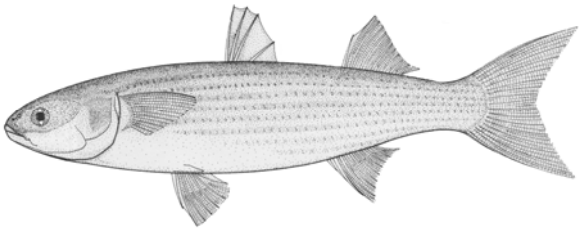
Sampling Procedures for Mullet Parasites

Kostadinova A., Ovcharenko M., Balbuena J.A. and Sarabeev V.L.



Evaluating the effect of an invasive species on local mullet communities in the Mediterranean: A parasite community approach

INTAS Project 03-51-5998

Linked mediafile on Youtube: https://www.youtube.com/watch?v=lHHNOhrAoqI&t=46s

This document describes a sampling protocol agreed by **all participants** in the project.

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I. Collection of hosts

All hosts should come from the same locality, and should not be pooled across localities. 30 specimens of each host are required representing the average age or size class for the population (see Table). Samples of 30 fish permit detection of parasites if the prevalence is 10% or more whereas detection of rare parasites requires greater sample size.

All hosts should be individually bagged and labeled with a code (Sea-Specific name-Locality, e.g. MCE1 = Mediterranean-Cephalus-Ebro Delta, specimen no. 1). Hosts should be examined within a few hours. Collection should be done twice annually (spring-early summer and late summer) because parasite populations can fluctuate seasonally.

II. Dissection sequence and laboratory protocol for macroparasites

1. Record host species, date caught, locality, condition, name of examiner (see data sheet).
2. Measure and weigh fish [Total length (TL cm), Standard length (SL cm), Height (H cm) and weight (W g) see data sheet].
3. Take a blood sample from the heart with a syringe or a Pasteur pipette, containing a droplet of heparin-saline solution. Prepare a smear (15 fish of each species).
4. Take a sample of mucus from gills and prepare a smear (15 fish of each species) for examination for microparasites.
5. Rinse external surface; collect rinse and examine with stereomicroscope for ectoparasites.
6. Examine external surface using stereomicroscope.
7. Open body cavity ventrally; record sex.
8. Examine cavity and surface of internal organs (heart, liver, spleen, gall bladder, digestive tract, gonads, kidney, swim bladder) for parasites. Then separate organs into Petri dishes with saline.
9. Separate oesophagus, stomach (cardiac and pyloric), pyloric caeca, and intestine. Open longitudinally and examine for parasites with stereomicroscope. For extensive gut contents, rinse into beakers, mix with sodium bicarbonate (one spoonful per litre) to remove mucus, and allow parasites to settle. Decant and examine residue with stereomicroscope.

10. Examine intestine and caeca first, then stomach and oesophagus. If the trematodes are not alive, collect immediately in alcohol, since they degrade very fast.
11. Remove gills and gill organ. Place the gill organ separately.
12. Rinse gills (4 gill arches, i.e. one side only). Examine each gill arch individually and the rinse with stereomicroscope.
13. Rinse buccal cavity; examine rinse with stereomicroscope.
14. Remove, dissect, and examine eyes (humor, retina, lens) with stereomicroscope.
15. Remove and dissect brain.
16. Remove otoliths.
17. Separate gall bladder, prepare a smear for examination for microparasites.
18. Cut organs and tissue (wall of stomach, pyloric caeca, intestine, liver, spleen, kidney, heart, gonads, brain, swimbladder) into smaller pieces, compress between glass plates or Petri dishes, and examine with stereomicroscope.
19. Rinse the body cavity; and examine rinse with stereomicroscope.
20. Thin-slice musculature (pectoral) and inspect for parasites.
21. Record number of parasites of each species and their location in the host on data sheet.
22. Each parasite species or type from each organ should be collected in a separate vial and labeled with host code and location in host (English, capital letters).

III. Laboratory protocol for microparasites

III.A. Blood smears (from fresh fish only)

Method 1: Take a blood sample from the heart with a syringe or a Pasteur pipette, containing a droplet of heparin-saline solution. Prepare a smear on a clean new slide. Allow to air dry, fix in absolute methyl alcohol for 3-5 min, and stain in Giemsa solution [10 volumes of Giemsa staining solution + 90 volumes 0.01 M phosphate buffer (pH 7.4) for 20 minutes. Rinse in distilled or de-ionized H_2O and air dry. Best results are obtained when Giemsa's stain is applied immediately after fixation. To remove extensive stain place the slide back in methyl alcohol. Examine under light microscope at high magnification (at least 400x). Immersion oil can be placed directly on the stained smear to use the higher power, oil immersion objectives.

Method 2: In low intensity infections spin with hematocrit centrifuge. Fill a heparinized capillary tube (0.05 ml) with blood. Seal one end with plasticine and spin in a hematocrit centrifuge for 4 min at 12 000 rpm. Then place the tube in a drop of immersion oil and examine at high magnification (400x). Trypanosomes are located between the layer of white blood cells and the clear plasma. If result positive, cut the tube above this layer, transfer to a slide, prepare a smear, fix in methyl alcohol and stain in Giemsa solution.

III.B. Smears of internal organs

Obligatory: gills. Take a sample of mucus, place in a drop of water on a microscope slide, cover with a cover slide and examine for microparasites. If positive, remove the cover slide, air dry and fix in absolute methyl alcohol. Ciliophora should be first silver impregnated, fixed in methyl alcohol and stained in Giemsa solution.

Cyst-like structures (if present)

Examine all internal organs for the presence of microparasite cysts. Smears from cysts from liver, spleen, kidney, gonads, intestine, muscle, brain, and scrapings of the gall bladder should also be made on microscope slides, and fixed in 95% methyl alcohol.

Preparations of myxosporidians

The cyst is smeared on a clean microscope slide, dried, a drop of glycerine jelly is then added, the slide is covered and heated. Later margins of the cover slide are covered with Canada balsam.

Preparations of microsporidins

1. If microsporidians are present in the initial smear: remove the cover slide, transfer part of the material in a drop of water on another slide, smear the rest, fix in absolute methyl alcohol and stain in Giemsa solution as described above.
2. Cover the drop on the other slide and add along margins of the cover slide liquid glycerin jelly or Canada balsam or mounting medium. This preparation can be then held for about a month.
3. Obligatory: TEM preparations are required for the species determination of microsporidians (as described below).

III.C. Material for histopathology (TEM examination)

Excise pieces (0.5-1 mm) of infected segments (close to the cyst observed) and fix in a 2.5% (v/v) glutaraldehyde in a 0.2 sodium cacodylate buffer (pH 7.2) for 3 hrs. Storage in eppendorf tube at 4ºC for c. 1 month.

General: Smears are examined for a fixed number of microscope fields (e.g. 50) or a fixed period of time (e.g. 5 min) with a compound microscope at 400x. The presence of parasites is recorded, and photographed for a permanent record.

Appendices

Appendix 1.

Approximate metrical data for the fish to be sampled in the Black and Azov seas.

Character	Species			
	Mugil cephalus	*Mugil soiuy*	*Liza aurata*	*Liza saliens*
Total length, cm	25-45	30-50	15-35	20-40
Weight, g	200-1200	350-1500	40-500	100-500

Approximate metrical data for the fish to be sampled in the Far East.

Character	Species
	Mugil soiuy
Total length, cm	15-35
Weight, g	50-400

Mullet Parasite Data Sheet

Species:
Collection code & number:
Total Length (TL, cm):
Standard Length (SL, cm):
Body Height (H, cm):
Weight (W, g):
Sex:

Sea:
Locality:
Date of collection:
Date of examination:
Condition: (fresh, held, refrigerated, frozen)
Examiner:

<table>
<tr><td>Blood smear:</td><td>Oesophagus:</td></tr>
<tr><td>Gill smear:</td><td>Stomach:</td></tr>
<tr><td rowspan="2">Gills:</td><td>Pyloric caeca:</td></tr>
<tr><td rowspan="3">Intestine:</td></tr>
<tr><td>Gill organ:</td></tr>
<tr><td>Eyes:
L:
R:</td></tr>
<tr><td>Gall bladder:</td><td>Muscles:</td></tr>
<tr><td>Liver:</td><td>Brain:</td></tr>
<tr><td>Heart:</td><td rowspan="3">Remarks:</td></tr>
<tr><td>Kidney:</td></tr>
<tr><td>Spleen:</td></tr>
<tr><td>Swim bladder:</td><td>Otoliths recovered? yes
no</td></tr>
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