Swimming behavior of Daphnia: its role in determining predation risk

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Abstract. Individual swimming behavior of zooplankton can play an important role in determining how planktivorous fish select their prey. Although several studies have documented the effect of prey size, contrast or degree of pigmentation, escape ability, encounter rate and abundance in determining predation risk, the importance of individual behavior has received relatively little attention by aquatic ecologists. Recent advances in the technology of video recording and computer analysis of motion have allowed us to collect digitized three-dimensional video records of free-swimming zooplankton such as Daphnia. We found that Daphnia clones, including those within a single species, exhibit a wide range of swimming behaviors as measured by swimming speed. The individual behavior of a species cannot be adequately described by looking at one clone. We also show that different behavior observed in live Daphnia can play an important role in determining attractiveness to visual predators. Given a choice between two clones of equal size and visibility contrast, fish selected individuals from the faster swimming clone. Our results suggest that current models of prey selection would be improved by the incorporation of individual swimming behavior because it is an important factor determining overall prey visibility.

Introduction

Visual predators, primarily fish, play an important role in structuring zooplankton communities through their ability as selective predators (Hrbdcek et al., 1961; Brooks and Dodson, 1965; Carpenter et al., 1985; Christoffersen et al., 1993). Upon encounter, prey selection by visual predators is governed by a combination of factors that include the following: visibility as determined by size, contrast or degree of pigmentation, and movement (Kerfoot, 1980, 1982; Buskey, 1994); escape ability (Drenner and McComas, 1980); and distribution and abundance (Ivlev, 1961; Werner and Hall, 1974). Of these factors, the importance of prey motion in determining predation risk has received relatively little attention by aquatic ecologists.

Two lines of evidence, described in detail below, strongly suggest that prey motion is important in determining predation risk: (i) work with a variety of predator–prey systems has shown that differences in the behavior of prey strongly affect predation risk and (ii) several studies with zooplankton as prey and fish as selective predators have not satisfactorily explained selectivity solely by considering static aspects of prey (i.e. size and visual contrast). In some of the most extensive investigations of how differences in behavioral aspects of prey affect vulnerability to predators, Prete and co-workers (Prete, 1993; Prete and Mahaffey, 1993; Prete et al., 1993) have demonstrated that preying mantis (Sphodromantis lineola) selectively attack prey that display certain behavior patterns. Their experiments.
with both artificial and computer-simulated prey show that prey preference varies with prey velocity as well as with 'jumpiness' in the path. Similarly, Freed (1988) found that treefrogs (*Hyla cinerea*) preferentially attack prey with greater linear velocity. In experiments with kestrels (*Falco sparverius*), Sarno and Gubanich (1995) found that the birds select the more active of two mice regardless of their size.

In several studies of prey selection by fish, researchers have concluded that the selectivity observed in fish predation cannot be explained solely by differences in static characteristics of prey. Consequently, some have proposed that differences in prey motion may affect prey selection. Zaret (1980) found that the fish *Melaniris* selectively preyed upon a bosminid species that had both the smallest body size and the smallest eye spot. He concluded that the fish may be using a search image of prey motion to focus on the smaller species. Confer and Blades (1975) found that reactive distance of pumpkinseeds (*Lepomis gibbosus*) to the copepod *Meso-cyclops edax* was greater than that predicted by observations of fish feeding on other copepod species, and suggested that its characteristic movement was a factor. Finally, Buskey and co-authors (Buskey *et al.*, 1993; Buskey, 1994) hypothesized that prey with 'active and irregular' motion patterns may be more attractive to planktivorous fish than prey with 'smooth' behavior. Govoni *et al.* (1983) have observed this in an experiment where larval fish fed on *Artemia*. Although these studies suggest that characteristic differences in prey motion among species are important in determining prey vulnerability, none were designed to, nor do they present data to test this hypothesis explicitly. Buskey *et al.* (1993) proposed that by designing fish predation experiments using prey of similar size and visibility contrast, it may be possible to evaluate the importance of prey motion patterns in determining predation risk (Buskey *et al.*, 1993; Buskey, 1994).

In one experiment using fish as predators, Brewer and Coughlin (1996) demonstrated that bluegill (*Lepomis macrochirus*) select virtual zooplankton, rendered on a computer monitor, that swim twice as fast as their neighbors 70% of the time. Dodson *et al.* (1994) showed that *Daphnia* that were heavily dosed with carbaryl would swim fast and with a high turning angle. Fish would select these erratically swimming individuals over normally swimming *Daphnia*. While this result shows that fish selectivity can depend on swimming behavior, the induced behavior does not likely represent behavior which fish would encounter in the field. Other studies with fish selecting zooplankton prey have tested the hypothesis that fish tend to strike moving rather than stationary individuals (Kerfoot, 1982; Wright and O'Brien, 1982, 1984), but these studies do not explicitly address the question of selection between individuals that move differently.

The goal of our study was first to quantify the swimming behavior of different *Daphnia* clones and determine whether differences in behavior actually exist within a single species. We addressed the question of whether *Daphnia* swimming behavior is clone specific or species specific, since previous studies of *Daphnia* swimming behavior have only examined a single clone from a species. To determine whether variability existed within a species complex, we selected five clones from the *pulex* species complex, and two clones from outside the *pulex* species complex for comparison. We predicted that behavior would be clone specific,
meaning that clones from the same species complex would not all swim the same. The alternative hypothesis is that all clones from a species complex swim the same, so observing the behavior of one clone would be sufficient to describe the behavior of that species complex. Inter-clonal variation within a species is high for both morphological development (Parejko and Dodson, 1991) and vertical migration (DeMeester et al., 1995). We expected to find this same degree of variation when comparing the swimming behavior of clones in the same species or species complex.

After quantifying swimming behavior, we determined whether the quantifiable differences that we measured in swimming behavior were biologically meaningful. We selected clones that exhibited dramatically different swimming behavior, but were similar in morphology and pigmentation for a fish choice experiment. We tested the hypothesis that fish would choose the faster swimming *Daphnia* when given a choice between two clones that exhibit different behavior. Brewer and Coughlin (1996) showed that fish significantly chose the faster swimming individual when given a choice of computer-generated virtual zooplankton on a monitor. Here we describe a complementary experiment using live, free-swimming prey. We also conducted an experiment with a darkly pigmented clone and a lightly pigmented clone to determine how pigmentation differences would affect fish choice for species with different swimming behavior.

**Method**

**Choice of clones**

We chose distantly related species within the genus *Daphnia*, including several clones within the common and widespread *pulex* species complex. The two *D.pulicaria* and three *D.pulex* clones are all members of the *pulex* species complex within the subgenus *Daphnia* (Colbourne and Hebert, 1996). The single *D.lumholtzi* and single *D.magna* clones used are different species in the subgenus *Ctenodaphnia*. We define clones as asexual offspring from a single female. The clones were selected from several different locations that represent different predation regimes.

The *D.pulex* clone plx1 was clone K from Dead Dog Pond (Columbia Co., WI), which is a permanent fishless pond (Black, 1991). *Daphnia pulex* clone plx2 was clone 9A-23 from a fishless temporary pond in Ontario, Canada (collected by D.Innes). *Daphnia pulex* clone plx3 was clone DISP4 from another fishless temporary pond in Ontario, Canada (collected by D.Innes). *Daphnia pulicaria* clone plc1 was clone PULON95 from Oneida Lake (Madison Co., NY), a relatively shallow eutrophic lake which supports perch and other planktivores and only occasionally stratifies (collected by C.Cáceres). *Daphnia pulicaria* clone plc2 was clone G from Lake Mendota (Dane Co., WI), a relatively deep and stratified eutrophic lake that supports cisco, yellow perch and other planktivores (collected by M.Brewer). This clone was darkly pigmented with melanin. Both *D.magna* clone mag and *D.lumholtzi* clone lum were from the culture facility at the Max Planck Institute for Limnology (Plön, Germany). Clone mag was originally collected from Grosser Binnensee, a shallow, unstratified, eutrophic lake in
Germany that supports a planktivore population (Lampert, 1994). Clone lum was originally collected from Fairfield Reservoir in Freestone Co., TX, which supports a planktivore population (Sorensen and Sterner, 1992). All of these clones had rounded helmets and similar overall morphology.

**General experimental procedures**

We maintained cultures of *Daphnia* in 4 l jars using artificial hard water (AHW; Mount and Norberg, 1984). Cultures were fed daily with a 50:50 mixture of *Chlamydomonas reinhardtii* (UTEX 90) and *Selenastrum capricornutum* (UTEX 1648) at an approximate concentration of 2.0 mg C l⁻¹. The algae were cultured in COMBO medium (S.S.Kilham and C.E.Goulden, personal communication).

**Quantification of Daphnia swimming behavior**

We used a video camera and motion analysis system to quantify *Daphnia* swimming speed. The evening prior to filming (between 17:00 and 19:00 h), *Daphnia* of a single clone and of a restricted size range were selected using Nitex sieves. A culture was poured through a 1.3 mm mesh sieve that was placed on an 800 µm sieve. We found that this method allowed us to collect efficiently adults in a relatively narrow size range. A total of 135 individuals that passed through the 1.3 mm sieve, but not the 800 µm sieve, were placed in the filming chamber overnight. This filming chamber was 20 cm on each side and 40 cm high. It contained 9 l of AHW with an initial concentration of 5.0 × 10⁴ cells ml⁻¹ *Chlamydomonas*. It was in a room with a light cycle that began at 9:00 h and ended at 22:00 h. Temperature was maintained at 22°C throughout the experiments.

*Daphnia* filming began between 10:00 and 11:00 h, and lasted for 2 h.

We used a two-camera filming system with a video frame splitter which allowed us to collect a record of the three-dimensional positions of individual *Daphnia* every 1/30 of a second. This system was similar to that used by Ramcharan and Sprules (1989), but we have made several modifications. We used Vicon, IR-sensitive, CCTV cameras fitted with National CCTV manual zoom lenses, with macro, and focal length set at 20 mm. The cameras focused on the center 4 × 4 × 8 cm section of the filming chamber. Usually, 1–4 individuals could be seen swimming through the field of view at any one time. Depending on their swimming speed, individuals typically remained in view for between 1 and 30 s. Both visible light from an incandescent bulb and IR light were used to illuminate the filming chamber. An array of 16 IR diodes (880 nm) covered with opaque plastic backlit the animals. Since *Daphnia* are not sensitive to IR light (Smith and Macagno, 1990), we predicted that it would not affect their swimming behavior. We found no phototropic response to IR lights when they are turned on or off (unpublished data). An incandescent bulb (2.8 µE m⁻² s⁻¹ as measured in the filming chamber) provided overhead lighting. Although this light level was relatively low, fish can see and easily feed on *Daphnia* under these light conditions.

We haphazardly selected 30 *Daphnia* swimming tracks from the 2 h video record and digitized each for a maximum of 5 s. If an individual selected for digitizing
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swam out of the field of view in <5 s, at least 1 s of swimming was recorded. The tracks were digitized using a Motion Analysis Expert Vision System which provided us with the three-dimensional coordinate data for each 1/30 s interval.

Our digitizing protocol consisted of haphazardly choosing one individual in the field of view and recording its behavior for 5 s. We repeated this process 30 times with each 2 h tape, so it was possible that we taped some individuals twice. Filming individual Daphnia in a smaller container where they can be easily tracked results in container effects (S.I.Dodson, unpublished data), and placing a single individual in a large container is not time efficient because it takes a single individual several minutes to come into view of the cameras. Therefore, it is possible that the true variance for swimming speed is higher than we measured.

We calculated swimming speed as the sum of the total distance traveled (measured at 1/30 s intervals) divided by time. Swimming speed is scale dependent; values depend on the spatial and temporal scale selected for measurement. In this study, we calculated swimming speed from coordinate data collected every 1/30 s, but if we had analyzed the same tracks using coordinate data collected every 1 s we would have obtained slower estimates of swimming speed because swimming paths would be smoothed, resulting in a reduction in measured displacement per unit time (Brewer, 1996).

We tested for normality and homogeneity of variance in the swimming speed. Normality was assessed by plotting a cumulative frequency distribution curve for each clone and with a Kolmogorov-Smirnov test (Systat, 1992). The data showed significant deviation from normality by the Kolmogorov-Smirnov test, but the cumulative frequency distribution curves were generally linear. Data were log transformed because distributions were skewed right with the mean greater than the median. We used a Model I ANOVA and a Tukey HSD test to make pairwise comparisons of differences among clones (Systat, 1992).

Fish choice experiments

To determine whether differences in swimming behavior affect predation risk, we conducted a series of experiments in which fish were offered a choice between two clones that differed in swimming speed, body pigmentation, or both. Two 40- to 50-mm-long bluegills (L.macrocirus) were collected from Lake Wingra (Dane Co., WI). These fish are efficient planktivores and common predators of Daphnia. The fish were allowed to swim freely in a 35 × 45 cm arena with water 5 cm deep. Paired fish tended to show more normal feeding behavior than solitary fish that were used in preliminary trials.

Fish were lured to one randomly selected corner of the arena with an empty pipette, and then two adult Daphnia of the same size, one of each clone, were released in the far corner from separate pipettes held 1 cm apart and in a random position. One of the fish typically detected the Daphnia within 5–20 s, focused on one of the two individuals, and then consumed it. In the first set of experiments, we offered the fish a choice between plx2 (fast swimmer) and plc1 (slow swimmer) or plc2 (fast swimmer) and plc3 (slow swimmer). It was easy to keep track of the two Daphnia with the unaided eye and determine which one the fish...
selected first. Trials in which a fish detected the *Daphnia* in <5 s were discarded as it was not always possible to release the two *Daphnia* at exactly the same moment, which resulted in a fish choosing the one that was released first. Trials in which the fish detected the *Daphnia* after >20 s were also discarded because the two *Daphnia* tended to swim away from each other to different parts of the arena, where fish could not see both at once.

To control for possible differences in morphological features of the clones, we conducted another set of trials with *Daphnia* anesthetized with carbonated water. These trials were conducted as described above with two equal-sized individuals representing the different clones. If a daphnid became caught in the surface tension or sank to the bottom before the fish noticed it, the trial was not scored.

To investigate effects of pigmentation on selection of clones with different swimming speeds, we conducted a final set of feeding trials in which the fish were given a choice between a slow-swimming clone (plc2) that was more darkly pigmented than a lightly pigmented slow swimmer (plcl) and a lightly pigmented fast swimmer (plx2). We conducted these feeding trials first with live *Daphnia* and then with anesthetized *Daphnia* as described above.

To evaluate fish choice, we used a normal approximation to the binomial test with a correction for continuity (Zar, 1996) to calculate the two-tailed probability that the fish behaved randomly in their selection of *Daphnia* clones.

**Results**

*Daphnia* swimming behavior

*Daphnia* exhibited two basic behavior patterns: 'hop-and-sink' and 'zooming' (Figure 1). Individuals that swam by hop-and-sink initiated a power stroke with their second antennae that created rapid vertical acceleration. Because they operated at low Reynold's number (~15), the *Daphnia* quickly came to a stop and sank passively for ~1/5 s. Individuals that zoomed did so by rapid flicks of their second antennae with no passive sinking. Zooming individuals tended to swim at speeds >15 mm s\(^{-1}\), while hop-and-sink individuals tended to swim at speeds <10 mm s\(^{-1}\).

Individuals from clone plx2 were nearly always observed zooming, and only 10% of the digitized paths displayed the hop-and-sink behavior commonly observed in the other clones. Except for one individual from the lum clone and two individuals from the plx1 clone, *Daphnia* from the other clones swam with hop-and-sink behavior. The average swimming speed (Figure 2) differed significantly among the seven clones (Model I ANOVA, \(F_{6,20} = 69.87, P < 0.001\)). Swimming speed for clone plx2, which nearly always swam with zooming behavior, was significantly different from that for all other clones (Tukey, \(P < 0.001\)). The clone plc1, which displayed the slowest and most consistent hop-and-sink behavior, was significantly different from all other clones (Tukey, \(P < 0.001\) for lum, mag, plxl and plx2, and \(P < 0.05\) for plc3) except for the slightly faster clone plc2 (Tukey, \(P > 0.1\)).
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Fig. 1. Two swimming tracks of individual Daphnia that illustrate regular hop-and-sink behavior (plcl) and zooming behavior (plx2). Each point represents the relative vertical position every 1/30 of a second. The plx2 individual traveled at 22 mm s⁻¹ and showed consistent acceleration with no time spent passively sinking. The plcl individual traveled at 7 mm s⁻¹ and accelerated upward with a power stroke and then sank passively.

Fish choice

In the trials with free-swimming individuals, fish given a choice between plx2 and plcl chose the faster swimming plx2 at a ratio of 4:1 (Table I). When given a choice between anesthetized individuals of these same two clones, the fish did not choose one over the other (Table I). Similarly, the fish chose the faster plx2 over the slower plx3 when both clones were free swimming, but not when they were anesthetized (Table I).

When the fish were given a choice between a slow-swimming but more darkly pigmented clone (plc2) and either of the two more lightly pigmented clones (plc1, a slow swimmer and plx2, a fast swimmer), the fish consistently chose the darkly pigmented clone (Table I). This same trend was observed in the trials with anesthetized Daphnia, indicating that physical differences were more important than behavioral differences, and were responsible for the fish selection of the dark clone.
Fig. 2. Swimming speed and turning angle for the seven clones used in this study as measured at the 1/30 s temporal scale (N = 30 for each clone). The error bars represent the SEM. The lower case letters designate significantly different groups (Tukey, $P < 0.05$).

Table I. Fish choice experiments. Results of the experiment to determine the effects of swimming speed and pigmentation on fish choice. In each trial, fish were given a choice between two different clones. The percentage values represent the proportion of the trials in which a given clone was selected first by the fish.

<table>
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<tr>
<th>Clone characteristics</th>
<th>Fish choice</th>
<th>Free swimming</th>
<th>Anesthetized</th>
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<td>Swimming speed</td>
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<td>plx2 (fast)</td>
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<td>80</td>
<td>40</td>
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<td>plc1 (slow)</td>
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<tr>
<td>plx2 (fast)</td>
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<td>71</td>
<td>34</td>
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<td>plx3 (slow)</td>
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<td>29</td>
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<td>Pigmentation</td>
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<td>plc2 (dark–slow)</td>
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<td>67</td>
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<td>plc2 (light–fast)</td>
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* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Discussion

The principal result of this study is that differences in small-scale individual behavior observed among naturally occurring zooplankton species (and clones) play an important role in determining overall predation risk from visually guided predators. This small-scale behavior is qualitatively different from other behavior strategies that zooplankton use to limit predation risk, such as diel vertical migration. While the migration of populations and escape behavior of individuals have both been examined before, very few experimental studies have considered the small-scale activity of zooplankton. Our results highlight the importance of considering what live animals do.
We conclude that it is not adequate to describe the swimming behavior of a species by only measuring a single clone. This finding agrees with previous work in which others have found that analysis of a single clone does not adequately describe the characteristics of a species (Parejko and Dodson, 1991; DeMeester, 1995). We have demonstrated that *Daphnia* clones within the same species complex can have very different swimming behaviors. It is important to note that our results are based on selecting clones from different environments.

The swimming behavior of the *pulex* clone plx2 stands out as unique in this study. Other *Daphnia* clones have been measured at fast swimming speeds and with similar behavior to our plx2. Both Larsson and Kleiven (1996) and Dodson et al. (1997) have measured *D.magna* clones that swim between 15 and 20 mm s$^{-1}$. Most clones, however, swim with the more typical hop-and-sink behavior at ~6–8 mm s$^{-1}$ (Dodson and Ramcharan, 1991; Dodson et al., 1994; this study). We do not know what the advantage of fast swimming is. Even if a fast swimmer comes from a fishless environment, we predict that it would still have a greater encounter rate with invertebrate predators (Gerritsen and Strickler, 1977). We propose three hypotheses for the advantage of fast swimming, related to feeding, escape from invertebrate predators, and mating.

Faster swimming may be related to feeding behavior. Larsson (1996) presents evidence from a single clone of *D.magna*, which swims as fast as 30 mm s$^{-1}$, that fast swimming with few hops is a food-searching behavior. Our *Daphnia* were all filmed in water with the same food concentration, but different clones may have different threshold food concentrations at which they make the switch to fast swimming. Another possibility is that faster swimmers can generate more power which allows them to escape from invertebrate predators. Finally, it is possible that swimming speed is related to mating behavior. While faster swimming speed may increase encounter rate with invertebrate predators, it could also increase encounter rate with potential mates. This could be important for a clone such as plx2 which comes from a small temporary pond. Males were often present in our cultures of plx2.

Naturally occurring differences in swimming behavior can be ecologically significant, in that they affect prey selection by fish, which either prefer or more readily detect the faster moving individuals. Our results support the conclusion reached by Main (1985) that all aspects of prey visibility should be included in models of prey selection. Although current models do acknowledge the importance of motion itself, particularly as it affects encounter probability (Gerritsen and Strickler, 1977), they do not incorporate characteristics of small-scale movement that affect the selection of prey by fish. Scott (1987) points out that regular omission of an important factor such as prey motion in models of prey selection (e.g. Werner and Hall, 1974; Egger, 1977) may result in false conclusions. He presents evidence that *Daphnia* and *Cyclops* activity differences are the dominant factor in determining selection by fish, and not capture efficiency as traditionally assumed.

While we have concentrated primarily on prey motion in the current study, this is clearly not the only factor that influences predation risk among *Daphnia* clones. We found that the more darkly pigmented clone plc2 was selected over more
transparent clones that swam at the same speed (plcl) and at a faster speed (plx2). This suggests that visibility contrast may be more important than just the characteristics of movement when considering selectivity by zooplankton. Among equally pigmented clones, however, movement is clearly important. It is likely that size, morphology, visibility contrast and motion all vary in complex ways among clones of a single species, as well as among different zooplankton species, making it difficult to isolate one single factor.

Comparing clones within the genus Daphnia has obvious advantages because clones can be matched with respect to size and visibility contrast. We expect that a more robust understanding of the role that prey motion plays in determining predation risk could be gained by considering the behavior of other common zooplankton species. A virtual zooplankton system, similar to the one designed by Brewer and Coughlin (1996), provides us with a potentially powerful tool which can help us efficiently design models that address questions of how important movement is in determining vulnerability to predation. Studies such as the present one on live zooplankton prey are necessary, however, to determine the range of different behavior variables actually seen in real zooplankton and to test models from the virtual plankton system.

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