PREDATOR-INDUCED DIAPAUSE IN DAPHNIA

Miroslaw Slusarczyk

Diapause is generally believed to be an adaptation to allow temporal avoidance of adverse conditions (Cohen 1966, Levins 1968, Venable and Lawlor 1980, Ellner 1985, Venable and Brown 1988). Passive dispersal of dormant stages can also enable a spatial escape from local adversity (Janzen 1970) and colonization of a new territory (Platt 1976, Howe and Smallwood 1982). Dormant stages are usually better equipped to withstand harsh environmental conditions than active individuals (Danks 1987, Schwartz and Hebert 1987). Diapause typically occurs before living conditions deteriorate (Taylor 1980, Hairston and Munns 1984). This anticipation is achieved by responding to token environmental stimuli that do not necessarily themselves influence fitness, but that are reliable predictors of future environmental change. Photoperiod and temperature are the most commonly recognized cues controlling diapause, which avoids adverse periods correlated with the seasons (Danks 1987, Stross 1987). The timing of seasonal dormancy is usually a species- and place-specific feature and in

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many species is highly heritable (Danks 1987). When the environmental cues are not tightly correlated with the appearance of unfavorable conditions, the reaction (e.g., diapause) to signals closely related to a hazardous agent should be favored instead (Harvell 1990, Pijanowska 1993).

Although both abiotic and biotic factors can be equally catastrophic, the role of abiotic factors in dormancy induction is better documented (Frey 1982, Danks 1987, Stross 1987). A possible role of predation in diapause induction has been hypothesized but poorly investigated. Most if not all of the evidence for this phenomenon has come from the freshwater environment, indicating an adaptive function of copepod diapause to invertebrate (Hairston 1987) or vertebrate (Nilssen 1977, Hairston and Olds 1984, Hairston 1987, Hairston et al. 1990) predation. Before any evidence for antipredator function of prey diapause was found, Strickler and Twombly (1975) had suggested that chemical stimuli released into the environment by a predator could play a role in the induction of prey diapause. Although postulated and tested for (Hairston and Olds 1984, Hairston et al. 1990), it has not yet been demonstrated. In contrast the results presented here show that exudates released into the water by fish predators can directly induce diapause in a population of the planktonic cladoceran *Daphnia magna* Strauss. J. Pijanowska and G. Stolpe (personal communications) have made similar observations.

Most freshwater Daphnidae alternate between asexual parthenogenetic and sexual modes of reproduction (Hebert 1987, Zaffagnini 1987). They can produce two types of eggs: numerous, parthenogenetic ones that immediately develop into females or males, and sexual ones, usually only two, that hatch into females after a certain time of dormancy. Under favorable conditions, usually only females are parthenogenetically produced. Males and sexual egg production commonly precede deterioration of environmental conditions. After fertilization, sexual eggs are deposited within protective thickened carapace walls called an ephippium. The ephippium is released into water with the next molt and can remain viable in lake sediments for many years (Carvalho and Wolf 1989). Ephippial eggs are resistant to harsh environmental conditions (Schwartz and Hebert 1987); they can even pass intact through predators’ intestines (Melior 1975).

In lakes of the temperature zone, cladoceran diapause usually occurs in late fall or early winter before the environment actually changes (Frey 1982). Occasionally, however, dormant stages are also observed in summer in spite of seemingly favorable environmental conditions (Frey 1982, Lampert 1991). Several factors are known to induce the transition from asexual to sexual reproduction and formation of resting eggs in cladocerans: short-day photocyte (Stross 1987), low temperature (Mortimer 1935), low food concentration (Banta 1939, D’Abramo 1980), and unknown chemical factors produced by crowding animals (Banta 1939, Carvalho and Hughes 1983).

In this study I have attempted to eliminate the possible effects of all known diapause-inducing factors for *Cladocera*, except for exudates released into water by predatory fish. The experimental conditions were: high water temperature, long-day light regime, and moderate food concentration, which together simulate the situation in summer in a lake in the temperate zone. Sexual reproduction and/or the subsequent formation of diapausing eggs in cladocerans has never been experimentally induced under similar conditions.

### Methods

A clone of *Daphnia magna* from Binnensee (Schleswig Holstein, Northern Germany), a shallow, eutrophic lake where *D. magna* coexists with fish (Lampert 1991), was used in this experiment. *D. magna* can be only occasionally found in reservoirs inhabited by fish. The relatively large size of *D. magna* makes it extremely vulnerable to visually feeding fish predators. The clone used in the experiment responds to chemical signals released by fish predators by changes in behavior (Dawidowicz and Loose 1992) and life history (Weider and Pijanowska 1993) that are regarded as anti-predatory adaptations.

A modified flow-through system (Dawidowicz and Loose 1992) was used in the experiment to maintain stable and fully controlled temperature, light, and nutritional conditions (Fig. 1). It consisted of 10 experimental and 10 control flow-through chambers placed in a water bath at 22°C. The bottoms of each of the 25-cm-long glass tubes were closed with a wire mesh (100 μm diameter) to prevent animals from escaping.

### Table 1. Number of offspring in five subsequent broods (columns indicated by Roman numerals) for 10 control *Daphnia magna* (without fish) and 10 individuals exposed to water containing fish exudates (with fish). A = aborted clutch, 0 = instar without eggs, E = ephippial eggs, Φ = female offspring, ι = male offspring.

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or being washed out. A multichannel peristaltic pump maintained a constant flow rate of 450 mL per chamber per day. The system was illuminated from above with three fluorescent lamps, producing an approximate photon flux density of 4 μmol·m⁻²·s⁻¹. A long-day (19L:5D) photocycle was applied. The culture medium for experimental animals was prepared from distilled water with the addition of soluble inorganic salts, according to Aachener Daphnien Medium recipe (Klütgen et al. 1994). In the fish treatment, the medium was conditioned by keeping one 7-cm-long bleak (Alburnus alburnus L; a small European cyprinid fish) for 24 h in 7 L of medium. The fish was fed daily with D. magna or Chironomidae larvae, about half an hour before the medium was prepared. To reduce the possible influence of chemical substances associated with the fish food, half of the water (≈100 mL) from a beaker containing the food portion was added to the control medium each time. Fresh medium was prepared once a day. Prior to use, the water was filtered through a 0.45-μm membrane filter and a suspension of unicellular green algae, Scenedesmus acutus, was added to obtain a final carbon concentration of 1.0 mg/L).

The animals used in the study came from the second generation of a clone originating from a single foundress. Ten females from the second brood were placed in a single, 250-mL, flow-through chamber, flushed with water free from fish cues. Food was maintained at a high concentration (carbon content >1 mg/L). Offspring were removed until each female had the eggs of her third brood in the brood pouch. Five females were then transferred to an identical chamber, with the same food concentration, but subjected to water containing fish exudates. As soon as the third brood was released, the synchronized (within 12 h) cohort of young was used to begin the experiment. Two females from the original (fish-cue-free) tube were placed in each of 10 flow-through tubes, together with a 5-d-old male to constitute the control group. For the rest of the experiment these animals received fish-free water. Similarly, two females from the tube pre-treated with the fish cue were placed with a male in each of the 10 experimental tubes, which were flushed with water containing fish exudates for the rest of the experiment. Two females were initially used in cases where there was any mortality during early instars, but no females died with the first 3 d. On the 4th d of the experiment, before females released the eggs of their first brood, one of the two females chosen at random was removed from each chamber, leaving one female with a single male. The chambers were inspected 5 times per day; neonates were removed and counted, and later their sex was examined. The experiment was continued for ≈3 wk, until all females released their fifth brood.

Results

In the control chambers, all broods were parthenogenetic and only female offspring were produced (Table 1). In the presence of chemical signals released by fish, all females produced diapaus ing eggs. Though the first brood was exclusively parthenogenetic, 9 of 10 second broods were ephippial. Only one female produced ephippia twice, and two females in the fish treatment produced male broods. The average brood size
immediately following ephippium formation was significantly smaller than the first brood in the fish treatment (Wilcoxon signed-rank test for pairs, $P < 0.005$), while in the control treatment the average clutch size increased with the brood number (Fig. 2).

**Discussion**

In temperate-zone lakes inhabited by fish, fish abundance and their impact on zooplankton changes seasonally, usually with a summer maximum, as well as between years (Hairston 1988, Jacnher 1989). Ephippial egg production shortly before or during the period of intensive predation may result in higher fitness for *Daphnia magna* females. It may be more advantageous to produce two well-protected ephippial eggs than more numerous parthenogenetic offspring that have little or no chance of surviving intense predation. These ephippial eggs can survive the complete summer extinction of the free-swimming *Daphnia* population, and together with older "winter" ephippial eggs, as yet unhatched, they can re-establish the population in the future, when biotic and abiotic conditions become more favorable.

Diapause, to be effective as a way to escape predation, should be initiated at the appropriate time. Too-early dormancy would reduce females' reproductive potential; too-late formation of resting eggs may not be completed in time (Taylor 1980, Hairston and Munns 1984). A cue that reliably informs animals of present or future predation intensity is thus necessary to trigger diapause. Existing evidence indicates that formation of resting eggs by a copepod species—*D. sanguineus*—is controlled by photocycle and temperature (Hairston et al. 1990). The timing of *D. sanguineus* diapause is regarded as a genetically fixed trait, apparently adapted to a local predation pattern and abiotic conditions (Hairston and Olds 1984). An adjustment of the timing of *D. sanguineus* diapause to new conditions can be achieved due to a genotypic shift within its population, caused by a change of the selective regime (Hairston and Dillon 1990).

My results indicate that unlike the dormancy of *D. sanguineus*, *D. magna* diapause is triggered by exudates released by predatory fish, which might better announce the real danger of predation. The advantages and disadvantages of inducible reaction to fish predation were discussed by Pijanowska (1993). My findings challenge the view that phenotypic responses to rapidly fluctuating selective agents cannot consist of complex life-history defense mechanisms (Bradshaw 1965; Pijanowska 1993). Bradshaw (1965) assumed that the development of complex defense traits is usually too slow to face the danger in time. However, if the responding individuals are somehow protected against the selective agent until the change takes place, this might not be the case. In *D. magna*, the small sizes of the young individuals help protect them temporarily against visual predation (Lampert 1991). Hence, if a complicated response is initiated during an early stage of ontogenesis, it can be completed before that individual becomes highly vulnerable to predation.

In my experiment ephippial broods occurred only in the early mature instars of animals exposed to fish exudates, and were not repeated in later instars, despite continued presence of the cues. Under an unpredictable predation regime and selective mortality of large-sized individuals, formation of ephippial eggs early in ontogenesis could have been favored by natural selection (surprisingly, ephippia were not formed in the very first brood). Similar responses may not be adaptive for late stages, since older and bigger females have little chance to form ephippial eggs in time.

An alternative hypothesis for the decline of ephippial production with age under my experimental conditions could be that females gradually lost their sensitivity to the constantly applied fish exudates. In natural reservoirs the concentration of fish exudates probably fluctuates due to horizontal and vertical diurnal fish migration (Gliwicz and Jacnher 1992).

Under stable favorable conditions, the clutch size of Daphnidae usually increases in subsequent broods. This is still true in the presence of fish exudates (Stibor 1992, Weider and Pijanowska 1993). The recorded reduction in offspring number in the broods immediately following ephippia (Table 1, Fig. 2) suggests a high cost for ephippia formation.

Fecundities measured in this study are low for a food carbon level of 1 mg/L (Weider and Pijanowska 1993).
This could be because algae tended to settle in the pumping system and in the water containers. Although the actual food level in the flow-through chambers may have been reduced, it should have been maintained constant over time and equal in both treatments. Food conditions were sufficient for parthenogenetic reproduction and food conditions alone did not induce diapause in the control chambers.

The proposed mechanism of inducible diapause as a way to escape predation is consistent with field data from Binnensee, collected during 1987 (Lampert 1991). Besides the commonly observed periods of ephippial production by D. magna in the late fall, other ephippial events were also recorded in spring and summer. They were apparently not induced by short photoperiod or low temperature. They cannot be fully explained by either overcrowding or low food concentration (Sommer 1989). The spring and summer ephippial periods were followed or accompanied by high death rate of Daphnia probably caused by intense fish predation (Lampert 1991). Induced ephippial production as a defense mechanism against fish predation could help explain this seasonal pattern.

The predator-induced diapause can be largely responsible for a rather uncommon phenomenon of persistence of D. magna in lakes with fish. Other reported cases of summer diapause of various cladoceran species (Frey 1982) could also reflect mechanisms to escape predation. Perhaps this phenomenon is more widespread than is currently recognized.

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