8.10 PROTOCOL FOR SAMPLING PARASITES IN FISH

Overview

All organisms to be examined for any particular survey should come from the same habitat and should not be pooled across habitats. Twenty to 30 organisms are required for a general parasite survey from the average age or size class for the population. For best results, analysis of data by age, size, sex, or season requires 30 host animals in each class. Samples of 25-30 fish permit detection of parasites if the prevalence is 10% or more. Detection of rare parasites requires greater sample size. Preferably, host organisms should be examined fresh for parasites or organisms should be frozen as soon as possible after capture.

Hosts fixed in preservative are of little use for parasitological examinations. Fish can be euthanized by pithing if small, a blow on the head if large, by cervical dislocation, or by an overdose of anesthetic such as tricaine methanesulfonate (MS 222). All hosts should be individually bagged to prevent loss of ectoparasites and labeled with collection data (date, sampling site, collector). Host organisms returned to the laboratory alive should be examined within a few hours. Otherwise, parasites with direct life cycles may spread between hosts or increase on infected hosts. Hosts kept in captivity for prolonged periods may lose many parasites. Loss of ectoparasites also may occur with certain methods of capture such as gill-netting. Parasite surveys should be done in spring-early summer and late summer because populations can fluctuate seasonally. If only one survey is scheduled, sample in July.

Sources

EMAN (Undated b)

At a glance

1 Capture fish using any of the methods outlined in the Manual.

2 Record host species, date caught, site sampled, method of collection, name of collector, name of examiner.

examine rinse

3 Measure and weigh fish. Rinse external surface; collect rinse and examine with stereomicroscope for ectoparasites. Examine external surface using stereomicroscope.

4 Remove gills, rinse. Examine each gill arch individually and the rinse with stereomicroscope.

5 Rinse buccal cavity; examine rinse with stereomicroscope.

6 Remove, dissect, and examine eyes (humor, retina, lens) with stereomicroscope.

7 Remove otoliths, fins, or scales for aging, if required.

examine

8 Remove fins and examine with stereomicroscope.

9 Open body cavity ventrally; record sex. Examine cavity and

organs

surface of internal organs (heart, liver, spleen, gall bladder, digestive tract, gonads, kidney, urinary bladder) for parasites. Separate organs into Petri dishes with water.

10 Separate stomach, pyloric caeca, and intestine. Open longitudinally and examine for parasites with stereomicroscope. For extensive gut contents, rinse into beakers, mix with sodium bicarbonate (1 spoonful per litre) to remove mucus, and allow parasites to settle. Decant and examine residue with stereomicroscope.

11 Cut organs and tissue (wall of stomach, pyloric caeca, intestine, liver, spleen, kidney, heart and large blood vessels, gonads, gall bladder, urinary bladder, brain) into smaller pieces, compress between glass plates, and examine with stereomicroscope.

12 Rinse the body cavity and examine rinse with stereomicroscope.

13 Thin-slice musculature and inspect for parasites.

14 Record number of parasites of each species and their location in the host on data sheet.

15 Fix all live parasites in hot or warm fixatives to kill them rapidly and at the same time avoid muscular contractions by the parasites, which then distorts their shape when fixed. For living, small monogeneans firmly attached to the gills, freeze a section of tissue with parasite attached overnight in water or 0.7% saline solution. The parasite will detach from the tissue and relax. It can then be thawed, retrieved, and fixed in 10% buffered formalin. Other helminths (cestodes, trematodes, acanthocephalans) should be heat-fixed in 70% ethanol, or relaxed in tap water (if alive) and fixed in 10% buffered. Nematodes should be fixed in hot (not boiling) 70% ethanol with 5% glycerol. Berland's fluid may also be used for nematodes and platyhelminths. Encysted parasites can be removed from their cysts by careful dissection with fine needles or forceps or gentle pressure with a coverslip on a slide. If these techniques fail, place the cyst in 0.5% trypsin and heat to 37-40^{o}C. Encysted acanthocephalans found in the viscera can be placed in tap or distilled water in the refrigerator overnight to stimulate eversion of the proboscis. Fix in 70% ethanol, 10% buffered formalin or AFA.

use hot or warm fixatives

careful dissection

16 Anesthetize arthropods in carbon dioxide bubbled through water, and then fix them in 70% ethanol.

17 Narcotize leeches to avoid contracting when fixed. Carbon dioxide bubbled through water can be used to anesthetize leeches after which they can be fixed in 10% buffered formalin.

18 Place each parasite species or type from each organ in a separate vial and label with host species and host number, geographic locality, date of capture, location in host, fixative

used, and date of examination. Formalin- or AFA-fixed specimens should be transferred to 70% ethanol after 1-7 days, and definitely for a few days prior to staining.

19 Stain monogeneans, trematodes, cestodes, and acanthocephalans in acetocarmine and mount them on permanent slides. Acanthocephalans should be pricked in a few places with a fine needle prior to staining.

20 Clear nematodes by evaporation in glycerol in 70% ethanol, letting the alcohol evaporate in the case of small worms, or gradually reducing the alcohol content and increasing the glycerol content of the mixture with large worms (>1 cm). Examine arthropod parasites whole.