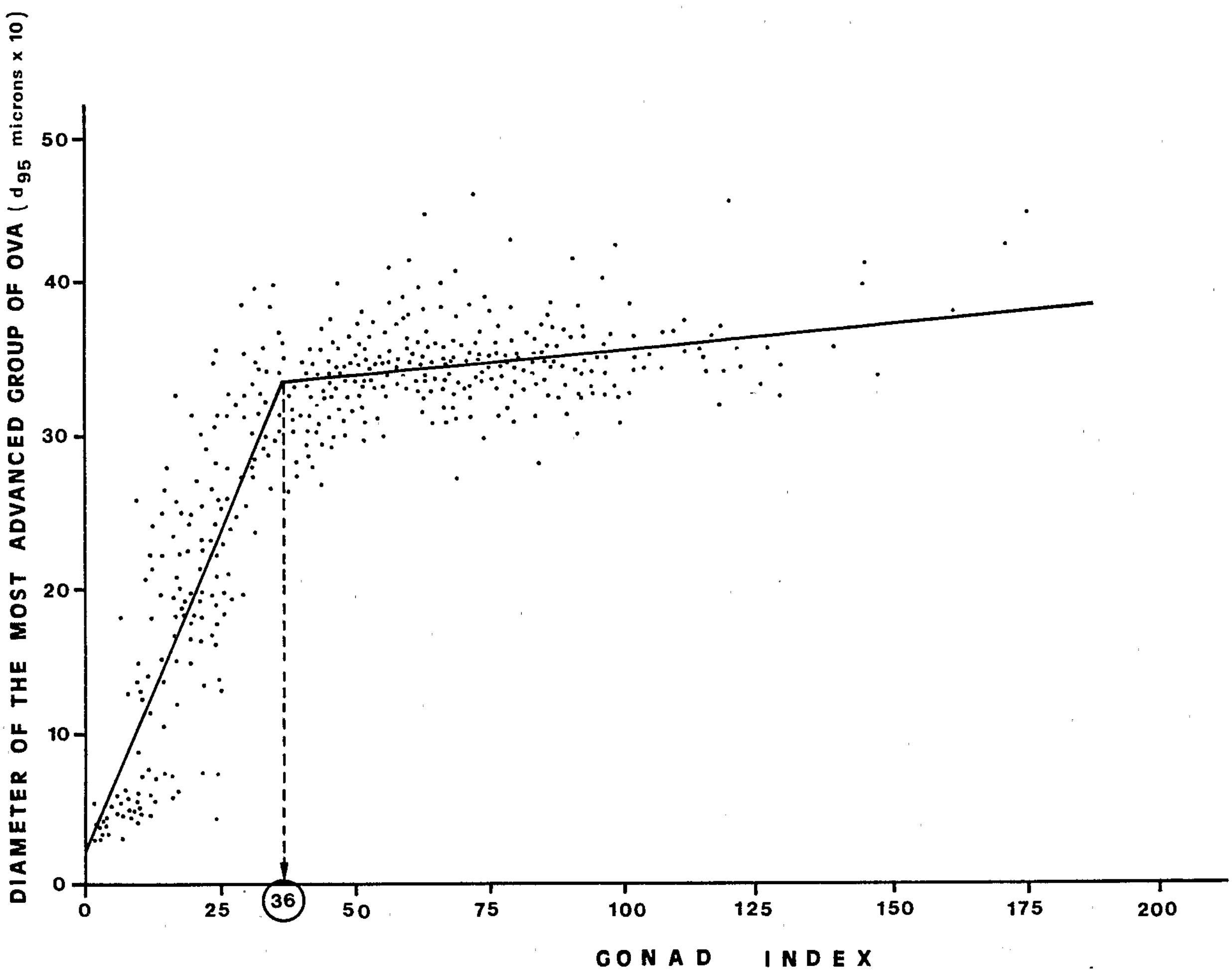

Fig. 1. *Katsuwonus pelamis.* Relationship between Gonad Index (GI) and the diameter of the most advanced oocytes (d_{95}, see text)

In order to make the function: $d_{95} = f(GI)$ continuous, the equality: $b_1C + a_1 = b_2C + a_2$ has been imposed. All parameters of model (1) are estimated by fitting the data with the least squares method using the GENSTAT[2] statistical package on an IBM 4331 computer. This method is a modified Newthon method (Ross, 1970).

After studying the distribution of the residuals, we carried out another adjustment of model (1); this time we minimized the sum:

$$\sum_{k=1}^{k=449} W_k^2 \cdot \varepsilon_k^2,$$

where W_k = is a raising factor.

The efficiency of model (1) in describing the relationship GI-d_{95} was evaluated using analysis of variance, with an effect of GI:

$$d_{95ik} = d_{95i.} + \varepsilon_{ik}.$$

The index i corresponds to the entire part of the GI values, with this index varying from 1 to 175 in our sample.

The index k designates each fish and $d_{95i.}$ is the mean value of the d_{95} for each given entire value of GI (from 1 to 175).

The deviation, ε_{ik} is then estimated by the difference between the d_{95} of an individual (k) and the average of the d_{95} of all the individuals having the same GI and noted $d_{95i.}$.

Results

GI value and maturity stage

The shape of the plotted points of GI–diameter of the biggest oocytes leads us to note first of all, as Yoshida (1966)[3], that:

– for a range of maximal oocyte sizes (d_{95}) between 300 and 500 μ the GI varies randomly between values as different as 30 and 175 (Fig. 1);

– beyond a value close to 30 the GI is no longer characteristic of different maturity stages.

[2] GENSTAT: A general statistical program (Rothamsted Experimental Station, Harpenden Hertfordshire, AL5 2 J Q, UK)

[3] This author was studying the comparisons between GI values and diameters of the largest oocytes sampled from skipjack in the central Pacific

On the contrary, it can be noted that the GI and the oocyte diameters show a linear relationship (for which we will give the equation later) for values of d_{95} smaller than 300 μ (or GI < 30) (see next section). It should, however, be pointed out that there is a strong variability in the GI for a given maturity stage (or a d_{95}) corresponding to these small sized oocytes.

Adjustment GI-d_{95}

The results from the adjustment by least squares ($\Sigma \varepsilon_k^2$) using model (1) are shown in Table 1 A. It can be noted in particular that the separation value of the GI (called C) is 36.008.

The statistics described (Table 1 A) suppose that the residuals, ε_k, are distributed according to normal independent distributions.

It can be seen from the graph of the residuals (Fig. 2A), that small GI values correspond to a wider dispersion of the residuals. It can accordingly be noted that the variance of the residuals corresponding to the 156 fish with a GI < 36 is 37.86, while that corresponding to the other 287 fish (whose GI > 36) is only 9.40. The ratio of these two values (4.03), under the hypothesis of equal variance, should approximatively follow Fisher's law: $F_{155,286}$; this hypothesis can be rejected with an error risk < 1%. It therefore shows that, for fishes whose GI < 36, there is a strong variability in the maximum oocyte size (d_{95}), and thus of the maturity stage corresponding to any value of the GI.

Table 1A. Adjustment of model (1), $D_{95}=B_1$ (IGS)$+A_1$ if IGS < C or $D_{95}=B_2$ (IGS)$+A_2$ if IGS > C, with a minimization of the residual sum of squares without application of a weighting factor

Parameters	Estimated value	SD	Correlation factor			
A_1	1.575	0.83	1.00			
B_1	0.890	0.04	−0.91	1.00		
B_2	0.036	0.01	−0.00	0.00	1.00	
C	36.008	0.99	0.49	0.74	−0.42	1.00
	d.f.	S.S.	M.S.			
Residual	439	8 558	19.49			

Table 1B. Adjustment of the model (1) after minimization of the weighted residual sum of squares (see text)

Parameters	Estimated value	SD	Correlation factor			
A_1	1.578	1.17	1.00			
B_1	0.890	0.05	−0.91	1.00		
B_2	0.036	0.01	−0.00	0.00	1.00	
C	36.010	1.23	0.55	−0.83	−0.24	1.000
	d.f.	S.S.	M.S.			
Residual	439	441	1.00			

The results from the adjustments of the model made by minimizing the squares:

$$\sum_{k=1}^{442} W_k^2 \cdot \varepsilon_k'^2 \quad \text{with} \quad W_k^2 = \frac{1}{37.86}, \quad \text{if } GI_k \leqq C$$

$$W_k^2 = \frac{1}{9.40}, \quad \text{if } GI_k > C$$

are shown in Table 1 B; the graph of the residuals as a function of the GI has been plotted (Fig. 2 B).

The fact that the parameters obtained from the two different adjustments (Tables 1 A and 1 B) are found to be very similar points out the robustness of the model.

Because we chose model (1) we wished to test its ability to reflect correctly the relationship between GI and d_{95} through analysis of variance. The average residual square obtained from this analysis of variance was 21.34, which is superior to that obtained from the adjustment of model (1), which was 19.49. Model (1), which was the model adopted here, therefore satisfactorily represents the relationship between GI and d_{95}.

Discussion

Justification of the choice of model

Several models of type: $d_{95}=f(GI)+\varepsilon$, could describe the relationship GI–d_{95}. We have chosen model (1), since it presents the ability to estimate directly the value of GI which corresponds to the breaking point for the set of pairs of values GI– size of the largest oocytes (d_{95}). Moreover, the comparison with the analysis of variance clearly indicates that the model chosen well fits the observed data. In any case we considered the breaking point C, estimated by the model, as a biological characteristic of the species involved here; this does not imply that other models would not describe the relation between GI and d_{95} as satisfactorily, but they would define the breaking point very poorly.

Comparison with previous results

The value (36) of the GI, which corresponds to the breaking point for the set of points in two different parts, was calculated using the objective criteria of the diameter of the 5% of the most advanced oocytes. This method, which is undoubtedly more objective than that used by Cayré and Farrugio (1983) for the determination of a critical GI value, is in fact not very different in principle; these authors have in fact taken as reference the females which are at an advanced stage of sexual maturation close to the spawning stage; these females were characterized by the presence of a last group of oocytes clearly separated from the others in the size frequency distributions of the maturing oocytes. It thus appears that the GI breaking point value calculated here and the critical GI value

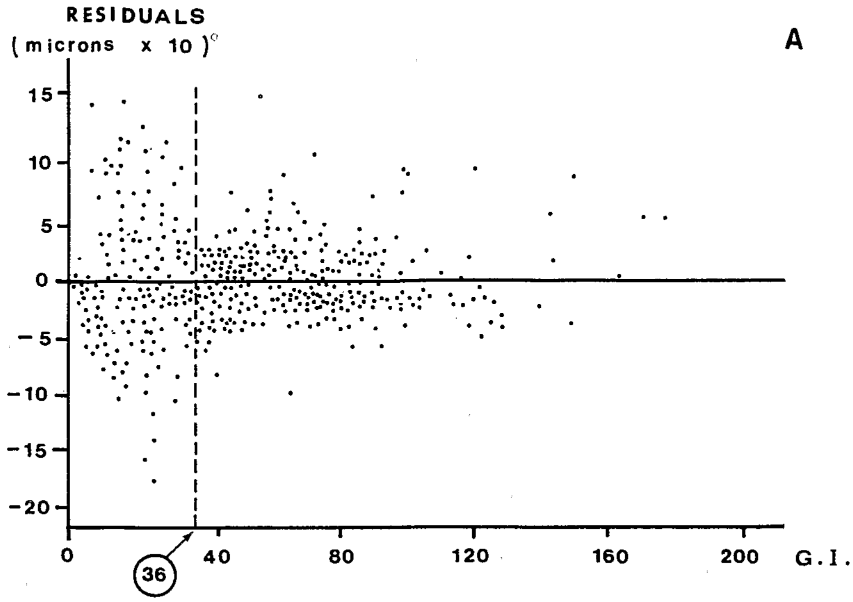

Fig. 2A. Repartition of the residuals after adjustment of the model by the least squares method (without application of a weighting factor), as a function of the Gonad Index (GI) values

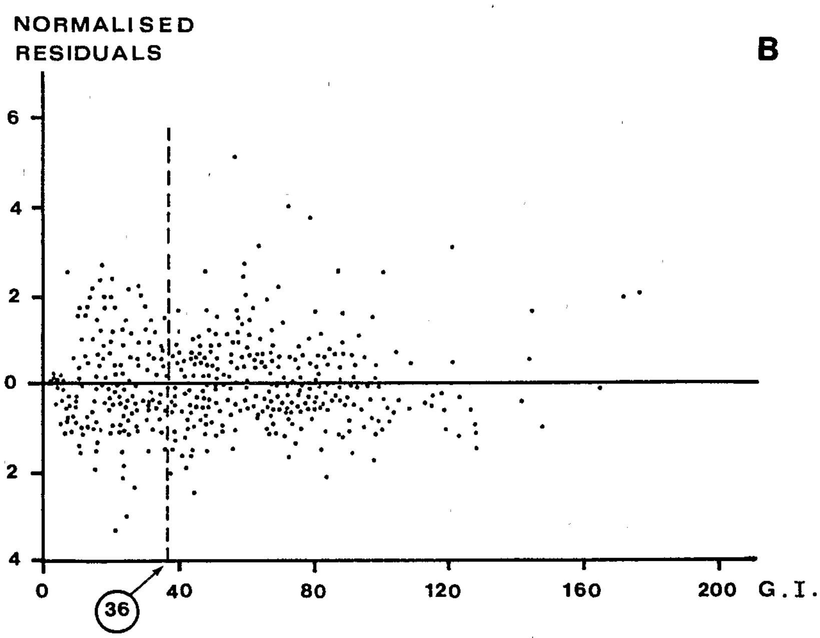

Fig. 2B. Repartition of the residuals after adjustment of the model by the "weighted" least squares method, as a function of the Gonad Index (GI) values

calculated by these authors, have the same meaning; although it has been calculated by a more objective method here, the GI critical value (36) found is similar to that calculated by Cayré and Farrugio (i.e. 35); this similarity of results tends to prove the biological reality and the reliability of the GI critical value.

The critical value that we have chosen to express in terms of GI could very well have been expressed in terms of value of d_{95} (here 336 μ); the more current use, the ease of calculation and the practical aspect of the use of the GI value have motivated this choice.

Relationship between GI and diameter of the most advanced group of oocytes (d_{95}) for advanced maturity stages

It can be noted (Fig. 1) that for a GI value > 36, calculated here (or of an oocyte size corresponding to 336 μ), the relationship between GI and d_{95} tends to be a straight line with a slope nearly equal to zero. The use of d_{95} instead of the actually observed modal value for the diameter of the most advanced oocytes can, at least partially, explain this fact. In fact, it can be noted that, when random measurements of oocytes in a sample are made, the oocytes which correspond to the most advanced mode represent a small proportion of the total number of oocytes of all sizes; if this proportion were smaller than 5%, that would be enough to have oocytes (with sizes well inferior to those of the last mode of oocytes) included in the calculation of d_{95} that do not really belong to the last mode; in this case the d_{95} value would systematically underestimate the modal size of the last group of advancing oocytes.

As a verification we redid our calculations using the value of d_{98}, which corresponds to the lower limit of the two percent of the largest oocytes. In this case the model would be:

$$d_{98} = a'_1 + b'_1\,GI + \varepsilon_k;\ \text{if } GI \leqq C'$$
$$d_{98} = a'_2 + b'_2\,GI + \varepsilon_k;\ \text{if } GI > C'$$

where $a'_1 + b'_1 C' = a'_2 + b'_2 C'$. The parameters estimated in this case are:

$$C' = 35.81;\ a'_1 = 2.36;\ b'_1 = 0.91 \text{ and } b'_2 = 0.03$$

These parameters are not different from those obtained using d_{95} (Table 1A), and do not modify the shape of the relationship GI-diameter of the largest oocytes.

The fact that the GI beyond a value of 36 seems to grow without any apparent relation to the size of the biggest oocytes could also be explained by the fact that these big oocytes, which are more fragile than the others, might be destroyed during the different manipulations (stirring in Gilson liquid) preceding the measurements. However, this does not seem to have been proved as yet.

If we exclude the hypothesis of the destruction of the biggest oocytes, and since the use of the parameters d_{98} does not modify the conclusions obtained using d_{95}, it still has to be explained why the GI can grow so much beyond a certain value (36) without a corresponding growth in the size of the oocytes. Without being able to give a precise response to this question since no supplementary research has been carried out, we can nevertheless try to suggest an explanation:

– the GI could not "only" express the increase in the size of the oocytes, but also a number of other biological phenomena which are directly related to sexual maturity; these phenomena, which could provoke an increase in the gonad weight without any relationship to the size of the largest oocytes, would explain the increase noted in the GI.

However, it is obviously by histological studies of the gonad tissue itself and/or of the oocytes that it will be possible to find the precise biological explanation of the changes observed in the relationship between GI and oocytes sizes.

The GI could therefore prove to be a possibly accurate, but difficult to interpret, index of the sexual maturity and of the numerous phenomena and their materialization related to sexual maturation; trying to evaluate the maturation process by only one parameter, although precise (for example the size of the oocytes) could lead to an inaccurate caricature. We can thus question the ability of the oocyte size to translate the sexual maturation (in a broad sense of the term) reliably.

Conclusions

The observation of the relationship between GI and diameter of the largest oocytes shows that beyond a certain maturity stage (in particular for *Katsuwonus pelamis* with GI values $\geqq 36$), it becomes unrealistic to try to distinguish several maturity stages whose definitions are based on the diameter of the largest oocytes. The process of sexual maturation, which is very rapid for skipjack, supports this point of view and this new concept of the relationship between GI and oocytes diameter.

Sexual maturation is a more or less continuous phenomenon, whose complexity is difficult to comprehend from the measurement of only one of its factors (for example: size of the oocytes); the gonad index (GI) could in fact give a true indication of the multiple phenomena which together constitute what we mean by the general term of sexual maturation. It can therefore not be expected that such an index exactly translates maturity stages defined as a function of only one parameter, or expression, of the maturation phenomenon. The strong variability of the GI values within each stage, and the overlapping of their values from one stage to another, tend to prove the arbitrary aspect of the imposed limits for each of these maturation stages.

It is then clear that the idea of the "critical index" and the determination of its value are interesting for those species which present the same type of relationship (maturity index, size of the oocytes or any other maturity parameter) as the skipjack. The "critical value" of the GI permits one to distinguish the individuals which are ready for reproduction from those in a resting stage of maturation, beginning of maturation or post-spawning stage.

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Date of final manuscript acceptance: September 16, 1985.
Communicated by O. Kinne, Oldendorf/Luhe