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Registration of Ectolesions of Redfish from *Sebastes* Genus in the North Atlantic (Methodical Guidelines)

by

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### Abstract

Method for studying the redfish from *Sebastes* genus in the North Atlantic, infested by *Sphyrion lumpi* and having pigmented patches on the body, is described, as well as the methods for examining fish, recording of results in a special format and for primary statistical data processing, etc. Scale of conventional maturity stages of the copepod *Sphyrion lumpi* is suggested. The method can be applied for studying other fish species infested by ectoparasites.

### **Material and Methods**

1. EXAMINATION OF FISH INFESTED BY SPHYRION LUMPI

# 1.1. Brief characteristics of the parasite

Sphyrion lumpi (Krøyer, 1845) is a representative of parasitic copepods from the Sphyriidae family. Females of the parasite, the mature specimens of which attain 10 cm length, and rarely above, settle on the fish body. The body is divided into the cephalothorax with large lateral convexities, a narrow "neck" and a broad dorsoventrally flattened trunk. Two long (to 5 cm) egg sacs, filled up with the small eggs arranged in several rows, attached to the trunk. After extrusion of the eggs by a female, the sacs fall off, the trunk shrinks, the parasite dies and also falls off. The ulcers, formed on the fish skin, are later cicatrized, and the cephalothorax, becoming brown in colour, remains in the fish body (0,8 cm in width and 2 cm in length). These remains of the parasitic copepode remain in the fish body for a long time (probably, during several years), gradually decreasing in size and resolving. In the North Atlantic, during different years *S. lumpi* occurred in 17 fish species referring to 13 genera and 8 families (Priebe, 1986; Ho, 1989; Bakay, 1989). Infestation by *S. lumpi* is of occasional pattern for the fish species mentioned. In our opinion, the exception is the redfish from *Sebastes* genus; in this case, the parasite gives absolute preference to *Sebastes mentella*, occurring over the entire area of distribution of mature *S. mentella*, i.e. from Canada to the Barents Sea. Infestation of *S. mentella*, inhabiting the Irminger Sea pelagial and the Labrador Sea adjacent area, is known to be the highest and stable.

### 1.2. Selection of samples

To adequately characterize the parasitological situation in a specific area, as our studies on *S. mentella* in the Irminger Sea showed, it is necessary to examine 2,5-3,0 thou. fish per month. In this case, it is necessary that the number of fish should be examined not at the same time, but throughout a whole month and from different catches taken at different depths. In practice, it can be done by means of parasitological analysis of 100 fish specimens taken daily from a specific trawl.

The method for selecting the parasite sample should provide a principle of random sampling. It is an admissible that only large specimens predominate in samples if the small-size fish are also available. Besides, sex ratio should also be considered in the samples, especially when the infestation rate is different for males and females (for instance, in the redfish from the Irminger Sea area). The method for a simple random sampling will allow to avoid such undesirable moments. If the catch is sufficiently large, the parasite sample of 100 fish specimens is selected and the results obtained are recorded in two special cards (form No. 1S), (50 individ. in each). If the catch consists of less than 100 individuals, all the fish are examined in it.

# 1.3. Parasitological analysis of fish

Data on infestation by *Sphyrion lumpi* and on pigmented patches in redfish are collected during standard ichthyological studies (field analysis for feeding, age sample, etc.). The 1S-form card allows to make compactly and clearly registrations of common ichthyological information in it, i.e. length, weight, sex, maturity stage, stomach fullness and food composition in it, as well as data on localization and characteristics of *S. lumpi* lesions (alive parasites, old cephalothoraxes, etc.) and pigmented patches (colour, size). The vessel's name, cruise and haul number, date, area, position, fishing depth, species of the fish and parasite examined are given in the top of the card.

Fish are thoroughly examined from both sides; alive parasites of *S. lumpi* (AP), remains of the parasite presence, frequently having a shape of "small ulcers" (U) and "old" lesions (OC), less visible on the body surface due to cicatrization of the small ulcers, are counted on it. The data are recorded in the appropriate columns AP, U and OC (form N. 1S) separately for different parts of the body according to the scheme given in the bottom of the card and in Fig.1., i.e. dorsal and tail (I), ventral (II), head (III), anus area (IV) (see example of filling up the card). To make sure of availability or absence of *S. lumpi* "old" lesions, the muscular tissue should be dissected in the area of the parasite expected localization. The muscles can be examined by means of transverse inclined dissections (6-8 mm) using a sharp knife. The total number of *S. lumpi* lesions ( $\sum AP + \sum U + \sum OC$ ), found in the fish examined, are recorded in column 13.

It is necessary that the stages of *S. lumpi* development be considered by the scale suggested (Fig.2). The stage is numerically recorded in AP column as the degree of the number of parasites at this stage (see the example of filling up the 1S form).

# Conventional stages of development of *Sphyrion lumpi*

- STAGE I the parasite recently settled on the fish body; the body is semitransparent, like glassy, poorly visible on the fish skin; the fish body length is 1,0-1,5 cm; " the neck" is relatively elongated, filiform; its body is oblong (5 mm long).
- STAGE II the neck and the trunk are thicker, compared to the stage I, is not transparent, whitish, conjugate branchy organ is at the the trunk end; the trunk length is 2-3 cm.
- STAGE III the length body is 3,5-4,0 cm, its morphology and colour are close to mature stage, however no egg sacs are available.
- STAGE IV mature form, short (1-3 cm long) egg sacs are present, which are filled up with small (0,1-0,3 mm diameter) eggs, the length of the body, including the egg sacs, is on the average 4,5-6,0 cm.
- STAGE V mature "prespawning" form, the egg sacs (4-6 cm long) are filled with ripe (0,4-0,5 mm in diameter) eggs ready for extrusion and hatching of embryos; the chephalothorax and branchy organ are much developed, the trunk is dorsoventrally slightly thickened.
- STAGE VI "spawning" and "postspawning" forms; the egg sacs are opened and partially or completely free of eggs or fallen off; the trunk is dorsoventrally flattened. Further, the trunk shrinks, dies off and as a rule falls off, breaking in the neck area, with the parasite cephalothorax remaining in the fish body.

When developing the scale of conventional stages of the parasite development, results from previous (Squires, 1966) histological analyses of *S. lumpi* reproductive system were considered. The scale was mainly developed based on the *S. lumpi* morphological features for convenience of staging the parasite during trips.

#### 2. EXAMINATION OF FISH WITH PIGMENTED PATCHES

#### 2.1. Characteristics of pigmented patches

In the redfish from *Sebastes* genus (mainly in *S. mentella* from the Irminger and Labrador Seas), pigmented patches of greyish-black or reddish-orange colour occur on their skin. The patches can be of both colours. It was established (Bogovski, Bakay, Karasev, 1986; Bogovski, Bakay, 1989ab) that the patches of greyish-black colour form as a result of accumulation of melanocytes - the cells containing black pigment (melanin), on the fish skin. In the patches above 10 cm<sup>2</sup> this phenomenon is usually a deep melanosis, when the melanocytes occur in the entire skin thickness. In the patches below  $10 \text{ cm}^2$ , a superficial melanosis occurs most frequently, at which the melanocytes are available only in the upper dermis. Patches of reddish-orange colour are formed as a result of accumulation of pterinophores in the skin - the cells containing red pigment.

These pigmented patches are not tumours. They can be considered as diffusive and nidal proliferation of pigmented tissue. Rarely in the background of large patches, the tumours (melanomas or pterinophoromas) can arrise 5-7 mm high over the surface of the fish body, having a friable structure and free of scales. All the tumours studied by us were benign, localized only on the skin and did not possess a marked infiltrative growth in hypodermic muscular tissue (Bogovski, Bakay, 1989a). The black, pigmented patches should be differentiated from the dark grey sites of the skin, typical of redfish, which, as a rule, localize in the upper area of the gill cover and at the dorsal fins.

Availability of the melanocytes between the muscle fibers (mainly between the first dorsal and pectoral fins), that determines the greyish-black colour of muscular tissue, is not related to a tumourous growth (Bogovski, Bakay, 1989a), and they should be differentiated from those described above for the dermal pigmented patches. To define a presence of melanocytes in the muscular tissue, the muscles are dissected into the pieces of 6-10 mm thick using a sharp knife at angle  $30-45^{\circ}$  with respect to the body surface in the site of their localization. The melanocytes are visually determined in the muscular tissue, as minimum, of 25 fish specimens from each sample by a 3-point scale (1 - rare melanocytes, 2 - mean intensity of melanocytes presence, 3 - high intensity of melanocytes presence).

#### 2.2. Registration of pigmented patches

Results from the registration of pigmented patches are recorded in columns 14 and 15 (1S form). The figure denoting a zone of the pigmented patch localization is recorded in column 14 according to the scheme given in the bottom of the card (1S form) and in Fig.1. Area (cm<sup>2</sup>) of a pigmented patch, visually determined as a square or a rectangle, is recorded in column 15, including its colour. The latter is denoted as follows, i.e. B - (Black), R - (Red), B + R (if the patch consists of the sites of black and red colour), A - a presence of tumours.

If two or more patches were found in one of the zones of localization, the data on one of them (on that with larger area and with tumour) are necessary to be recorded. If it is necessary to make registration of pigmented patches on the left and right sides of the fish body separately, the data on their localization, size ( $cm^2$ ) and colour are recorded in the left and right parts of the columns 14 and 15, respectively.

The degree of intensity (from 1 to 3) of occurrence of melanocytes in the redfish muscles is recorded in column 16 (Fig. 3).

# 3. PRIMARY STATISTICAL DATA PROCESSING

Primary statistical processing of the data from the analysis includes determination of the parasite prevalence, intensity of infestation, abundance index of infestation by *S. lumpi*, occurrence of fish with skin pigmented patches, concretions of melanin in the fish muscles, as well as the total infestation of redfish from one or several samples.

The parasite prevalence is the frequency of occurrence of the specific species parasite in the sample examined, i.e. a proportion of fish, infested by the parasite, of the total amount of the fish examined and expressed in percent.

Intensity of infestation is the minimum, maximum or mean amount of parasites of a specific species, found on a specific infested specimen of a host.

Abundance index is the mean number of parasites of a specific species, per one fish examined, and it is established according to the formula:

$$I = \frac{\Sigma x i}{N}$$

where:  $x_i$  - is the number of parasites of a specific species found in one fish examined; N - is the sample size (number of the fish examined).

Mean prevalence of parasite is the mean number of parasites of a specific species, per one fish infested, and it is established by the following formula:

$$C = \frac{\sum xi}{n}$$

where: n - is the number of fish infested by a specific parasite in a sample.

Occurrence of pigmented patches is the proportion of fish with pigmented patches (patches, tumours) on the fish skin of the total number of the fish examined and expressed in percent.

Total infestation is the proportion of fish, both infested by copepod and with pigmented patches on skin, of the total amount of the fish examined, in percent.

Occurrence of melanocytes in the muscles is the proportion of fish with concretions of melanin in the muscles of the amount of the fish examined and given in percent.

Based on the data derived, infestation by *S. lumpi* and occurrence of fish with pigmented patches on the skin is determined for the catch, aggregation, area and population.

# Conclusions

When developing these methodical guidelines, results from the experiments performed by researchers from different countries, as well as the authors' long-term experience (1983-1999), accumulated in studying the beaked redfish and other species of redfish from *Sebastes* genus in different areas of the North Atlantic and Barents Sea, were considered. According to these methodical guidelines, it is suggested that the data on the infestation by *S. lumpi* and pigmented patches in redfish should be collected during standard ichthyological works (field analysis of feeding, age sample, etc.). The card (1S form) allows to simultaneously make compact and clear registration of both the ichthyological data (length, weight, sex, maturity stages of fish, stomach fullness, food composition) and the data on characteristics of the infestation by *S. lumpi* (alive parasites at different stages of their development, remains of the copepod presence), pigmented patches (colour, size) and their localization. Using of natural (biological, parasitological) tags (Sindermann, 1959, 1961; Bakay, 1988), such as registration of alive parasites of *S. lumpi* (indicating their stages of development), remains of *S. lumpi* presence and occurrence of pigmented patches, will be an additional basis for studying the intraspecific structure of redfish from *Sebastes* genus in the North Atlantic. Results from the analysis of the data collected by the method suggested will allow to reveal the centres of *S. lumpi* invasion and occurrence of pigmented patches, that, in combination with the peculiarities of occurrence and geographical variability of the phenomena studied, will be a pre-condition for studying the routes of migrations and intraspecific structure of redfish.

The scheme of sites of localization and registration of parasites and pigmented patches was developed with allowance for traditional technological methods of processing the fish (Fig.1). The investigations on beaked redfish (*Sebastes mentella*) in the Irminger and Labrador Seas in 1994-1999 indicate that applying of the unjustified large (10 from the left and 10 from the right side of fish body) number of lesions on the redfish body, suggested by the Icelandic scientists (Magnusson J.V., 1992. ICES Working Document, submitted to the meeting of the Study Group in 1992), results in a considerable complication of collection and registration of biological data, as well as of their subsequent statistical data processing. Moreover, the registration of lesions and pigmented patches on the redfish body on the left and right sides separately complicates not only collection and processing of the data, but is unnecessary also. Thus, the results from our

investigations, as well as from other researchers (Bakay, 1989; Bogovski, Bakay, 1989a; Kerstan, Barhs, Moller, 1989) indicate the nearly similar occurrence of *S.lumpi* copepod and pigmented patches on the left and right sides of the fish body.

To solve problems of the registration, storage, statistical data processing and analysis of all the biological data, it is suggested that the specialized programme, developed by PINRO (Murmansk, Russia) based on EXCEL spread sheet for IBM PC/AT, should be applied.

The methodical guidelines suggested for studying the ectolesions of the redfish from *Sebastes* genus is relatively universal and can be applied for studying any fish species infested by other ectoparasites (*Copepoda, Isopoda, Hirudinea*), as well as those having other anomalies on their body.

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Fig. 1. Parcelling the body surface out the zones of lesion localization: I – dorsal ("fillet ") and caudal part, II – ventral part, III – head, IV – anal part



Fig. 2. Conventional stages of development (I – VI) of copepod Sphyrion lumpi.

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