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CURLING BEHAVIOR IN A PTERONARCID STONEFLY NYMPH

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Timothy Voskuil

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ABSTRACT

The research described in this thesis examined the question of curling in pteronarcid stonefly nymphs. Two major hypotheses were identified: (1) that curling functions as an anti-predator behavior, and (2) that curling functions as a position behavior, enabling nymphs to control their location while drifting in the stream current.

Experimental methods varied with hypothesis tested. Curling's function as a position behavior was primarily tested in the laboratory in a series of experiments examining how nymph behavior changed with experimental variable. Curling's function as an anti-predator behavior was tested in a series of predator-prey experiments in which the reaction of several fish predators to curled and uncurled (open) nymphs was recorded. Laboratory research was supported by field observations.

Results from the experiments examining curling's function as a position behavior showed that small (younger) nymphs curled more frequently than large (older) nymphs. Curled nymphs were also shown to sink more rapidly than open nymphs.

Results from the experiments examining curling's function as an anti-predator behavior indicated that some fish predators did reject nymphs, that nymphs always curled when attacked by fish, and that surviving, curled nymphs sustained fewer injuries than surviving, open nymphs when attacked, but rejected, by fish. Wide variation in fish behavior, both between species and within species, made interpretation of these results difficult.

From these results it was concluded that curling may function as both a position behavior and an anti-predator behavior.

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CHAPTER 1: INTRODUCTION

Aquatic insect morphology often reflects the pressures exerted by the stream environment. Ambuhl (1959) was the first to observe that the dorso-ventral flattening of many lotic insect nymphs enables them to move in the boundary-layer of passive water extending a few millimeters from the substrate and, thereby, to escape the turbulence of the main flow.

Behavioral adaptations may also be very important. For example, unusual posturing by larvae of the predatory mayfly Pseudiron centralis in areas of rapid flow and loose sand creates a vortex that exposes small invertebrate prey (Soluk and Craig 1990). Hence, asking questions about the possible advantages of a common behavior in a lotic insect may be an important step in understanding the survivorship and distribution patterns of that insect.

The specific behavior examined in this study is a water-column behavior of a pteronarcid stonefly nymph: these nymphs often exhibit curling behavior while in the water column. Curling behavior is defined in this thesis as behavior in which the nymph is curled so that its ventral surface is concave (i.e., surrounded by its head, thorax, and abdomen), similar to a curling sowbug or armadillo.

In preliminary tests it was found that curled nymphs were rejected by normally aggressive centrarchid predators (red-breasted sunfish [Lepomis auritus] and largemouth bass [Micropterus salmoides]) when introduced into the fish's aquaria. Did the curling behavior serve as a predator defense? Or did the curling behavior benefit the nymphs in some other way? Identifying potential advantages of curling behavior became the major goal of this study. Pertinent aspects of the drift and pteronarcid biology are examined in this introductory chapter. In addition, the major question of this research are identified and possible hypotheses discussed.

The Drift

One of the methods by which lotic invertebrates deal with the patchy lotic environment is the drift, or downstream transport of aquatic invertebrates in the current of streams and rivers (Brittain and Eikeland 1988). Invertebrates may passively enter the drift when catastrophic events, such as floods, pull them from the substrate (e.g. Tsuda 1972; McLay 1968; Crisp and Robson 1979; Bird and Hynes 1981). Invertebrates may also passively, and accidentally, enter the drift when exposed to stream currents while feeding (Waters 1965; Elliott 1967, 1968).

Invertebrates may also enter the drift actively. Hypotheses attempting to explain active entry include: (1) to escape benthic predators (e.g. Peckarsky 1979, 1980; Walton 1980; Corkum and Clifford 1980) and (2) to colonize, for a variety of reasons, new regions within the stream (Elwood and Cushman 1975; Otto 1976; Bohle 1978). The relative importance of these mechanisms in contributing to the drift are debated at present (Brittain and Eikeland 1988).

Part of the difficulty in understanding the drift is that invertebrates are assumed to run very real risks when entering the drift. Entering the drift is a balance between the advantages accrued in moving to a new location and the risks run while travelling to that location.

A primary danger is that visual fish predators will feed on the invertebrates as they drift downstream. Feeding studies of lotic fish show that a large percentage of their diet comes from drifting insects, both aquatic and terrestrial (e.g. Chapman and Demory 1963; Cada et al 1987).

Drifting at night is one defense against visual fish predators. Many studies have found that the drift does increase at night (e.g. Waters 1962; Muller 1963; Walton 1980; Allan 1978, 1984; Skinner 1985; Malmqvist 1988). There are exceptions to this rule. For example, Anderson (1966) found that drift increased at night on nights with a

new moon, but not on nights with a full moon. Of course the light of the full moon may have illuminated drifting nymphs, and Anderson's study can actually be used to support night-time drift as a predator avoidance behavior.

These studies did not attempt to differentiate between passive and active drift. Elliott (1968) tried to ascertain if nymphs are more active at night and thereby increase the drift through passive entry. His laboratory research indicated that mayfly nymphs were more active at night. Casey (1987) found, using direct observation of lotic nymphs, that most (but not all) taxonomic groups were more active at night.

Prey-preference research indicates that visual predators preferentially select the larger individuals to the smaller individuals within a given invertebrate taxonomic group (Metz 1974; Devonport and Winterbourn 1976; Newman and Waters 1984). Drift research supports this observation. For example, some studies have found that taxa dominated by small individuals, such as Chironomidae, do not show any diel pattern in drift frequency (Anderson 1966; Anderson and Lehmkuhl 1968). Other studies have found that the frequency of night drift increases with nymph size within some taxa. These taxa include Plecoptera (e.g. Anderson 1966; Elliott 1967; Anderson and Lehmkuhl 1968), Ephemeroptera (e.g. Anderson 1966; Muller 1966; Elliott 1967; Anderson and Lehmkuhl 1968; Malmqvist 1988), Chironomidae (e.g. Cowell and Carew 1976), Simuliidae (e.g. Anderson 1966), Trichoptera (e.g. Anderson 1967), and Amphipoda (Holomuzki and Hoyle 1990). In addition, Malmqvist (1988) found increased nocturnal drifting among a mayfly species in waterways with introduced trout but found no diel periodicity in waterways without trout. In response to data such as the above, Allan (1978) hypothesized that predation risk for aquatic insect nymphs increases with increasing nymph-size and that the greatest risk of predation for larger nymphs should be in the daylight hours. Therefore, larger benthic prey should be more constrained to nocturnal activity and drift than smaller individuals.

One assumption in this argument is that drifting invertebrates have no defenses other than to drift at night or to be really small. This thesis examined that assumption by testing the water column defenses of a pteronarcid stonefly. If this stonefly is shown to have defenses against fish, than perhaps other invertebrates do also and another variable must be considered in the description of diel drift patterns. Thus, the hypothesis that curling is an anti-predator behavior based on maximizing the effectiveness of the exoskeleton as a mechanical barrier or defense was the major hypothesis of this research.

The morphology of pteronarcids supports this hypothesis. The thoracic segments of the nymphs possess wing-like projections that appear to be more exposed when the nymph curls, thereby posing a greater deterrent to fish predators. The exposed, external gills, assumed to be vulnerable to damage, are also protected when the nymph curls.

A balancing drift-related risk that should also be considered is that invertebrates may drift too far downstream and into an unsuitable habitat (i.e. drifting from a small stream into a large stream). Research has shown that drift lengths increase across pools that are unsuitable habitat to lotic insects (Leudtke and Brusven 1976; Vinikour 1981). It is possible that curling is a behavior that enables pteronarcid nymphs to exert some control over their position in the water column. For example, Otto and Sjostrom (1986) demonstrated that three plecopteran species sank faster when showing a "u-form" behavior. Thus, the possibility that curling is a "position behavior" became the other major hypothesis that this research addressed.

These two major hypotheses are not mutually exclusive and could function simultaneously. Each could also assume primary importance at a different stage of nymph development. In addition to these two primary hypotheses, other hypotheses are described in the following section.

Question and Hypotheses

The primary question of this thesis is: What advantage(s) do(es) P. scotti gain from curling while in the water column? In addition to the two main hypotheses already identified (see "The Drift" above), other potential hypotheses should also be considered. There are four main classes of identified hypotheses:

1. Curling is a nonaptation, or "neutral."
2. Curling is a protective behavior.
3. Curling is a position behavior that increases the nymphs ability to control its water column position.
4. Curling is an anti-predator behavior.

Curling as a Nonaptation

Gould and Vrba (1982) defined a nonaptation as a feature not now contributing to an organism's fitness, but not necessarily with a maladaptive impact (i.e., "neutral"). The research conducted for this thesis was not constructed to test for nonaptation and so this hypothesis will not be addressed directly. However, nonaptation remains a possible explanation of curl behavior. Furthermore, failure of thesis data to support the other identified hypotheses does not necessarily support a nonaptation explanation: the proper hypothesis might simply not have been identified.

Curling as a Protective Behavior

Edmunds (1974) separated protective behaviors from defensive behaviors by noting that protective behaviors protect an organism from physical, chemical, or biological dangers in the environment while defensive behaviors are a subcategory of protective behaviors that protect prey from predators. Accordingly, curling may be a

protective behavior to minimize damage to the external gills from collisions with stationary or floating particles while the nymphs are drifting.

This hypothesis was not tested in the present research. However, field observation did have some application to this hypothesis and will be mentioned briefly in Chapter 3.

Curling as a Position Behavior

Curling also may be a means of escape from the drift. Otto and Sjostrom (1986) demonstrated that three plecopteran species sank faster when showing a "u-form" behavior. Accordingly, curling may be a position behavior that allows nymphs to control (to a limited but important extent) their vertical location in the drift.

This hypothesis was tested in a series of laboratory experiments and the laboratory results were supported by field observations. The laboratory experiments are described in Chapter 2 and the field observations in Chapter 3.

This thesis expanded on the research of Otto and Sjostrom (1986) by comparing variation in curl behavior among age groups and by examining potential advantages of curling as an anti-predator behavior.

Curling as an Anti-Predator Behavior

Three hypotheses were identified that considered curling an anti-predator behavior:

1. Curling is a defensive behavior based on maximizing the effectiveness of the exoskeleton as a mechanical barrier and defends drifting nymphs against fish predators.
2. Curling is a defensive behavior based on "thanatosis" and defends drifting nymphs against fish predators.

3. Curling is a camouflage behavior that minimizes fish attacks because the nymph does not appear to be food.

Obviously, curling could function as an anti-predator behavior in all three ways. Edmunds (1974) identified primary and secondary defense mechanisms. Primary defensive mechanisms function to decrease the probability of a predator-prey encounter, while secondary defenses function during a predator-prey encounter to increase the probability of prey survival. Hypothesis #3 would operate as a primary defense while hypotheses #1 and #2 both operate as secondary defenses. Curling as thanatosis is similar to curling as a camouflage behavior because, in general, the efficiency of both is maximized if the potential prey does not move. These two hypotheses are separated in that camouflage functions prior to predator-prey encounter while thanatosis functions during a predator-prey encounter.

The primary anti-predator hypothesis tested in this research was that curling functions as an anti-predator behavior by maximizing the effectiveness of the exoskeleton as a mechanical barrier or defense. Experiments conducted to test this hypothesis are described in Chapter 4. Curling as thanatosis was not directly tested in this research, but the research of Moore and Williams (1990) will be compared against present results and this hypothesis will be also be discussed. Due to the structure of the experiments, this thesis does not address curling as a camouflage behavior.

Pteronarcid Biology

The study animal was Pteronarcys scotti Ricker of the family Pteronarcyidae (order Plecoptera). This species was selected because it exhibits curl behavior and because of substantial populations near Knoxville, Tennessee.

The family Pteronarcyidae is composed of two genera: Pteronarcella, limited to western North American, and Pteronarcys, extant across North America. Pteronarcys was subdivided into two sub-genera (Pteronarcys and Allonarcys) by Ricker (1952) and these sub-genera were later given generic status by Illies (1965). Zwick (1973) also argued for a three genus taxonomy. More recently, however, these genera were rejected on cladistic grounds and rejoined into Pteronarcys (Nelson and Hanson, 1971; Stark and Szczytko, 1982; Nelson, 1988). The two genus taxonomy appears to be the accepted status currently (see Stewart and Stark, 1988).

The study animal was tentatively identified to P. scotti. Identification of pteronarcid stonefly nymphs to species is difficult; Brigham et al (1982) recommended that nymphs be raised to adults for conclusive identification. This did not prove feasible in the present study. The identification of the nymphs in this study was based on Ricker's key (1952). This permitted fairly positive identification to P. proteus or P. scotti. Identification beyond this was based on location: Ricker first described P. scotti and the type specimens came from near the study sites. The nymphs will be referred to as P. scotti in this thesis.

Pteronarcid stoneflies are readily identified by their large size (a length of 50 mm+ in final instars), their dark coloration, and the external gills located on the venter of their thorax and first two abdominal segments. They are detritivores ("shredders" in the sense of Cummins 1974) and feed on decomposing leaves and other plant materials that fall into streams and rivers. They may also feed on bacteria growing on the surface of this decomposing vegetation. Most studies of pteronarcid ecology have focused on the role they play in recycling carbon in the stream ecosystem (e.g. McDiffet 1970; Cummins et al 1973).

Pteronarcid species have a two to three year life cycle. Most of this time is spent as nymphs, with adults living only a few weeks. Both P. scotti (McDiffet 1970; Folsom

and Manuel 1983) and P. proteus (Holdsworth 1941a, 1941b) have been reported to have a two year life cycle.

Pteronarcids are sexually dimorphic. Holdsworth (1941a, 1941b) found P. proteus females to be larger than the males, and McDiffet (1970) found late instar P. proteus females to weigh two to three times as much as the males. Mature stonefly nymphs are easily sexed; females possess a mid-ventral notch on the eighth abdominal segment (Brigham et al 1982; Folsom and Manuel 1983). This character was used to separate sexes in this research.

Pteronarcids have interesting defensive mechanisms. Benfield (1974) found that adult P. proteus autohemorrhaged to protect themselves from arthropod predators. Autohemorrhaging in insects is the exuding of hemolymph from the intersegmental membranes of the legs. Benfield found that the exuded hemolymph stuck to ants, along with sand grains, and the ants would release their grip immediately, giving the adult time to escape. This defense was not effective against vertebrate (mice) predators.

Moore and Williams (1990) reported autohemorrhaging by P. dorsata nymphs when the nymphs were attacked by crayfish (Cambarus robustus). The hemolymph would adhere to the crayfish and, while the crayfish were cleaning themselves, the nymphs would move to safety. They reported this defense to be 100% effective.

When attacked by fish predators (rainbow trout [Oncorhynchus mykiss], sculpins [Cottus bairdi], and suckers [Catostomus commersoni]), Moore and Williams (1990) found that the nymphs would not autohemorrhage. Rather the nymphs would either freeze (i.e., cease moving in whatever position they were upon attack) or "feign death in a curled position." This latter defense they termed thanatosis. Thanatosis was not as effective a defense against fish (67% rejection frequency) as autohemorrhaging was against crayfish (100% rejection frequency).

The thanatosis that Moore and Williams (1990) described is equivalent to the curl behavior of this thesis. Other researchers have also described a similar type behavior in stoneflies. Otto and Sjostrom (1986) described a curl behavior in three non-pteronarcid stonefly species, but called the behavior "u-form" behavior. Blum (1989) described a "c" posture in nemourid stoneflies analogous to the present curl behavior.

Thesis Organization

Chapters are organized by the experiments grouped around a specific hypothesis; each chapter contains the methods, results, discussion, and conclusions for those experiments. Chapters 2 and 3 describe the experiments and observations that primarily relate to curling as a position behavior. Chapter 2 presents experiments conducted in the laboratory; Chapter 3 presents observations conducted in the field. Chapter 4 describes the experiments that primarily relate to curling as an anti-predator behavior. Chapter 5 contains an overall summary of the research and the conclusions drawn.

CHAPTER 2: LABORATORY BEHAVIOR EXPERIMENTS

The main hypothesis that the laboratory behavior experiments addressed was curling as a position behavior. In addressing this hypothesis, data were collected that tested whether behavior and nymph size affected sinking time. In addition, frequencies of water column behaviors were examined for significant associations with nymph size, test conditions, and stream of origin.

The hypothesis that curling is an anti-predator behavior was also examined. Here, changes in nymph behavior were examined in the presence and absence of fish scent in the test water. This test assumed that if curling was a defensive behavior, nymphs would curl in the presence of predators or signs (scent) of predators.

The laboratory behavior experiments consisted of two main sets of experiments: (1) experiments identified as "lab-kept" experiments in which the nymphs were tested several months after their capture and (2) experiments identified as "fresh-caught" experiments in which the nymphs were tested within days of capture. These two sets of experiments were not statistically tested against each other, but, rather, were used mainly to examine if behavior patterns found in the lab-kept experiments were repeated with fresh-caught nymphs.

In addition to the data and the experiments described above, size class distributions were examined. Although not a test of the actual hypotheses identified in Chapter 1, size class structures formed an underlying assumption of these tests and, thus, some knowledge of these structures became useful in forming conclusions.

Collection and Care of the Nymphs

During the summer of 1989 over a period of 3 weeks more than 400 P. scotti nymphs were collected from two streams, Norton Creek and Reed Creek. Both are low

order streams in eastern Tennessee just outside of the Great Smoky Mountains National Park. Because Norton Creek runs through a private development and Reed Creek is in a Tennessee State Wildlife Area, both streams probably experienced lower human impact than many neighboring streams. Norton Creek is a tributary to the Little Pigeon River and Reed Creek is in the Little River system, both in the upper Tennessee River drainage. Two distinct size classes of nymphs were visually apparent.

Citico Creek was the source of some of the nymphs used in the size distribution studies and all of the nymphs used in the predator experiments (Chapter Four). Because the size distributions are discussed in this chapter, a short description of Citico Creek follows. Citico Creek is a low order stream in the northern portion of the southern District of Cherokee National Forest. For most of its length it runs through Monroe County, Tennessee. Citico Creek is a tributary of the Little Tennessee River and is in the upper Tennessee River drainage. Nymphs were collected from its main channel and from minor tributaries.

At all sites, nymphs were collected from natural leaf or stick packs. These packs were wedged under or caught on rocks, logs, and other stable features of the substrate. Packs were pulled from the stream by hand, placed in a net, and sorted for nymphs. Nymphs were held in stream water until they arrived at the lab, where they were transferred to the dwelling tanks.

Nymphs were restricted to areas of high flow. The most densely occupied packs were often found lodged between neighboring rocks in natural "dams" or chutes where the water level dropped several inches. Leaf packs in which the leaves were less compact and in which twigs and small sticks created spaces also seemed more productive. Both of these habitat characteristics are presumed to reflect the high oxygen demands of the nymphs.

Once in the laboratory, nymphs were segregated according to the two size classes observed in the field and kept in four forty-liter aquaria (the dwelling tank) in the lab. The water was kept at a temperature of 16-19° C. They were fed a diet of decaying maple leaves. Aeration was supplied by mechanical pumps and the diel cycle was the natural cycle from light coming through the lab windows. Throughout their captivity, the nymphs showed low mortality and appeared healthy when examined. Laboratory care attempted to recreate natural conditions as nearly as resources permitted. Mimicking natural conditions is a common goal of aquatic invertebrate care when they are brought into the laboratory (e.g., Peckarsky and Penton 1988; Blum 1989).

Methods

Methods will be discussed for the experiments with lab-kept nymphs, the experiments with fresh-caught nymphs, and, finally, for the collection of size class data.

Experiments with Lab-kept Nymphs

The experiments were conducted in a 3-way crossed design. The three factors tested were:

1. Size class - whether the nymphs had been judged to be "small" or "large" when captured.
2. Origin - whether the nymphs came from the dwelling tank or a holding tank. The dwelling tank was the tank in which the nymphs were kept while in the laboratory, while a holding tank was a one-liter, aerated tank in which the nymphs were held for short periods of time prior to experimental tests.
3. Fish scent - whether or not the test aquarium was partially filled with water from a fish aquarium.

Thus, there were eight treatments:

1. Small nymphs taken from a holding tank and tested in unscented water.
2. Large nymphs taken from a holding tank and tested in unscented water.
3. Small nymphs taken from a holding tank and tested in fish scented water.
4. Large nymphs taken from a holding tank and tested in fish scented water.
5. Small nymphs taken from their dwelling tank and tested in unscented water.
6. Large nymphs taken from their dwelling tank and tested in unscented water.
7. Small nymphs taken from their dwelling tank and tested in fish scented water.
8. Large nymphs taken from their dwelling tank and tested in fish scented water.

Twenty-five nymphs were tested per treatment for a total of 200 individual tests. These treatments are displayed visually in Figure II-1.

	From Holding Tank	From Dwelling Tank
Without Fish Scent	25 small nymphs 25 large nymphs	25 small nymphs 25 large nymphs
With Fish Scent	25 small nymphs 25 large nymphs	25 small nymphs 25 large nymphs

Figure II-1. Experimental design for the lab-kept behavior experiments.

Nymph behavior in the water column and on the substrate was recorded. The following water column behaviors were observed:

1. Curl Behavior - The nymph sank through the water column in a curled position without moving.

2. Swim Behavior - The nymph swam in the water column by moving its legs in a rowing fashion. The nymph was always on its back while swimming.

3. Open Behavior - The nymph drifted through the water column without swimming, but also without exhibiting curling behavior; i.e. it was "open".

4. Back Arch Behavior - The nymph arched its back in such a way that the head and the posterior tergites were extended dorsally away from the venter. The legs were at least partially extended. This behavior is the opposite of curling and maximizes the spatial sweep of the legs.

In addition, the following substrate behaviors were observed:

1. Bottom Curl Behavior - The nymph lay on the substrate in a curled position without moving. Frequently, the nymph slowly relaxed from the curled position to the open position while lying on the bottom substrate. Because it was impossible to tell exactly when the behavior changed from a curled position to an open position, no differentiation was made between the two behaviors when a slow transition occurred.

2. Bottom Open Behavior - The nymph lay on the substrate without moving but also without exhibiting curling behavior; i.e. it was "open". By definition, this behavior had to be separate and unique and could not evolve from a bottom curl behavior, for reasons outlined above.

3. Crawl Behavior - The nymph crawled upright on the substrate.

4. Bottom Swim Behavior - The nymph swam as above, but it propelled itself along the substrate rather than in the water column. This behavior occurred only after one of the other substrate behaviors.

All behaviors were timed to the nearest tenth second using a hand-held Acutrak (model SC-504) stopwatch. In the laboratory behavior experiments, nymphs usually only

curled or swam while in the water column. Both behaviors were recorded and timed, but the swimming data were not analyzed statistically. This was due to the small size of the test aquarium: swimming nymphs would often change behavior upon contact with the aquarium sides.

Experiments were conducted in the lab where the nymphs were kept. They were conducted in a 40 liter aquarium in water at room temperature (i.e., the same temperature as that in the nymphs' dwelling aquaria). All experiments were run between 2 and 5 PM from October 3, 1989 to November 15, 1989. Experimental protocol was as follows:

1. The nymphs were removed from the dwelling tank. They were then either placed into a small (1 liter), aerated holding tank for subsequent testing or immediately tested, depending on experimental factor.
2. The nymphs were dropped into the experimental tank where their behavior was observed. Individual experiments were terminated when the nymphs had crawled on the substrate for at least 10 seconds. The water in this tank was still (non-aerated).
3. The nymphs were placed in a dish containing Alka-Seltzer. The carbon dioxide effectively anesthetized them so steps (4) and (5) could follow.
4. The nymphs' head capsule width was measured using the ocular scale of the dissecting microscope. The raw numbers recorded were later converted to millimeters by comparing the ocular scale to a micrometer and then calculating the proper ratio to transform the data.
5. The nymphs were reintroduced to the experimental tank, where their anesthetized sinking time was recorded. This time was used to compare uncurled sinking time versus curled sinking time. The water in this tank was still (non-aerated).

6. The nymphs were released into the "experienced" dwelling tank. Early in the series of experiments, nymphs were released in a second, aerated holding tank and observed overnight. When it was determined that nymphs were not adversely affected by the experimental treatment this step was discontinued.

Alka-Seltzer was the agent of anesthesia throughout the experiments in this research. High concentrations of CO_2 produced by the Alka-Seltzer temporarily anesthetized the nymphs. Other researchers have also used Alka-Seltzer to anesthetize arthropods (Nicolas and Sillans 1989). In this research, anesthetized nymphs were found to revive in five to ten minutes after being placed in a recovery tank. Preliminary tests indicated that Alka-Seltzer did not kill the nymphs. Other research has found that high CO_2 concentrations can affect insect behavior (see Nicolas and Sillans 1989). However, no behavior tests in this research followed anesthesia. The nymphs were anesthetized to collect size data and to measure the sinking times of open nymphs.

Experiments with Fresh-caught Nymphs

The behavior experiments with fresh-caught nymphs were similar to the behavior experiments with lab-kept nymphs. Because the nymphs in the lab-kept experiments had been kept in the laboratory for several months before being tested, the fresh-caught nymphs experiments were conducted to test two assumptions of the lab-kept experiments: (1) that prolonged captivity did not impact nymph behavior and (2) that creek of origin did not affect nymph behavior. Furthermore, preliminary examination of

the data indicated that fish-scent did not affect behavior and so these tests were discontinued. There were three goals in these experiments:

1. To test if stream of origin affected nymph behavior.
2. To test if tank of origin affected fresh-caught nymphs behavior.
3. To test whether size classes affected fresh-caught nymphs behavior.

Because the goals of the test were different from the lab-kept experiments, the experimental design was also different. The experiment still took a three-way factorial design with eight treatments, but the factor of "Fish Scent" was replaced by "Creek of Origin." Tank of Origin and Size were still factors. Because of the failure to catch equal numbers of large nymphs from Norton Creek and Reed Creek (see next paragraph), experiment cells had unequal sample size. The treatments in the fresh-caught laboratory experiments are visually displayed in Figure II-2.

	From Holding Tank	From Dwelling Tank
Nymphs from Reed Creek	13 small nymphs 13 large nymphs	12 small nymphs 12 large nymphs
Nymphs from Norton Creek	12 small nymphs 13 large nymphs	13 small nymphs 0 large nymphs

Figure II-2. Experimental design for the fresh-caught behavior experiments. The sample sizes represent actual sample sizes and not designed sample sizes.

The nymphs were caught on December 11, 1989 in the manner described previously under "Collection of Nymphs." About 30 large nymphs and more than 30 small nymphs were caught from Reed Creek. About 15 large nymphs and about 30 small

nymphs were caught from Norton Creek. To maintain equal sizes of large nymphs for statistical analysis, Norton Creek was resampled on December 12, 1989. Unfortunately, high water caused by overnight rains prevented capture of more nymphs. Sample sizes of large nymphs from Reed Creek (25) therefore differed from sample sizes for large nymphs from Norton Creek (13). Nymphs were segregated by stream of origin both in the field and in the lab.

The experiments with small nymphs were conducted December 14, 1989 from 2:30 to 7:05 PM. The experiments with large nymphs were conducted December 15, 1989 from 2:30 to 5 PM. Water temperature was 17° C both days. Treatment and stream of origin were tested each day. Size classes were tested on different days because stream of origin and treatment effect were the factors of primary interest. The only change in Experimental Procedures was that the anesthetized sinking time (step #5 in "Experimental Procedures" under "Behavioral Experiments with Lab-Kept Nymphs") was not recorded. The data from the experiments with the lab-kept nymphs were more than adequate to determine whether curling decreased sinking time.

Size Classes

As mentioned earlier, two size classes were visually apparent in the nymphs as they were collected from the stream. These size classes became one of the test factors. To test whether these classes were valid, head capsule width was recorded in the manner described earlier and subsequently tested using ANOVA.

The question of size class distribution is important because nymph size is generally assumed to be a good measure of nymph age. Therefore, if two distinct size classes are found, the nymphs are assumed to have a two-year life cycle. Head capsule width is a common method of measuring aquatic insect size (e.g. Holdsworth 1941a, 1941b;

Harper and Pilon 1970; Harper 1973), and Folsom and Manuel (1983) found head capsule width to be the best way to determine pteronarcid nymph age.

Visual examination of the head capsule width data from the lab-kept experiments revealed a trimodal distribution. Three possible hypotheses were identified for this size structure:

1. The trimodal distribution represented a three-year life cycle as nymphs.
2. The trimodal distribution represented a sexual dimorphism in size for the second year of the nymphs life.
3. The trimodal distribution represented a laboratory artifact.

In order to evaluate these hypotheses, all nymphs subsequently captured were sexed and sized. Most of these nymphs were the nymphs captured for the predator experiments, but one sample occurred simply to collect size distribution data. The nymphs from the predator experiments came from Citico Creek and its tributaries, while nymphs from the other sampling date came from Reed Creek. While comparisons between creeks introduced an experimental unknown, it was felt that continued sampling from Reed Creek might introduce a harvesting effect on that population, thus affecting not only size distribution analysis, but the health of the population itself. Collection locations and dates for which both sex ratios and size distributions were measured are listed below:

1. Norton and Reed creeks; December, 1989.
2. Reed Creek; March, 1990.
3. Citico Creek; May, 1990.
4. Citico Creek; May/June, 1990.
5. Citico Creek; June, 1990.

Because the experimental design assumed two size classes, statistical analyses were based on two size classes and not the three size classes indicated in the size distributions from the laboratory experiments.

Results

Because size class gave supporting data for assumptions built into the design of the lab-kept and fresh-caught experiments, size class will be discussed first. Other results will be discussed by type of data collected.

Size Classes

Although the experimental design assumed two size classes, examination of the head capsule frequencies from the lab-kept and fresh-caught data revealed three peaks of frequency in each set of experiments (Figures II-3 and II-4a). Both groupings, two size classes and three size classes, were found to explain significant variation when tested with ANOVA. For the lab-kept nymphs both two size classes and three size classes showed a $P < 0.001$ (F-ratio = 999.999 for both two and three size classes). For the fresh-caught nymphs both groupings also showed a $P < 0.001$ (F-ratio = 668.269 for two size classes; F-ratio = 760.655 for three size classes).

Examination of size class data from other population samples failed to show a three peaked graph (see Figs. II-4b through II-4e). Although the data from Citico Creek in June, 1990 (Fig II-4e) do show three peaks, the two larger peaks are not very distinct. However, examination of size distributions by sex reveals distributions more similar to those in the laboratory experiments. Following, data from each collection location and collection date are discussed separately.

The Reed Creek data of March, 1990 were limited by small sample size, particularly in the older, larger size classes. Nymphs may have begun to emerge at this

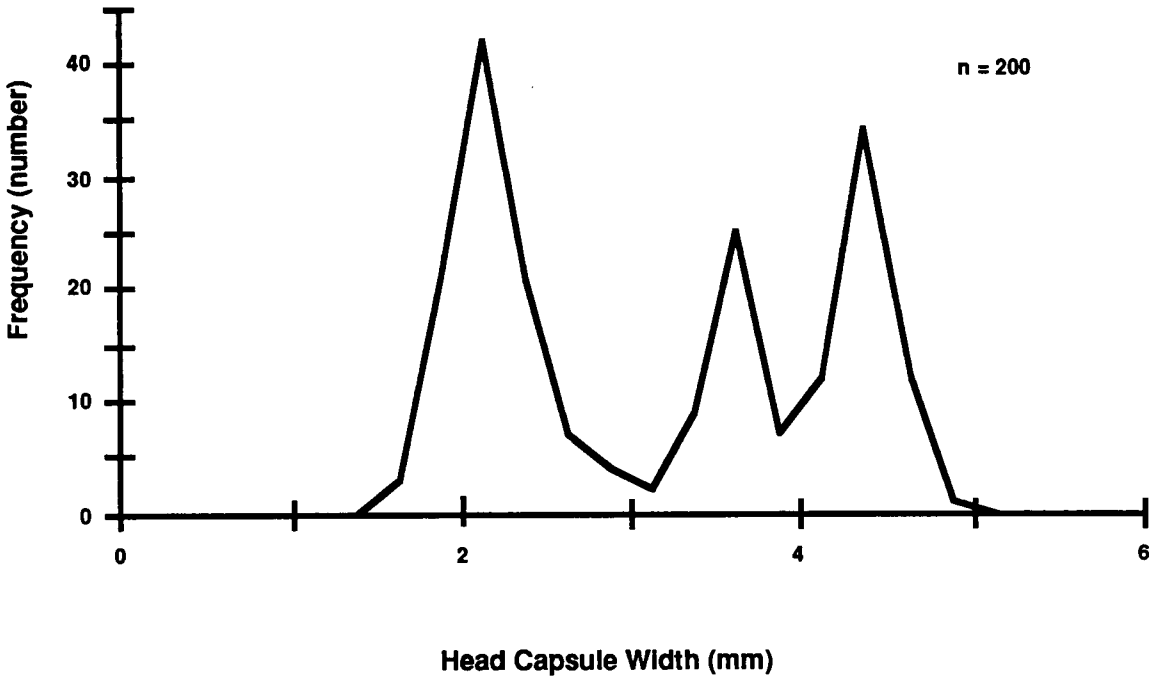


Figure II-3. Head capsule width distribution of the nymphs used in the lab-kept experiments.

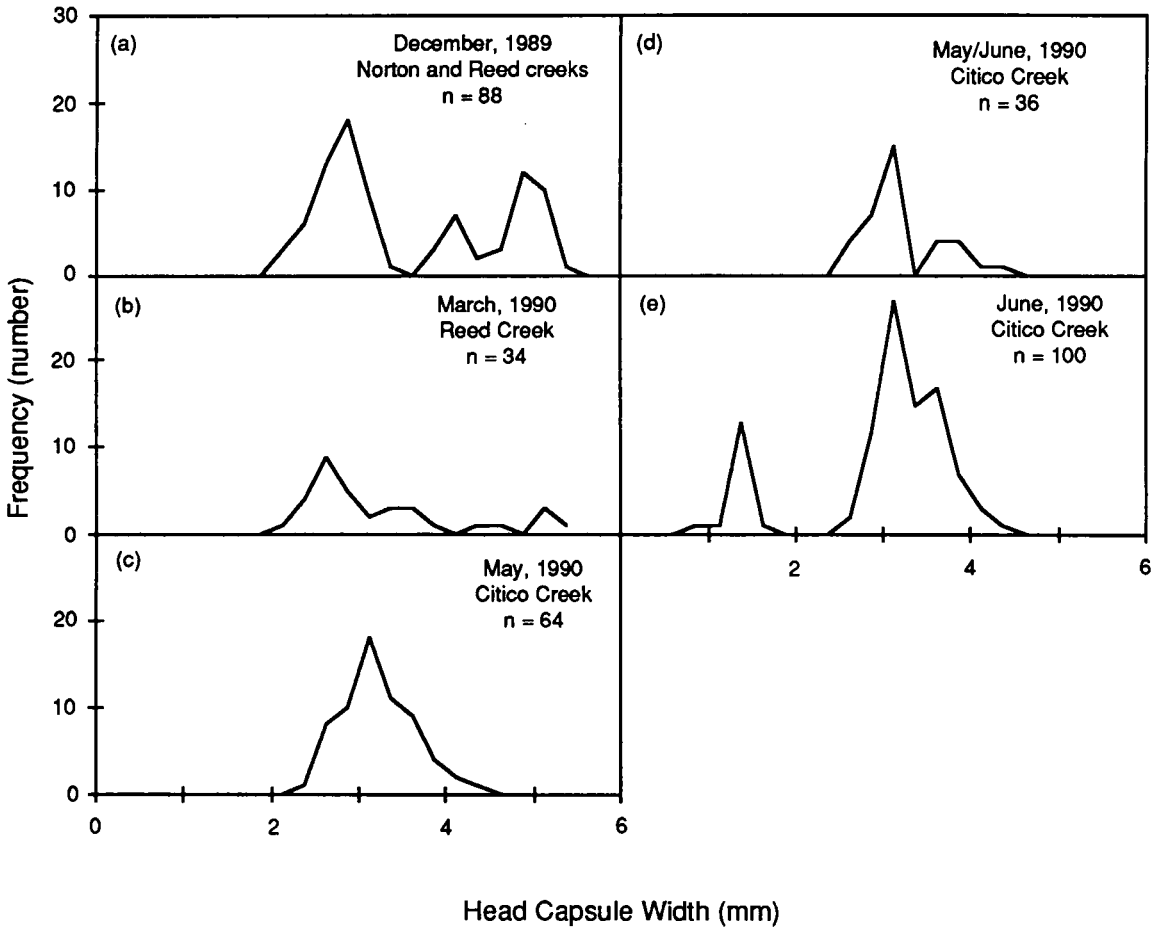


Figure II-4. Head capsule width distribution of nymphs from five sampling dates. Nymphs emerge in March and April.

time, resulting in reduced densities of large nymphs. It is also possible that previous sampling for the experiments impacted the population's size distribution. Examination of the distribution by sex (Fig. II-5) shows that females are larger than males and that the sexes appear to represent two distinct groups within the larger size class.

The single-peaked distribution of Citico Creek of May, 1990 does not show the newly hatching nymphs. The newly hatched nymphs were first observed in late May, and only in very low densities ($n < 5$). Examination of the distribution by sex (Fig. II-5b) shows that within the single peak, a bimodal distribution was developing, with females larger than males. If the hatching nymphs are included in the data from Citico Creek in late May (Fig. II-5d), a three peaked distribution is revealed.

Finally, the larger peaks of Citico Creek in June, 1990 (Fig. II-4e) become more distinct when the sexual distribution is analyzed (Fig. II-5d). (Note: the newly hatched nymphs were too small to sex at this time.)

In general, examination of the data showed that sexual dimorphism played an important role in shaping the size distributions. It appears that a two-year life cycle is more likely than a three-year life cycle. Furthermore, it appears that sexual dimorphism in size probably becomes distinct around the end of the first year/beginning of the second year. This conclusion is complicated by the inability to sex small nymphs.

Although it appears that the three-peaked size distributions in the behavior tests were probably explained in large part by sexual dimorphism, only two size classes were used in statistical tests of the laboratory behavior experiments. Sexual variations in behavior were not examined. This was because the experimental design assumed two size classes, and because it was not known whether the size classes accurately represented the sexes.

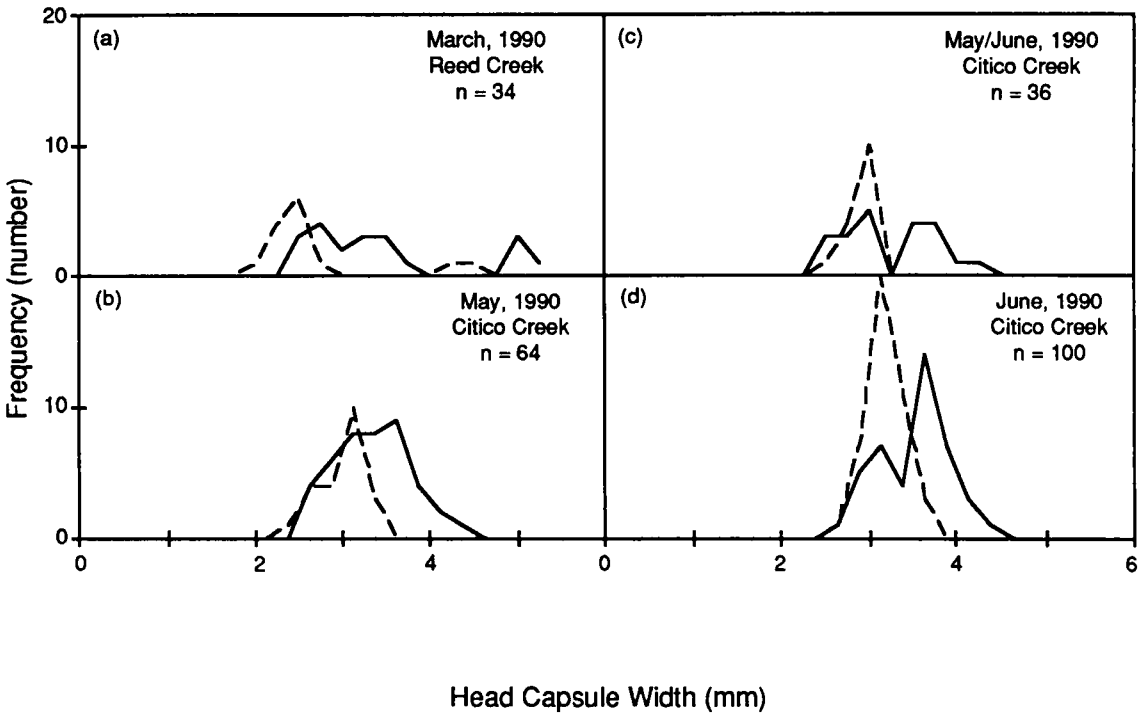


Figure II-5. Head capsule width distribution of nymphs from five sampling dates, by sex. Solid lines represent female distributions, and dotted lines represent male distributions.

Sinking Time

Effects of Size. The effects of size on sinking time were tested in two ways: the sinking times of large, open nymphs were compared with the sinking times of small, open nymphs and the sinking times of large, curled nymphs were compared with the sinking times of small, curled nymphs.

Open nymphs were anesthetized and then timed as they sank. Since nymphs were not anesthetized in the fresh-caught experiments, only the data from the 200 nymphs in the lab-kept experiments were used in this analysis. Results indicated that large, open nymphs sank more quickly than small, open nymphs ($t = -22.713$, $n = 200$; $P < 0.001$).

The sinking times of curled nymphs were timed prior to being anesthetized. Only the data from the lab-kept experiments were used to compare sinking times of curled nymphs. Sample size was limited to 116 nymphs because only the data from those nymphs which curled all the way to the bottom could be used in the analysis. Results indicated that curled, large nymphs sank more quickly than curled, small nymphs ($t = -9.526$, $n = 116$; $P < 0.001$).

Effects of Behavior. To test the impact behavior had on sinking times, the sinking time of curled nymphs (unanesthetized) was compared with the sinking time of open nymphs (anesthetized). However, not all nymphs curled all the way to the bottom in the unanesthetized test. To avoid biasing the results, only the data from nymphs that did curl all the way to the bottom were used. Because anesthetized data were not collected in the fresh-caught experiments, only the data from the lab-kept experiments were used.

It was found that curling decreased the time it took for a nymph to sink the depth of the aquarium. This was true in every individual test run. The means for the curled sinking times also differed significantly from the means for the open sinking times. This held for tests within size classes and for the pooled data (Table II-1).

Table II-1. Effect of behavior on sinking times in the lab-kept experiments. All tests were t-tests.

Nymph Size/ Behavior	Mean Sinking Time	Standard Deviation	t-value, Probability
Small nymphs (n = 74)			
curled	5.6	0.7	
			-14.110, < 0.001
open	7.5	0.9	
Large nymphs (n = 42)			
curled	3.8	0.5	
			-8.348, < 0.001
open	4.9	0.7	
Pooled (n = 116)			
curled	4.9	1.1	
			-9.526, < 0.001
open	6.5	1.5	

Count Data

Several behaviors were recorded for their presence or absence. These included: (1) whether the nymph curled for longer than one second upon entering the test tank (INI_1); (2) whether the nymph curled all the way to the bottom (CUR2BT); and, (3) whether the nymph curled on the bottom of the test tank (BOTCUR). The capital letters in parentheses in the previous sentence will be used to refer to the corresponding data sets in the following text.

The count data were analyzed by using a chi-square goodness of fit test in a two-by-two contingency table. The chi-square test used in this way tests for independence (or, conversely, "association") of two variables (Sokal and Rohlf, 1981). In this research, the chi-square test tested for independence between count data and nymph size class. Chi-square values that proved to be significant indicated that behavior count data and nymph size class were not independent; i.e., that small nymphs were behaving differently than large nymphs. An alpha level of 0.05 was chosen for significance in all tests.

In general it was found that behavior was associated with the size of the nymph. Presence or absence of fish scent in the aquarium was not found to be associated with count data in any test. The other factors in the experimental design, creek of origin and treatment, associated with behavior only inconsistently.

Effects of Size Class. Size was significantly associated with INI_1 data, CUR2BT data, and BOTCUR data in both sets of experiments (Table II-2).

Effects of Tank of Origin. In general, tank of origin was not associated with behavior (Table II-3). It did associate ($P < 0.05$) with CUR2BT data from the lab-kept experiments (Table II-3).

The tests from the lab-kept experiments were broken down by size classes and large nymphs were found to be the group showing an impact of tank of origin on behavior (Table II-4). The large nymphs curled to the bottom twice as frequently in the

Table II-2. The effect of nymph size class on count data in the lab-kept and fresh-caught experiments. All tests were chi-square goodness of fit of two-by-two contingency tables. Significance taken at alpha < 0.05. Positive responses indicate the number of nymphs showing the indicated behavior; negative responses indicate the number of nymphs not showing that behavior.

Data	Positive Responses	Negative Responses	Chi-square value, Probability
Lab-kept Experiments			
INI_1			
small	95	5	28.125, < 0.001
large	65	35	
CUR2BT			
small	74	26	21.018, < 0.001
large	42	58	
BOTCUR			
small	74	26	18.603, < 0.01
large	44	56	
Fresh-caught Experiments			
INI_1			
small	46	4	14.300, < 0.001
large	22	16	
CUR2BT			
small	41	9	22.915, < 0.001
large	12	26	
BOTCUR			
small	43	7	34.684, < 0.001
large	9	29	

Table II-3. Effect of tank of origin on count data in the lab-kept and fresh-caught experiments. "Holding" and "dwelling" refer to the two possible tanks of origin. See text for details.

Data	Positive Responses	Negative Responses	Chi-square value, Probability
Lab-kept Experiments			
INI_1			
holding	79	21	
			0.125, NS
dwelling	81	19	
CUR2BT			
holding	50	50	
			5.255, < 0.05
dwelling	66	34	
BOTCUR			
holding	55	45	
			1.323, NS
dwelling	63	37	
Fresh-caught Experiments			
INI_1			
holding	37	14	
			1.541, NS
dwelling	31	6	
CUR2BT			
holding	30	21	
			0.100, NS
dwelling	23	14	
BOTCUR			
holding	27	24	
			1.898, NS
dwelling	25	12	

Table II-4. Effect of tank of origin on count data for the lab-kept experiments, by size class. "Holding" and "dwelling" refer to the two possible tanks of origin. See text for details.

Data	Positive Responses	Negative Responses	Chi-square value, Probability
INI_1, large nymphs			
holding	32	18	
			0.044, NS
dwelling	33	17	
INI_1, small nymphs			
holding	47	3	
			0.211, NS
dwelling	48	2	
CUR2BT, large nymphs			
holding	13	37	
			10.509, < 0.05
dwelling	29	21	
CUR2BT, small nymphs			
holding	37	13	
			0.0, NS
dwelling	37	13	
BOTCUR, large nymphs			
holding	18	32	
			2.597, NS
dwelling	26	24	
BOTCUR, small nymphs			
holding	37	13	
			0.0, NS
dwelling	37	13	

experiments run directly from the dwelling tank as in the experiments run from the holding tank. Large nymphs also showed increased frequency of INI_1 and BOTCUR in the dwelling tank tests, although the trends were not statistically significant. Small nymphs showed exactly the same frequency of CUR2BT data in both the dwelling tank and holding tank treatments.

Effects of Fish Scent. Fish scent was tested only in the lab-kept experiments. Presence or absence of fish scent did not associate with any category of count data (Table II-5).

Effects of Creek of Origin. Creek of origin was tested only in the fresh-caught experiments. It was significantly associated only with BOTCUR data (Table II-6). In this test, nymphs from Norton Creek bottom-curled more frequently than did nymphs from Reed Creek. The Norton Creek data did have a higher percentage of small nymphs than did that of Reed Creek. This was due to the unequal sample sizes of large nymphs as explained under "Experiments with Fresh-caught Nymphs."

Discussion

Results are discussed by the two hypotheses tested in laboratory experiments: curling as a position behavior and curling as a defensive behavior. A brief discussion of possible sources of experimental bias will follow the discussion of the hypotheses.

Curling as a Position Behavior

This discussion will consist of two parts: the factors that affect sinking times and the factors that affect changes in behavior.

Factors Affecting Sinking Times. Size had an important effect on sinking times; large nymphs sank faster than small nymphs. These results were straightforward and are not surprising. The faster sinking time of large nymphs is assumed to be due to a simple

Table II-5. Effect of fish scent on count data in the lab-kept experiments. "With" and "without" refer to the presence or absence of fish scent. See text for details.

Data	Positive Responses	Negative Responses	Chi-square value, Probability
INI_1			
without	76	24	
			2.000, NS
with	84	16	
CUR2BT			
without	56	44	
			0.328, NS
with	60	40	
BOTCUR			
without	54	46	
			2.067, NS
dwelling	64	36	

Table II-6. Effect of creek of origin on count data in the fresh-caught experiments. "Norton" and "Reed" refer to the two creeks from which nymphs were captured for the behavior tests. Creek of origin was tested only in the fresh-caught experiments.

Data	Positive Responses	Negative Responses	Chi-square value, Probability
INI_1			
Norton	29	9	0.035, NS
Reed	39	11	
CUR2BT			
Norton	26	12	1.875, NS
Reed	27	23	
BOTCUR			
Norton	28	10	5.892, < 0.05
Reed	24	26	

surface area to mass phenomenon: the large nymphs have a smaller surface area to mass ratio and so overcome the resistance of water more easily. Campbell (1985) reported similar results with baetid mayflies.

Behavior also had an important effect on sinking times; nymphs that curled sank faster. This was true in every individual test. The mean of the sinking times for the curled nymphs was also significantly less than the mean of the sinking times for the open nymphs. The mechanism for this again appears to be the result of a surface area to mass relationship: curling decreases resistance by decreasing the surface area to mass ratio. Otto and Sjostrom (1986) reported similar results.

Factors Affecting Changes in Behavior. Behavior varied significantly between size classes. The small nymphs were found to curl more frequently (INI_1) and for longer durations (CUR2BT and BOTCUR) than the large nymphs. These differences were generally consistent whether using nymphs from the holding tank or dwelling aquaria, whether fish-scent was present or absent, or whether the nymphs were from Norton Creek or Reed Creek. The behavior frequencies seen in these experiments should not be interpreted as indicative of the absolute frequencies typical of the stream. Rather, they serve as a relative measure for comparison of nymph behaviors among size classes.

Variation in behavior with size class has been frequently reported in the literature for a wide variety of aquatic insect nymphs. Colbo and Moorhouse (1979) found that early instars of three species of blackflies (Simuliidae) drifted from quiet reaches of mature rivers to rapids. They also found microdistributional differences between instars: the late instars occurred in faster current than the early instars. Wotton et al (1979) found that small blackfly nymphs drifted more frequently than large nymphs. As mentioned in Chapter 1, researchers have found a number of taxa, including stonefly and mayfly species, in which larger nymphs tend to drift more frequently at night, but in which small nymphs did not exhibit a diel pattern (e.g., Anderson 1966; Allan 1978).

Campbell (1985) found that mayfly nymphs changed their water-column behavior with size. Peckarsky (1987) found that large ephemerellid nymphs were more likely to "scorpion posture" than small nymphs when touched by a stonefly predator.

The effect of tank of origin on nymph behavior is more difficult to interpret than the effect of size (see Table II-3, p. 30). In general, differences were consistent between large and small nymphs within this treatment. However, large nymphs from the dwelling aquaria did tend to curl longer (but not more frequently) than the large nymphs taken from the holding tank. This difference was only true of the lab-kept nymphs. The large fresh-caught nymphs did not show this trend, but small sample size hampered the interpretation of this data. These results could indicate that the behavioral differences between small and large nymphs, when dislodged from leaf packs in the stream environment, may not be as great as the experimental data from the laboratory show. However, due to consistent indications in the opposite direction, it is felt at this point that the unusual results with the large lab-kept nymphs is an aberration and not an important pattern. Furthermore, despite this variation within the large nymph test group, the tests between small and large lab-kept nymphs still showed significant variation, the main question of this section of research.

Curling as an Anti-predator Behavior

The fish-scent test was the experimental factor in the laboratory experiments testing whether curling is an anti-predator behavior. This test made two important assumptions: (1) that there was indeed a chemical in the fish aquaria that was transferred to the test aquarium with the transfer of water and (2) that the nymphs had the sensory receptors to detect these chemicals. As a result of these assumptions, this test was only able to test for a positive result. If there had been differences between presence and absence of fish scent, then it would support a predator defense

mechanism. The absence of a difference, however, does not mean that curling is not a defensive mechanism. It could be evidence against curling as a defensive mechanism, but absence of a difference could just as easily mean that either (or both) of the two assumptions had been violated.

The first assumption is supported by several studies. Todd (1971) found that the behavior of sub-dominant bullheads changed when aquarium water from dominant bullheads was introduced into their aquaria. Others have found that amphipods were less active in the presence of fish-conditioned water (Williams and Moore 1985; Holomuzki and Hoyle 1990).

Assumption two appears to have been violated, however. Motyka et al (1985) concluded that the nymphs they tested (Pteronarcys picteti and Pycnopsyche guttifer [a caddisfly]) relied on contact chemoreceptors to find food. Peckarsky (1982), in a review article, concluded that there was little evidence of chemoreceptors in aquatic insect nymphs.

Given the above discussion, it is assumed that P. scotti nymphs do not have sensory mechanisms to detect fish scent and that the results of the fish-scent test provide neither evidence for nor against curling as a predator behavior.

Potential Sources of Experimental Bias

Performing these experiments in an aquarium may have impacted nymph behavior. Most lab experiments dealing with drift behavior are conducted in an artificial stream (e.g. Madsen 1968, 1969; Blum 1989).

Testing the nymphs in a lighted room may also have biased the results. Pteronarcids are negatively phototactic (McDiffet 1970), as are many aquatic insects (Peckarsky 1982). Research on stoneflies is frequently conducted under red lights (Allan et al. 1986; Casey 1987).

The use of aquaria instead of artificial streams and room light instead of red light is not believed to have affected the results significantly for two reasons: (1) differences between large and small nymphs were consistent regardless of test condition; and (2) these differences were observed in the field (see Chapter 3).

Conclusions

From the sinking results it is apparent that curling could function as a position behavior. Curled sinking times and open sinking times were significantly different. Furthermore, since large nymphs sank more rapidly than small nymphs, large nymphs would not have to curl as frequently or as long to sink as quickly as small nymphs. What is missing in addressing curling as a position behavior is information on the selective pressures these nymphs face in the stream. For example, which is more important? To get out of the drift and escape possible predation? Or to remain in the drift for long periods of time and thereby increase the probability of reaching suitable habitat? From the variation in curling behavior with age, it is tempting to argue that these pressures change with developmental stage and drive the ontogenetic variation in behavior observed.

There is indeed evidence for ontogenetic variation in P. scotti nymph behavior. Small nymphs curled more frequently than large nymphs; large nymphs swam more frequently than small nymphs (see Table II-2, p. 29). Other experimental factors did not exert an important influence on nymph behavior.

The reasons for these differences are unclear. Given what is known from the sinking experiments, it is possible that small nymphs are benefitted from a rapid return to the safety of the substrate. Large nymphs, conversely, may benefit from remaining in the water column. Possibly, they remain in the downward flow as a dispersal tool; remaining in the drift until suitable habitat is found. Interestingly, many Tennessee Valley

Authority capture records for pteronarcid nymphs are in April and May (Bob Terry, Tennessee Valley Authority, personal communication), about the time these insects emerge. This could indicate that the nymphs are dispersing prior to emergence. However, these records may be biased by sampling technique and it is difficult to say anything firm, except that they point the way to further questions and hypotheses.

As described under **"Discussion,"** there appears to be neither evidence for nor against curling as an anti-predator defense. This conclusion is derived mainly from the fact that certain key assumptions appear to have been violated and thereby render firm conclusions impossible.

Summary

Pteronarcys scotti nymphs were tested under laboratory conditions for factors affecting their water column behavior. Behavior and size were found to influence sinking speed. Additionally, size (and therefore age) was found to play a significant role in influencing behavior. Possible explanations for this behavior pattern were explored, but the data did not allow a tight hypothesis. Other experimental factors, including tank of origin, presence or absence of fish scent, and creek of capture, were not found to play a significant role in influencing behavior.

Although only indirectly important to the study question, size and sex records were kept of three natural populations for various sampling dates. These data indicated a two-year life cycle with noticeable sexual size dimorphism in the second year. This conformed to previously reported studies.

CHAPTER 3: FIELD OBSERVATIONS

Field observations were made to address limitations of the laboratory investigations, both behavioral (Chapter Two) and predator (Chapter Four). The primary hypotheses tested included curling as an anti-predator behavior and curling as a position behavior. Some comments concerning curling as a protection behavior (hypothesis #2, p. 9) will also be made. Significant predator-prey interactions were not observed, but important behavioral patterns were observed. In addition, a behavior not observed in the laboratory behavior experiments was observed and is described.

Field Site

Field observations were conducted October 6, 1989 in Citico Creek of Cherokee National Forest in Monroe County, Tennessee. Citico Creek was chosen because of its substantial and visible population of dominant fish predators (rainbow trout [Oncorhynchus mykiss], red-breasted sunfish [Lepomis auritus], and smallmouth bass [Micropterus dolomieu] among them) and its high densities of P. scotti. The water was high, but clear, and its temperature was 16.5° C.

Methods

About 25 large nymphs (visually classified) and about 40 small nymphs were collected from 10 AM to noon. The nymphs were held in stream water in a styrofoam ice chest until the afternoon observations were conducted. The nymphs were released one at a time and their behavior observed from 2 PM to 4 PM that afternoon. Observations were accomplished using a wetsuit, diving mask, and snorkel.

There were two goals to these observations: (1) to observe predator-prey (fish-stonefly) interactions under natural conditions and (2) to observe nymph behavior under

natural conditions. The observations can be separated into three groups: (1) stationary observations in which the goal was to observe predator-prey interactions; (2) nonsystematic observations of downstream drift; and (3) systematic observations of downstream drift at a pool/riffle interface.

Stationary Observations

The first set of behavioral data was recorded while attempting to make predator-prey observations. For these, the observer was stationary and observations continued until the nymph was out of sight (generally 2-3 meters). The shyness of the dominant predators made it impossible to observe their behavior. Schools of minnows (composed mainly of whitetail shiners [Notropis galacturus] and warpaint shiners [Notropis coccogenis]) followed the snorkeler. On two occasions minnows attempted to eat released nymphs, but neither minnow was big enough to eat even a small nymph. Even though predator response did not occur in a consistent fashion, nymph behavior was recorded. Ten small nymphs were observed in these tests.

Nonsystematic Observations

Once it was concluded that the predation experiments would not work, stationary observations were abandoned. At this point, nymph behavior in the drift was observed by releasing the nymphs one-by-one and then observing them by floating in the current and following the nymphs downstream. In this way, it was possible to observe drift behavior over a much longer distance (20-30 meters in some cases). Individual observations were continued until the nymph reached the bottom or until visual contact was lost in turbulent stream conditions. These observations encompassed a wide range of time durations and stream habitats (in terms of current velocity and substrate type). The behavior of seven large nymphs and seven small nymphs was observed in these

tests. Unfortunately, the complexity of the observation technique did not permit association of nymph behaviors and habitat.

Systematic Observations

After conducting nonsystematic observations, it was decided to conduct systematic observations for more robust comparisons of the drift behavior of large versus small nymphs. These observations were conducted in the downstream region of a pool as it flowed into a riffle (Figure III-1). Nymph behavior in the pool/riffle current interface was a primary focus of these observations. At the pool/riffle interface the stream current increased from medium to fast (relative to each other, no measurements were taken) and the depth changed from about 30-100 cm to about 15-30 cm. The substrate in both the pool and the riffle was dominated by cobble (rocks 10-30 cm in diameter), but also contained small boulders (rocks greater than 30 cm in diameter).

Observations were made by sequentially releasing individual nymphs about 10 meters upstream from the riffle. They were actively followed and observed until they reached the substrate in the riffle or until visual contact was lost in the riffle (generally 2-3 meters into the riffle). Because a behavior of interest occurred at the pool/riffle interface, nymphs that reached the substrate before entering the riffle were pulled off by the observer and re-released into the current. Eleven large nymphs and ten small nymphs were observed in these tests.

Results

Results will be discussed by predator-prey interactions, impact of nymph size on behavior, and back arch behavior.

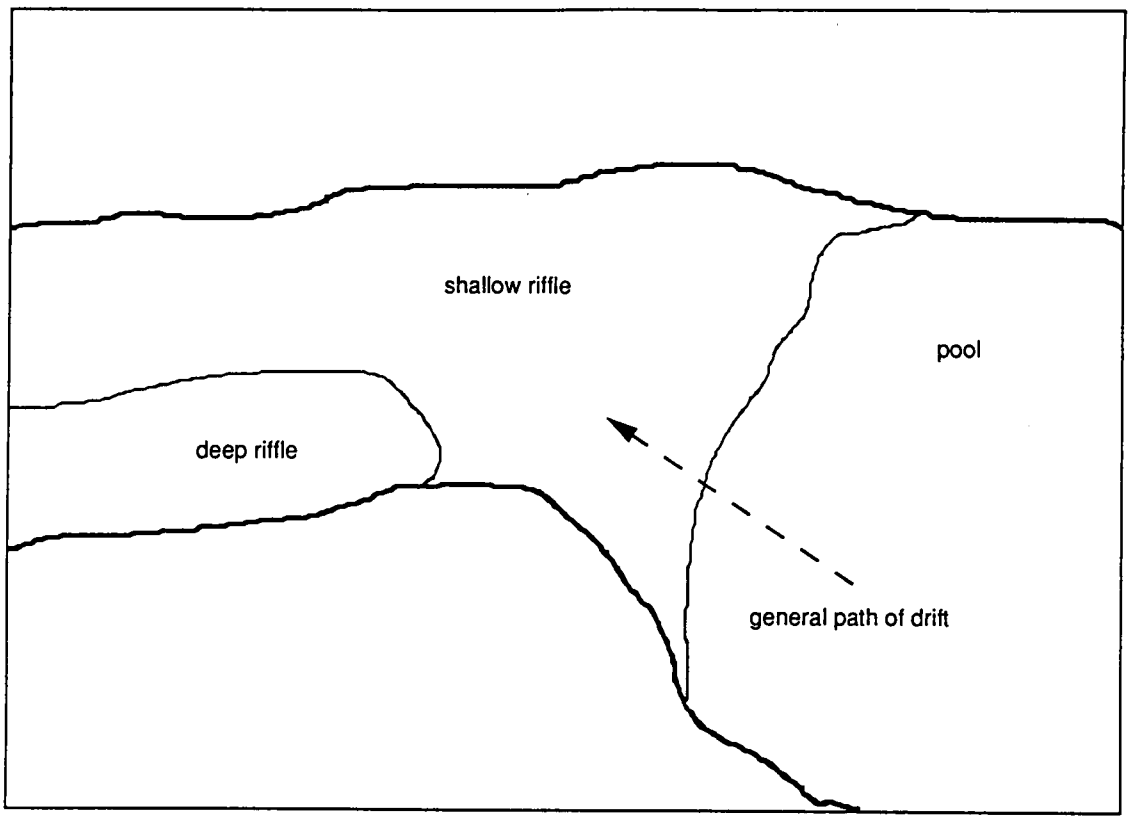


Figure III-1. Map of pool/riffle current interface where systematic field observations were conducted.

Predator-Prey Interactions

As stated in **"Methods,"** predator-prey interactions with dominant predators were not observed in any trial. Predator-prey interactions with minnows (N. galacturus and N. coccogenis) as predators were observed on two trials during the predator observations. In the first of these trials, the nymph's behavior changed from an open behavior to a curl behavior after being mouthed by the fish. In the second trial, the nymph's behavior changed from a mixed open/curl to a definite curl after being mouthed by the fish.

Impact of Nymph Size on Behavior

Trends among size classes can be observed if the behavioral data from the three groups of observations are pooled (Table III-1). In general, the small nymphs are more likely to curl or be open and less likely to swim, and the large nymphs are more likely to swim and less likely to curl or be open.

Back Arch Behavior

Eleven large nymphs and ten small nymphs were observed in the systematic drift observations at the pool/riffle interface (Figure III-1). Nymph behavior in the pool (before entering the riffle) varied between large and small nymphs. Seven of the large nymphs showed only swim behavior; one showed a mixture of swim and curl behaviors; and three showed only curl behavior. None of the small nymphs showed swim behavior. Five of the small nymphs showed only open behavior; five showed only curl behavior.

Consistent between size classes was the "back arch" behavior. The back arch consists of the arching of the nymph's back in such a way that the head and the posterior tergites extend dorsally away from the venter. The full extension of the legs is also part of this behavior. Thus, the space which the legs contact is maximized. This behavior occurred 100% (n = 19) of the time at the interface of the smooth, slower,

Table III-1. The impact of size on nymph behavior during field observations. This table shows the summary data of field observations in Citico Creek. The last three rows are mutually exclusive and sum to the total observed.

Classification	Small Nymphs	Large Nymphs
total observed	27	18
exhibited only curl behavior	13	4
exhibited only open behavior	8	0
exhibited only curl or open, or mix of two	23	4
exhibited only swim behavior	1	12
exhibited mix of swim and curl or open	3	2

pool current and the turbulent, faster, riffle current. (Only nineteen observations of riffle behavior were made as visual contact with two of the large nymphs was lost at the pool/riffle interface.)

Back arch behavior also occurred in these trials when large nymphs contacted the substrate. For example, if a nymph in the water column bumped a rock prior to entering the riffle, but remained in the slower eddies near the rock without exiting the water column, the nymph always showed back arch behavior. This behavior was observed in each of the five trials where a large nymph contacted the substrate. Two of these large nymphs contacted the substrate twice before reaching the riffle and both exhibited back arch behavior with each contact. Since no small nymphs contacted the bottom prior to the riffle, this behavior was not observed under this condition for small nymphs.

Discussion

The discussion will proceed by the three hypotheses to which the field observations applied: curling as a position behavior, curling as an anti-predator behavior, and curling as a protective behavior.

Curling as a Position Behavior

In general, the field observations strongly supported the lab investigations: small nymphs were more likely to curl and less likely to swim while in the water column than large nymphs. However, the frequency of behaviors observed in the field was consistently lower than the frequencies observed in the laboratory (Table III-2).

There were important differences between the method in which the data were collected in the field and in the laboratory: nymphs experienced a range of flow conditions in the field but only still water in the lab, and nymphs were observed for much longer periods of time in the field. Furthermore, sample sizes were much greater in the

Table III-2. Comparison of the behavior frequencies for the laboratory experiments and the field observations. Sample sizes are in parentheses. INI_1 data was used for "curled at least once" in the laboratory experiments. CUR2BT data was used for "only curled" in the laboratory experiments.

Behavior	Laboratory Experiments	Field Observations
large nymphs (N)	(138)	(18)
curled at least once	63%	33%
only curled	39%	22%
small nymphs (N)	(150)	(27)
curled at least once	94%	67%
only curled	83%	48%

laboratory experiments. Because of these differences, these two groups of data were not analyzed statistically.

Perhaps the most important difference was that the small nymphs were much more likely to exhibit open behavior in the field: 30% (8 of 27) showed only open behavior and 41% (11 of 27) showed open behavior part of the time (Table III-2). Open behavior did not occur frequently enough to be recorded in the lab experiments.

The presence of the back arch behavior may indicate that pteronarcids do use behavior to control their position in the drift. Back arch behavior has previously been described for both mayflies (Campbell 1985; Otto and Sjostrom 1986) and stoneflies (Madsen 1969; Blum 1989). Campbell (1985) named a behavior found in baetid mayflies "parachuting behavior." He found that parachuting was a behavior for remaining in the water column. Otto and Sjostrom (1986) found that three different mayfly species exhibited a form of back arch behavior, which they described as a "scorpion-like posture" (after Peckarsky's (1980) work with benthic mayfly defenses). They found that the mayflies exhibiting scorpion-like posture exited the water column more quickly than dead mayflies, but did not describe how this occurred. Madsen (1969) described a behavior equivalent to the back arch in the stonefly Brachyptera risi. Blum (1989) described and illustrated a series of similar behaviors in nemourid nymphs (four species in two genera were tested). He identified two "a" postures. The "a_2" posture differed from the "a_1" posture by the increased degree of back arch. The "a_1" posture was found to be typical of sinking in calm water, while the "a_2" posture was found to be typical of the nymphs when in fast currents.

What is the function of the back arch behavior in P. scotti? The field observations were not designed to test this question, but it is likely that the back arch behavior facilitates exiting the water column. Nymph behavior consistently changed from behavior typical of its size class to back arch behavior in two situations: (1) when the

nymph approached closely to the substrate and (2) when the nymph entered the pool/riffle interface and the current changed velocity. In the first situation only the large nymphs were observed and extension to the small nymphs is not possible. In the second situation behavior was consistent across size classes. Furthermore, the behavior maximized the spatial sweep of the legs and thus maximized potential contact with the substrate.

It is interesting to note that the riffle is the preferred habitat of P. scotti nymphs. Possibly, by changing to a back arch behavior, the nymphs are increasing their probability of exiting the water column in a preferred habitat. Unknown at this time is whether the absolute velocity causes the behavior switch or whether the velocity of the riffle relative to the pool velocity causes the behavior switch.

Curling as an Anti-predator Behavior

Although the sample size was small, it should be noted that in both predator-prey interactions, the nymph changed to definite curl behavior upon being mouthed by the minnow. This change to curl behavior upon predator attack was also observed during the predation tests. Discussion of its significance will occur in Chapter 4.

Curling as a Protective Behavior

One of the hypotheses identified in the first chapter stated that curling may be a protective behavior. While this hypothesis was not tested in this research, the stimuli (beginning of a riffle) for the back arch behavior can be used to indirectly address this hypothesis. If curling is a protective behavior, then one would expect curling to be common in areas where the chances of physical injury are relatively high. A riffle would be expected to be such an area: the nymph would be travelling at a higher velocity than in the pools and would be more likely to collide with the substrate (much closer to the

entire current flow volume in the riffle). Yet, far from curling in a riffle, the nymphs actually behave in the opposite fashion; they back arch and maximally expose their ventral region. Given the above argument, the likelihood that curling is a protective behavior seems remote.

Conclusions

The field observations address the hypothesis that curling is a position behavior in two important ways: (1) they supported the laboratory results indicating ontogenetic variation in pteronarcid behavior (Chapter Two) and (2) they showed that P. scotti nymphs may use behavior to manipulate their position in the water column.

While the ontogenetic behavior results from the laboratory tests were supported by field observation, the same difficulty existed in interpreting field observations as existed in interpreting the laboratory behavior experiment results: information concerning the selection pressures that might trigger these differences was lacking.

The significance of the back arch behavior is that it shows that pteronarcids may very well use behavior to control their position in the stream. While this does not prove that curling functions as a position behavior, it does indicate that there is a potential for curling to operate in such a fashion. Otto and Sjostrom (1986), referring to the fact that scorpion-posturing mayflies exited the water column more quickly than dead nymphs, felt the "behavioral component of drift may not express itself by any activity, but a change in shape may suffice" (p. 80).

Due to limited observations, the stream experiments can give no strong support for or against the hypothesis that curling is an anti-predator behavior. Observations that were made do parallel results in the laboratory predator-prey experiments (Chapter Four); the results of the stream observations applicable to curling as an anti-predator behavior will be discussed in that chapter.

Summary

The behavior of P. scotti nymphs was observed in the field. Results supported the ontogenetic variation in behavior reported in the laboratory experiments. Furthermore, a new behavior, the back arch, was observed and described. The back arch is very likely a behavior for escaping from the water column. This behavior is significant because it indicates that the nymphs may indeed use behavior to manipulate their water column position.

CHAPTER 4: PREDATION EXPERIMENTS

Experiments were conducted with several species of predators to test the hypothesis that curling may be a defensive behavior based on increasing the effectiveness of the exoskeleton to mechanically withstand predator attack. These predators included banded sculpin (Cottus carolinae) and four species of centrarchids: red-breasted sunfish (Lepomis auritus), rock bass (Ambloplites rupestris), bluegill (Lepomis macrochirus), and warmouth (Lepomis gulosus). In addressing the hypothesis that curling is a defensive behavior, data were collected on the following: (1) reaction of various fish species to pteronarcids--this because curling might be effective against some predators but not others; (2) reaction of predators to different pteronarcid behaviors; (3) comparative size data of nymphs and fish--because a nymph behavior that might be considered an effective defense might actually be due to a gape limitation of the fish predators; and (4) injury data--because curling might be a survival mechanism for rejected nymphs. There were three groups of predation experiments: (1) "sculpin experiments" using sculpins as predators; (2) "centrarchid experiments" using four species of centrarchids, including bluegills, as predators; and (3) "bluegill experiments" using only bluegills as predators.

Methods

Experimental methods varied among experimental groups and the three sets will be discussed separately. Nymphs for the predation experiments came from the Citico Creek drainage for fear of adversely impacting the health of the pteronarcid populations in Reed and Norton creeks.

Sculpin Experiments

Four sculpins were used in these experiments (Table IV-1). Three of the sculpins were seined from Pond Creek and were not experienced with pteronarcids. One sculpin was seined from the Little River in an area sympatric with known pteronarcid populations.

Table IV-1. Sizes of predators and prey used in the sculpin experiments. The number in parentheses represents the number of nymphs tested with the individual sculpin.

Fish	Total Length (mm)	Weight (g)	Capsule Width of Nymphs	
			median (mm)	range (mm)
A (9)	81	7.3	2.6	1.8 - 3.9
B (5)	94	9.0	2.4	2.3 - 3.8
C (6)	102	13.5	2.9	2.7 - 3.7
D (6)	91	8.4	2.7	2.3 - 2.9

Experiments were conducted from February 16 to February 21, 1990. All experiments were conducted in the morning (between 8:30 AM and 11:30 AM). Three sculpins were kept alone in separate 40 liter aquaria during the experiments. Due to space limitations, the fourth sculpin was kept with a small yellow bullhead (Ameiurus natalis), also in a 40 liter aquarium. This last sculpin proved to be the most aggressive individual.

By the time the experiments began, sculpins had already been observed to eat P. scotti in the laboratory. The primary goal of these experiments was to test if sculpins preferentially selected open nymphs to curled nymphs. A secondary goal was to see if there was a size limit at which sculpins no longer ate nymphs. Accordingly, head capsule width was recorded prior to the tests.

Individual tests were conducted by simultaneously dropping two nymphs into the tank of a sculpin. Both of the nymphs had previously been anesthetized using Alka-Seltzer and their capsule width had been measured. One nymph was anesthetized approximately 15 minutes prior to the test and then allowed to recover "consciousness" in an aerated holding tank. The second nymph was anesthetized just prior to the test and not allowed to recover "consciousness." The second nymph was rinsed to remove Alka-Seltzer residue. The nymphs given the chance to recover exhibited normal nymph behaviors: some curled and some swam. The anesthetized nymphs always showed open "behavior." Nymphs used in an individual test were approximately the same size. The nymphs ranged in head capsule width from 1.8 - 3.9 mm.

Centrarchid Experiments

Eight individual fish predators were tested in the centrarchid experiments (Table IV-2). Four species of fish in two categories were used in these experiments. The categories were: (1) probable experience with Pteronarcys and (2) improbable experience with Pteronarcys. Rock bass (Ambloplites rupestris) and redbreast sunfish (Lepomis auritus) were caught using hook-and-line from the Little River in an area with known populations of pteronarcids. These predators were potentially experienced with Pteronarcys. The other two species were bluegill (Lepomis macrochirus) and warmouth (Lepomis gulosus). These were electroshocked from the Tennessee River at I.C. King Park in Knox County. Because the habitat was so dissimilar to typical Pteronarcys habitat, it was assumed that these predators were not experienced with Pteronarcys. Two fish of each species were used in the experiments. Nymphs in these experiments (and the bluegill experiments) were collected from the Citico Creek system.

Anesthetized nymphs and unanesthetized nymphs were used to test the impact curling had on predator behavior. Unanesthetized nymphs were generally curled while

Table IV-2. Sizes of predators and prey used in the centrarchid experiments.

Fish	Length (mm)	Weight (g)	Capsule Width of Nymphs	
			median (mm)	range (mm)
Rock Bass #1	141	51.4	3.5	2.8 - 4.1
Rock Bass #2	143	46.3	3.4	2.5 - 4.4
Redbreast #1	137	41.9	3.2	2.8 - 3.5
Redbreast #2	150	60.2	3.2	2.9 - 4.0
Bluegill #1	134	33.3	3.1	2.6 - 3.4
Bluegill #2	137	36.6	3.2	2.6 - 3.7
Warmouth #1	138	52.3	3.0	2.6 - 3.7
Warmouth #2	123	35.5	2.9	2.4 - 3.3

they fell through the water column, but some swam and some were not attacked until they were on the bottom. Anesthetized nymphs were open, but several recovered before being attacked and exhibited normal, unanesthetized behaviors. Earthworms, a preferred food item, were used as controls in these tests. Planned statistical tests were (1) among fish behaviors towards types of prey (nymphs and worms), nymph behaviors, and nymph size classes and (2) among species of fish.

The centrarchid tests lasted four days, from May 20 to May 23, 1990. Tests were run twice a day, once in the morning and once in the afternoon. Each fish was tested with either an anesthetized or an unanesthetized nymph per test. Each nymph type was tested once per day. Order was randomized, but both anesthetized and unanesthetized nymphs were tested twice in the morning and twice in the afternoon. Each fish was also tested with one worm per test. In four of the tests the worm was introduced first and in four of the tests the nymph was introduce first. Again, the order was randomized.

Fish appeared hungry (i.e. they adopted an alert pose and positioned themselves face forward in the aquarium) at all times throughout the tests. Satiation was assumed to not play an important role in the experiments. Several of the fish ate every organism used in the tests and appeared ready for more.

Fish behaviors fell into two categories: simple behaviors and behavior sequences. Simple behaviors consisted of one clearly identifiable behavior. Behavior sequences, on the other hand, consisted of a sequence of simple behaviors that were part of a larger, more complicated task. Recorded simple behaviors included:

1. Orient Behavior - the fish positioned itself so that its head was pointed toward the nymph. The fish would continue to orient as the nymph fell through the water or crawled along the bottom.

2. Bump Behavior - the fish approached the nymph and bumped it with its nose without opening its mouth or taking the nymph into its mouth in any way. Bumping was very rare; pecking was the more common method for fish to explore possible food items.

3. Peck Behavior - the fish approached the nymph and took part, but not all, of the nymph into its mouth. This behavior was of short duration.

4. Inhale Behavior - the fish took the nymph entirely into its mouth. All fish used in these experiments used negative pressure to engulf food.

Behavior sequences included:

1. Attack Sequence - an attack was a series of behaviors in which the fish approached the nymph and made physical contact in some fashion. An attack could consist of one inhalation ending when the fish swallowed the nymph or an attack could consist of a series of inhalations and pecks ending in the ultimate rejection of the nymph. An attack was considered over when the fish swallowed the nymph or rejected and failed to orient or oriented but failed to re-attack for greater than twenty seconds.

2. Handling Time - was the amount of time the fish was in physical contact with the nymph before it rejected it or swallowed it. Handling time was considered a behavior sequence because all the inhalations of one attack were summed in the handling time of one test. A fish was judged to have swallowed a nymph when it re-oriented toward the front of the aquarium and/or the surface of the water.

Data collected in these experiments included:

1. Nymph behavior: this includes the behaviors described in Chapter 2.
2. Fish behavior: this includes the behaviors described above.
3. Temporal duration of fish behavior. The most important were time to attack, length of attack duration, interval between attacks, and handling time.
4. Capsule width, weight, and sex of all nymphs.
5. Weight of all earthworms.
6. Length and weight of all fish. These were measured at the end of the experiment to avoid "spooking" the fish prior to the tests.

The protocols for individual test sessions are given below. Test session protocol for the earthworms was as follows:

1. Anesthetize the earthworm in water with Alka-seltzer.
2. Rinse the earthworm off.
3. Record the weight of the earthworm.
4. Wait several minutes for the earthworm to revive.
5. Conduct the feeding experiment with the earthworm.

Test session protocol for the nymphs was as follows:

1. Anesthetize the nymph in water with Alka-seltzer.
2. Record capsule width, sex, and weight of the nymph.
3. (a) If running an anesthetized test, rinse the nymph off and immediately conduct the feeding experiment.

   (b) If running an unanesthetized test, allow the nymph to revive in mechanically aerated water until active (at least five minutes) and then conduct the feeding experiment.

At first, nymph sizes were randomized for the various fish. However, size was manipulated between species as patterns emerged early on in the experiment. For example, when it was observed that warmouth would not eat Pteronarcys nymphs, size of test nymphs was decreased to test for gape limitations as a possible explanation. Similarly, when it was observed that rock bass and redbreast sunfish would eat the nymphs, size of test nymphs was increased to test if there was a size limitation to what they could handle.

Bluegill Experiments

It was discovered in the centrarchid experiments that bluegills were the only species to exhibit a variety of behaviors toward pteronarcid nymphs. The centrarchid experiments were repeated using bluegills as predators in order to increase the sample size of bluegills. A first set of experiments was discontinued when a power outage raised room temperatures and stressed the nymphs. A second set of experiments was conducted with a new set of five bluegill predators (Table IV-3) and freshly caught pteronarcid nymphs. One fish (Fish #8) was too shy to test at the start of the experiment and was started on the third day of the experiment; it was only tested four times. The second set of experiments was conducted from June 20, 1990 to June 23, 1990. The bluegills were caught using hook-and-line from Fort Loudon Lake at I.C. King Park in Knox County.

Table IV-3. Sizes of predators and prey used in the bluegill experiments.

			Capsule Width of Nymphs	
Bluegill #	Length (mm)	Weight (g)	Median (mm)	Range (mm)
2	156	68.3	3.1	2.9 - 3.7
3	159	63.5	3.0	2.9 - 3.6
5	161	69.6	3.1	2.9 - 3.9
6	144	53.1	3.2	2.6 - 3.6
8	165	63.5	3.1	3.0 - 3.2

The bluegill experiments were conducted in the same way as the centrarchid experiments with two exceptions. The first exception was that all nymphs rejected during a test were examined after the test for injuries. By comparing the injuries of open, rejected nymphs to the injuries of curled, rejected nymphs, the survival benefits that curling might bring could be examined. Data were collected by examining rejected nymphs under a dissecting microscope and recording injuries on a data sheet (Figure IV-1). A simple scale of injury was developed (Table IV-4) in order to compare the injury data collected in this fashion.

The second exception was that all tests were terminated at the end of the first attack. This change was for two reasons. First, variables beyond initial nymph behavior entered the tests after the first attack. For example, the behavior of the nymph often changed after the first attack. Secondly, because rejected nymphs were examined for injuries, comparisons between nymphs rejected after different numbers of attacks would not be valid.

Fish :
Test :
Cap Wid :
Behavior @ Attack :

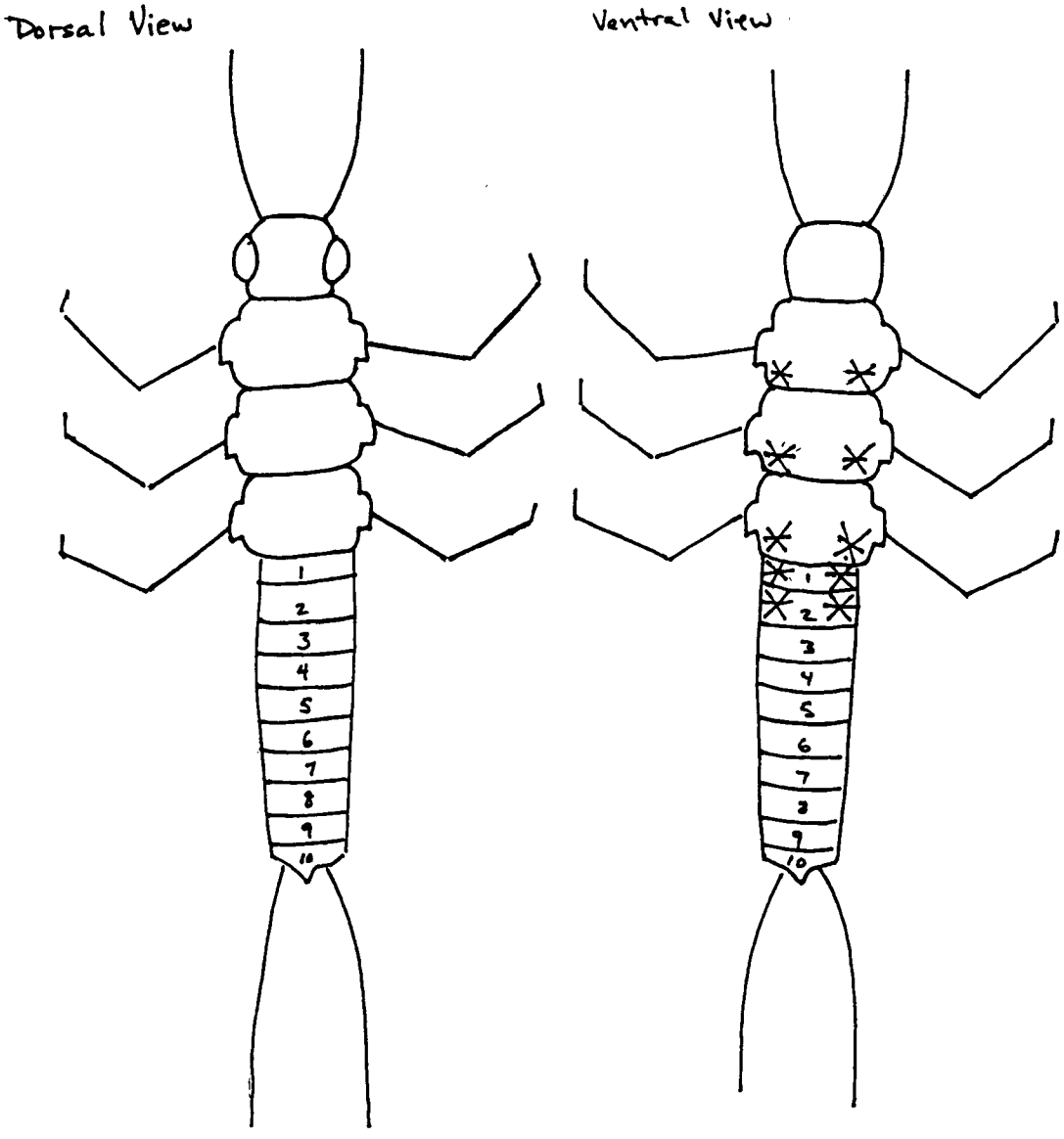


Figure IV-1. Data sheet for recording injury data in the bluegill experiments. Location of injuries was indicated on diagrams and explanatory comments were noted.

Table IV-4. Scale used to evaluate the injuries sustained by rejected nymphs in the bluegill experiments.

Injury Scale:

0 = no injury visible.

1 = (a) mucous visible; and/or
(b) slight abdominal rupture; or
(c) slight limb injury.

2 = (a) massive mucous visible; and
(b) severe rupture in abdominal tergites; and/or
(c) moderate limb injury.

3 = died in the holding tank within a week of the test.
4 = dead upon removal from tank where the test occurred.

Definitions:

Abdominal injuries:

Slight - swelling at or slight separation of one or two of the abdominal segments joints. One or two tooth holes in the abdominal exoskeleton.

Severe - severe separation of one or more of the abdominal segments so that internal organs were sticking out.

Limb injuries:

Slight - one to three tarsi missing.

Moderate - one or two limbs missing below the limb/thorax joint.

Severe - part of thorax damaged in loss of limb.

Results

Results will be discussed by the three experiment groups: the sculpin experiments, the centrarchid experiments, and the bluegill experiments.

Sculpin Experiments

Because the sculpins generally would not strike at the nymphs while in the water column, the results from the sculpin experiments were inconclusive and the experiments were stopped shortly after they were begun. Eleven pairs of nymphs were tested and two additional large nymphs were tested individually to find a size limitation.

In general, the sculpin would orient toward the nymphs and watch the nymphs as they sank through the water column. Once the nymphs touched bottom, the sculpin would remain in an alert position (fins erect and head slightly up). If the nymph moved the sculpin would orient toward it (if it wasn't already so oriented), and if the nymph moved again the sculpin would eat it. Only the sculpin housed with the bullhead struck at nymphs while they still were in the water column. Three pairs of nymphs were tested, and four of the six nymphs were eaten before they contacted the bottom. In the first test the nymph was eaten while curling. In the second test, the nymph was eaten while open. In the third test, the nymphs (one curled and one open) sank through the water column in close proximity and were simultaneously struck by the sculpin and subsequently eaten.

All sizes of nymphs were eaten except for one. The smallest sculpin (81 mm in length) was unable to eat a nymph 3.9 mm in head capsule width. The sculpin struck twice as the nymph crawled on the bottom, but was unable to subdue and eat the nymph. Eventually the nymph crawled under the filter, escaping to safety. In one test, the largest sculpin (102 mm in length) had no trouble eating two nymphs 3.7 mm in head

capsule width. Later it was discovered that sculpins were able to subdue and eat nymphs rejected by larger centrarchid predators.

Centrarchid Experiments

In the following discussions, unless otherwise indicated, all data refer to the first attack of the fish on the nymph, even though additional data were collected. Again, there was concern that uncontrolled variables, such as changes in nymph behavior, would make later comparisons difficult or invalid.

Effects of Prey Type. Prey type was significantly associated with frequency of prey eaten; the fish ate more worms than nymphs (Table IV-5). Handling time was also significantly longer for eaten nymphs than for eaten worms (Table IV-6).

Effects of Predator Species. The most striking results were the differences between fish in their response to the nymphs. Warmouth did not eat a single nymph throughout the experiment. Bluegill ate several nymphs, but rejected more than they ate. Redbreast sunfish and rock bass ate every nymph presented, with two exceptions (see "Redbreast Sunfish and Rock Bass" below). A detailed discussion of trends within species follows.

WARMOUTH. Although the warmouth did not eat a single nymph, they aggressively attacked all that were presented. In all tests the nymphs were inhaled in the first attack and held in the fish's mouth from 0.5 to 8.5 seconds. In six of the sixteen tests, nymphs were inhaled more than once. The maximum number of inhalations in the first attack was three. Frequently, the warmouth would initiate a second attack after the first attack if the nymph was allowed to remain in the aquarium until it began to move. The second attack was similar to the first, except that only one inhalation ever occurred per attack. Additionally, in three separate tests, warmouth #1 showed inhalations of over ten seconds in the second attack. These long inhalations had

Table IV-5. Comparison of the number of eaten worms and the number of eaten nymphs in the centrarchid experiments.

Prey	Eaten	Rejected	Chi-square value, Probability
worms	64	0	
			37.161, < 0.001
nymphs	34	28	

Table IV-6. Comparison of handling time for eaten worms and eaten nymphs in the centrarchid experiments.

Prey (n)	Handling Time (s)	Standard Deviation	t-value, Probability
eaten worms (56)	42.1	63.1	
			3.870, < 0.001
eaten nymphs (34)	262.7	420.3	

a duration of 10.1, 14.0, and 15.3 seconds. There was no trend in nymph behavior prior to these long inhalations.

A mucous-like substance was observed in the water after the warmouth attacks in three of the sixteen tests. Additionally, mucous was observed on five of the eight nymphs that were examined under the dissecting scope after the tests were run. Warmouth #1 was observed to eject mucous after it pecked, but didn't inhale, a crawling nymph.

Handling time of worms did not differ significantly between warmouth and the pooled data for rock bass and redbreast (Table IV-7). Neither did worm handling time differ between the two individual warmouth (Table IV-7).

BLUEGILL. There was a striking difference in the behavior of the individual bluegills. Both bluegills ate approximately the same number of nymphs, but their reaction to the nymphs differed. Bluegill #1 ate the first three nymphs tested, but then refused to eat any more nymphs the rest of the experiment. In the fourth test, Bluegill #1 spent 11 minutes and 39 seconds attempting to subdue the nymph over a series of five attacks before finally rejecting the nymph. The nymph in this test measured 3.1 mm in head width, a small to average size for the nymphs tested. In the last four tests, bluegill #1 inhaled nymphs in two tests (2.9 and 4.5 seconds) and only pecked at nymphs in the other two tests.

Bluegill #2 ate four nymphs, and, in general, was more persistent throughout in attempting to eat the nymphs. In the second test, bluegill #2 took 12 minutes and 52 seconds to subdue and eat the nymph (head capsule 3.7 mm). In the third test, bluegill #2 spent between 53 and 73 minutes attempting to subdue the nymph (head capsule 3.7 mm), but eventually rejected it. (The exact time of rejection was not observed: the nymph was found crawling on the substrate.) In two later tests, bluegill #2 took between 7 and 8 minutes to subdue and eat the nymphs (head capsules 3.2 and 3.6

Table IV-7. Comparisons of handling time for worms eaten by warmouth in the centrarchid experiments.

Test/ Predator Group (n)	Handling Time (s)	Standard Deviation	t-value, Probability
warmouth vs rock bass and redbreast, pooled			
warmouth (14)	30.9	18.3	
			0.317, NS
pooled (28)	29.2	15.5	
warmouth 1 vs warmouth 2			
warmouth 1 (7)	23.2	9.6	
			-1.698, NS
warmouth 2 (7)	38.7	22.2	

mm). In the final test, bluegill #2 spent over 24 minutes before finally rejecting the nymph (head capsule 3.7 mm).

Bluegill #2 spent significantly more time handling worms than other species (Table IV-8). Bluegill #1, on the other hand, spent significantly less time handling worms than other species. Handling time for worms was accordingly significantly different between the two bluegill.

A major part of the increased handling time of worms for bluegill #2 was that it repeatedly spat out the worm and re-inhaled it in four of the eight tests. This occurred with increasing frequency as the tests continued. In the final test bluegill #2 spat out the worm eleven times over a period of 2 minutes and 40 seconds before finally swallowing it.

REDBREAST AND ROCK BASS. Redbreast sunfish and rock bass ate every nymph presented, with two exceptions. Both of these exceptions were in redbreast sunfish tests and both occurred early in the experiment. In both cases, the redbreast sunfish huddled in the corner of the aquarium and refused to attack the nymph. The redbreast sunfish were initially very shy, and they never became as bold as the other species.

The nymphs these species did eat were the largest of the nymphs tested. The rock bass ate nymphs as large as 4.4 mm in head size. The redbreast sunfish ate nymphs as large as 3.6 mm in head size during the experiment and 4.5 mm in head size after the experiment.

The main difference between the redbreast and the rock bass was that the redbreast spent a much longer time handling both the worms and the nymphs than the rock bass (Table IV-9).

Effects of Nymph Behavior. The data from the first attack were analyzed statistically. The data showed a slight tendency for the fish to eat nymphs exhibiting non-curling behavior and to reject nymphs exhibiting curling behavior (Table IV-10).

Table IV-8. Comparisons of handling time for worms eaten by bluegills in the centrarchid experiments.

Test/ Predator Group (n)	Handling Time (s)	Standard Deviation	t-value, Probability
bluegill #1 vs warmouth, rock bass, and redbreast, pooled			
bluegill #1 (7)	16.9	6.5	
			-2.057, < 0.05
pooled (42)	29.8	16.3	
bluegill #2 vs warmouth, rock bass, and redbreast, pooled			
bluegill #2 (7)	141.1	146.2	
			5.011, < 0.001
pooled (42)	29.8	16.3	
bluegill #1 vs bluegill #2			
bluegill #1 (7)	16.9	6.5	
			-2.246, < 0.05
bluegill #2 (7)	141.1	146.2	

Table IV-9. Comparisons of handling time for prey eaten by rock bass and redbreast in the centrarchid experiments.

Test/ Predator Group (n)	Handling Time (s)	Standard Deviation	t-value, Probability
rock bass vs redbreast, worms			
rock bass (14)	22.0	8.7	
			-2.765, < 0.05
redbreast (14)	36.5	17.6	
rock bass vs redbreast, eaten nymphs			
rock bass (16)	52.9	19.8	
			-2.467, < 0.05
redbreast (14)	410.1	580.4	

Table IV-10. Effect of nymph behavior on nymph survival of first attack in the centrarchid experiments.

Test Group/ Behavior	Eaten	Rejected	Chi-square value Probability
all data			
curled	13	13	
			0.758, NS
non-curled	22	14	
bluegills only			
curled	0	6	
			3.484* NS*
non-curled	6	4	

* P = 0.062 with Yates correction (for sample sizes less than twenty, Sokal and Rohlf, 1981).

Curling behavior included bottom curling for these comparisons; all other behaviors were lumped. Confounding the data are the fish that exhibited monolithic behavior in tests: rock bass, redbreast sunfish, and warmouth. If just the bluegill are considered, the effect of behavior still does not become significant, but a trend does become apparent: no curled nymphs were eaten by bluegill (Table IV-10). Small sample size made interpretation of the bluegill data difficult.

Handling time did not differ significantly between prey behaviors in these experiments (Table IV-11).

Effects of Nymph Size. Size did not exert a significant impact on handling time. This was true overall for eaten nymphs and rejected nymphs (Table IV-12), and also true for eaten nymphs within the rock bass and redbreast sunfish tests, and for rejected nymphs within the warmouth tests (Table IV-13). Neither did size exert a significant effect on whether or not bluegill ate the nymphs they did (Table IV-14). Again, small sample size made interpretation of the bluegill data difficult.

There were trends in the handling time data. Fish tended to spend more time eating nymphs larger than the median than nymphs smaller than the median (Tables IV-12 and IV-13). This trend was particularly obvious in the redbreast handling times (Table IV-13). Two unexpected trends appeared: fish tended to spend more time attacking nymphs larger than the median before rejecting them (Tables IV-12 and IV-13) and fish spent more time handling curled nymphs before rejecting them. However, large standard deviations prevented these trends from being statistically significant. These large standard deviations represent the extreme variation in feeding behavior between species, within species, and within individuals (particularly within individual bluegills).

Table IV-11. Impact of nymph behavior on predator handling times in the centrarchid experiments.

Test Group/ Factor Tested (n)	Handling Time (s)	Standard Deviation	t-value, Probability
rejected nymphs			
curled (10)	15.2	36.9	0.815, NS
other (17)	7.4	11.0	
eaten nymphs			
curled (13)	150.4	279.7	-1.235, NS
other (21)	332.1	480.9	

Table IV-12. Impact of nymph size on predator handling time in the centrarchid experiments.

Test Group/ Factor Tested (n)	Handling Time (s)	Standard Deviation	t-value, Probability
rejected nymphs (median = 3.0)			
$\leq$ median (14)	3.9	2.9	-1.493, NS
> median (13)	17.2	33.3	
eaten nymphs (median = 3.3)			
$\leq$ median (17)	131.6	144.3	-1.888, NS*
> median (17)	393.7	554.0	

* $P < 0.10$.

Table IV-13. Impact of nymph size on handling time for nymphs eaten by rock bass and redbreast sunfish and nymphs rejected by warmouth, all from the centrarchid experiments. Nymphs were grouped by whether they were larger or smaller than the median head capsule width.

Predator Group/ Nymph Size Class (n)	Handling Time (s)	Standard Deviation	t-value, Probability
rock bass, eaten nymphs, (median = 3.5)			
≤ median (8)	49.9	21.1	
			-0.585, NS
> median (8)	55.8	19.3	
redbreast, eaten nymphs, (median = 3.2)			
≤ median (7)	115.6	79.8	
			-2.145, NS*
> median (7)	704.5	722.0	
warmouth, rejected nymphs, (median = 3.0)			
≤ median (9)	4.2	2.3	
			-1.818, NS*
> median (7)	7.8	5.4	

* Significant at $P < 0.10$.

Table IV-14. Effect of nymph size on nymph survival of first attack in the bluegill tests of the centrarchid experiments. Nymphs were grouped by whether they were larger or smaller than the median head capsule width (3.1 mm).

Size Class	Eaten	Rejected	Chi-square value, Probability
≤ median	2	6	
			0.267, NS*
> median	4	4	

* P = 0.606 with Yates correction (for sample sizes less than twenty, Sokal and Rohlf, 1981).

Bluegill Experiments

The main result in the bluegill experiments was the detection of differences in behavior among individual bluegills. Only two of the five fish tested actually ate a nymph, and only one of these ate a curled nymph. However, nymph behavior was not significantly associated with whether or not the nymph was eaten.

Effects of Prey Type. Prey type was significantly associated with frequency of prey eaten (Table IV-15); the fish ate more worms than nymphs. Handling time, however, was not significantly longer for eaten nymphs than for eaten worms (Table IV-16). Small sample size of eaten nymphs (n = 7) biased these results. Furthermore, one fish ate six of the seven eaten nymphs.

Effects of Individual Predator. Differences between fish will be discussed first with worms and then with nymphs.

WORMS. Bluegill #2 showed the greatest variation in the handling time of worms. Bluegill #2 spent greater than three minutes to handle the worm in four tests and less than 45 seconds in the other four tests. The worm masses did not differ significantly from each other in these two groups (P = 0.85, t-test).

Bluegill #6 spent an average of 330 seconds handling worms. This was the longest average of any fish and significantly different (Table IV-17) than the worm handling average of the other bluegills in the bluegill tests. There was an interesting pattern in the handling times of bluegill #6: it spent an average of 436 seconds handling worms in the first six trials and only 12 seconds in the last two. Masses were not significantly different between these two groups (P = .21, t-test). A dark mark was observed near the mouth and may have been a hemorrhage. It is possible that this represented an injury from capture (hook-and-line) that healed by the last two tests. This hypothesized injury did not appear to impact patterns of behavior with the nymphs (see below).

Table IV-15. Comparison of the number of eaten worms and the number of eaten nymphs in the bluegill experiments.

Prey	Eaten	Rejected	Chi-square value, Probability
worms	36	0	
			7.754, < 0.01
nymphs	7	29	

Table IV-16. Comparison of handling time for eaten worms and eaten nymphs in the bluegill experiments.

Prey (n)	Handling Time (s)	Standard Deviation	Probability
eaten worms (36)	120.0	191.5	
			1.933, NS
eaten nymphs (7)	265.4	111.5	

Table IV-17. Comparison of the worm handling time of bluegill #6 with other bluegills, pooled, in the bluegill experiments.

Predator Group	Handling Time (s)	Standard Deviation	t-value, Probability
bluegill #6 (8)	330.0	290.8	
			4.309, < 0.001
pooled (28)	60.0	94.0	

The other three fish (bluegills #3, #5, and #8) were all very efficient and only once spent longer than 45 seconds handling a worm (bluegill #8 took 48 seconds in its first trial). Most handling times (85%) for these fish were less than 30 seconds.

NYMPHS. Of the five bluegill tested, only two ate nymphs. Bluegill #2 ate six of the eight nymphs presented. These were equally divided between behaviors: three nymphs were open and three nymphs curled. This bluegill ate all four of the nymphs in the first four tests, but only two in the last four. It rejected a curled nymph in less than one second, and spent 30+ seconds before rejecting an open nymph in these latter tests. Fish #8 was the other bluegill to eat a nymph. Fish #8 was started on the third day of the experiment (it was too shy to test at the start of the experiment) and was tested four times. The only nymph it ate was the first nymph presented. This nymph was open and the fish spent 391.4 seconds to subdue it. The nymphs were curled in the next two trials and the bluegill spent less than 2 seconds in both trials before rejecting the nymph. In the final trial, the nymph was open, and the fish spent 41.4 seconds before rejecting the nymph.

Three fish rejected all nymphs presented. Bluegill #6 inhaled an open nymph in the first trial and spent a total of 8.0 seconds handling it before rejecting it. In subsequent trials, it inhaled a nymph only once, and that briefly. In the last four trials, the nymph

was never inhaled, but only pecked. In the second to the last trial, bluegill #6 only bumped the nymph.

Bluegill #5 showed decreased persistence with time in its attacks.

Bluegill #3 did not show consistently decreasing persistence, but, rather, was most persistent on days one and four and least persistent on days two and three.

Effects of Nymph Behavior. The impact of nymph behavior will be analyzed in three ways: whether behavior protected the nymph from being consumed, whether behavior changed handling times, and whether behavior protected the nymph from serious injury.

CONSUMED. Due to the swamping effect of individual monolithic behavior, the data failed to show whether behavioral mechanisms protected nymphs from being eaten (Table IV-18).

HANDLING TIME. The handling times for open, rejected nymphs were significantly lower than the handling times for curled, rejected nymphs (Table IV-19). Handling times for eaten nymphs were not tested because of small sample size.

INJURY. The results showed that nymphs attacked while open sustained greater injuries than those that were attacked while curled (Table IV-20). Of the 15 nymphs attacked while curled, 12 (80%) sustained no visible injury. Of the 14 nymphs attacked while open, only 7 (50%) sustained no visible injury. Furthermore, the 3 injured, curled nymphs were only injured slightly, while 3 of the 7 injured, open nymphs died as a result of their injuries.

Effects of Nymph Size. Size did not appear to play a role in determining whether tests resulted in successful predation. Capsule widths did not differ significantly between anesthetized and unanesthetized nymphs (Table IV-21). Neither did handling times differ significantly between rejected nymphs smaller than the median and rejected nymphs larger than the median (Table IV-22).

Table IV-18. Effect of nymph behavior on nymph survival of first attack in the bluegill experiments.

Behavior	Eaten	Rejected	Chi-square value, Probability
open	4	14	
			0.177, NS
curled	3	15	

Table IV-19. Comparison of handling time for curled, rejected nymphs and open, rejected nymphs in the bluegill experiments.

Behavior (n)	Handling Time (s)	Standard Deviation	t-value, Probability
open (14)	2.4	2.1	
			-2.400, < 0.05
curled (14)	12.3	15.2	

Table IV-20. Comparison of injuries for open, rejected nymphs versus curled, rejected nymphs in the bluegill experiments. Severity of injuries increases as ranking increases: zero represents no injury and four represents nymphs dead upon rejection.

Nymph Behavior	Injury Ranking				
	0	1	2	3	4
Open	7	2	2	2	1
Curled	12	3	0	0	0

Injury Scale: 0 = no injury visible; 1 = mucous visible and/or slight abdominal rupture or slight limb injury; 2 = massive mucous visible and severe rupture in abdominal tergites and/or moderate limb injury; 3 = died in the holding tank within a week of the test; 4 = dead upon removal from tank where the test occurred. For a more complete description of injury rankings, please see Table IV-4 (p. 62).

Table IV-21. Comparison of the head capsule width of anesthetized nymphs and unanesthetized nymphs in the bluegill experiments.

Nymph Class	Capsule Width (mm)	Standard Deviation	t-value, Probability
anesthetized (18)	3.2	0.4	
			-0.536, NS
unanesthetized (18)	3.1	0.3	

Table IV-22. Impact of nymph size on handling time for rejected nymphs in the bluegill experiments. Nymphs were grouped by whether they were larger or smaller than the median head capsule width. The median capsule size was 3.1 mm.

Nymph Size Class	Handling Time (s)	Standard Deviation	t-value, Probability
$\leq$ median (16)	8.4	14.3	
			0.536, NS
> median (12)	5.9	7.5	

Discussion

The hypothesis that these experiments tested was whether curling is a defensive behavior based on maximizing the effectiveness of the exoskeleton against visual fish predators while the nymphs are drifting.

It is apparent that Pteronarcys nymphs have effective, but not foolproof, defenses against fish predators. In all experiments, they were eaten at significantly lower rates than worms. The mechanism of this defense is unclear from these experiments. With sculpins size may have been the primary defense; with others predators, the primary defense may have been the armored, spinous exoskeleton. This latter seems to have been augmented by behavior (bluegill) in some instances and possibly by chemical defense (warmouth) in others.

Effects of Curling

Curling does appear to be a defensive behavior. Curling did not present any sort of defense against sculpins, redbreast sunfish, or rock bass, and it was unnecessary for warmouth. Only with bluegill did it seem to be of any importance, and this only in certain instances. The reasons that curling is argued to be a defensive behavior are outlined below.

(1) Behavior did appear to play a slight role in defense against bluegills. Chi-square goodness of fit tests failed to find an association between curling of nymphs and rejection of nymphs by bluegills. On the other hand, throughout the experiments, four bluegill were observed to eat nymphs, but only one bluegill was observed to eat a curled nymph.

(2) The data from the injury observations also support this conclusion. It shows that curled nymphs were less likely to be damaged in an attack than open nymphs. Curling, therefore, becomes a method that increases the chance of survival if rejected

and only secondarily a method that increases the frequency of rejection. Rejection in the field could be the result of fish size, fish condition (e.g. mouth injury), environmental conditions, etc., and may be independent of nymph defenses. In this scenario, survival of random (to the nymph) rejections could be more important than induced rejection. Curling would increase the chance of surviving random rejection.

(3) Curling was a consistent nymph behavior upon attack by fish. Thus, the conditions of curl behavior argues that curling is a defensive mechanism. The fact that nymphs consistently changed to a curl behavior upon attack argues that curling is a secondary defense, or a defense that functions during a predator-prey encounter to increase the probability of prey survival. Unfortunately, the fact that curling may be a secondary defense does not help differentiate between the two competing anti-predator hypotheses; both hypotheses are secondary defenses.

(4) Moore and Williams (1990) reported that 18 out of 24 P. dorsata survived rainbow trout attacks, whereas zero of twenty Agnetina capitata (Plecoptera: Perlidae) survived. They attributed this to thanatosis behavior (equivalent to the present curling behavior) in the P. dorsata nymphs. (More will be said concerning curling as thanatosis in Chapter 5.)

Effects of Nymph Size

Nymph size appeared to have no impact on the success or failure of rock bass or redbreast sunfish predation. There was not a size of nymph that these species were unable to eat. Moore and Williams (1990) also failed to find a size impact on trout predation. However, this conclusion cannot necessarily be applied to the field. There would be smaller (and larger) predators in natural predator populations. These smaller individuals would not be expected to eat all sizes of nymphs.

There were several indications that nymph size did discourage predation. Large nymphs were protected from sculpins. In the field, minnows were observed to attack and reject several nymphs. Certainly, in the streams where many of the nymphs were captured, fish predators would be small and gape limitation could be an important predator defense.

Predation Pressure

Important to understanding the role curling plays as an anti-predator behavior under natural conditions are questions concerning the predation pressures to which pteronarcid nymphs are exposed. Three components are important to identifying the pressure that predators place on pteronarcid nymphs: (1) behavioral variation among predator species; (2) variation within predator guilds between habitats; and (3) vulnerability of pteronarcids to fish predators. Of these three, the present research can only directly address the first; the other two can only be addressed indirectly.

Variation in Predator Behavior. Whatever defenses Pteronarcys nymphs do have, their effectiveness is strongly tempered by variation in predator behavior. This variation occurs between species, within species, and within individuals through time.

SCULPINS. Although sculpins were the smallest fish tested, they readily ate Pteronarcys nymphs. They are well known as aggressive predators. Because they live under rocks in riffles, habitat similar to Pteronarcys habitat, and because they are nocturnal, as are Pteronarcys, sculpins are potentially important predators of Pteronarcys, probably primarily when pteronarcid nymphs are not drifting.

REDBREAST SUNFISH. It was surprising that redbreast sunfish did not reject any nymphs. Of the five predators tested, they had the second smallest mouth size per body size (after the bluegill). Furthermore, in earlier, uncontrolled tests, redbreast sunfish had rejected Pteronarcys nymphs. Possibly the redbreast in the experiments learned that

pteronarcid nymphs were food early on in the tests and subsequently ate nymphs larger than they normally would. Redbreast sunfish did spend quite a bit of time (mean of 410.1 s) handling the nymphs they ate. Small sample size may not have given a true representation of individual variation within the species.

ROCK BASS. Rock bass, on the other hand, commonly eat crayfish (Pflieger 1975), and this feeding strategy may well prepare them for feeding on heavily armored, curled Pteronarcys nymphs. Certainly, their average handling time (52.9 s) was very much lower than that of the other fish species.

WARMOUTH. That warmouth did not eat Pteronarcys is very surprising. They have a mouth morphology similar to rock bass and also commonly eat crayfish (Pflieger 1975). It is interesting that mucous from the nymphs was commonly observed in their tests but not in the tests of other species. This mucous was not tested chemically, but matches the description of pteronarcid hemolymph exuded in attacks by crayfish (Moore and Williams 1990). Locations of mucous concentrations on nymphs examined after attacks also matched reported sources of hemolymph. Possibly the patch of teeth on the warmouth tongue, unique among the species tested (Etnier pers. comm.; see also any standard fish key, e.g. Pflieger, 1975), mechanically triggered the release of the hemolymph. There are indications that the hemolymph is triggered mechanically when crayfish attack (Moore and Williams 1990). This hypothesis begs an explanation of why warmouth would be sensitive to the hemolymph when other fish are not. It is hard to imagine that a defense that works mechanically by sticking to the exoskeleton of arthropod predators would deter a fish predator. Hemolymph has not been found to be effective against vertebrate predators in other research (Benfield 1974; Moore and Williams 1990).

BLUEGILL. The bluegill were only variably successful at eating nymphs. The main defensive mechanism appears to be the armored exoskeleton. Curling appeared to play a

minor role. The small size of the bluegills mouths probably played a key role in the failure to consistently eat nymphs.

ALLOPATRIC AND SYMPATRIC FISH PREDATORS. Interestingly, it was only the predator populations allopatric from Pteronarcys that rejected the nymphs. What is it about the sympatric species that permitted them to eat Pteronarcys? Were individuals within the sympatric species truly experienced with Pteronarcys? Is experience of particular importance in feeding on these species? Is there a co-evolutionary process ongoing? It is unlikely that it is a food recognition problem as all fish attacked nymphs initially. The present research was not designed to test these questions, and cannot adequately address them. However, it is now known that Pteronarcys species are very likely sympatric with populations of all the predator species tested (see next section).

Experience does not always affect predator behavior in predictable patterns. Hepburn et al (1973) found that fish predators learned to overcome the distastefulness of benzoic acid. In their experiments, fish initially rejected a higher frequency of food pellets contaminated with benzoic acid than they did as the experiment progressed. This was despite the fact that the initial benzoic acid concentrations were the lowest and that benzoic acid concentrations increased as the experiment progressed.

Variation in predator guilds. Evolution of defensive behavior can occur only if the predators are actually applying predation pressure on the nymphs. In many of the streams where nymphs were collected fish predators were rarely observed, and when fish were observed, they were limited to small species such as sculpins, darters, and minnows. In these small streams fish predators may not be major predators of pteronarcid nymphs.

Bob Terry (personal communication) reported that the Tennessee Valley Authority had records of Pteronarcys species from a number of larger rivers in east Tennessee. Pteronarcys dorsata was recorded at mile 65 of the Powell River, and also in the Elk

River and the Sequatchie River. Unidentified Pteronarcys species were found well below Douglas Dam in the French Broad River, and also in the Holston and Little Tennessee rivers. Additionally, two unidentified (to species) exuviae were collected by this researcher from the surface of the French Broad River (miles 5-9) on April 13, 1990. Given these ranges, Pteronarcys species are sympatric with a wide variety of fish predators and would face a wide range of predator pressures. Included as potential predators would be all the fish used in the experiments.

Predation pressure would vary greatly among habitats. In the small streams in which they were collected, nymphs would probably face their greatest pressure from benthic predators. These predators could possibly include amphibians, such as hellbenders (observed to eat pteronarcid nymphs in the laboratory), and arthropod predators, such as crayfish. Insect predators, such as the large perlid stoneflies, may present the greatest predator pressure in the nymphs' first year. Moore and Williams (1990) suggested that autohemorrhaging may be effective against perlids, but this has gone untested so far. Crayfish also pose a potential threat to pteronarcid nymphs. In at least two instances, crayfish were found in the same leaf pack as collected pteronarcid nymphs (pers. obs.). Moore and Williams (1990) showed that the autohemorrhaging defense was extremely effective against crayfish predators. In uncontrolled lab tests, crayfish were observed to eat pteronarcid nymphs (pers. obs.). More work remains to untangle the arthropod defenses of pteronarcid nymphs.

In small creeks, important fish predators would include minnows, darters, and sculpins. Here size may be an effective deterrent to many predators.

In small rivers, such as the Little River and Citico Creek, fish predators would possibly form the greatest predation pressure. Here potential predators would include smallmouth bass, other centrarchids, and rainbow trout. Catfish, such as channel catfish

and yellow bullhead, also are potential predators. All of these species attain a size which would allow them to handle any pteronarcid nymph.

The pteronarcid populations in the large rivers would face a whole host of warm-water fish predators, from large, aggressive benthic feeders that search using chemoreceptors, such as blue catfish, to a variety of warm-water feeders that search visually.

Variation in Exposure. Also key to the present research is the frequency with which the nymphs are exposed to predation by fish. Pteronarcid nymphs do have important primary defenses minimizing predator contact (Edmunds 1974). Their dark coloration and secretive behavior decreases the frequency of contact with many potential fish predators. In the leaf packs, under large rocks, nymphs are protected from a variety of predators. Here only arthropod predators, possibly sculpins, and a few unusual predators (e.g. hellbenders) are able to find and attack them.

Nymphs would be most exposed to visual fish predation while drifting. Published records do not mention pteronarcids as a major component of the drift (e.g. Chapman and Demory 1963; Cada et al 1987). Jerry Freilich (personal communication) and Bill Ensign (personal communication) both reported that their data showed that pteronarcids only infrequently drift. Whether this represents data from areas of low pteronarcid densities or a low drift rate in pteronarcids is unknown.

The TVA records (Bob Terry, personal communication) of pteronarcid occurrences were from May and April. Interestingly, this is the approximate time of their emergence. Whether these records represent emerging adults, larval migration prior to emergence, or a sampling artifact is unknown.

Given the above data, it becomes even more difficult to determine the predator pressure that drifting pteronarcids might face.

Conclusions

Based on the research described in this chapter, curling does appear to be a defensive behavior that functions by maximizing the efficiency of exoskeleton against visual fish predators. The advantages that curling do present are highly species specific and situation specific (survival of a random rejection) but may present enough of an advantage to be important in the survival of P. scotti. Complicating interpretation are questions about the nature and frequency of predation pressure that were not addressed by the present research. Furthermore, it is also possible that curling is a behavioral remnant of previous predation pressures (Peckarsky and Penton 1988).

Summary

The reaction of predators to P. scotti nymphs was explored. Reaction varied among predator species. Sympatric species, rock bass and redbreast, ate all nymphs. Bluegill, an allopatric species, displayed various reactions to nymphs. Warmouth, another primarily allopatric species, would not eat any nymphs.

Curling behavior was not found to be the primary defensive mechanism used against any of these predators. However, there was evidence that it did play an important secondary defensive role. This evidence included the reaction of bluegill to curled nymphs, the consistent timing of curling behavior upon attack, and the higher survival rates of rejected, curled nymphs. Previous research with trout (Moore and Williams 1990) also supports this interpretation.

CHAPTER 5: CONCLUSIONS

In this chapter, the hypotheses initially identified in Chapter 1 will be listed and the conclusions derived from this research will be summarized. In addition, the following subjects will be addressed: a hypothetical stream drift model for pteronarcids and questions developing from this research.

Summary of Hypotheses and Conclusions

The primary question of this thesis was: What advantage(s) do(es) P. scotti gain from curling while in the water column? Two hypotheses were tested in this research:

1. Curling is a position behavior that increases the nymphs ability to control its water column position.
2. Curling is a defensive behavior based on maximizing the effectiveness of the exoskeleton and defends drifting nymphs against fish predators.

Additionally, observations pertinent to the following hypothesis were made and discussed in relation to the research of Moore and Williams (1990):

3. Curling is a defensive behavior based on "thanatosis" and defends drifting nymphs against fish predators.

Curling as a Position Behavior

It appeared that curling could very well be a position behavior. Factors arguing in support of this hypothesis included:

1. Curled nymphs sank more quickly than open nymphs.
2. The back arch behavior appeared to be a behavior to facilitate exiting the water column in preferred habitat. While this does not argue directly that curling is a position behavior, back arch behavior does present a line of

argument that if behavior is used as a position behavior in one situation, the likelihood increases that another behavior (such as curling) also functions as a position behavior.

3. The ontogenetic variation in nymphs behavior also argues that in some fashion, curling behavior provides the nymph with some advantage. The assumption is that variation in behaviors at different nymph ages represent responses to different selective pressures at different nymph ages. What these pressures are was not tested in this research and can only be guessed at. The ontogenetic variation in behavior then doesn't so much support the hypothesis that curling is a position behavior, as lend support to the overall assertion that curling is in some way advantageous.

Curling as a Defensive Behavior

The two hypotheses that treat curling as a defensive behavior will both be addressed in this section. However, the first question in discussing this hypothesis is whether pteronarcids have a defense against fish at all. Important indications that pteronarcids do have effective defenses against fish are well documented, including:

1. The failure of warmouth to eat any nymphs, and the relatively high percentage (when compared with worms) of nymphs rejected by bluegill.
2. The successful defenses that pteronarcids exhibited against trout, suckers, and sculpins as reported in the research of Moore and Williams (1990).

The second question to be addressed is whether curling presents a defense against fish predators while the nymphs are in the water column. The primary result supporting curling as a defensive behavior is the fact that the nymphs curled consistently upon attack by fish, as reported both in this thesis and in the research of Moore and Williams

(1990). Given the consistency with which the stimuli (fish attack) elicited the behavior (curling), curling appears to be a defensive behavior.

Having established that curling is likely a defensive behavior, the final question is whether it is a defense based on maximizing the effectiveness of the exoskeleton or a defense based on thanatosis. Here the evidence is not so clear. Curling, both as physical protection of the nymph and as thanatosis, functions as secondary defense during an encounter with a predator. The function of a secondary defense is to increase the chance of an individual surviving an encounter with a predator (Edmunds 1974, p. 136). Moore and Williams (1990) and Otto and Sjostrom (1986) both named the behavior "thanatosis." Moore and Williams (1990) presented evidence that thanatosis does increase the chances of an individual nymph surviving an attack by a trout predator. However, their control was a perlid stonefly, which lacks the exoskeleton projections of pteronarcids, so possibly it was not behavior that was the defense.

The primary evidence that curling is a defensive behavior based on maximizing the exoskeleton is the injury data reported in this thesis. This provides strong evidence that curling maximizes the effectiveness of the thick, spiny exoskeleton by enabling pteronarcids to survive attacks in which fish inhale but later reject the nymphs.

Most likely curling functions in both ways. By not moving, pteronarcids deflect possible attacks by the visual predators, such as trout and centrarchids, that are common predators in streams. It was observed in the present study that fish would often lose interest in rejected nymphs that curled and did not move, but would re-attack nymphs that began to move while curled. When attacked, pteronarcids have the additional defense of a heavily armored exoskeleton that is augmented by curling to protect the vulnerable, exposed gills. The injury data from this research show that the nymphs are better able to survive failed attacks if they are curled.

In summary, it appears that pteronarcid stoneflies are equipped with some defenses against visual fish predators. Thus, the potential that drifting nymphs possess defenses against fish predators must be considered by researchers when explaining drift patterns seen in the stream.

Stream Behavior Model

The obvious question when discussing the ontogenetic variation in stonefly behavior is: "why do small nymphs curl more frequently than large nymphs?" Perhaps a more insightful question in understanding the ontogenetic behavior of pteronarcids is: "why do large nymphs swim more frequently than small nymphs?" Assuming that exiting the water column is advantageous, and given that curling does decrease sinking time and does offer some predator protection, one would expect all nymphs to curl.

The assumption that exiting the water column is always advantageous is not necessarily valid in all scenarios. For example, a rapid exit from the water column may be disadvantageous if in non-ideal habitat. Here, nymphs would swim across pools and back arch in riffles, the preferred habitat.

Frustrating a clear interpretation of this behavior is the wide variation in predation pressure between habitats. In the streams where pteronarcids were found in their highest densities, many of the predators may be strongly gape limited. The chance of coming upon a large predator while in the drift may be small compared with the potential gain of staying in the water column until preferred habitat is found. In this scenario, various selective pressures would operate at different strengths on different sized nymphs. Small nymphs would run a greater risk of predation because of their small size and would curl out of defensive necessity. Large nymphs would be large enough to escape most predators and finding suitable habitat would be the primary pressure. This model contradicts the drift research which has reported predation pressure is greatest for

large nymphs (Allan 1978, 1984; Malmqvist 1988). However, Malmqvist (1988) reported that large nymphs did not show any diel periodicity in waterways without trout, but that large nymphs did tend to drift more frequently at night in waterways with trout. Here it is seen that changes in predator pressure can cause variation in drift behavior.

Given that pteronarcids most likely do not posses long-distance chemoreceptors (Motyka et al 1985), sensory input for finding preferred habitat could be through detection of changes in current. Upon detection of a change in current velocity, the nymph would change to an exiting behavior (the back arch) and thus increase the probability of latching onto a rock.

Directions of Future Research

Questions posed by these results follow:

1. Do pteronarcids from different habitat types (stream orders) have different behaviors? Are these behaviors hard-wired, or are they triggered by environmental cues? Key to this question would be an analysis of predator guild and the predation pressures that these nymph face. Possibly, the Schall and Pianka (1980) hypothesis could be examined using aquatic insect nymphs as a test class of organisms.

2. Nature of the defense that deters warmouth. Studies have shown that noxious taste does play a role in defense in aquatic invertebrate-vertebrate prey-predator interactions (Kerfoot 1982; Benfield 1972).

3. Trigger for the behavioral change in the drift. Is it the absolute velocity of the current, or is it the relative change in current speed?

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