Abstract.—Gonad weights and results of histological analyses from 85 swordfish, *Xiphias gladius*, were used to develop a validated method for classification of the reproductive activity of female swordfish based on gonad indices (GI's). The validated method provides a significant improvement over previously published (unvalidated) methods. The method was shown to be independent of the length of individual fish, important when length is used as a criterion for selection of individuals from which summary statistics based on GI are being developed. Female swordfish were found to be in a reproductively active condition when GI = ln(gonad weight in gm)/ln(eye-fork-length in cm) ≥ 1.375. Classification methods for species with comparable reproductive habits and characteristics may be alike, and it is speculated that results for other billfishes would be similar to those described for swordfish.

Use of gonad indices to estimate the status of reproductive activity of female swordfish, *Xiphias gladius*: a validated classification method

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We describe a validated classification method that uses gonad indices (GI's) to determine accurately the reproductive condition of female swordfish, *Xiphias gladius*. This study uses previously unpublished data as well as histological analyses detailed in Taylor and Murphy's (1992) study of the reproductive biology of swordfish captured in the Straits of Florida. It is standard practice to use GI's to identify regions and times of active spawning in studies of the distribution and structure of stocks of many species of fish, including swordfish (e.g. Kume and Joseph, 1969; Shingu et al., 1974; Miyabe and Bayliff, 1987; Sosa-Nishizaki, 1990; Nakano and Bayliff, 1992; Arocha and Lee, 1993; Arocha et al., 1994; Gouveia and Mejuto, 1994; Arocha and Lee, 1995; Hinton and Deriso, in press). Data on the reproductive activity of swordfish are costly and difficult to obtain but essential to studies such as those noted; full use should be made of all available information. Our classification method overcomes problems of published methods (e.g. Miyabe and Bayliff, 1987), which have the potential to reduce the information database of the researcher by over 50%. To our knowledge, it is the first method applicable to female swordfish to have been validated with data obtained from histological analyses, which provide a verifiable measure of the reproductive status of individual swordfish.

The standard practice (Gouveia and Mejuto, 1994) in studies using values of GI for female swordfish has been to estimate $GI = 10^4 \times GW/EFL^3$, where GW = gonad weight in grams, and EFL = length from the posterior edge of the orbit to the fork of the tail in centimeters (we note that without loss of generality, other length measurements, such as lower-jaw fork length (LJFL), have been used) (Kume and Joseph, 1969). The latter assumed that "[females] with gonad indices equal to or greater than 3 are about to spawn." Miyabe and Bayliff (1987) modified their method by assuming that "only females with gonad indices of 7.0 or greater were [about to spawn]." Arocha and Lee (1995) modified the method of Kume and Joseph (1969) when they noted that females with GI's greater than 4.0 were in prespawning condition. In certain applications of these methods, e.g. comparison of average GI's for different regions or time periods,

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it is necessary to ensure that the averages being compared are for individuals with comparable reproductive potential or maturity. Thus, it is standard practice to use a minimum length (e.g. Miyabe and Bayliff, 1987; Sosa-Nishizaki, 1990; Nakano and Bayliff, 1992, Arocha et al., 1994; Arocha and Lee, 1995) to decide which data to include in estimates of average values of GI, making it important to document that minimum-length criteria have no impact on methods used to estimate reproductive status (Cayré and Laloë, 1986).

Data and methods

Details on data collection and histological analyses, other than estimation of the values of individual gonad indices, may be found in Taylor and Murphy (1992). Female swordfish were assigned to eight developmental classes (Murphy and Taylor, 1990) based on the appearance of histological features (Wallace and Selman, 1981). These classes and mean observed oocyte diameters were 1) immature, < 20 µmm; 2) developing, 71 µmm; 3) maturing, 160 µmm; 4) mature, 434 µmm, 5) gravid, 723 µmm; 6) spawning or partially spent, 823 µmm; and 7) spent, 181 µmm. Individuals in class 8 (recovering) were observed but not described in Taylor and Murphy (1992). Gonads of swordfish in class 8 exhibited signs of having spawned in the previous season and were undergoing maturational, prespawning development for subsequent reproductive efforts.

The preferred formulation for GI may be determined by examining the relation of gonad weight to measures of body size (de Vlaming et al., 1982). The formulation chosen should meet the underlying assumptions (de Vlaming et al., 1982) for use of GI as an index of reproductive status. In addition to examining the previously described "standard" expression of GI (hereafter referred to as GI(1)), we examined $GI = \ln(GW)/\ln(EFL)$, hereafter referred to as GI(2), and $GI = GW/EFL$. Stepwise analysis of covariance (ANCOVA) was used to examine how well these formulations for GI met the underlying assumptions (de Vlaming et al., 1982) for use of GI as an index of the reproductive status of female swordfish. Values of GI were determined for the fish for which histological data had been obtained. For those individuals for which measurements of EFL were not obtained, measurements of LJFL were used to estimate EFL as follows: $EFL = -8.259 + 0.930 \times LJFL$ [n=316, r^2=0.996, P<0.001] (Taylor and Murphy, 1992). Of the over 400 fish examined by Taylor and Murphy (1992), there were 85 individuals (Table 1) with measurements (40) or estimates (45) of EFL ranging from 73 to 253 cm, for which there were GW's and data from

Table 1

The status of reproductive activity (R) of swordfish determined by histological analyses [Taylor and Murphy, 1992]), EFL = eye fork length (cm), and GW = gonad weight (gm).

R	GW	EFL	R	GW	EFL	R	GW	EFL	R	GW	EFL
2	3	77	2	100	113	3	752	169	6	8,740	221
2	13	86	2	110	147	4	530	161	6	8,840	208
2	14	88	2	135	115	4	780	182	6	9,920	206
2	15	94	2	140	118	4	800	187	6	10,180	188
2	30	96	2	140	128	4	1,320	186	6	10,430	227
2	30	95	2	150	121	4	2,140	219	6	11,340	223
2	30	92	2	220	93	4	2,888	201	6	15,140	253
2	35	97	2	225	73	5	2,690	169	8	446	167
2	35	99	2	255	148	5	3,950	184	8	540	172
2	35	106	3	100	121	6	1,240	181	8	600	166
2	35	106	3	105	130	6	1,540	202	8	640	164
2	37	101	3	110	123	6	1,760	174	8	730	183
2	45	114	3	200	137	6	2,270	182	8	800	190
2	50	99	3	240	139	6	3,650	181	8	980	179
2	50	96	3	300	168	6	3,780	171	8	995	197
2	55	104	3	353	166	6	3,850	208	8	1,325	200
2	70	103	3	437	120	6	3,900	155	8	1,340	222
2	70	105	3	470	184	6	4,000	180	8	1,360	249
2	80	104	3	500	181	6	4,700	191	8	1,500	211
2	80	112	3	620	185	6	4,720	197	8	1,790	191
2	90	113	3	680	186	6	6,034	154			
2	100	128	3	680	174						

histological analyses. According to the results of the histological analyses, swordfish were considered to be in spawning condition, i.e. spawning was in progress or imminent in the region in which the fish was captured, if they were either "gravid" or "spawning or partially spent" (classes 5 and 6 of Taylor and Murphy [1992]). Individuals in these conditions were classified as in an active (A) reproductive status. All others were classified as quiescent (Q).

In our study the results of histological analyses represent the known reproductive status of individual swordfish, and the H(i) are various hypothesized classification methods (Table 2) for placing swordfish into categories A or Q. In some cases, these H(i) indicate minimum-length criteria used to determine which data from individual swordfish should be included in estimates of average values of GI. To facilitate the examination of impacts of minimum-length criteria on classification methods, these criteria were treated as a component of the H(i) in our analyses. We do not attempt to define minimum-length criteria for size at maturity in this study (cf. Taylor and Murphy [1992]); our concern was to determine if classification methods based on GI were independent of such criteria. The hypotheses identified in Table 2 as "Present study" are representative of a multitude of hypotheses we examined.

The optimum value (OV) and confidence intervals for the value of GI to be used as criteria, GI*, to estimate the reproductive condition of individual female swordfish were determined by using maximum-likelihood estimation procedures and the following model:

Let $R = 1$ if a swordfish is reproductively active (classes 5 and 6 of Taylor and Murphy [1992]), otherwise $R = 0$. Then for individual fish, i, selected at random,

P (a swordfish is reproductively given its gonad index) $= \pi(GI)^R \times (1 - \pi(GI))^{(1-R)}$.

Maximum-likelihood estimates of $\pi(GI)$ are given by the number of successes in the series of trials determined by the value of GI^* in the data, divided by the number of trials. Thus, for our data set: $[GI_1, ..., GI_k^*, ..., GI_n]$ and $[R_1, ..., R_k, ..., R_n]$ = [the observed values of GI] and [the respective measure of reproductive status determined by histological analyses] for individual fish, these estimates are given by

$$\pi(GI) = \begin{cases} \dfrac{(\text{number of individuals with } R = 1 \text{ and } GI < GI_k^*)}{k-1} & \text{for individuals with } GI < GI_k^* \\ \dfrac{(\text{number of individuals with } R = 1 \text{ and } GI \geq GI_k^*)}{n-k+1} & \text{for all others} \end{cases}$$

It follows that the optimum value, GI^*, is that which maximizes the following log-likelihood function ($LKLHD$):

$$LKLHD = \sum\left[R \times \ln(\pi(GI)) + (1-R) \times \ln(1-\pi(GI))\right]$$

Results and discussion

Results of classifying individuals as either A or Q based on the results of the histological analyses and from application of the various H(i) (treated in each test as the null hypothesis) are given in Table 3. It is clear that H(2) and H(3) fail to classify individuals correctly according to reproductive status as determined by histological analyses. Under H(2) and H(3), individuals below the minimum-length criteria are not classified. About 75% of the individuals whose lengths were above the size restrictions stated in these hypotheses were classified correctly, but only 48% of the individuals in this group that were reproductively active were correctly classified which represents a significant type-1 error that may be extremely costly in terms of loss of information on the distributions of reproductively active swordfish. However, this is a result of the value of GI included in the hypotheses and not a result of restrictions placed on lengths of individuals included in the analyses, as is evidenced by the results for the other H(i). We also note that length was not found to be a signifi-

Table 2

Levels of gonad indices (GI) used to classify the reproductive activity of female swordfish and minimum eye fork length (EFL) criteria used to standardize statistics for comparison among areas and times, as employed by various researchers. Formulations for GI(1) and GI(2) are given in the text.

H(i)	Classification method	Author
1	GI(1) ≥ 3.0	Kume and Joseph
2	GI(1) ≥ 7.0 and EFL > 150 cm	Miyabe and Bayliff
3	GI(1) ≥ 7.0 and EFL > 160 cm	Sosa-Nishizaki
4	GI(1) ≥ 4.0 and EFL > 131 cm[1]	Arocha and Lee
5	GI(1) ≥ 6.0	Present study
6	GI(2) ≥ 1.37	Present study

[1] Authors used lower-jaw fork length >150 cm.

Table 3

Comparison between the correct (from histological analyses [HA] of the ovaries) and estimated (from GI's) classification of reproductive status of female swordfish. Individuals were classified as reproductively active (A) or quiescent (Q). Asterisks designate incorrect classifications based on GI's, IC is the percentage of all individuals [n determined by H(i)] classified correctly, and AC is the percentage of reproductively active individuals, within the n individuals, that were classified correctly.

H(i)	n	HA	GI A	GI Q	IC	AC
1	85	A	19	2*	95.3	90.5
		Q	2*	62		
2	48	A	10	11*	77.1	47.6
		Q	0*	27		
3	46	A	10	11*	76.1	47.6
		Q	0*	25		
4	52	A	17	4*	92.3	81.0
		Q	0*	31		
5	85	A	15	6*	92.9	71.4
		Q	0*	64		
6	85	A	21	0*	95.3	100.0
		Q	4*	60		

cant term in logistic regressions that included EFL as a classification variable.

Under H(1) and H(6), 95% of the 85 individuals were classified correctly (Table 3); further, under H(6) all individuals that were reproductively active were correctly classified, which was significantly (see following discussion) more than the 91% of the reproductively-active individuals correctly classified under H(1) and the 71% to 81% correctly classified under H(5) and H(4), respectively. Hypothesis H(6) placed about 4.7% of the individuals that were quiescent in the active category, and H(1), about 2.5%, both of which represent relatively low rates of type-2 error.

Although length-cubed is often the choice to standardize GW, as in the "standard" expression for GI, length is also frequently used. Further, GW may be exponentially related to body size (de Vlaming et al., 1982), in which case log transformation as in GI(2) is indicated. We examined these hypotheses in formulations of GI for female swordfish. The results of ANCOVA revealed significant (P<0.01) heterogeneity among slopes and intercepts of the regressions of GW on EFL and on EFL^3 for reproductive classes (2, 3, 4, [5, 6] and 8) of Taylor and Murphy (1992). At the same time, ANCOVA on the log-transformed data yielded only one significant (P<0.01) coefficient, that for the intercept of class (5, 6); however this coefficient was only about 28% of the estimated intercept of the overall regression. Thus, the formulation of the gonad index that best conformed to the underlying assumptions (de Vlaming et al., 1982) was GI(2). In addition, the maximum-likelihood test based on the values of LKLHD for the difference between methods showed that model GI(2) provided a significant ($\chi^2(1)$, P=0.033) improvement over model GI(1). The estimate of OV obtained from maximum-likelihood analyses for $GI(2)$ was ($1.366 < \text{OV} < 1.375$). Note that although GI(2) has a continuous distribution, the interval estimate of OV is a function of the distribution of values of GI(2) in the sample data, and thus any hypothesized value in this range would yield LKLHD and tabled results identical to those shown for H(6). Further, because the solution for the function LKLHD is so knife-edged, the estimate of OV includes the 90% confidence interval, and the 95% confidence interval for the estimate of OV, ($1.357 < \text{OV} < 1.375$), differs only in the lower bound.

Two points should now be clear. First, the classification methods that are based on GI(1) that have been used and published in studies requiring estimates of the reproductive status of female swordfish do not meet the underlying assumptions (de Vlaming et al., 1982) for use as an indicator of reproductive status, and they may also be viewed in some instances as overly restrictive, in that they may have excluded significant amounts of usable data from analyses that were already hampered by limited information. This resulted, at least in part, from using values of GI that corresponded to a fully ripe and running condition of the gonad. Second, the classification methods, both previously published and described herein, were not impacted by minimum-length criteria which may be required to standardize comparisons of statistics that are based on gonad indices.

Our results are conservative in the following respect. We have no knowledge of the frequency of spawning of female swordfish. Thus, because hydration of eggs may occur over a very short period of time, by not including individuals in class 4 ("mature ovaries" of Taylor and Murphy [1992]), some individuals that might be expected to spawn within a short period of time, and thus presumably within the general area of capture, may be excluded from consideration. The question of whether to include these individuals as reproductively active could be addressed by conducting a study of spawning frequency of female swordfish based on the condition of yolk development in the eggs, as has been done for yellowfin tuna, *Thunnus albacares* (Schaefer, 1996). Alternatively, it may be possible to determine whether or not class-4 individuals should be included by estimating the distribution of spawning using both a classification scheme that considers these individuals as reproductively active, and the classification developed herein, with subsequent testing by comparison of these distributions to other measures of spawning activity, such as distributions of larval fish or of male:female ratios.

Given the interval estimate for OV, and taking a conservative approach with respect to including individuals that are not reproductively active in estimates of the spatial and temporal distributions of spawning, we recommend that researchers requiring an estimate of the reproductive status of female swordfish adopt a method that classifies reproductively active female swordfish as those for which *GI* = ln(GW)/ln(EFL) ≥ 1.375, the upper limit of the interval. When additional information becomes available, further analyses should be undertaken.

The need to develop species-specific classification methodologies has been clearly documented (de Vlaming et al., 1982). However, methods for species with similar characteristics and reproductive habits may be similar (Cayré and Laloë, 1986). Merrett (1970) found that for sailfish (*Istiophorus platypterus*); striped (*Tetrapturus audax*), blue (*T. nigricans*), and black (*T. indica*) marlin; and spearfish (*T. angustirostris*), changes in ovaries through oogenic cycles were similar in all species, as was the shape of the gonads (with the exception of the shape of the spearfish gonad, which was Y-shaped rather than bilaterally symmetrical). These changes are similar to those observed in swordfish (cf. Taylor and Murphy, 1992). Thus, while we concur with de Vlaming et al. (1982) and strongly recommend that classification methods be developed and validated for each species of billfish, we would speculate that results for these species would be comparable to those shown herein.

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