

Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities

Cynthia S. Kolar*, Frank J. Rahel

Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, USA

Received: 23 October 1992 / Accepted: 7 April 1993

Abstract. We examined the response of benthic invertebrates to hypoxia and predation risk in bioassay and behavioral experiments. In the bioassay, four invertebrate species differed widely in their tolerance of hypoxia. The mayfly, *Callibaetis montanus*, and the beetle larva, *Hydaticus modestus*, exhibited a low tolerance of hypoxia, the amphipod, *Gammarus lacustris*, was intermediate in its response and the caddisfly, *Hesperophylax occidentalis*, showed high tolerance of hypoxia. In the behavioral experiments, we observed the response of these benthic invertebrates, which differ in locomotor abilities, to vertical oxygen and temperature gradients similar to those in an ice-covered pond. With adequate oxygen, invertebrates typically remained on the bottom substrate. As benthic oxygen declined in the absence of fish, all taxa moved above the benthic refuge to areas with higher oxygen concentrations. In the presence of fish mayflies increased activity whereas all other taxa decreased activity in response to hypoxia. Mayflies and amphipods remained in the benthic refuge longer and endured lower oxygen concentrations whereas the vertical distribution of caddisflies and beetle larvae was not influenced by the presence of fish. As benthic oxygen declined in the presence of fish, all but the beetle larva reduced activity over all oxygen concentrations compared to when fish were absent. As benthic oxygen continued to decline, mayflies and amphipods moved above the benthic refuge and were preyed upon by fish. Thus, highly mobile taxa unable to tolerate hypoxia (mayflies and amphipods) responded behaviorally to declining oxygen concentrations by migrating upward in the water column. Taxa that were less mobile (beetle larvae) or hypoxia-tolerant (caddisflies) showed less of a response. Taxa most vulnerable to fish predation (mayflies and amphipods) showed a stronger behavioral response to predator presence than those less vulnerable (caddisflies

and beetle larvae). Because invertebrates differ in their ability to withstand hypoxia, episodes of winter hypoxia could have long-lasting effects on benthic invertebrate communities either by direct mortality or selective predation on less tolerant taxa.

Key words: Hypoxia – Benthos – Invertebrates – Predator-intimidation – Behavior

Episodic disturbance and predation often interact to influence community structure. Typically, this involves loss or reductions in predators due to severe abiotic conditions and a subsequent restructuring of the community as species previously held in check by predators come to dominate the system. In alpine ponds, winter freezing of the water column eliminates amphibians and results in a zooplankton community dominated by large species: where amphibians persist, small zooplankton dominate (Dodson 1970). A brief period of unsuitable temperature-oxygen conditions during summer stratification can cause fish die-offs that in turn cause long-lasting changes in the phytoplankton community and ultimately water quality of eutrophic lakes (Vanni et al. 1990). Elimination of piscivorous fish by brief periods of winter anoxia can result in complete transformation of fish and zooplankton communities as changes in predator-prey interactions cascade through lower trophic levels (Tonn and Paszkowski 1986; Carpenter 1988; Hall and Ehlinger 1989).

Episodes of unfavorable, abiotic conditions can affect community interactions even though organisms may not be directly killed. Behavioral avoidance of unfavorable conditions may result in organisms migrating to new environments where they face increased predation or competition (Sousa 1984; Wolf and Kramer 1987). For example, young perch *Perca flavescens* leave cattail beds and move into areas with higher predation risk when dissolved oxygen concentrations in prairie ponds drop during summer nights (Suthers and Gee 1986). Estuarine

* Present address: National Fisheries Research Center-Great Lakes, United States Fish and Wildlife Service, 1451 Green Road, Ann Arbor, MI 48105, USA

Correspondence to: F.J. Rahel

fish migrate inshore during periods of summer hypoxia even though this may result in exposure to lethal temperatures or reduction in reproductive success (Coutant 1985; Breitburg 1992).

Benthic invertebrates may be particularly vulnerable to interactions between severe abiotic stress and predation. Seasonal or episodic occurrence of low dissolved oxygen concentrations (hypoxia) in the benthic region is common in both marine and freshwater habitats (Greenbank 1945; Rossignol-Strick 1985; Swanson and Parker 1988; Rahel 1990). Shallow lakes having extended periods of ice-cover are prone to winterkill, i.e. the development of low oxygen and associated conditions (e.g. elevated hydrogen sulfide levels) stressful to aquatic organisms. In extreme cases, the entire water column becomes anoxic and massive mortality of aquatic organisms results. Often, however, low oxygen is limited to the benthic region and mobile aquatic organisms can avoid mortality by migrating up in the water column (Nagell 1977; Magnuson et al. 1985).

Avoidance of low benthic oxygen by vertical migration to the ice-water interface has been observed among aquatic invertebrates (Sikorowa 1968; Nagell 1980; Brittain and Nagell 1981). This strategy works when fish are absent, but can result in substantial mortality from predation when fish are present (Rahel and Kolar 1990). Because benthic invertebrates differ in their ability to withstand low oxygen concentrations (Jacob et al. 1984; Davies et al. 1987) and in their vulnerability to fish predators (Crowder and Cooper 1982; Morin 1984), winter hypoxia could alter benthic community composition through both direct mortality and enhanced vulnerability to predation. Thus, interaction of an abiotic factor (hypoxia) and a biotic factor (predation) could have important consequences for benthic invertebrate assemblages in waters that develop dissolved oxygen gradients during winter ice-cover.

We examined how various benthic invertebrates balance the conflicting demands of avoiding low benthic oxygen and fish predation. We exposed benthic invertebrates having different locomotor abilities to hypoxia under simulated winter conditions in the presence and absence of fish. The mayfly, *Callibaetis montanus*, and the amphipod, *Gammarus lacustris*, represented invertebrates with good swimming ability; and the caddisfly, *Hesperophylax occidentalis*, and beetle larva, *Hydaticus modestus* (Nilsson 1981, suggested this species be called *H. aruspex*), represented non-swimmers. Our objectives were: 1) to rank benthic invertebrate taxa in their relative ability to survive hypoxia; 2) to document behavioral responses of the invertebrates to hypoxia and determine how predators modify these responses; 3) to rank the invertebrates in their relative vulnerability to predation by fish. With this information, we should be able to predict the responses of the benthic invertebrates to the combined stresses of low oxygen and predation risk in ponds that experience periodic winter hypoxia.

Materials and methods

Bioassay: Tolerance of anoxia

Bioassays were run to rank invertebrates by their ability to withstand hypoxia. Both the bioassay and behavioral experiments used a flow-through laboratory apparatus to remove the oxygen and chill experimental water to 4° C. The system created vertical temperature and dissolved oxygen gradients similar to those found during winter in shallow ponds with oxygenated, 0° C water under the ice and hypoxic, 4° C water near the bottom substrate (see Fig. 2 in Rahel and Kolar 1990). The system relied on the temperature-dependent density of water to maintain temperature and dissolved oxygen stratification within the experimental chamber and is described elsewhere (Rahel 1989). Oxygen was removed by bubbling nitrogen through the incoming water.

We modified the system described above for the bioassay. After water left the oxygen-stripping columns and the chilling tank, it flowed into a small Plexiglas tank (35 × 22 × 15 cm) placed in a 4° C water bath. A sodium sulfite solution (20–40 mg/L) dripped into this tank to further reduce the dissolved oxygen to about 0.15 ± 0.05 mg/L O₂. Similar concentrations of sodium sulfite did not alter fish behavior (Kramer and McClure 1982). Wolf and Kramer (1987) used sodium sulfite to produce hypoxia in a variety of laboratory and field studies with no apparent ill effects. The system was set up and allowed to run for 12 h until the dissolved oxygen concentration equilibrated. Dissolved oxygen concentration was measured with a Yellow Springs Instruments Model 57 dissolved oxygen meter (Yellow Springs Instruments, Anitoch, OH, USA). We collected invertebrates from fishless ponds at the Red Buttes Laboratory, about 20 km south of Laramie, WY. All organisms were kept in holding tanks at 4° C for 2 weeks prior to use in a 12-h light 12-h dark light regime. Each invertebrate was used only once. In the bioassay, invertebrates were subjected to a single low dissolved oxygen concentration for 48 h.

Ten individuals of each invertebrate species were placed in separate plastic mesh boxes, submerged in anoxic water and observations on survival were taken every 6 h over a 48 h period. The following invertebrate species were used: the amphipod *Gammarus lacustris*, the mayfly nymph *Callibaetis montanus*, the caddisfly larva *Hesperophylax occidentalis*, and the beetle larva *Hydaticus modestus*. Because these invertebrates become lethargic in anoxia, it was necessary to standardize survival determination. To do this, mesh boxes were removed from the experimental chambers and placed in normoxic water at every check period. An animal was considered dead when it did not respond to prodding after 30 s. The mesh boxes were then replaced in the tank. Two replicate bioassays were run. We submerged ten individuals of each species in plastic mesh boxes in 4° C normoxic water for the duration of the bioassay as controls. Survival of control individuals was monitored along with invertebrates submerged in water with low oxygen.

A repeated-measures ANOVA was used to test for differences in how the invertebrates responded to hypoxia (Neter et al. 1990). The repeated measure was time and the subject was species. Pairwise differences were examined with Scheffe's *F*-test.

Behavioral experiments: Response hypoxia and fish

To determine the effect of predators on the response of invertebrates to hypoxia we exposed the same four invertebrates used in the bioassay to reduced benthic oxygen concentrations with and without fish present. We began each trial by placing the four taxa together in the experimental chamber (55 cm × 26 cm × 55 cm; 78 l). The beetle and caddisfly larvae were predaceous but did not appear to influence the behavior of other invertebrates and were fed chironomid larvae prior to testing. We used eight individuals of each invertebrate taxon in our experimental aquaria resulting in densities of 56 individuals of each taxon/m². Though benthic macroinvertebrate densities vary with macrophyte density (Pennak

1978; Crowder and Cooper 1982), the densities used in our experiments were within the range of natural densities reported for taxa in lentic environments: mayfly nymphs 81–50/m² (Ökland 1964; Allison and Harvey 1988); caddisflies 57–200/m² (Thorp and Bergey 1981; Allison and Harvey 1988); beetles 18–29/m², (Thorp and Bergey 1981; Allison and Harvey 1988); and up to 10,000 *Gammarus* sp./m² (Pennak 1978). Beetle larvae were stocked at higher densities than reported in the literature to allow a sufficient sample size for data collection.

Brook trout (*Salvelinus fontinalis*; 80–90 mm total length) used as predators in the behavioral experiments were collected from Crow Creek in the Pole Mountains (15 km east of Laramie, WY). Twenty fish were kept in a common tank and fed live invertebrates for 2 weeks prior to experimentation (the same four taxa used in experiments were offered daily but only mayflies and amphipods were eaten). Fish were starved for 24 h prior to each experiment and were used in only one trial per day. One fish was randomly selected for each trial. To begin each trial, the experimental chamber was set up with a benthic refuge from fish predators and vertical temperature gradient of 0° C water at the top and 4° C at the bottom of the tank (Fig. 1). Ambient light conditions were 9.05 micromoles/s/m² (Li-Corp. spherical quantum sensor model number LI-193SA), which are typical of a dimly lit pond under ice-cover (Greenbank 1945). After this gradient was created, eight invertebrates of each species were gently pipetted into the tank below the layer of crushed ice.

We allowed invertebrates to acclimate for 1 h before beginning observations on activity and vertical distribution. Activity was measured as the proportion of individuals of a species moving at a point in time. As mayfly nymphs had two distinct ways of moving, their activity was divided into swimming and crawling motions. The proportion of each species beneath or attached to the underside of the screen was used as a measure of vertical distribution. In calculating the percent of invertebrates above the refuge and the percent active in trials with a predator the denominator used was the number remaining uneaten. Behavioral observations on invertebrate distribution and activity were made every 20 s through the experiment. A new rotation through observations of all species was begun every 3 min.

After the acclimation period, we gradually reduced oxygen within the refuge by bubbling nitrogen through the inflowing water. In

trials with a predator, a fish was added. Brook trout typically rested on the screen for about 20–30 min after addition; throughout the rest of the experiment, fish displayed aggressive predacious behavior (e.g. pecking at refuge screen), chased and captured prey. Dissolved oxygen concentrations within the benthic refuge declined from about 4.5 mg/L to 0.1 mg/L O₂ over 2 h. The benthic dissolved oxygen was noted each time a prey item was consumed. As the dissolved oxygen approached 1 mg/L O₂, sodium sulfite (20–40 mg/L) was added to further reduce the oxygen concentration (Wolf and Kramer 1987). Fifteen trials with fish and 15 without were performed in this manner.

To confirm that changes in invertebrate behavior were due to changes in oxygen concentration and not merely acclimation to the experimental chamber, we conducted preliminary trials where oxygen concentration remained unchanged over 2 h. No changes in invertebrate behavior or distribution were observed in the absence of declining benthic oxygen (Kolar 1992).

A repeated-measures ANOVA was used to test whether invertebrate activity or vertical distribution was effected by the presence of a fish predator and to test the effect of declining benthic oxygen on invertebrate behavior. A separate repeated-measures ANOVA was performed on each species. Oxygen concentration was the repeated measure and the subject was predator presence or absence. Oxygen concentrations were grouped into eight categories (≥ 4.5 , 4.5–3.01, 3.0–2.01, 2.0–1.51, 1.50–1.01, 1.0–0.51, 0.50–0.26, ≤ 0.25 mg/L O₂). As there was a missing value in the highest category of dissolved oxygen (≥ 4.5 mg/L) in three of the 30 trials, data from these trials were excluded from analyses. A non-parametric Wilcoxon signed rank test was used to examine the behavioral shift of mayfly locomotor activity when fish were present (Snedecor and Cochran 1980). A repeated-measures ANOVA was also used to examine between-species differences in the percent of invertebrates above the refuge in the presence and again in the absence of fish. The repeated measure was dissolved oxygen and the subject was species. Pairwise differences were examined with Scheffe's *F*-test. Unless stated otherwise, *F* values reported are results of repeated-measures ANOVAs.

Results

Bioassay: Tolerance of anoxia

Four invertebrate species were exposed to 0.1 mg/L O₂ for 48 h in the bioassay. Invertebrates became lethargic within 6 h of beginning the bioassay and migrated to the

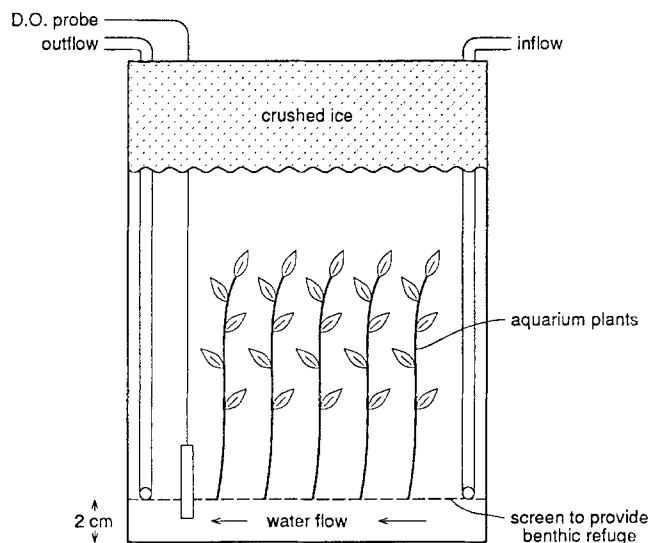


Fig. 1. Experimental tank used in the behavioral experiments. A 1.4 cm mesh screen was placed 2 cm above the tank bottom to serve as a benthic refuge from predators. Plastic plants provided cover for the invertebrates. A temperature gradient was produced by having a layer of crushed ice on top and 4° C water flowing across the tank bottom

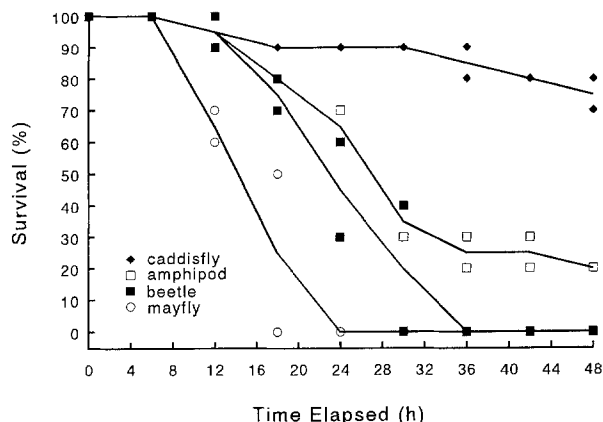


Fig. 2. Survival of invertebrates submerged in water with 0.1 mg/L O₂ for 48 h during the bioassay. Results of two trials plotted (each with 10 individuals per taxon); curves represent mean survival over both trials. Specific names given in text

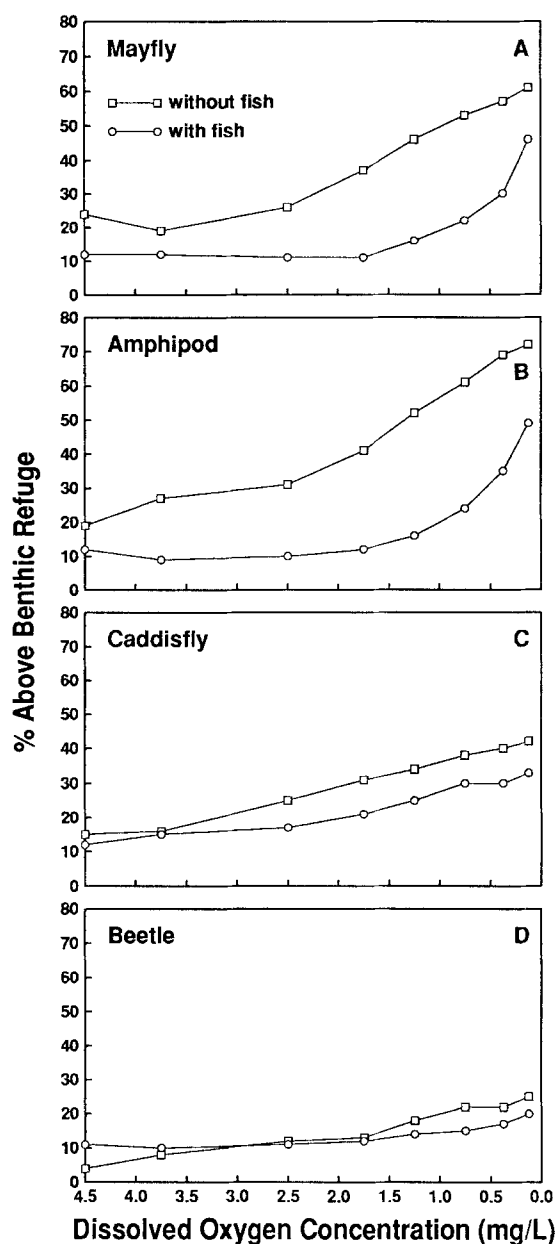


Fig. 3A–D. Percent of remaining invertebrates above the benthic refuge as a function of oxygen concentration and the presence/absence of fish. In trials with a predator, the denominator used to calculate percent above refuge was corrected for number of uneaten invertebrates. $N=15$ trials with and 15 without predators, 8 individuals per taxon. Specific names given in text

underside of the plastic mesh boxes. Survival was similar for each species between the two trials (Fig. 2). No mortality was seen in control trials. Taxa exhibited a wide range of tolerances to hypoxia and the repeated-measures ANOVA detected significant differences among species (between subject effects, $F=43.27$, 3,4 df, $P<0.01$). Based on mean survival at the end of the bioassay, the mayfly nymph and the beetle larvae were most sensitive to low oxygen, the amphipod was intermediate in tolerance, and the caddisfly was quite tolerant of hypoxia (Scheffe F -test; $\alpha=0.05$).

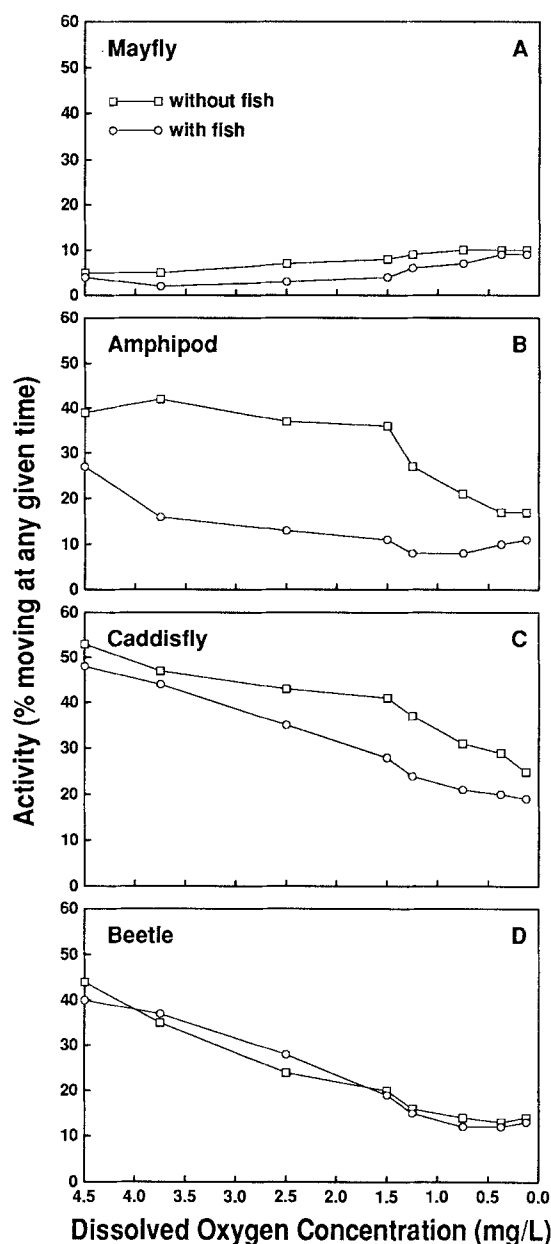


Fig. 4A–D. Invertebrate activity as a function of oxygen concentration and the presence/absence of fish is shown. In trials with a predator, the denominator used to calculate percent moving was corrected for number of uneaten invertebrates. $N=15$ trials with and 15 without predators, 8 individuals per taxon and trial. Specific names given in text

Behavioral experiments: Response to hypoxia and fish

In the absence of fish, all four taxa responded to declining benthic oxygen by moving off the bottom to areas with higher dissolved oxygen (within subject effects; Fig. 3A, $F=103.67$, 1,7 df, $P<0.01$; Fig. 3B, $F=168.04$, 1,7 df, $P<0.01$; Fig. 3C, $F=95.47$, 1,7 df, $P<0.01$; Fig. 3D, $F=50.88$, 1,7 df, $P<0.01$). In all four cases, significant interaction was present between dissolved oxygen concentration and the presence or absence of fish ($P<0.01$). Examination of Fig. 3 shows that this interaction is explained by a change in magnitude of response:

as dissolved oxygen concentrations declined, the effect of fish on the vertical distribution of invertebrates became greater.

Mayflies and amphipods endured lower oxygen concentrations in the presence of fish compared to when they were absent (between subject effects; Fig. 3A, $F=96.59$, 1,25 df, $P<0.01$; Fig. 3B, $F=88.65$, 1,25 df, $P<0.01$). Both taxa moved above the refuge in response to declining oxygen levels, but at any given oxygen concentration more of each taxa were found within the refuge when fish were present compared to when fish were absent. The vertical distribution of caddisflies and beetle larvae was unaffected by fish presence (between subject effects; Fig. 3C, $F=0.00$, 1,25 df, $P=1.00$; Fig. 3D, $F=1.55$, 1,25 df, $P=0.23$). Increased use of the benthic refuge in the presence of fish was due to avoidance of the upper portion of the tank and was not merely a result of the removal of invertebrates that strayed above the screen. In many cases, invertebrates moved above the benthic refuge but quickly returned to it after being chased by fish. By contrast, in the absence of fish, invertebrates that moved above the refuge often remained there. Further, in six replicates with a predator where few invertebrates were eaten (one amphipod and one or two mayflies), uneaten invertebrates still remained in the benthic refuge longer as oxygen declined just as in replicates where more invertebrates were consumed. Therefore, the increased percentage of invertebrates in the benthic refuge in the presence of fish was not solely because prey above the refuge was removed by predators but reflected avoidance of the riskier areas of the tank.

Invertebrates also altered their activity in response to declining benthic oxygen. Amphipods, caddisflies and beetles decreased overall activity as benthic oxygen declined (within subject effects; Fig. 4B, $F=39.15$, 1,7 df, $P<0.01$; Fig. 4C, $F=36.42$, 1,7 df, $P<0.01$; Fig. 4D, $F=58.63$, 1,7 df, $P<0.01$). Mayflies, however, increased activity as benthic oxygen declined (within subject effects; Fig. 4A, $F=13.44$, 1,7 df, $P<0.01$). Significant interaction was present between dissolved oxygen and predator presence or absence in the case of the amphipods and caddisflies ($P<0.05$). Again, Fig. 4 shows that this interaction is explained by a change in magnitude of invertebrate response. While fish were present, mayflies, amphipods and caddisflies were less active over

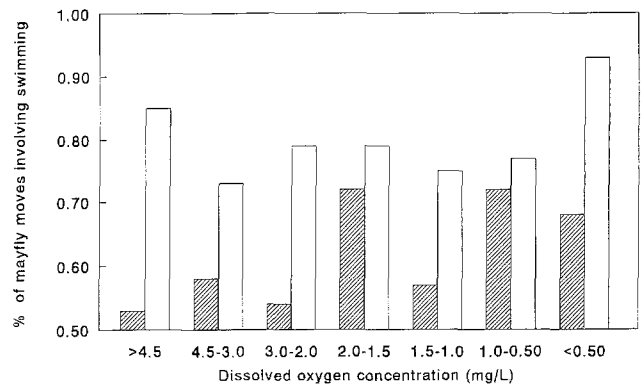


Fig. 5. Mayfly crawling movement as a function of oxygen concentration in the presence and absence of fish. Mayfly movement consisted of swimming or crawling. Symbols: ▨ with fish; □ without fish

all oxygen concentrations compared to when fish were absent (between subject effects; Fig. 4A, $F=12.62$, 1,25 df, $P<0.01$; Fig. 4B, $F=88.54$, 1,25 df, $P<0.01$; Fig. 4C, $F=13.26$, 1,25 df, $P<0.01$). Mayfly nymphs also altered their type of locomotion in response to predators. Without fish, mayflies often swam in the water column. With fish present, they decreased overall activity (Fig. 4A) but significantly increased proportion of crawling locomotion (Fig. 5; Wilcoxon signed rank tests, $P<0.05$). Unlike the other taxa, beetle larvae activity was not influenced by the presence of predators (between subject effects; Fig. 4D; $F=0.33$, 1,25 df, $P=0.57$).

Differences among invertebrate species with respect to the percent above the benthic refuge were significant both in the absence and presence of fish (between subject effects; $F=86.10$, 3,44 df, $P<0.01$; $F=18.88$, 3,56 df, $P<0.01$). Table 1 shows the mean percentage of invertebrates above the refuge as benthic oxygen declined in the presence and absence of fish. In the absence of fish, the rankings of invertebrates by mean percentage above the refuge remain the same over all oxygen categories: amphipods > mayflies > caddisflies > beetles. In the presence of fish, however, the invertebrate rankings differ by oxygen category. In each of the six oxygen categories greater than 0.50 mg/L O_2 , the mean percentage of caddisflies above the refuge was higher than other invertebrates (though not always significantly different; see

Table 1. Mean percentage of invertebrates above the benthic refuge as benthic oxygen declined in the behavioral experiments. Bars represent means that are not significantly different using Scheffe's

		Dissolved oxygen concentration (mg/L)							
		≥4.5	4.5-3.01	3.0-2.01	2.0-1.51	1.5-1.01	1.0-0.51	0.5-0.26	≤0.25
Predator Absent	A	19	A 21	A 31	A 41	A 52	A 60	A 69	A 72
	M	18	M 19	M 26	M 36	M 46	M 53	M 57	M 61
	C	15	C 16	C 25	C 31	C 33	C 38	C 40	C 43
	B	4	B 8	B 11	B 13	B 18	B 21	B 22	B 25
Predator Present	C	12	C 15	C 17	C 22	C 25	C 30	A 35	A 49
	A	12	M 12	B 11	B 12	M 16	A 24	M 30	M 46
	M	12	B 10	M 11	A 12	A 16	M 22	C 30	C 33
	B	5	A 9	A 10	M 10	B 14	B 15	B 17	B 20

F -test ($\alpha=0.05$). A = amphipod, B = beetle, C = caddisfly, M = mayfly. Specific names given in text

Table 2. Mortality due to fish predation as benthic oxygen declined. The percent eaten is based on 120 individuals of each species (eight individuals in each of 15 replicates). Of the total individuals eaten,

the number eaten at various oxygen concentrations are shown. Most of the predation occurred as oxygen concentrations declined below 1 mg/L. Specific names given in text

Taxon	Number eaten	% of Total Eaten	Number eaten at various Oxygen concentrations (mg/L)				Vulnerability to fish predators ^a
			>3.0	3–2	2–1	<1.0	
Mayfly	45	38	5	8	8	24	high
Amphipod	30	25	2	3	10	15	high
Caddisfly	0	0	0	0	0	0	low
Beetle	0	0	0	0	0	0	low

^a Vulnerability to young brook trout (80–90 mm TL)

Table 1). The rankings of the three remaining taxa change with oxygen category but the means are never significantly different. Below 0.50 mg/L O₂, the same rankings emerge as in the absence of fish (although the significant differences between means vary): amphipod and mayfly > caddisfly > beetle. Thus, the presence of fish inhibited the upward movement of amphipods and mayflies until benthic oxygen fell to ≤ 0.50 mg/L.

Amphipods and mayflies responded strongly to benthic hypoxia by moving off the bottom in the highest proportions and enduring lower oxygen concentrations in the face of predation risk. The oxygen concentration (mean $\pm$ SE) at which 50% of the surviving mayflies had left the refuge was 1.50 ± 0.10 mg/L when fish were absent but 0.20 ± 0.04 mg/L when fish were present (Fig. 3A; *t*-test for unequal variances, $t = 11.36$, $df = 14$, $P < 0.01$). Similarly, the oxygen concentration at which 50% of surviving amphipods moved off the bottom was 1.65 ± 0.13 mg/L O₂ in the absence of predators but 0.31 ± 0.05 mg/L when fish were present (Fig. 3B; *t*-test for unequal variances, $t = 10.03$, $df = 14$, $P < 0.01$). Comparable values could not be calculated for caddisflies and beetle larvae because the majority of these invertebrates did not move above the refuge in every trial, even during hypoxia (see Figs. 3C and D). Fish did seem to inhibit the upward movement of caddisflies, however. In 12 of the 15 trials without fish, 50% of the caddisflies were above the refuge for at least two observations made when oxygen concentrations were below 2.0 mg/L. In trials with fish, only three out of 15 trials had 50% of the caddisflies above the refuge for at least two observations.

The degree of invertebrate behavioral response to predators was related to the vulnerability of the taxa to fish predation. Table 2 qualitatively categorizes invertebrate vulnerability to predators based on the total number of invertebrates eaten during the behavioral experiments. Given that all four invertebrate taxa were offered to the fish daily in the holding tank but that only mayflies and amphipods were eaten, the caddisfly and beetle appeared invulnerable to the size of fish predators used. We believe that the lack of predation on caddisflies is a result of the size of brook trout used since the same species of caddisfly has been found in stomachs of larger brook trout near the pond where we collected invertebrates (F. Rahel, unpublished data). Beetles, however, appeared unpalatable in our experiments and were not found in the stomachs of larger trout though they were

available in the pond mentioned above (F. Rahel, unpublished data). Although mayflies and amphipods altered their behavior in the presence of fish, they still experienced higher predation as benthic oxygen declined, especially below 1 mg/L O₂ (Table 2).

Discussion

Oxygen depletion at the substrate-water interface affects the distribution and activity of benthic organisms and species-specific mortalities resulting from hypoxia have been noted. For example, Nagell and Fagerstrom (1978) found the mayfly *Cloeon dipterum* persisted during winter anoxia but the stonefly *Nemoura cinerea* was eliminated by winter anoxia in a small pond. In a similar study, Westwood et al. (1983) showed that mosquito larvae *Aedes* sp. survived 25 days beneath ice in pools where dissolved oxygen remained high, but died in pools where dissolved oxygen fell drastically. The effects of invertebrate winter die-offs from hypoxia can remain evident during subsequent seasons. For example, community level changes persisted into the summer after winter anoxia in Severson Lake, MN (Schindler and Comita 1972). Water transparency was greater due to a change in the zooplankton community and this allowed increased macrophyte growth. The historically common copepod *Diaptomus siciloides* was eliminated from the lake, while smaller zooplanktors such as rotifers were replaced by larger species such as *Daphnia pulex*. Since this anoxic event was severe enough to kill all fish and there was little inflow or outflow into the lake, the aquatic community was not expected to recover quickly.

If prey can tolerate conditions that predators cannot, a period of environmental harshness can create local refuges by eliminating predators (Meffe 1984). Tonn and Paszkowski (1986) found this to be true for mudminnow-perch assemblages in northern Wisconsin following severe winterkill of adult yellow perch. Mudminnow *Umbra limi* densities increased due to high survival and recruitment of yearlings in the absence of predation by perch. Similarly, taxon-specific invertebrate survival may be important in structuring benthic invertebrate communities following severely hypoxic conditions. Britt (1955a) found that a 2-day summer anoxic event in the western basin of Lake Erie in 1953 killed massive numbers of mayfly nymphs *Hexagenia* sp.; in some places the

entire *Hexagenia* population had been killed. The population had not recovered by 1954 (Britt 1955b) and remained low even 20 years later (Brinkhurst 1974). Thus, hypoxia is an important mechanism controlling benthic assemblages by selectively eliminating intolerant species or by eliminating fish predators.

Our experiments suggest that winter anoxia can influence community structure even if mortality due to anoxia does not occur. The mechanism would involve selective predation on taxa that respond to low oxygen conditions by moving up in the water column, thus becoming highly vulnerable to fish predation. Such upward migrations during benthic hypoxia have been observed in two species of mayflies in Swedish ponds (Sikorowa 1968) and in the four taxa we studied.

In most studies of predator intimidation, prey must respond to only one factor, namely the presence of predators. By contrast, in our experiments, prey had to respond to both the presence of a predator and stressful oxygen concentrations. The taxa we examined appear to make a trade-off between these conflicting stresses by remaining in low oxygen conditions longer in the presence of a predator. Such a trade-off would have physiological costs associated with low oxygen conditions, but these presumably are offset by the reduced probability of mortality due to fish predation. Ultimately, however, if oxygen concentrations become low enough, prey are forced to leave the benthic region and predation rates increase.

Because invertebrates differ in their ability to tolerate hypoxia, there should be a succession of species leaving the bottom substrate as oxygen levels decline. Based on the upward movement of invertebrates as benthic oxygen declined in the absence of fish (Fig. 3) and on their physiological tolerance of low oxygen (Fig. 2), we predict that as benthic oxygen declines, in the absence of fish, the mayfly and amphipod would move off the substrate first. As benthic oxygen declined further, the caddisfly would migrate next, followed by the beetle larva. These predictions are in agreement with the multi-species behavioral tests but seem at odds with the bioassay results in the case of the beetle larvae which were intolerant of low oxygen (Fig. 2) but showed little upward movement during hypoxia (Fig. 3). Though many dytiscid larvae require atmospheric contact (Merritt and Cummins 1984), Roughley and Pengelly (1981) report that larvae of the genus *Hydaticus* have a network of large trachea for respiration. Diffusion of oxygen into the tracheal system apparently was insufficient in hypoxic water (100% mortality after 36 h at 0.1 mg/L oxygen). Perhaps because of the beetle's slow, crawling locomotion it may have been more energetically efficient for the beetle to reduce activity and remain within the refuge as long as possible. Nagell and Fagerstrom (1978) suggested a similar rationale to explain why a mayfly nymph that swam well increased activity during hypoxia but a stonefly nymph that swam poorly decreased activity during hypoxia. They suggested that if an invertebrate's only recourse is to creep along a substrate to escape stressful conditions, it would be advantageous to remain motionless to keep energy expenditures low until conditions improve.

Given that invertebrates should act to minimize their risk of predation (Sih 1980; Main 1987), the order of species migration from the substrate as benthic oxygen declines may be altered by the presence of fish. We found that mayflies and amphipods left the substrate first in the absence of fish but that the caddisfly left first in the presence of fish (Table 1). Based on the upward movement of invertebrates as benthic oxygen declined in the presence of fish (Fig. 3; Table 1) and on their vulnerability to fish predation (Table 2), we predict that as benthic oxygen begins to decline in ponds and until it falls to around 0.5 mg/L, fish will inhibit the upward movement of the amphipod and mayfly and that the caddisfly would be the first to move upward in the water column. The caddisfly would be vulnerable only to larger fish. As benthic oxygen declines below 0.5 mg/L, the amphipod and mayfly would be forced off the bottom and would be highly vulnerable to fish predators. The beetle larva would be the last invertebrate tested to move off the bottom, not because of the presence of fish (as there was no effect of fish on beetle behavior) but because of the energetic benefits discussed above. Differential temporal migration by invertebrate taxa would lead to pulses of selective predation on the taxa vulnerable to fish predators. Thus, pulses of selective predation on macroinvertebrates during episodes of hypoxia could be an important influence on the structure of benthic macroinvertebrate assemblages.

In our experiments, mayfly nymphs, amphipods, and caddisfly larvae reduced their activity in the presence of predators. Such reductions in activity are a common behavioral response of invertebrates to predators (Table 3). We found that taxa most vulnerable to fish predation (mayflies and amphipods) showed the greatest response to the presence of fish. Taxa less vulnerable to fish predation (caddisflies and beetle larvae) showed no change in vertical distribution in the presence of fish. Further, of the three taxa at some risk to fish predation, the caddisfly was the least preferred (Table 1) and showed the smallest separation between the with fish and without fish curves describing use of benthic refuge (Fig. 3). Others also have noted that prey most vulnerable to predators show the greatest behavioral response to predators. Examples include crayfish (Stein and Magnuson 1976), shrimp (Main 1987), notonectids (Sih 1982) and mayflies (Peckarsky 1980; Kohler and McPeck 1989). The greater mortality of mayflies and amphipods was not merely because they spent more time above the refuge than caddisflies or beetle larvae. When all four taxa were presented to fish in the holding tank, only mayflies and amphipods were consumed.

Several potentially important aspects of the invertebrate migration and pulse predation scenario were not addressed within this study. To avoid predation during winter hypoxia, benthic invertebrates may migrate upward using downed trees or other avenues which provide some protection due to cryptic coloration from fish predators. Higher dissolved oxygen would generally be found at the underside of the ice or around macrophytes if conditions are suitable to allow photosynthesis (Sculthorpe 1967; Barko et al. 1988). Invertebrates that

Table 3. Studies in which an invertebrate prey organism reduced activity in the presence of a predator

Prey	Predator	Reference
Oligochaeta Tubificidae ^b	Creek chub- <i>Semotilus atromaculatus</i>	Gilliam et al. 1989
Crustacea Zooplankton various species	Predaceous copepod <i>Acanthocyclops vernalis</i>	Li and Li 1979
Isopoda <i>Lirceus fontinalis</i> ^b	Green sunfish – <i>Lepomis cyanellus</i>	Holomuzki and Short 1988
Amphipoda <i>Gammarus pseudolimnaeus</i> ^b <i>Gammarus pseudolimnaeus</i> ^b	Rainbow trout- <i>Salmo gairdneri</i>	Williams and Moore 1982
<i>Gammarus pulex</i> ^b	Eight species of fish (predators and nonpredators), stonefly, mayfly, caddisfly	Williams and Moore 1985
Shrimp <i>Tozeuma carolinense</i> ^a	Sculpin – <i>Cottus gobio</i> Pinfish – <i>Lagodon rhomboides</i>	Andersson et al. 1986 Main 1987
Decapoda <i>Orconectes propinquus</i> ^c <i>Astacus astacus</i> ^c	Smallmouth bass – <i>Micropterus dolomieu</i>	Stein and Magnuson 1976
Insecta		
Plecoptera <i>Phasganophora capitata</i> ^b <i>Agnetina capitata</i> ^b	Mottled sculpin – <i>Cottus bairdi</i>	Williams 1986
Ephemeroptera <i>Baetis tricaudatus</i> ^b <i>Callibaetis montanus</i> ^b	Mottled sculpin Mottled sculpin	Soluk and Collins 1988 Kohler and McPeck 1989
Odonata <i>Coenagrion puella</i> ^c <i>Ischnura verticalis</i> ^c <i>Ischnura elegans</i> ^c	Brook trout – <i>Salvelinus fontinalis</i> Sticklebacks – <i>Gasterosteus aculeatus</i> or <i>Pungitius pungitius</i> Backswimmer – <i>Notonecta glauca</i> Rainbow trout skin mucous	Rahel and Kolar 1990 Convey 1988 Dixon and Baker 1988 Heads 1985
Hemiptera <i>Microvelia australis</i> ^b	Green sunfish	Sih 1988
Diptera <i>Aedes aegypti</i> ^c <i>Culex pipiens</i> ^c	Backswimmer – <i>Notonecta undulata</i>	Sih 1986
Gastropoda <i>Thais lamellosa</i> ^a	Waterborne stimuli from crabs or conspecifics	Appleton and Palmer 1988

^a marine invertebrates ^b lotic invertebrates ^c lentic invertebrates

spend daylight hours clinging to the underside of vegetation, logs and other structure and spend night hours clinging to the underside of the ice may remain reasonably safe from fish predators while obtaining enough oxygen for survival. Invertebrates may be able to persist through the winter by moving into riskier oxygen-rich areas for short periods and then returning to low oxygen areas to avoid predators (though not quantified, this type of behavior was observed in the behavioral experiments). In our experiments, predation rates were high on invertebrates venturing above the screen, but predation rates would likely be lower in a pond situation where the habitat is more structurally diverse (Crowder and Cooper 1982) and where cryptic coloration lends protection. Emergence from and re-entry into the benthos poses another dilemma: invertebrates could spend minimal time in the riskier oxygen-rich areas and suffer a slowed growth rate or could spend minimal time in the stressful abiotic condition and increase their predation risk. For immature aquatic insects, enduring hypoxia to escape predation may translate to later emergence than individuals of the same species that risked predation.

Without the benefit derived from swarming to overwhelm predators, the late-emerging adult is at a greater risk of predation and also has reduced reproductive opportunities (Thornhill and Alcock 1983).

In summary, we found that benthic invertebrates differ greatly in their tolerance of hypoxia and in their vulnerability to fish predators. Highly mobile taxa sensitive to hypoxia altered behavior more than tolerant taxa; taxa most vulnerable to fish predation altered behavior more than taxa safe from fish predators. When presented with the combined stresses, invertebrates vulnerable to both low oxygen and fish predation attempted to balance these conflicting demands by remaining longer in low oxygen water in the presence of predators. Ultimately, these invertebrates were forced to leave the benthic region and experienced an increased predation rate. Invertebrates differ widely in their ability to withstand hypoxia and in their vulnerability to fish predators and these differences should be reflected in the benthic community assemblages of ponds that develop periodic hypoxia. The benthic community may be impacted directly through selective mortality of sensitive taxa or

indirectly by differential predation on invertebrates leaving the benthos in search of better oxygen conditions.

Acknowledgements. James DeStaso III and Ralph Baldwin collected the brook trout. Larry DeBrey aided in developing laboratory apparatus and Richard Wilkison helped in running the behavioral experiments. Lyman McDonald and Anthony Frank provided advice on statistical analyses. Jeffrey Lockwood, Jacqueline Savino, David Lodge, and an anonymous reviewer provided critical review of the manuscript. This research was supported by funds from the College of Arts and Sciences and the Office of the Vice President of Research at the University of Wyoming and a grant from the National Science Foundation to FJR (BSR-8807200).

References

- Allison WR, Harvey HH (1988) Composition, distribution and standing crop of the benthos in a shallow lake. *Verh Internat Verein Limnol* 23:241-245
- Andersson AG, Bronmark C, Herrmann J, Malmqvist B, Otto C, Sjöström P (1986) Presence of sculpins (*Cottus gobio*) reduces drift and activity of *Gammarus pulex* (Amphipods). *Hydrobiologia* 1133:209-215
- Appleton RD, Palmer AR (1988) Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in marine gastropods. *Proc Nat Acad Sci* 85:4387-4391
- Barko JW, Godshalk GL, Carter V, Rybicki NB (1988) Effects of submersed aquatic macrophytes on physical and chemical properties of surrounding water. Technical Report A-88-11, US Army Engineer Waterways Expt Station, Vicksburg, MS, USA
- Breitbart DL (1992) Episodic hypoxia in the Chesapeake Bay: interacting effects of recruitment, behavior and physical disturbance. *Ecol Monogr* 62:525-546
- Brinkhurst RO (1974) The benthos of lakes. Macmillan, London, England
- Britt NW (1955a) Stratification in western Lake Erie in summer of 1953: effects on the *Hexagenia* (Ephemeroptera) population. *Ecology* 36:239-244
- Britt NW (1955b) *Hexagenia* (Ephemeroptera) population recovery in western Lake Erie following the 1953 catastrophe. *Ecology* 36:520-522
- Brittain JE, Nagell B (1981) Overwintering at low oxygen concentration in the mayfly *Leptophlebia vespertina*. *Oikos* 36:45-50
- Carpenter SR (1988) Editor. Complex interactions in lake communities. Springer Verlag, New York, NY, USA
- Convey P (1988) Competition for perches between larval damselflies: the influence of perch use on feeding efficiency, growth rate and predator avoidance. *Freshwater Biol* 19:15-28
- Coutant CC (1985) Striped bass, temperature, and dissolved oxygen: a speculative hypothesis for environmental risk. *Trans Am Fish Soc* 114:31-61
- Crowder LB, Cooper WE (1982) Habitat structural complexity and the interaction between bluegills and their prey. *Ecology* 63:1802-1813
- Davies RW, Yang T, Wrong FJ (1987) Inter- and intraspecific differences in the effects of anoxia on erpobdellid leeches using static and flow-through systems. *Holarct Ecol* 10:149-153
- Dixon SM, Baker RL (1988) Effects of size on predation risk, behavioural response to fish, and cost of reduced feeding in larval *Ischnura verticalis* (Coenogronidae: Odonata). *Oecologia* 76:200-205
- Dodson SI (1970) Complementary feeding niches sustained by size-selective predation. *Limnol Oceanogr* 15:131-137
- Gilliam JF, Fraser DF, Sabat AM (1989) Strong effects of foraging minnows on a stream benthic invertebrate community. *Ecology* 70:445-452
- Greenbank J (1945) Limnological conditions in ice-covered lakes, especially as related to winterkill of fish. *Ecol Monogr* 15:343-392
- Hall RJ, Ehlinger TJ (1989) Perturbation, planktivory, and pelagic community structure: the consequence of winterkill in a small lake. *Can J Fish Aquat Sci* 46:2203-2209
- Hamrin SF (1987) Seasonal crayfish activity as influenced by fluctuating water levels and presence of a fish predator. *Holarct Ecol* 10:45-51
- Heads PA (1985) The effect of invertebrate and vertebrate predators on the foraging movements of *Ischnura elegans* larvae (Odonata: Zygoptera). *Freshwater Biol* 15:559-571
- Holomuzki JR, Short TM (1988) Habitat use and fish avoidance behaviors by the stream-dwelling isopod *Lirceus fontinalis*. *Oikos* 52:79-86
- Jacob U, Walther H, Klenke R (1984) Aquatic insect larvae as indicators of limiting minimal contents of dissolved oxygen. Part II. *Aquat Insects* 6:185-190
- Köhler SL, McPeck MA (1989) Predation risk and the foraging behavior of competing stream insects. *Ecology* 70:1811-1825
- Kolar CS (1992) Invertebrate response to low oxygen and predator presence. M.Sc. thesis, University of Wyoming, Laramie, Wyoming
- Kramer DL, McClure M (1982) Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fishes. *Env Biol Fish* 7:47-55
- Li JL, Li HW (1979) Species-specific factors affecting predator-prey interactions of the copepod *Acanthocyclops vernalis* with its natural prey. *Limnol Oceanogr* 24:613-626
- Magnuson JJ, Beckel AL, Mills F, Brandt SB (1985) Surviving winter hypoxia: behavioral adaptations of fishes in a northern Wisconsin lake. *Environ Biol Fishes* 14:242-250
- Main KL (1987) Predator avoidance in seagrass meadows: prey behavior, microhabitat selection, and cryptic coloration. *Ecology* 68:170-180
- Meffe GL (1984) Effects of abiotic disturbance on coexistence of predator and prey fish species. *Ecology* 65:1525-1534
- Merritt RW, Cummins KW (1984) An introduction to the aquatic insects, 2nd edition. Candle/Hunt Publishing Company, Dubuque, Iowa
- Morin PJ (1984) The impact of fish exclusion on the abundance and species composition of larval odonates: results of short-term experiments in a North Carolina farm pond. *Ecology* 65:53-60
- Nagell B (1977) Phototactic and thermotactic responses facilitating survival of *Cloeon dipterum* (Ephemeroptera) larvae under winter anoxia. *Oikos* 29:342-347
- Nagell B (1980) Overwintering strategy of *Cloeon dipterum* larvae. In: Flannagan JF, Marshall KE (eds.) *Advances in Ephemeropterological biology*. Plenum Press, New York, NY, USA, pp 259-264
- Nagell B, Fagerström T (1978) Adaptations and resistance to anoxia in *Cloeon dipterum* (Ephemeroptera) larvae under winter anoxia. *Oikos* 29:342-347
- Neter J, Wasserman W, Kutner MH (1990) Applied linear statistical models. Third Edition. Irwin, Inc. Homewood, IL, USA
- Nilsson AN (1981) The Fennoscandian species of the genus *Hydaticus* Leach (Coleoptera: Dytiscidae). *Ent Scand* 12:103-108
- Ökland J (1964) The eutrophic Lake Borrevann (Norway)-an ecological study on shore and bottom fauna with special reference to gastropods, including a hydrographic survey. *Folia Limnol Scand* 13, 337pp
- Peckarsky BL (1980) Predator-prey interactions between stoneflies and mayflies: behavioral observations. *Ecology* 61:932-943
- Pennak RW (1978) Fresh-water invertebrates of the United States, 2nd edition. John Wiley and Sons, Inc. New York, NY, USA, 803pp
- Rahel FJ (1989) Simulation of vertical limnological gradients. *J Freshwater Ecol* 5:247-252
- Rahel FJ (1990) Anomalous temperature and oxygen gradients under the ice of a high plains lake in Wyoming. *Limnol Oceanogr* 35:247-252

- Rahel FJ, Kolar CS (1990) Trade-offs in the response of mayflies to low oxygen and fish predation. *Oecologia* 84:39–44
- Rossignol-Strick M (1985) A marine anoxic event on the Brittany Coast, July 1982. *J Coastal Res* 1:11–20
- Roughley RE, Pengelly DH (1981) Classification, phylogeny, and zoogeography of *Hydaticus* Leach (Coleoptera: Dytiscidae) of North America. *Quaest Entomol* 17:249–309
- Schindler DW, Comita GM (1972) The dependence of primary production upon physical and chemical factors in a small, senescing lake, including the effects of complete winter oxygen depletion. *Arch Hydrobiol* 69:413–451
- Sculthorpe CD (1967) The biology of aquatic vascular plants. Edward Arnold Ltd London, England 609 pp
- Sih A (1980) Optimal behavior: Can foragers balance two conflicting demands? *Science* 210:1041–1043
- Sih A (1982) Foraging strategies and the avoidance of predation by an aquatic insect, *Notonecta hoffmanni*. *Ecology* 63:786–796
- Sih A (1986) Antipredator responses and the perception of danger by mosquito larvae. *Ecology* 67:434–441
- Sih A (1988) The effects of predators on habitat use, activity, and mating behaviour of a semi-aquatic bug. *Anim Behav* 36:1846–1848
- Sih A, Petranks JW, Kats LB (1988) The dynamics of prey refuge use: a model and tests with sunfish and salamander larvae. *Am Nat* 132:463–483
- Sikorowa A (1968) The behavior of *Chaoborus* (Licht) larvae under unfavourable oxygen conditions *Ekol Pol(A)* 16:185–192
- Snedecor GW, Cochran (1980) Statistical Methods. The Iowa State University Press. Ames, Iowa, USA
- Soluk DA, Collins NC (1988) A mechanism for interference between stream predators: responses of the stonefly *Agneta capitata* to the presence of sculpins. *Oecologia* 76:630–632
- Sousa WP (1984) The role of disturbance in natural communities. *Ann Rev Ecol System* 15:353–392
- Stein RA, Magnuson JJ (1976) Behavioral response of crayfish to a fish predator. *Ecology* 57:751–761
- Suthers IM, Gee JH (1986) Role of hypoxia in limiting diel spring and summer distribution of juvenile yellow perch *Perca flavescens* in a prairie marsh. *Can J Fish Aquat Sci* 43:1562–1570
- Swanson RL, Parker CA (1988) Physical environmental factors contributing to recurring hypoxia in the New York Bight. *Trans Am Fish Soc* 117:37–47
- Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard University Press, Cambridge, MA, USA, 547pp
- Thorp JH, Bergy EA (1981) Field experiments on responses of a freshwater, benthic macroinvertebrate community to vertebrate predators. *Ecology* 62:365–375
- Tonn WM, Paszkowski CA (1986) Size-limited predation, winter-kill, and the organization of *Umbra-Perca* fish assemblages. *Can J Fish Aquat Sci* 43:194–202
- Vanni MJ, Leucke C, Kitchell JF, Rentmeester Y, Temte J, Magnuson JJ (1990) Cascading trophic interactions and phytoplankton abundance in eutrophic lakes: effects of massive fish mortality. *Nature* 344:333–335
- Westwood AR, Surgeoner GA, Helson BV (1983) Survival of spring *Aedes* spp. mosquito (Diptera: Culicidae) larvae in ice-covered pools. *Can Entomol* 115:195–197
- Williams DD (1986) Factors influencing the microdistribution of two sympatric species of Plecoptera: An experimental study. *Can J Fish Aquat Sci* 43:1005–1009
- Williams DD, Moore KA (1982) The effect of environmental factors on the activity of *Gammarus pseudolimnaeus* (Amphipoda). *Hydrobiologia* 96:137–147
- Williams DD, Moore KA (1985) The role of semiochemicals in benthic community relationships of the lotic amphipod *Gammarus pseudolimnaeus*: a laboratory study. *Oikos* 44:280–286
- Wolf NG, Kramer DL (1987) Use of cover and the need to breathe: the effects of hypoxia on vulnerability of dwarf gouramis to predatory snakeheads. *Oecologia* 73:127–132