Biological Information on Target Species: Sex-Determination, Maturation Stages and Gravimetric Indexes

M. Gristina*, G. Morizzo, G. Bono and S. Ragonese

Abstract

Procedures for collecting data and information on the reproductive biology of target species taken during trawl surveys implemented in the framework of the new Italian Fishing Plan and the FAO MedSudMed Project is proposed. These procedures cover sampling, sex-determination, analysis of gravimetric indexes, and the identification of the maturation stage of individual organisms. The maturation stage is assessed through a macroscopic examination of the reproductive system and a synoptic scale is presented for bony and cartilaginous fishes, crustaceans and cephalopods.

Introduction

Analysing the reproductive biology is a crucial step in assessing the dynamics of any exploited populations (stocks). In fishery science, in particular, the monitoring of gonad development and cyclic changes in the maturation stages is normally used to determine the maturity ogive by age and size, the size at onset of reproduction ("first maturity"), the spawning period and areas. This information is essential both for further assessments (for example, estimation of spawning stocks) and for setting management regulations, such as "minimum catchable size" or "area/seasonal closure".

Materials and methods

Sampling

For each haul and target species, all specimens (or a sub-sample thereof in the event of a large catch) are collected. Sub-samples are gathered for special processing, such as histological analysis.

It is preferable to manipulate the samples when fresh (for measurement or for extraction of gonad samples and conservation), directly on board. Conversely, the material is deep-frozen (–40°C) for 24 h and subsequently stored (–18°C) on board, and later defrosted (overnight) and processed in the laboratory.

One female and one male (where foreseen) are picked from each haul catch by the following size-classes (in millimeters): <10, 10–20, >20 for bony and cartilaginous fishes (total length; TL), cephalopods (mantle length; ML) and crustacean decapods (carapace length; CL).

Sex-determination

Each sampled specimen is classified macroscopically (i.e. by naked eye) into one of three categories: Females (F; 1), Males (M; 2), and Indeterminate (I; 3). In case of hermaphrodites,

* MaLiRA-Group, CNR-IAMC, Via L. Vaccara 61, 91026 Mazara del Vallo TP(Italy); Tel.: 0923-948966; Fax: 0923-906634; michele.gristina@irma.pa.cnr.it

the sex is assigned on the basis of the predominant gonad. Unsexed specimens (U; 4) should be employed to characterize specimens for which the assignation of sex was not possible for various reasons (for example, whole specimen measured at the market). Particularly:

Bony fishes – Specimens are dissected in order to expose the internal body cavity and appreciate the shape and appearance of the gonads.

Cartilaginous fishes – Males are distinguished from females on the basis of the external presence of copulatory organs (pterygopods).

Crustacean decapods – The petasma (2nd pleopod) and thelycum (4th sternite) indicate males and females, respectively.

Cephalopods – Specimens are dissected in order to expose the internal body cavity and appreciate the shape and appearance of the gonads.

Maturation stages

The number of maturation stages on macroscopic keys varies from two (immature–mature) to more than fourteen. For monitoring surveys, four or five (4/5) stages (including "0" for the Indeterminate, as previously defined) can be considered acceptable. Two kinds of maturity scale can be considered: "universal" and "specific". In the "universal" type, the most frequently adopted scales are:

Bony fishes: 5/8-stage scale for partial/total spawners, respectively (Holden and Raitt, 1974).

Cartilaginous fishes: 4-stages for females and 3-stages for males, based on the degree of development of the pterygopods, gonads, ducts and shell glands.

Crustaceans decapods: 3/4-stage scale for females (Froglia, 1984; Ragonese, 1985), based on the colour of the gonads and the shape and the degree of development of the ovary lobes; a 3-stage scale for males (Ragonese, 1985) based on the petasma structure, rostrum length and the presence/absence of spermatic masses inside the seminal pockets (*ampullae*).

Cephalopods: 5/6-stage scale for females (Mangold, 1963; Lipinsky, 1979) and for both sexes of the Teuthoidea; 4- and 3-stage scales for the Octopoda (Ragonese, 1985) based on the appearance of the gonads, presence/absence of spermatophores in the Needham's sac (males) or inside the mantle (residuals; females), modification of the *hectocotylus* and the "relative" dimension (and appearance; "smooth" surface) of the eggs.

As concerns future planning, a general 4/5-stage scale (including "0") is proposed herein for each category (maturation stage) of organisms (Table 1). In particular:

Bony fishes: 5-stage scale (0 and from 1 to 4) based mainly on the relative volume of the gonads.

Cartilaginous fishes: 4-stage scale (0 and from 1 to 3) based mainly on the presence of embryos and on the egg dimensions (females), and on the pterygopods (males).

Crustacean decapods: 5-stage scale (0 and from 1 to 4) based on the appearance of ovary lobes (females), and 4-stage scale (0 and from 1 to 3) based mainly on the degree of fusion of

the petasma, presence/absence of the spermatic masses on seminal *ampullae* and the dimension of the rostrum (males).

Cephalopods: 5-stage scale (0 and from 1 to 4) based mainly on the degree of development of the reproductive apparatus, the dimension of the eggs and the presence of spermatozoa. Some particular attention should be given to assessing the gonad colour in most crustacean decapods (for red shrimps, for example, it is necessary to cut the dorsal part of the carapace and expose the tissue).

Gravimetric indexes

The gonad weight (GW) will be determined in sub-samples of females (to the nearest 0.1 g, for bony and cartilaginous fishes, and 0.01 g, for cephalopods and crustacean decapods), and expressed as a proportion of the "whole" body weight (BW) by computing the gonosomatic index (GSI=GW/BW). In bony fishes, the GW refers only to the well defined and predominant gonad. On the contrary, in cartilaginous fishes and cephalopods, the GW includes other reproductive organs. For the cephalopods, in particular, the GW includes the weight of the testis, Needham's sac, the spermatophoric complex and penis (in males), and nidamental and oviducal glands, accessory glands, ovary and oviducts (in females). Some particular remarks concern the determination of GW in shrimps. The extraction of such a structured ovary is very difficult, especially in small and immature females, and in any case is not routinely feasible; GSI should be determined using a sub-sample. Furthermore, the eggs extruded onto the pleopods (as is the case of the Norway lobster) are normally not included in the GW.

Other parameters

The mating condition (i.e. absence, 0, or presence, 1, of spermatophores on the thelycum) and the berried condition (absence, 0, or presence, 1, of the eggs among the pleopods) in crustacean decapods should be recorded on a separate field in the reporting form to help both in the sex-determination and in describing more precisely the reproduction status.

Table 1. Universal maturity stages by sex.

Sex	Maturity stages					Remarks
	0 Virginal	1 Immature	2 Maturing or resting	3 Mature	4 Post-spawning	
F	Impossible to determine sex by naked eye. Gonads small and translucid, almost transparent. Sex is indeterminate.	Pinkish and translucent ovary occupies about one-third of length of body cavity. Ova not visible to naked eye.	Pinkish and translucent ovary occupies about one-half of length of body cavity. Ova not visible to naked eye.	Orange-pink ovary occupies from two-thirds to full length of body cavity. Under light pressure, large transparent, ripe ova are expelled.	Ovary is shrunken to about one-half of length of body cavity. Ovary may contain remnants of darkened ova. Dormant state.	MEDITS: 4-point scale GRUND: 5-8-point scale CAMPBIOL 5-point scale
M		Whitish testis occupies about one- third of length of body cavity.	Whitish testis more or less symmetrical occupying about one-half of length of body cavity.	Whitish-creamy soft testis occupies from two-thirds to full length of body cavity. Under light pressure sperm is expelled.	Bloodshot and flabby testis is shrunken to about one-half of length of body cavity. Dormant state.	
F	Impossible to determine sex by naked eye. Sex is indeterminate.	Ovary is barely discernible, with small isodiametric eggs. Oviducts contain no eggs.	White eggs are visible in the ovary. The distal parts of oviducts (uterus) are well developed but contain no eggs.	The uterus has a sack shape and contains eggs or embryos.		GRUND: 3-point scale CAMPBIOL 3-point scale
M		Pterygopods are small and flaccid and do not reach the posterior edge of the pelvic fin.	Pterygopods are larger, and not ossified. They extend to the posterior edge of the pelvic fin.	Pterygopods extend well beyond the posterior edge of the pelvic fin and their internal structure is hard and ossified.		
F	Impossible to determine sex by naked eye. Sex is indeterminate.	Traslucid and stringy ovary, anterior and lateral lobes are poorly developed. No spermatophores on thelycum.	Ovary in the process of development. The colouring begins to be evident.	Ovary much developed, occupying all the dorsal portion. Colouring is more intense. The anterior and lateral lobes are much developed. Oocytes well visible.	Stage "Berried" for *Nephrops norvegicus:* presence of eggs on pleopods.	MEDITS: 3-point *Nephrops* scale GRUND: 3/4-point scale for female 2 point scale for males CAMPBIOL 4/5-point scale for females 2/3 point scale for males
M		Petasma is not much visible, and there are spermatic masses on seminal ampullae. Long rostrum.	Petasma appears visible and nearly or completly joined, but there are no spermatic masses in seminal ampullae. Long or intermediate rostrum.	The petasma is perfectly visible and completly joined; spermatic masses in seminal ampullae. Small rostrum.		
F	Impossible to determine sex by naked eye. Sex is indeterminate.	Small and translucid nidamental glands (NG). Stringy and translucid ovary. Eggs are very small.	Larger and opaque NG. Accessory nidamental glands (ANG) reddish coloured. Ovary with small eggs but visible to naked eye.	Very large reddish ANG and white- cream coloured NG. Bulky ovary and oviducts with many medium and big eggs. Presence of spermatophores in the mantle indicates that mating occurred.	Flaccid and less bulky NG. Smaller ovary with not many mature eggs. Oviducts contain many eggs in degenerative phase.	MEDITS: 3-point scale GRUND: 3-point scale CAMPBIOL 3-point scale On females it is important to measure the dimensions of the eggs (See specific tables in CAMPBIOL and GRUND).
M		Small and translucid testis. Spermatophoric complex (SC) not much visible. No spermatophores on Needham's sac. Deferens ducts with no spermatozoids. Starting differentiation of hectocotylus.	Larger whitish testis. Bright white SC. No or not many spermatophores on Needham's sac. Deferens ducts with spermatozoids. Hectocotylus is clearly recognizable.	Opaque big testis. Spermatophores on Needham's sac and penis. Deferens ducts are full of spermatozoa.	Long but thin testis. SC with many membranous corpuscles. No spermatophores.	

Discussion

Setting discrete categories and defining discrete reference points for continuous biological processes, such as the sexual maturation and reproduction of marine organisms, are a matter of convention. A wide variety of time- and space-scales, definitions of terms, and methods, both qualitative and quantitative, are found in the relevant technical literature and are determined by the context and the goals of different studies. In planning the routine collection of "maturity" data in a multipurpose seasonal bottom trawl survey with limited resources, however, a compromise must be found between the scientific requirements and the practical possibilities. The present proposal is incomplete and in some parts inadequate, but might allow the acquisition of information suitable for most of the available assessment procedures. The lacking measurements were, however, considered and are summarized here below.

Sex-determination

In crustacean decapods, the observation and description of the "male organ" were not considered, because the organ is too small for a macroscopic evaluation. In cephalopods, the presence/absence and development evaluation of the hectocotylus, the male arm modified for spermatophore transfer during mating, could help both in sex-determination (maturing and mature specimens) and in evaluating the adulthood condition, but the technique is prone to error and is time-consuming.

Maturation stages

The macroscopic determination of the maturation stage of a marine organism is a powerful tool for routinely monitoring the "maturation schedules" of marine organisms; whatever the scale (of the stages relevant to a given species) chosen, it is important to remember that the maturation stage assigned to a given specimen is relative to its adulthood condition. An "adult" shrimp (i.e. a shrimp that has spawned at least once) caught and analysed macroscopically in a period different from its reproductive season can, in fact, have a resting (or spent) gonad that is similar in appearance to that of a juvenile shrimp. The number of stages and their definition must, consequently, be determined in such a way as to minimize the risk of misclassification, a problem which makes the fitting of the maturation ogive at full maturity problematic. Furthermore, specific histological study remains the only way to validate the adopted macroscopic stages, whether universal or specific.

Gravimetric indexes

An estimation of the energy investment in gonad tissue, even if only rough by means of the computaion of the GSI, could be a useful tool, not only for the interpretation of the macroscopic data, but also for the establishment of the relationships between GSI and the life-cycle (for example, the link with the natural mortality, at least in bony fishes). In this context, somatic weight (SW; i.e. body weight excluding all the viscera) might have been a better alternative to body weight (BW) itself, but only the BW was considered, for practical reasons.

Other parameters

In planning trawl surveys (Bianchini *et al.,* in the present Technical Document), many other morphological and gravimetric measurements might have been considered. As examples of the morphological measurements, the most attractive would be the length of the right propodite of Norway lobster males (to figure out the transition between the juvenile and adult condition), or the length of the nidamental gland in cephalopods. As concerns gravimetric measurements, the hepato-somatic index (HSI=100LW/BW, where LW denotes the weight of

the liver) could be a precious piece of integrative information, especially for female cartilaginous fishes, since this index is often "in phase" with the corresponding GSI. Also with respect to the cartilaginous fishes, important information (especially as an aid in discriminating sub-populations) could be obtained by measuring and weighing the egg capsules and the embryos inside them.

As a matter of fact, many measurable body parts (for example, the hectocotylus length and testis diameter indexes in cephalopod males) could be relevant either to the assessment of the maturation pattern of an exploited population or to their implications for determining, for example, maximum girth in fish. Whatever the protocol adopted, however, it must be maintained unchanged until experimental evidence disproves its validity.

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