Influence of Collection Methods on the Occurrence of Alimentary Canal Helminth Parasites in Fish

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ABSTRACT: The effect of collecting trauma on the metazoan parasites in the alimentary canal of French grunts, Haemulon flavolineatum (Desmarest) (Perciformes: Haemulidae), was evaluated by comparing the number and species of parasites in 10-fish lots that were identical except for collecting technique. Collecting techniques included speared (dead), speared through the caudal peduncle (live), trapped, dipnetted at night, and ostracitoxin exposed. Dead, trapped, and toxin-stressed fish had no alimentary canal parasites, whereas speared-live and night-dipnetted fish had comparable numbers of parasites. Fish collected by using apparently traumatic techniques quickly expelled their alimentary canal metazoan parasites. Our results cast doubt on the reliability of traditional fish parasite surveys, studies on population dynamics of fish parasites, and experiments that employ these traumatic collecting methods.

We have found that physical trauma to fish during collection may drastically reduce the number of alimentary canal parasites found and have attempted to examine this effect in a series of experiments that may have broad implications in traditional studies of marine fish parasites.

We examined a collection of 20 living specimens of French grunts, Haemulon flavolineatum (Desmarest) (Perciformes: Haemulidae), that were captured with a minimum of physical force and contact and were held for less than 1 hr. The number, variety, and locations of parasites in the alimentary tract of these fish (mean density of all alimentary canal metazoan parasites/host = 18.2; species recovered = 8) contrasted greatly with former results from speared, dead individuals (n = 32) of this fish species (x̄ = 3.7; species recovered = 4) (Dyer et al., 1985). More than half (n = 19) of the spear-dead fish samples had no alimentary parasite.

We were concerned by this apparent effect of host-collecting techniques on alimentary canal parasites, but the variety of collection situations in our spear-dead data made this comparison inconclusive. To test this apparent effect, we conducted a series of experiments to evaluate the effects of various collecting techniques on the number and variety of parasites collected in necropsies.

In experiment 1, 10 French grunts were speared through the head (spear-dead) (Table I, 2A) and placed underwater within 30 sec to 2 min in individual plastic bags. An additional 10 were speared through the caudal peduncle and held alive in a diver-carried bait-bucket, perforated with additional 0.5–1.0-cm holes to improve circulation, and were transported to the laboratory alive and pithed immediately prior to necropsy (spear-live) (Table I, 1B).

Experiment 2 was conducted with 10 living specimens from wire-mesh Puerto Rican arrow fish traps (trap-live) (Table I, 2A), and 10 spear-live from the same location on the same day (Table I, 2B). The trap-live French grunts had been held for no more than 24 hr, and probably less, underwater in the trap. All trap-live specimens had suffered abrasions and contusions on the face prior to examination. Some also had
Table I. Percentage of fish (French grunts, *Haemulon flavolineatum* (Desmarest)) infected and mean density for each metazoan parasite in each alimentary canal, using different host-collecting methods at La Parguera, Puerto Rico.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Collection method</th>
<th>Number of fish</th>
<th>Digenea species</th>
<th>Other parasites*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1A</td>
<td>Spear-dead†</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1B</td>
<td>Spear-live‡</td>
<td>10</td>
<td>60.0/4.7</td>
<td>40.0/2.2</td>
</tr>
<tr>
<td>2A</td>
<td>Trap-live§</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2B</td>
<td>Spear-live§</td>
<td>10</td>
<td>80.0/4.1</td>
<td>70.0/2.6</td>
</tr>
<tr>
<td>3A</td>
<td>Net-live¶</td>
<td>10</td>
<td>80.0/4.5</td>
<td>40.0/2.8</td>
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<tr>
<td>3B</td>
<td>Spear-live¶</td>
<td>10</td>
<td>70.0/5.1</td>
<td>30.0/1.9</td>
</tr>
<tr>
<td>4A</td>
<td>Ostracitoxin‖</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4B</td>
<td>Net-live‖</td>
<td>10</td>
<td>10.0/0.1</td>
<td>100/17.2</td>
</tr>
</tbody>
</table>

* C, cestode; N, nematode.
† Enrique Reef, western end of fore-reef, 24 September 1976.
‡ Insular shelf edge southeast of La Parguera, 16 September 1976 (dive #162).
§ Media Luna Reef, fore-reef, near western end, 18 September 1976.
‖ Media Luna Reef, shoreward of western end, 10 December 1976.

abrasions and missing scales on the body and eroded or bloody fins. All fish were transported to the laboratory alive and pithed immediately prior to examination.

In experiment 3, 10 French grunts were collected by blinding them at night with underwater dive lights, picking them up in dipnets, and placing them in a live-bucket, with scuba divers (net-live). These were compared as described above, to spear-live samples from the same geographic location at different times on the same day (Table I, 3).

During these experiments, a fortuitous accident (dive #177, 10 December 1976) established an opportunity for an additional stress-related experiment (designated experiment 4). An underwater collector, unaware of the toxic properties of trunkfishes, placed a dipnetted 12.4-cm (standard length) smooth trunkfish, *Lactophrys triquetra* (Linnaeus) (Tetraodontiformes: Ostraciidae), in a live-bucket with 5 net-live, French grunt specimens. The 5 French grunts were exposed to the trunkfish for approximately 15 min underwater. Once in the boat, the trunkfish was identified, measured, and released alive. The living, toxin-exposed French grunts were isolated from the 10 other net-live specimens, but otherwise they were treated the same as described above.

Fish sizes (14–18 cm standard length), collection locality and time, holding times (1–3 hr), and examination techniques were held as close to identical as possible within each experiment. Necropsy and parasite preparation techniques followed Dyer et al. (1985). Numbers of parasite species per host used for comparisons include 1 species of cestode and 1 species of nematode that are not included numerically in Table I because their occurrence was very low.

In experiment 1, no alimentary canal parasite occurred in the spear-dead samples (Table I, 1A), whereas the mean density of alimentary canal parasite specimens was 17.6 and the number of species collected was 7 in the spear-live samples (Table I, 1B). In experiment 2, parasites were not found in the trapped fish (Table I, 2A), compared with an $\bar{x} = 15.8$ and species recovered = 8 in the spear-live sample (Table I, 2B). In experiment 3, the results of both collection techniques were very similar ($\bar{x} = 19.3$ vs. 16.5, species recovered = 8 vs. 8). In experiment 4, parasites were not found in the toxin-exposed fish (Table I, 4A) whereas the mean density of parasites of the net-live hosts was 33.1 and species recovered was 6.

The percentages of hosts infected (A vs. B) in experiments 1, 2, and 4 were significantly different (experiment-wide confidence level of 0.05; 2 $\times$ 2 contingency table using G-test) (Sokal and Rohlf, 1981). The number and species of alimentary canal parasites found in spear-live and net-live samples were very similar, whereas parasites were not found in the spear-dead, trap-live, and ostracitoxin samples. Dead or dying French grunts seemed quickly to expel most, if not all, of their alimentary canal
metazoan parasites. Similar results in hosts experiencing physical trauma in traps or by exposure to toxins seem to suggest that these severe stresses may cause hosts to expel their parasites. Various surveys and studies on the population dynamics of alimentary canal metazoan parasites of marine fishes have assumed fishes obtained by traumatic methods (spearing, traps, or hook and line) represent normal alimentary canal parasite species and numbers. Our results suggest that this may not necessarily be the case.

We do not have information concerning the fate of the alimentary canal parasites that apparently were lost during collection. We suspect that collection techniques that traumatize the host cause expulsion of these parasites. Fishes speared through the head (Table I, 1A), ideally, should die immediately, as if pithed in the brain. There should be no host reaction to affect their alimentary canal parasites. Unfortunately, such precision spearing almost never occurs. None of our head-speared specimens died immediately, and we assume all experienced severe trauma accompanied by erratic thrashing movements. If the speared host could be enclosed in a plastic sample bag immediately, even expelled alimentary canal parasites could be collected. Unfortunately, speed is restricted in scuba divers, coral reefs shred plastic bags, and the fish must be retrieved and the spear removed before it can be placed in a bag. The 30 sec to 2 min lag between the impact of the spear and the sealing of the host in a bag seems to have been a sufficient time for reactions of the injured and dying fish to expel their alimentary parasites. Our examinations included the decanting and examination of the sediments of these bags, but worms were not recovered. Occasionally, we have collected Digenea from the sediment in the confining bags with other marine fishes similarly examined. Possibly those fishes died immediately when speared.

These experiments identify which collection techniques may be used to examine fishes with a minimal amount of disturbance to their alimentary canal parasites. They also may indicate which techniques may be more traumatic to the host. Before these experiments we would have suspected that tail-spearing would be a very traumatic technique and that trapping would be less so. We would have assumed also that the numbers of parasites of hosts speared through the head would not change.

We examined 235 additional French grunts that had been collected by using the net-live or spear-live techniques. None of these specimens lacked canal alimentary parasites, and the mean density of parasites was 15.4. This is further evidence that these methods consistently allow the collection from this fish species of the metazoan alimentary canal fauna that was in the host prior to the collection, or at least much more of it than encountered from using more traumatic collecting methods.

Trunkfishes confined in aquaria or live-wells are known to secrete an ostracitoxin when stressed that will kill themselves and any other fishes confined with them (Thresher, 1980). Thresher (1980) found the action of this toxin on fishes to be almost immediate, irreversible, and fatal. We cannot explain why the toxin did not kill the fish we collected. The diver-carried live-bucket with additional perforations for increased circulation may have diluted the toxin sufficiently to reduce its effect.

We have examined other trap-live specimens of French grunts from different locations and at different times. Many of them appeared less physically damaged than the experimental lot and had levels of alimentary canal parasites intermediate to comparable to the spear-live samples. Circumstantially, this suggests that the more injuries to the hosts, or the higher the level of host activity in response to injury in the traps, the fewer parasites remain. Unfortunately we have no satisfactory method for ranking the level of trauma.

Expulsion from or abandonment of traumatized or injured hosts may inadvertently be a reproductive advantage for helminths by allowing for quick dispersal of the eggs of adult parasites that might otherwise be lost. We described a similar burst release of cymothoid isopod reproduction from female isopods on traumatized hosts (Williams and Williams, 1985).

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LITERATURE CITED

