EFFECTS OF THE TROMATODE **UVULIFER AMBLOPLITIS** ON JUVENILE BLUEGILL SUNFISH, *LEPOMIS MACROCHIRUS*: ECOLOGICAL IMPLICATIONS

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ABSTRACT: Field and laboratory studies were conducted to assess the effects of *UVulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*, both at the individual host level and at the host population level. Neither natural mortality of the parasite, selective predation by largemouth bass, nor selective predation by kingfishers could account for the observed elimination of heavily parasitized (>50 cysts/fish) bluegill in the field. Field and laboratory experiments showed that heavily infected fish died when exposed to decreasing water temperature, but not if temperature remained elevated (20-25°C). Parasitism significantly reduced total body lipid and body condition of bluegill in the laboratory and was significantly correlated with reduced lipid and body condition among bluegill in the field. Newly acquired metacercariae significantly stimulated the oxygen consumption of artificially infected fish for up to 60 days post-infection; these changes were correlated with the encapsulation process by the host, after which no further metabolic consequence of parasitism was evident. Bluegill held at warm temperatures continued to feed and recovered from previous lipid depletion. However, declining water temperatures in the fall suppressed feeding and caused heavily infected fish (>50 cysts/fish) to begin winter in a state of lipid depletion. The extent of depletion and associated mortality were directly proportional to parasite intensity, the critical point for host survival was reached when total body lipid decreased to approximately 5% dry weight or less. These data provided a clear example of mortality in a natural population of fishes due to the direct effects of parasitism. In Reed's Pond, parasite-induced host mortality accounts for the elimination of 10-20% of the young-of-the-year bluegill population each winter.

The concept of mutual regulatory interactions between host and parasite populations has been promulgated by many theoreticians since the pioneering models first proposed by Crofton (1971a, b). No one, however, has stated the case with greater certainty than May and Anderson (1982). They said parasites are as important as predators and insect parasitoids in the regulation of animal populations. The serious difficulty with this sweeping generalization, especially for host-parasite systems in aquatic habitats, is that there is very little evidence to support it. Holmes (1983) recently asserted that most parasite-induced host mortality is compensatory, not regulatory, or additive.

Meyer (1958), Kakonge (1972) and Harrison and Hadley (1982) have suggested that infections of *Neascus* spp. may be a regulatory factor for host populations through the mortality of heavily infected fish. Studies on various aspects of the population biology of *UVulifer ambloplitis* in juvenile bluegill within a small farm pond in the Piedmont area of North Carolina suggested the possibility of parasite-induced host mortality (Lemly and Esch, 1984b). The purpose of the present report is to describe the effects of *U. ambloplitis* on juvenile bluegill, *Lepomis macrochirus*, and to assess the impact of the parasite at the level of the host population.

MATERIALS AND METHODS

Field studies

**Study site**: The site for the study was Reed’s Pond, a small (2.0 ha) eutrophic impoundment located in the Piedmont area of North Carolina. Average depth is about 2.5 m and maximum depth about 10 m. Several physical and chemical characteristics were measured monthly during the course of the study (Lemly, 1983a), but all except temperature were consistent. Maximum surface temperatures of 31-32°C were recorded in July and August and minimum temperatures of 0-1°C in January.

**Behavior of Megacercus Alecyn**: Observations were made on the behavior of the definitive host of *U. ambloplitis*, the belted kingfisher (*Megacercus Alecyn*), at Reed’s Pond. The number of sightings of kingfishers (from December 1979 through November 1982) was recorded as well as the number of feeding attempts made (dives into the water). The total amount of time spent in this census was recorded as number of observation days (12 hr/day).

**Predation by largemouth bass (Micropterus salmoides)** on parasitized bluegill. To examine the possibility of selective predation by largemouth bass on parasitized bluegill in Reed’s Pond, the stomach contents of 75 largemouth bass were analyzed; all were collected during July, August, and September 1980.

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The fish were collected within 3 hr after sunrise using a 40 × 2 m (1.5-5.0 cm³ mesh) gill net. Fish were removed from the net, anesthetized in 1.0% MS-222 (metacaine tricaine), and immediately eviscerated. Stomachs were placed on ice and returned to the laboratory where their contents were examined. Bluegill present in the stomachs were dissected and the number of cysts of *U. ambloplitis* per fish was recorded. The percentage of heavily parasitized bluegill (>50 cysts/fish) in the stomachs was compared to the percentage of heavily parasitized bluegill in the monthly sweep samples from the pond (Lemly and Esch, 1984b). The age of each largemouth bass was determined via scale analysis to allow an assessment of feeding behavior according to age class.

**Body condition, total body lipid, and survival of parasitized bluegill.** A coefficient of body condition (K) was calculated for each bluegill from Reed's Pond on which parasite counts were made. The coefficient is expressed as:

$$K = \frac{100 \times \text{weight (g)}}{\text{standard length (cm)}}$$

and is a reflection of a fish's general state of health (Baggalis, 1978).

Total body lipid of 180 bluegill was measured in fish collected from Reed's Pond between November 1981 and October 1982 (15 fish/month). All of the fish were either lightly parasitized (<15 cysts/fish) or uninfected. The lipid extraction procedure used was that of Bighoff and Dyer (1979). An additional 180 bluegill from the sweep samples of November 1981 to October 1982 were analyzed for total body lipid. In addition, all of the bluegill which died during the course of the October 1982-January 1983 livebox experiment (described below) were analyzed for total body lipid.

Four field experiments were conducted to study the effect of parasitism by *U. ambloplitis* on survival of bluegill through the winter. The first experiment was conducted from October 21, 1979 until April 1, 1980 and involved holding 18 bluegill ≤70 mm TL (collected from Reed's Pond) with various densities of parasites in an unheated, outdoor aquarium. The aquarium was examined daily and, if a dead fish was found, the number of cysts present was counted and the date of death recorded. The fish in this experiment were fed a commercial flake food (TetraMin) *ad libitum*. The other 3 experiments involved holding Reed's Pond bluegill (≤70 mm TL) in the Litoral zone within liveboxes throughout all or part of the winter (October 1, 1980—April 1, 1981; October 1, 1981—January 1, 1982; October 1, 1982—January 1, 1983). A total of 60 fish were held in the 1980–81 experiment, 60 fish in the 1981–82 experiment, and 54 fish in the 1982–83 experiment. The bluegill in these 3 overwintering experiments were distributed evenly among 3 liveboxes. The boxes were checked at 1–3 day intervals. Any dead fish were removed, dissected, and the number of cysts and approximate date of death recorded. After dissection, the remains of the fish which died during the 1982–83 experiment were frozen (−30 C) in separate airtight containers for subsequent lipid analysis. At the end of each experiment, all remaining bluegill were killed and examined for cysts. Data on body condition, total body lipid, and survival of bluegill over the winter were compared and their relationship to the intensity of parasitism by *U. ambloplitis* determined.

**Laboratory studies**

**Maintenance of experimental animals:** Bluegill used in the laboratory studies were collected from the field as needed (either from Reed's Pond or from uninfected stock in another pond) and acclimated indoors at 20 C for a minimum of 14 days prior to the initiation of experiments. During the acclimation period, fish were held in 150 liter aquaria exposed to natural lighting (windows). Tetracycline (0.1 mg/liter) was added to prevent bacterial infections. Commercial flake food (TetraMin) was supplied *ad libitum*, both during the acclimation period and throughout the course of the experiments. Only animals which appeared alert and healthy at the end of the acclimation period were selected for use in the various studies.

**Establishment of artificial infections of *Uviolifer amboplitis***: Several of the laboratory experiments required the use of bluegill for which the date of infection with *U. ambloplitis* was known. These fish were infected under laboratory conditions. The procedure consisted of confining bluegill in a small aquarium (6 litre) with 5 *Helisoma trivolvis* that were shedding cercariae of *U. ambloplitis* and examining the fish for the presence of cysts 21 days later. By varying the duration of exposure, fish acquired different intensities of parasites. A number of preliminary trials were made to determine the precise exposure time needed to produce a particular range in intensity of infection. Exposure periods tested were 2, 4, 6, 8, 16, and 24 hr. The 16 and 24 hr exposures were not suitable because of acute mortality, probably due to direct effects of cercariae penetration and migration (Krull, 1934). The 2, 4, 6, or 8 hr exposure periods did not cause mortality and were sufficient to produce a final intensity of cysts comparable to that present in the bluegill in Reed's Pond. Therefore, depending on the intensity of parasitism required for a specific experiment, either 2, 4, 6, or 8 hr exposure periods were used. Artificially infected bluegill were also used for comparison with naturally parasitized fish in studies on survivorship, body condition, and total body lipid.

**Enumeration of cysts of *Uviolifer amboplitis***: For the laboratory and field experiments, cysts were counted in the manner described by Lemly and Esch (1984b). Several of the studies required that counts be made on fish that were to remain alive for use in one or more experiments, and then be killed and examined for the final determination of the number of cysts. A livebox system was designed (Lemly, 1983b) to allow inspection of live fish using a dissection microscope. Bluegill examined using this system were kept for up to 1 hr while making counts of cysts. No mortality or apparent injury resulted.

**Survivorship of parasitized bluegill:** Three experiments were conducted to study the effects of *U. ambloplitis* on the survivorship of bluegill. On October 1, 1980, 90 bluegill from Reed's Pond (≤70 mm TL), with various intensities of infection with *U. ambloplitis*, were placed into 2, 150 liter aquaria and maintained at a constant temperature (20 C) until April 1, 1981. The aquaria were exposed to natural lighting and were examined daily. Any dead fish were removed, the date
of death recorded, and the number of cysts counted. On April 1, all surviving fish were killed and the number of cysts determined. The study was repeated the following year (October 1, 1981–January 4, 1982) using 80 bluegill.

The remaining 2 experiments were conducted in a constant temperature (4 C) room. Bluegill were held in 20 liter aquaria in which temperature was manipulated so that the fish were exposed to constant or variable temperatures from 4–25 C. Fluorescent lighting, regulated to maintain a light/dark cycle equivalent to natural conditions, was used in both of the experiments. In November 2, 1981, 90 bluegill from Reed's Pond (30 = 80–110 mm t.l., 60 ≤ 70 mm t.l.), with various intensities of U. ambloplitis, were brought to the laboratory and placed in aquaria at 20 C. The water temperature was then decreased to 4 C at a rate which approximated the onset of winter in Reed's Pond (2 C per week). The water temperature was then held constant (4 C) until January 7, 1982, after which all surviving fish were killed and the number of cysts/fish was counted. Fish that died during the experiment were removed, the number of cysts each harbor was counted, and the date of death recorded. This study was repeated the following summer (June 2–August 3, 1982) using artificially infected bluegill (<70 mm t.l.). The final experiment was conducted from April 15 until June 19, 1982. Thirty fish were artificially infected on February 15 and held at 20 C until April 15. The water temperature was then decreased to approximate the onset of winter conditions. Mortality and parasite intensity were examined in the manner previously described. After the cysts were counted, the remains of each fish that died during the course of the experiments were placed into an airtight container and frozen (<30 C) for subsequent lipid analysis.

Effect of Uvulifer ambloplitis on body condition of bluegill: A coefficient of body condition (K) was calculated for bluegill which died during the June 2–August 3, 1982 survivorship experiment. The coefficients were grouped by the length class of the fish (<30 mm, 31–50 mm, 51–70 mm t.l.) and relationships between the intensity of parasitism and body condition on the day of death were determined.

Two experiments were conducted in which the body conditions of artificially infected bluegill were monitored over a period of 4 mo, with K determined immediately before infection and at approximately 30 day intervals thereafter. One experiment utilized 30 individuals from Reed's Pond (conducted July 16–November 16, 1981) and the other utilized 30 uninfected individuals from another pond (conducted July 22–November 15, 1981). The fish were held at a constant temperature (25 C) in 2, 150 liter aquaria that were exposed to natural lighting. The intensity of cysts was determined for any fish that died and for all fish at the end of the experiments.

Effect of Uvulifer ambloplitis on oxygen consumption of bluegill: Oxygen consumption of bluegill was monitored with a YSI Model 5420 stirring polarographic oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, OH). A 1.0 liter plexiglass box was used as a test chamber. A fish was placed in an isolation cage in the chamber (to control its activity and prevent contact with the stirring rod) and was immersed in air-saturated water. The chamber was then sealed, the oxygen probe was inserted, and the stirrer mechanism engaged. The system was allowed to equilibrate for 30 sec, after which the concentration of dissolved oxygen was monitored for 10–20 min (the longer times were used with the smaller fish). The rate of oxygen consumption (QO2, in mg/kg/hr) was subsequently calculated.

Three experiments were conducted in which the QO2 of artificially infected bluegill was compared to that of uninfected individuals of the same size class. On July 14, 1981 the oxygen consumption of 15 uninfected bluegill (5 of each length size class, ≤30 mm, 31–50 mm, 51–70 mm t.l.) from Reed's Pond was measured. The fish were then artificially infected (on the same day) and held at 25 C. The QO2 of these fish was subsequently determined on August 18 and on September 10. A group of uninfected bluegill from another pond was also maintained at 25 C and their QO2 was determined on July 20, August 3, and October 3. The third experiment involved 60 bluegill from Reed's Pond (<70 mm t.l.) whose rate of oxygen consumption was measured on July 23, immediately after which the fish were artificially infected. The QO2 of these infected fish was then determined on August 23. At the end of these experiments the number of cysts in each fish was counted. Of all the fish were then placed into separate airtight containers, frozen (<30 C), and kept for lipid analysis.

Effect of Uvulifer ambloplitis on the lipid content of bluegill: Total body lipid was measured for the 60 bluegill used in the third oxygen consumption experiment (described above). Total lipid was also measured for the bluegill that died during the course of the experiments in which water temperature was decreased to mimic the onset of winter. In addition, 64 uninfected bluegill were starved to death at 5 C (n = 32) and 25 C (n = 32). Total body lipid at the time of death from starvation for these uninfected fish was compared to that at the time of death of infected fish.

Data analysis

Data that were found to be heteroscedastic (nonnormally distributed) when compared to a normal probability plot were transformed (log or square root transformation) prior to analysis in order to meet the assumptions of normality required by parametric statistical tests.

Single classification and factorial analysis of variance (ANOVA) were used to compare body condition, oxygen consumption, total body lipid, and survivorship of bluegill. Prior to each ANOVA, variances were examined for similarity using the Fmax-test. Spearman's coefficient of rank correlation (r) was used to determine associations between parasite intensity and body condition, total body lipid, and survivorship of bluegill. The Spearman coefficient was also used to determine correlations between total body lipid and body condition, and between total body lipid and survival. Any statistical probability less than 0.05 was considered significant.

RESULTS

Field studies

Behavior of Megaceryle allon: A total of 89 observation days were spent studying the visi-
Table I. Prevalence and intensity of *Urobilis ambloplitis* in bluegill (<70 mm T.T.) recovered from stomachs of largemouth bass from Reed's Pond during July, August, and September 1980.

<table>
<thead>
<tr>
<th>Month</th>
<th>Age class</th>
<th>N. with bluegill</th>
<th>N. of bluegill</th>
<th>Cysts per fish</th>
<th>% Heavily infected*</th>
<th>% Heavily infected in Reed's Pond</th>
<th>P for pooled vs. stomachs</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0+</td>
<td>6</td>
<td>5</td>
<td>14</td>
<td>21</td>
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<td>6</td>
<td>3</td>
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<td>55</td>
<td>155</td>
<td>114</td>
<td>114</td>
<td>11</td>
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</table>

* Fish with >50 cysts.

- Predation by largemouth bass on parasitized bluegill: Analysis of the stomach contents of 75 largemouth bass indicated that they were feeding on bluegill which had a wide range of *U. ambloplitis* cysts (Table I). An age-specific trend was noticed in the feeding behavior by bass, with age 0+ to 3+ fish accounting for a majority of the bluegill ingested. On an age-specific and month-specific basis, the bass occasionally contained a significantly greater percentage of heavily parasitized bluegill (>50 cysts/fish) than were simultaneously present in Reed's Pond. However, there was no discernable pattern to these observations. Thus, when data were pooled to include all age classes of bass and all months, a significantly higher percentage of bass actually contained lightly parasitized or uninfected bluegill as compared with heavily infected (>30 cysts) fish.

- Body condition, total body lipid and survival of parasitized bluegill: There was a significant negative correlation between the intensity of parasitism by *U. ambloplitis* and body condition of bluegill from Reed's Pond (Fig. 1). Differences in body condition between heavily parasitized (>50 cysts/fish) and lightly parasitized (<25 cysts/fish) individuals were significant for every month in which comparisons were made (F values ranged from 4.99—9.96 and, in all cases, \( P < 0.05 \)).

A significant negative correlation was also found between the intensity of parasitism and total body lipid (Fig. 2). Lipid content was significantly different between heavily parasitized and lightly parasitized fish, regardless of length class (for fish <30 mm, \( F = 48.44, P < 0.001 \); for fish 31—50 mm, \( F = 79.60, P < 0.001 \); for fish 51—70 mm, \( F = 51.70, P < 0.001 \)), but was not different between the sexes (\( F = 2.11, P > 0.05 \)). In lightly parasitized bluegill, total body lipid was lowest during February and highest in May (Fig. 3). Total body lipid gradually declined from May through August, rose slightly in September, and then sharply decreased from November into February. Lipid content was significantly lower in February than from April through December (F values ranged from 1.07 to 21.30 and \( P = 0.05 \)). Total body lipid of fish that died during the October 1, 1982—January 1, 1983 survivorship experiment was consistently 3.1 to 5.1% of dry weight; no correlation
was evident between the intensity of parasitism and total body lipid at the time of death. A significant positive correlation did, however, exist between body lipid and body condition for bluegill collected from Reed’s Pond (Fig. 4).

Livebox studies showed that heavily parasitized bluegill did not survive through the winter, and seldom lived past the point at which the water temperature reached 4°C. However, most of the fish with <50 cysts survived. Almost all of the heavily infected fish died when water temperature was ≤10°C. The intensity of parasitism and the length of time until death following the onset of declining water temperature were negatively correlated (Fig. 5). Differences between the survival time of lightly vs. heavily parasitized fish were also significant (Table II).

**Laboratory studies**

*Survival of parasitized bluegill.* Artificially infected bluegill which carried large numbers of *U. ambloplitis* (>50 cysts/fish) did not survive when subjected to slowly decreasing water temperature. However, almost all of the fish with <50 cysts lived, producing a significant negative correlation between intensity of parasitism and longevity (Fig. 6). Almost all of the heavily infected fish died when water temperatures fell below 10°C. Differences in the survivorship of heavily and lightly infected fish were significant (Table II). If bluegill were artificially infected, held for 60 days at 20°C, and then subjected to decreasing water temperature, there was no difference in survival regardless of the intensity of parasitism (Table II). Similarly, intensity of parasites did not affect the survival of fish that were held at constant temperature (Table II), or fish that were 80–110 mm t.l. and subjected to lower temperatures (Table II).

*Association between Uvulifer ambloplitis and body condition of bluegill.* Body condition at the time of death was not significantly correlated with intensity of parasites for bluegill that died during the June 2–August 3, 1982 survivorship experiment. Differences in mortality between heavily and lightly parasitized fish exposed to decreasing
Figure 2. Association between parasite intensity and total body lipid for bluegill from Reed’s Pond, November 1981 through October 1982. All fish (≤70 mm total length) were naturally infected with *Umbilicaria ambiguus*. $n = 182$, correlation coefficient ($r$) $= -0.963$ ($P < 0.001$). The dotted line indicates the maximum intensity observed for bluegill that overwintered successfully in Reed’s Pond.

Figure 3. Seasonal variation in total body lipid of bluegill from Reed’s Pond, November 1981 through October 1982. All fish were lightly infected (≤25 cysts per fish) or uninfected. $n = 15$ fish per month (all ≤70 mm t.l.). The lines connect mean values.
water temperature were also not significant. However, the body conditions of bluegill that were artificially infected and subsequently held at 25°C were significantly depressed by approximately 30 days post-infection (Figs. 7, 8). Body condition remained significantly lower for another 30 days, after which it gradually recovered to the pre-infection level. This pattern held for all 3 length classes of fish.

**Association between Uvulifer ambloplitis and oxygen consumption of bluegill:** The rate of oxygen consumption of bluegill that were artificially infected and subsequently held at 25°C was significantly elevated at 30 days post-infection (Fig. 9). By day 60, however, O2 had returned to pre-infection levels. This pattern occurred consistently among the 3 length classes of fish.

**Table II. Statistical summary for ANOVA of longevity between bluegill with >50 cysts of Uvulifer ambloplitis and those with <50 cysts following various treatments.**

<table>
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<th>Experimental conditions</th>
<th>df</th>
<th>F</th>
<th>P</th>
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</thead>
<tbody>
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<td>Outdoor aquaria—water</td>
<td>1,17</td>
<td>72.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Outdoor livebear—water</td>
<td>1,17</td>
<td>364.12</td>
<td>&lt;0.001</td>
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<td>Indoor aquaria—constant temperature</td>
<td>1,169</td>
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<td>1,15</td>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
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<td>1,25</td>
<td>2.11</td>
<td>&gt;0.05</td>
</tr>
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<td>80–110 mm (L) fish</td>
<td>1,29</td>
<td>3.11</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* All fish were >70 stem total length unless otherwise stated.
† Natural infections of Uvulifer ambloplitis.
‡ Experimental infections of Uvulifer ambloplitis.
§ Fish were experimentally infected with Uvulifer ambloplitis, held for 60 days at 25°C, and then subjected to declining water temperature.
Figure 5. Association between parasite intensity and survival following onset of declining water temperature for bluegill from Reed's Pond during fall and winter 1980, 1981, and 1982. Data are for fish held in outdoor aquaria at ambient temperature (n = 18) or in liveboxes in the littoral zone (n = 174). All fish were naturally infected with *U. ambloplitis* (≤70 mm total length). Correlation coefficient (r) = -0.940 (P < 0.001). The dotted line indicates the maximum intensity observed for bluegill that overwintered successfully in Reed's Pond.

Figure 6. Association between parasite intensity and survivorship following onset of declining water temperature for bluegill from Reed's Pond held in aquaria indoors. All fish were naturally infected with *U. ambloplitis* (≤70 mm total length). n = 120, correlation coefficient (r) = -0.864 (P < 0.001). The dotted line indicates the maximum intensity observed for bluegill that overwintered successfully in Reed's Pond.
Figure 7. Effects of parasitism by *U. ambloplitis* on body condition of bluegill from Reed's Pond held at constant temperature (25 C). All fish were uninfected when captured (July 2) and were experimentally infected with cercariae of *U. ambloplitis* on July 16. Resulting artificial infections were 69–167 cysts per fish. The body condition measurements for July 16 were conducted prior to exposure to cercariae. n = 5 fish per data point. Experimental values were significantly different from the control for each size class of bluegill on August 7 and September 1 (P < 0.01) but not on October 2 or November 16.

**Lipid of bluegill.** When uninfected bluegill died, their total body lipid ranged from 3.0–6.0% dry weight (Fig. 10) and was not significantly affected by water temperature. Total body lipid at the time of death of parasitized fish which died during exposure to decreasing water temperature was not significantly correlated with the intensity of parasitism. Death among those fish also consistently occurred when body lipid was between 3.0% and 6.0% of dry weight, regardless of the length of the fish. Differences between heavily and lightly parasitized individuals also were not significant. However, the lipid content of bluegill that were artificially infected and subsequently held at a constant temperature of 25 C decreased significantly by 30 days post-infection; remained significantly lower through day 60, but by day 90 returned to pre-infection levels. Relationships among the intensity of parasites, total body lipid, and oxygen consumption on day 30 are shown in Figure 11.

**Discussion**

Effects of *U. ambloplitis* on oxygen consumption, total body lipid, and body condition of bluegill

It is evident that artificial infection of bluegill with *U. ambloplitis* was accompanied by an increase in metabolic activity. Concomitant decreases in total body lipid suggest that the energetic requirements of these infected fish were not adequately met by feeding; hence, they must utilize stored energy reserves.

The elevated rate of oxygen consumption and utilization of body lipid of newly infected bluegill may, in part, be a consequence of 2 aspects of the infection. First, detailed studies by Davis (1936), Lewert and Lee (1954a, b), and Erasmus (1960) have shown that migration of strigeid cercariae within infected fish results in cellular destruction, internal bleeding, edema, an intense local inflammatory response, and even death in some hosts. Similar host responses have been
described for infections with *U. ambloplitis* (Krull, 1934; Hunter and Hamilton, 1941). Trauma from any source is well known to induce a non-specific stress response in fishes, resulting in a variety of behavioral and physiological changes (Wedemeyer et al., 1976; Ali, 1980; Pickering, 1981). Elevated oxygen consumption is a universal component of the stress response. Therefore, the trauma induced from tissue damage caused by migrating cercariae, in conjunction with the resulting inflammatory response, may cause an increase in metabolic activity.

A second and perhaps more important consequence of parasitism by *U. ambloplitis* is the energetic cost during the formation of the host cyst. Once a cercaria has become embedded in host tissue, it quickly (within a few hours) secretes a cyst wall, presumably as protection from attack by cellular and non-cellular components of the host's immune system. After approximately 1 day, host macrophages appear in the inflamed area surrounding the parasite and the fish forms a fibrous capsule around the parasite (Hunter and Hamilton, 1941). The mobilization of fibroblasts and formation of the cyst wall continue for up to 3 wk; the final phase of cyst formation includes melanization of the fibrous capsule (Hunter and Hamilton, 1941). The energetic cost of synthesizing fibrous capsules around many dozens of parasites would constitute a metabolic demand to the host. Moreover, the increased utilization of body lipid by infected bluegill may have also contributed to elevated oxygen consumption since more oxygen is required to completely catabolize lipid than carbohydrate or protein (Love, 1970).

Since cercaria migration and encystment, and the initial stages of the host's immune response, are complete within 5 days of infection (Hunter and Hamilton, 1941; Hoffman and Putz, 1965), the metabolic demands and associated lipid depletion of the host after 5 days are most likely a consequence of the formation of the host cyst. This is especially so since the metacercariae rely
Figure 9. Effects of parasitism by *Uvulifer ambloplitis* on oxygen consumption of bluegill from Reed's Pond held at constant temperature (25°C). All fish were uninfected when captured (July 1) and were experimentally infected with cercariae of *Uvulifer ambloplitis* on July 14. Resulting artificial infections were 59–167 cysts per fish. The oxygen consumption measurements for July 14 were conducted prior to exposure to cercariae. *n* = 5 fish per data point. Experimental values were significantly different from the controls for each size class of bluegill on August 18 (*P* < 0.001), but not on September 10.

Figure 10. Total body lipid present at time of death for uninfected bluegill from Reed's Pond following starvation. *n* = 33 for each water temperature; all fish were ≤70 mm total length. Correlation coefficient (r) = −0.211 for 5°C (*P* > 0.05); r = −0.364 for 25°C (*P* > 0.05).
on stored energy reserves to support their own metabolism and do not directly impose an energetic demand on their host (Hoffman and Putz, 1965; Vernberg and Vernberg, 1971). Thus, the initial impact of cercaria penetration is inadequate to account for the sustained elevation in oxygen consumption observed 30 days post-infection and the lipid depletion observed up to 60 days post-infection. Therefore, the major energetic impact of *U. ambloplitis* on bluegill, beyond the point of cercaria migration and secretion of the inner cyst wall by the parasite, is probably a non-specific metabolic stress stimulated through the generation of new tissue to be incorporated into host cysts. Once *U. ambloplitis* is established and the cysts have been fully formed, the parasite probably constitutes little energetic demand upon its host.

The extent of stress imposed on bluegill by both artificial and natural infections of *U. ambloplitis* appears to be directly proportional to the intensity of parasites. However, because cercariae are recruited over a period of weeks in nature (Lemly and Esch, 1984a, b) rather than a period of hours as in artificial infections, the period of elevated oxygen consumption and decreased total body lipid would likely be much longer for bluegill in a natural setting such as Reed's Pond. This hypothesis is supported by the observation that body lipid of naturally infected bluegill, as indicated by body condition, was reduced throughout much of the period of parasite recruitment. Thus, the metabolic effects of *U. ambloplitis* on juvenile bluegill in the laboratory and in the field seem to be similar.

Some controversy exists in the literature concerning the effects of black grubs or black-spot parasites on the body condition of fishes. Hunter and Hunter (1938) reported that *U. ambloplitis* caused weight loss and, thereby, a reduction in the body condition of artificially infected smallmouth bass. However, Rabideau and Self (1953) found no such relationship for green sunfish and orange-spotted sunfish in a natural environment; their conclusions were, however, based on comparison of 1-4 year old fishes which had been pooled into a single group. The body conformation of centrarchids changes dramatically with age (Carlson, 1977), resulting in a high length-weight ratio for young individuals and a much lower ratio for older individuals. Therefore, a comparison of body conditions across widely different age classes is invalid for studies on centrarchids.

In the present study, data from laboratory and field investigations indicated a direct relationship between changes in lipid content and the quantitative effects of *U. ambloplitis* on bluegill. Comparable data have seldom been obtained in other investigations which have made use of body
condition to make inferences about fish health. One notable exception is the study by Caulton and Bursell (1977) in which changes in body condition were closely paralleled by changes in various chemical constituents within the tissues. Comparisons in the present study were confined within specific size classes so that size-related bias in body condition would be minimized. Correlations between body condition and total body lipid were highly significant and indicate that, in this case, length-weight relationships and body lipid provided a very good assessment of fish health. Similarly, correlations between total body lipid and body condition in relation to parasite intensity indicate that differences in body condition were paralleled by physiological and biochemical changes in the fish. Because of the similarity between laboratory and field data, it can be firmly stated that the observed differences in body condition among juvenile bluegill in Reed’s Pond were linked to parasitism by *U. ambloplitis*. This notion is further supported by the observation that the artificial infections used to generate the laboratory data were not exceptionally high, unlike studies by Knill (1934) and Hunter and Hunter (1938), but were actually quite moderate as compared to intensities commonly found in bluegill from Reed’s Pond (60–140 cysts/fish in the laboratory vs. 100–225 cysts/fish in the field). In addition, the absence of any other internal or external parasite associated with these fish precludes the possibility of bias in the data from possible synergistic effects of multiple parasitic infections. Thus, body condition can be useful in assessing the effects of parasitism if the inherent error due to conformational changes in body shape with age is adequately considered.

**Effects of *Uvulifer ambloplitis* on survival of bluegill**

Perhaps the most notable observation made at the outset of this study was that bluegill heavily parasitized by *U. ambloplitis* (>50 cysts/fish) were very common in Reed’s Pond in the fall, but were absent the following spring. From the studies by Fischthal (1949) and from data generated in our earlier study (Lemly and Esch, 1983), it was evident that the parasites and cysts were not being lost during the winter, but rather that the heavily infected fish were disappearing. Three hypotheses were developed to explain the elimination of heavily parasitized fish. These were: 1) selective predation by the definitive host, 2) selective predation by largemouth bass, and 3) parasite-induced host mortality.

Attempts to study the behavior and feeding of kingfishers at Reed’s Pond were fruitless (Lemly and Esch, 1984b). Although the seasonal pattern of infection in *H. trivolis* suggested that visitation by kingfishers was most frequent in the spring (Lemly and Esch, 1984a), no direct evidence was obtained to suggest that the definitive hosts were responsible for the removal of the 10–20% of the young-of-the-year bluegill which were heavily parasitized and failed to overwinter successfully. There was also no evidence that heavily parasitized fish were more vulnerable to predation (after extensive observations in the laboratory, no difference in feeding or swimming behavior of infected and uninfected bluegill was apparent). Observations on infected bluegill in the field also did not reveal any peculiar behavior (e.g., side-swimming, surfacing, etc.) such as that which occurs in other fishes in response to other parasites (Holmes and Bethel, 1972; Radabaugh, 1980; Brassard et al., 1982). Thus, selective predation by kingfishers was unlikely to account for the disappearance of heavily infected bluegill.

Largemouth bass, which were abundant in Reed’s Pond, could have selectively eliminated heavily parasitized bluegill (Lemly, 1980). However, data from the studies conducted to test this hypothesis did not confirm it.

The final hypothesis, namely that of parasite-induced host mortality, was confirmed by both laboratory and field studies. In laboratory survival experiments involving water temperatures similar to those which occur in Reed’s Pond during winter, essentially all of the heavily parasitized bluegill died. On the other hand, most lightly parasitized or uninfected individuals lived for the duration of the studies. The laboratory data were confirmed by field experiments which showed similar survivorship patterns for parasitized fish during the winter. Thus, all of the direct evidence indicated that *U. ambloplitis* was responsible for the mortality of heavily parasitized bluegill.

By plotting the data for survivorship in the laboratory and livebox experiments against parasite intensity, it is possible to predict the percentage of hosts expected to survive through the winter in Reed’s Pond, given a particular level of parasitism. The curves (Fig. 12) indicate that survivorship of bluegill should be essentially unaffected by parasite intensities of <20 cysts/fish. However, for fish with a range of 20–50 cysts,
survivorship should decrease from about 90% to essentially zero. Thus, the complete range of survivorship of infected bluegill can be accounted for by an increase of only about 30 parasites/fish (from 20/fish to 50/fish), with median survival occurring at an intensity of 38 parasites/fish. These predictions were confirmed by direct evidence from over 4 years of data on the seasonal dynamics of parasitized bluegill in Reed's Pond. Thus, none of the fish with >50 cysts survived the winter (Lemly and Esch, 1984b). Moreover, the pattern of survival did not change during the course of these studies. Analysis of the experimental data has, in this case, provided a very accurate model of host-parasite interactions and regulation in the field.

As discussed previously, it is clear that infection of bluegill with *U. ambloplitis* results in the mobilization and breakdown of lipid, increased metabolism, and a concomitant decrease in body condition of the fish. However, there also appears to be a close relationship between the time of lipid depletion and the probability of associated mortality. In the survivorship experiments when the water temperature decreased, mortality of heavily parasitized fish occurred. However, at constant temperature (20–25 °C), no differences in mortality among heavily infected and uninfected or lightly infected fish were noted. Similarly, if fish were artificially infected, held for a sufficient period of time to allow the formation of cysts and recovery of lipid reserves, there was no increase in mortality, even during subsequent exposure to low temperature. Thus, the depletion of body lipid only influences mortality rate in conjunction with low temperature.

The association between lipid depletion and temperature-dependent mortality is apparently correlated with the ecology of the fish. A large database is available which shows that small fishes, notably centrarchids, are almost totally dependent upon stored lipid as an energy source.
during winter (Oliver, 1977; Salamon, 1979; Oliver et al., 1979; Toney and Coble, 1980; Pierce et al., 1980). As water temperatures decline, the activity and feeding of these fishes decreases substantially, sometimes reaching a condition of total dormancy in mid-winter. Therefore, feeding is environmentally suppressed by the late fall and the fish must rely on stored energy reserves for overwintering. Reliance upon stored energy reserves is well illustrated by data from the present study which show a sharp decline in body lipid during the winter when feeding is suppressed, followed by a rapid rise the following spring as feeding resumes. It therefore follows that fish must accumulate a lipid reserve by fall in order to survive through the winter. Fish that do not acquire this critical level of lipid by the time water temperatures begin to decrease will not be able to overwinter successfully (Salamon, 1979; Oliver et al., 1979; Toney and Coble, 1980).

Parasitism by *U. ambloplitis* clearly stimulates mobilization of the reserve supply of lipid. A concomitant decrease in water temperature would suppress feeding and the fish would be forced into winter in a less favorable energetic condition. If lipid depletion was extensive enough, a fish would not be expected to survive the winter. With sufficient time between the end of parasite recruitment and the onset of winter, bluegill in Reed’s Pond would be able to feed and accumulate sufficient lipid reserves to overwinter successfully. However, this is not the case since parasite recruitment and decreasing water temperature overlap temporally (Lemly and Esch, 1984a, b). Feeding is thus environmentally suppressed before a sufficient recovery of lipid reserves can occur among heavily infected bluegill.

Both laboratory and field data indicate that infected bluegill died when total body lipid approached, or fell below, 5%. Similarly, uninfected bluegill that were starved had a lipid content of about 5% at death. Therefore, a minimum total body lipid necessary to survive the winter appears to be approximately 5% dry weight. The actual cause of death to a fish which experiences severe lipid depletion is probably a combination of acidosis and accumulation of ammonia (from protein catabolism) and ketone bodies that arise during lipid catabolism (Love, 1970).

The potential for parasite-induced host mortality is evidently restricted to bluegill <70 mm t.i. since, beyond this length, differences in survivorship were no longer apparent. Bluegill larger than 70 mm t.i. move into open water away from the littoral zone in Reed’s Pond. Consequently, their parasites were probably acquired when the fish were smaller and frequented shallow water. Moreover, these individuals would probably recruit fewer parasites over their remaining life span because of lower exposure probabilities in deeper water.

Although many fish parasites are considered harmful (Hoffman and Meyer, 1974), and many studies deal only with negative effects, it appears that mortality due to *U. ambloplitis* may be beneficial to Reed’s Pond, certainly at the population level. It is well known that bluegill tend to overpopulate many small lakes and ponds. The condition results in stunted growth and large numbers of small, adult bluegill (Bennett, 1970; Cooper et al., 1971). In such situations, the growth and productivity of largemouth bass may decrease, eventually leading to reductions in successful reproduction and standing crop (Stroud and Clepper, 1975). Maintaining a balance between bluegill and bass is not easily accomplished and, where fishing pressure is light, removal of excess bluegill, or lake drawdown to reduce reproduction, have been advocated as the best methods to use in restoring a balance (Anderson and Funk, 1974; Novinger and Dillard, 1978). In Reed’s Pond, it is evident that bluegill are not stunted (Lemly, 1979); growth rates are among the most rapid and adult size among the largest reported in the literature (Carländ, 1977). Because reproduction is much greater by bluegill than largemouth bass (Lemly and Esch, 1984b), and since almost no fishing pressure has been exerted over the past 2 decades, it appears that the population balance between bass and bluegill and the excellent growth by both species in this pond is most unusual. Since 10–20% of the young-of-the-year bluegill are apparently eliminated each winter because of the effects of *U. ambloplitis*, the potential for overcrowding in Reed’s Pond is kept in check by this parasite. Thus, *U. ambloplitis* may effectively “crop” the excess in reproduction that would ultimately lead to overcrowding, stunting, and population imbalance commonly associated with the bass-bluegill combination in small lakes and ponds.

Although the possibility of parasite-induced host mortality has been considered in great detail by many theoreticians (Croston, 1971a, b; Anderson, 1976, 1978, 1979, 1980, 1982; May, 1977, 1983), almost no direct evidence has been developed to show that such mortality occurs in
natural populations of fishes. Even among the few studies which have reported mortality, death was almost always related to changes in the behavior of the host (i.e., they become more vulnerable to predation) rather than to direct negative effects of the parasite (Dence, 1958; Arme and Owen, 1968; Penney, 1971; Wilson, 1971; Brassard et al., 1982). A number of other studies are difficult to evaluate because more than 1 species of parasite was present (Hoffman and Dunbar, 1961; Becker and Brunson, 1967; Davies et al., 1973; Betterton, 1974; Sweeting, 1974). Because direct evidence of parasite-induced host mortality is often difficult, or impossible, to obtain, indirect assessments based on extrapolations from laboratory results, or relationships between observed and theoretical frequency distributions, have become increasingly popular methods for developing inferences regarding regulatory interactions between host and parasite populations (Penney; 1971b; Kakonge, 1972; Lopukhina et al., 1973; Lester, 1977; Rau and Gordon, 1978; Harrison and Hadley, 1982; Brassard et al., 1982; Gordon and Rau, 1982; Lemly and Esch, 1984b). It is in the light of these investigations that the value of direct evidence from the present study is realized. These data provide clear evidence of mortality in a natural population of fishes due to the direct effects of parasitism. The study is also significant in that parasite-induced host mortality was demonstrated through both laboratory and field evidence. Finally, simple cause and effect relationships between mortality and parasitism were established by physiological and biochemical data generated from both field and laboratory experiments.

While data from the present study clearly indicate that {U}. {amboinensis} is having a pronounced impact on the population biology of juvenile {L}. {macrochirus} in Reed’s Pond, we feel the situation may be unique to this habitat. A careful survey of 43 other ponds, lakes and streams in the same area indicates that both the prevalence and intensity of parasitism in juvenile centarchids is quite low (Lemly and Esch, 1984c); indeed, much lower than in Reed’s Pond. If we accept the observation that intensity levels of {U}. {ambloplitis} must be >50 cysts/fish in order to cause mortality among overwintering juvenile centrarchids, then it is also clear from Lemly and Esch (1984c) that the host-parasite relationship in Reed’s Pond is exceptional. Indeed, we would conclude that mutual regulatory interaction involving {U}. {ambloplitis} and juvenile {L}. {macrochirus} is the exception and not the rule.

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