Environmental plasticity of fish avoidance diapause response in *Daphnia magna*

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ABSTRACT

Organisms cope with harsh environmental conditions in various ways: either by tolerating environmental stress (through physiological adaptations), or by avoiding it in space (through migration) or time (diapause). Some species rely on a single strategy while others may choose from an array of options when facing different environmental stressors. Planktonic crustaceans may utilise different active (morphological, behavioural, life-history) or passive (diapause) defences to survive periods of high risk of fish predation. Recent evidence has indicated that resting egg production could be induced in Daphnia magna *by chemical cues associated with fish predation. This suggests that contrary to most known cases of diapause, which are triggered well in advance of catastrophic events (here termed "predictive diapause"), fish avoidance diapause in D. magna may exhibit a "responsive nature" and be initiated only after intensive predation appears. Experimental evidence discussed here indicates that the reaction of D. magna to chemical signals of fish predation could be conditional and determined by key environmental conditions, which in nature affect relative gains of activity vs dormancy. At high risk of fish predation, the decision of* Daphnia *to produce resting eggs was disfavoured by high food concentration. This reaction was claimed adaptive since high food allows for higher reproductive rates and better survival of offspring. All this may assure higher benefits due to activity despite some risk of predation (once predation pressure is not fatal to all active descendants) and disfavour resting eggs production. Moreover, at moderate food conditions the decision of* Daphnia *to produce resting eggs was disfavoured by the availability of a dark refuge from fish visual predators and thus likely lowering the risk of being preyed upon. Furthermore, when food was at a moderate level and a dark refuge was not present the decision of* Daphnia *to produce resting eggs was favoured by low water temperature. This could be explained as an adaptive reaction again, once low water temperature (due to its effect on a rate of metabolism) should have affected the gains derived from active life and reproduction more seriously than ones of inactive stages. The evidence presented here indicates that a responsive diapause allows* D. magna *to maximise reproductive output by taking advantage of opportunities presented by an unpredictable environment.*

Keywords: chemical communication, defence reaction, fish avoidance, conditional response, predictive vs *responsive diapause,* Daphnia
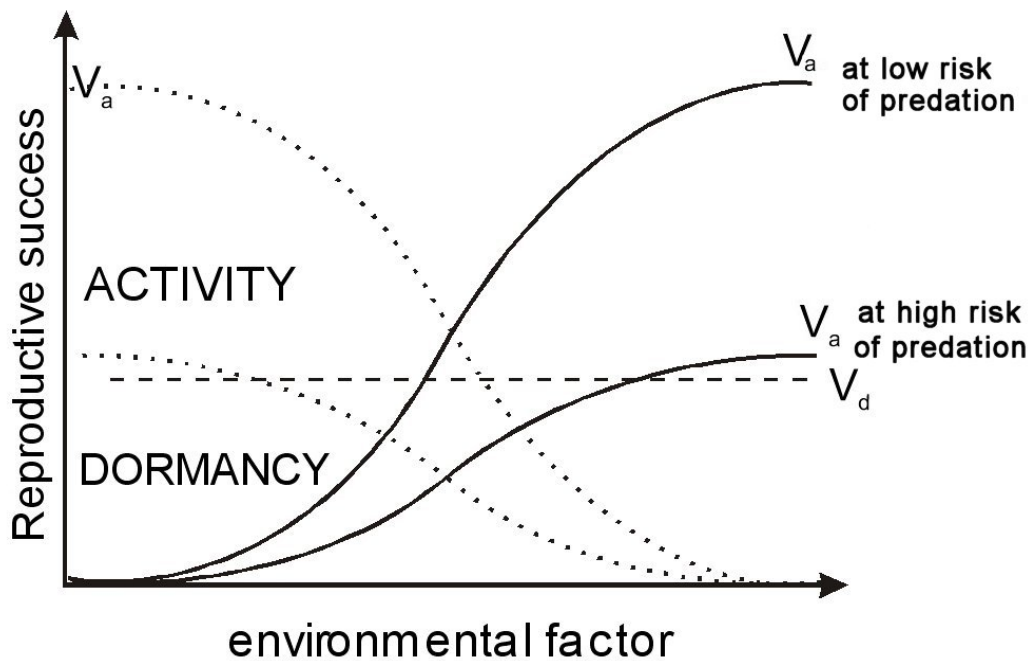
1. INTRODUCTION

Organisms cope with harsh environmental conditions in various ways: either by tolerating environmental stress (through physiological adaptations), or by avoiding it in space (through migration) or time (diapause). Some species rely on a fixed mode of defence, while others may choose from an array of options as they face different environmental stressors. Having a choice, organisms should employ that mechanism which maximises their reproductive success.

Most organisms cope with predation through various defensive responses. There are, however, environments that feature an exceptionally high risk of predation, e.g. open water habitats that do not offer many places to hide from visually orienting predators and expose whole populations of pelagic specimens to extermination (Gliwicz & Pijanowska 1988). There we can find organisms, which besides the widely used active anti-predatory defences produce resting stages to assure genome protection in periods of high risk of predation (Hairston 1987; Pijanowska & Stolpe 1996). Thanks to specific adaptations associated with slowed-down metabolism, dormant forms may tolerate a wider range of environmental conditions than active individuals, e.g. surviving passage through the digestive track of predators (Melllors 1975; Saint-Jean & Pagano 1995) or being deposited in places not accessible to predators and active individuals, e.g. the anoxic hypolimnion. Nevertheless, diapause would seem to be a costly defence, since, by entering it, individuals suspend propagation of their genotypes. Furthermore, although diapause may assure greater tolerance to harsh conditions, the survival of dormant forms may be far from complete for several reasons. The self-healing mechanisms that characterise active individuals are typically switched off or turned down in diapause. Thus, even the small injuries readily healing in active individuals may emerge as terminal in dormant specimens. Equally, as most resting stages are not able to control their position, their fate may be a matter of chance. They may be readily displaced into sites exposed to fatal conditions. Ultimately, successful survival of any hostile conditions must be rounded off by a resumption of development at the proper place and time. The perception of such favourable conditions may, however, be a challenge to dormant individuals when these are exposed to false signals, or else displaced into sites isolated from the cues that break dia-

Fig. 1. Relative benefits of active life and reproduction (V_a) *vs* Diapause (V_d) at different levels of hypothetical environmental factor that affects chances for survival and reproduction of potential prey when exposed to different levels of predation (more details in the text.)

pause (Caceres & Tessier 2003). In consequence, dormant forms may thrive in diapause terminally or break dormancy spontaneously in an unfavourable place or season (Danks 1987). For all these reasons diapause seems to be a last-resort defence utilised when the protective mechanisms of active individuals prove ineffective.

1.1. *Predictive* vs *responsive diapause*

The mechanism underpinning the induction of fish-avoidance diapause may be initiated either in advance of, or after, the onset of a deterioration in the environment. The first mode will be termed here "predictive diapause", the second "responsive diapause". The diapause in the calanoid copepod E. sanguineus may be an example of the first kind. The species enters diapause well in advance of any intensification of predation pressure on the part of fish in a lake, in that the first individuals in a population enter diapause one generation or more beforehand (Hairston 1987), when informed of oncoming seasonal predation by a specific combination of factors involving photoperiod and temperature (Hairston & Dillon 1995).

The mechanism by which fish avoidance diapause is induced in the cladoceran D. magna could be used as an example of the other kind, i.e. of "responsive diapause". D. magna may produce resting eggs in response to a combination of fish kairomones and the alarm substances of injured conspecific prey (Ślusarczyk 1999), which appear in the environment when predation is already occurring. Both means by which

diapause is induced have their flaws and advantages, and these will be discussed in a later section.

The large sizes of individuals of *D. magna* makes this species so extremely vulnerable to fish predation that it is typically found in small water bodies free of fish. However, thanks to a set of defensive responses, involving life-history (Weider & Pijanowska 1993), diapause (Ślusarczyk1995) and changes in behaviour (e.g., through diel vertical migration (Ellert & Dawidowicz 1993), swarming and enhanced vigilance (Pijanowska & Kowalczewski 1998)), *D. magna* has been successful in coping with fish in the shallow, coastal, eutrophic waters of the Grosse Binnensee in North Germany (Lampert 1989).

2. PLASTICITY OF DIAPAUSE INDUCTION

2.1. *The hypothesis*

The effectiveness of active defences, and hence the chances of *Daphnia* surviving and leaving viable offspring when facing predation, may be limited by various environmental conditions. If so, the limiting factors could potentially affect *Daphnia* decisions as regards the employment of the last-resort diapause response.

Let us consider a single hypothetical environmental factor which increases (solid line) or diminishes (dotted line) the chances for survival and reproduction of active individuals under predatory pressure in an asymptotic way (V_a lines) (Fig. 1) when it intensifies. It can affect the reproductive success of active individuals at various levels of predation (two levels of predation are actually shown on the graph). If we assume that the

benefits of diapause (V_d) are small, and independent of wide ranges in the limiting factor (the rate of proliferation is low in diapause due to suspended development, while tolerance to various unfavourable conditions is commonly enhanced). For clearer presentation, a single V_d line is shown, and applies to both predation levels, though its value may in fact be somewhat affected by predation. Considering the given intensity of predation, there might be entirely different situations, depending on the level of the limiting factor. While individuals would gain more by continuing in active life and with reproduction at a high level (considering the solid lines on the graph), greater gains may derive from the formation of resting eggs when the level is low, i.e. when below V_d line. It seems that potential prey having a choice should apply that type of reproduction (active *vs* dormant eggs) which promises greater reproductive success. Predation level may shift the equilibrium point of benefits due to activity and dormancy to a different value of the limiting factor.

2.2. Testing the hypothesis

Here, I present briefly the experimental evidence to support the idea that the responsive diapause is flexible in nature, and go on to discuss its potential benefits. A detailed description of the studies invoked may be found elsewhere (Ślusarczyk 2001), or else will be published soon (Ślusarczyk *et al.*, unpublished manuscript; Ślusarczyk, unpublished manuscript).

Feeding conditions seem to represent the key environmental factor determining chances for survival and reproduction of individuals in the active state. At low food levels, a substantial decline is typically observed in growth and/or reproduction, and/or expenditure on defence. As a result, individuals grow more slowly and mature later, experiencing limitations on their chances of successful reproduction in a period of danger, giving rise to descendants that are few in number or of poor quality, and exposing themselves to a higher risk of predation where defences are not exhibited. Equally, when the food supply is not limited, the high reproductive effort of a well-fed, fast-growing prey individual displaying various defensive responses may ensure the survival of at least some descendants to reproduce - even when there is substantial risk of predation - and hence imply that the limited benefits of producing resting eggs are exceeded. To sum up, the production of resting eggs may offer greater benefits where feeding conditions are poor, only to give way to the more favourable production of active offspring at times when the food situation is better.

The probability of the production of ephippial eggs in *D. magna* exposed to a simulated threat of fish predation was determined in relation to food concentration in a batch culture experiment (Ślusarczyk 2001). In "fish-free" conditions, no production of resting eggs was observed with either low (0.4 mg C l^{-1} ≈ 20 µg C ind^{-1}) or high (1.6 mg C l^{-1} ≈ 80 µg C ind^{-1}) food availability, whereas in "fish" treatments, resting eggs were produced by various proportions of females in line with food concentration. With the limited food (20 µg C ind^{-1}) there was production of ephippial (dormant) eggs in the early broods of almost all females in the "fish" treatment. Where food concentrations were higher, proportions of ephippial females in the population were progressively lower, with females postponing the formation of resting eggs until later broods. At the highest food concentration (80µg C ind^{-1}), only about 17% of females produced ephippia when exposed to fish cues.

Water temperature resembles food in being able to affect both the quantity and quality of descendants. At low temperature (within tolerable range) ectothermic individuals typically produce larger offspring at rates a few-fold lower than when thermal conditions are optimal (Atkinson & Sibly 1997). Water temperature should not affect the benefits of dormancy where the metabolism of dormant forms and survival seem temperature-independent over a broad range (Danks 1987). Reproductive success in individuals producing active offspring may thus be lower at low water temperature, but higher under optimal thermal conditions, than that in individuals producing resting eggs when facing a high risk of predation. We might thus expect low water temperature to favour production of dormant offspring. On the other hand, water temperature may determine the energetic demands of fish predators, and thus the predatory pressure imposed on prey, which could affect the reproductive success of active individuals in the opposite direction. The overall effect of water temperature on the decision of potential prey to produce resting eggs could thus be ambiguous. Experimental evidence has in fact revealed a disfavouring effect of water temperature on the frequency of resting-egg formation in *Daphnia* exposed to a simulated threat of fish predation (Ślusarczyk, unpublished manuscript). At "low" water temperature (18 °C), all experimental females produced resting eggs in the early broods when facing the risk of fish predation and experiencing moderately good feeding conditions (0.25 mg C l^{-1} in a flow-through system). With increasing temperature (22 °C and 26 °C), and hence faster reproduction, but matching food conditions, the proportions of females producing resting eggs were significantly lower (44% *vs* 7%, respectively). The production of resting eggs in environments barren of chemical cues of predation was either observed at a very low level or was not present at all.

The disfavouring effect of water temperature on the frequency of occurrence of ephippial females may indicate either that water temperature does not increase the risk of fish predation as fast as it promotes the rate of *Daphnia* reproduction, or else that the risk of predation is anticipated by *Daphnia* in line with a signal other than water temperature, e.g. in accordance with

the concentration of chemical cues associated with fish predation (Reede 1995).

Chances of prey surviving in the face of visually-oriented fish predators may be affected by light conditions, since the effectiveness of such predation may be limited at low light intensity (Blaxter 1970). As light levels attenuate quickly in a water column, deep or non-transparent lakes may offer a dark refuge from visually-feeding fish. *Daphnia magna*, like many other pelagic organisms, seeks to reduce rates of encounters with fish engaging in visually-oriented predation by spending daylight hours in the relatively safe, dark bottom zone, if this is accessible (De Mester *et al.* 1999). Individuals ascend to the warm and food-abundant subsurface water after sunset to enjoy a higher food concentration and the likely accelerated metabolism. However, the effectiveness of this diurnal mechanism may be influenced by various environmental conditions. Transparency of water or water level may change in the course of a season, determining the presence or absence of a safe refuge from fish predators in aquatic environments. When a dark refuge is not present, *Daphnia* must utilise an alternative defence, e.g. diapause, to survive. Indeed, the presence of a dark refuge seems to affect the decision of potential prey as regards their producing resting eggs. In laboratory batch culture experiments, a diapause response to chemical cues of fish predation in *D. magna* was tested for at various light intensities which in natural conditions determine the potential vulnerability of *Daphnia* to visually-orienting planktivorous fish. At a moderate light intensity which allows for effective predation by fish (1.4 μmol m^{-2} s^{-1}), the proportion of females producing resting eggs was found to be significantly higher than in dim light conditions (of 0.001 μmol m^{-2} s^{-1}), which are not favourable to the visual detection of prey (62% *vs* 8.7% of experimental females respectively produced ephippia). Production of resting eggs was not observed in complete darkness, nor in jars missing chemical cues of fish predation, irrespective of the tested light conditions (Ślusarczyk *et al.*, unpublished manuscript).

So far, three key environmental factors have been tested in this way: food concentration, water temperature and light intensity. All three have been shown to affect the probability of resting eggs being produced by *Daphnia* under a simulated threat of fish predation, but equally appeared non-influential in environments free from cues regarding predation. The list of modifying factors may likely be extended to include a few other conditions (e.g., food quality) that affect differentially the reproductive success of potential prey producing active *vs* dormant offspring. Could they affect the mechanism of induction of the predictive diapause in a similar way?

3. ADVANTAGES AND DISADVANTAGES OF PREDICTIVE AND RESPONSIVE DIAPAUSE

Typically, the predictive diapause is utilised to avoid highly predictable seasonal catastrophes (winter, dry seasons, etc). It is often initiated well in advance of unfavourable conditions, thereby allowing most individuals to enter diapause in time. The optimal time for diapause initiation seems to be one generation time or little more prior to the mean period of cyclical occurrence of harsh conditions in a population of unsynchronised individuals with a short generation time (Taylor 1980; Hairston & Munns1984). The life cycles of species of longer lifespan are commonly attuned to predictable environmental change, such that they may break the one generation time constraint and initiate diapause shortly before the environment deteriorates. Photoperiod or temperature are commonly used to trigger this. Since both environmental cues shift cyclically along with seasonal changes, the values for these signals that may be selected for are those that precede the appearance of any seasonal catastrophe with whatever degree of advancement may be adaptive. However, indirect signals do not inform organisms of the state of the environment at the time, but rather of the typical situation confronted by ancestors at that time of the year in the past. As a result, diapause may be initiated unnecessarily when a catastrophe does not take place incidentally, or else may not be employed in time when lethal conditions occur earlier then usual. Once selected, the strength of response and optimal period of diapause initiation may be adjusted to a new pattern of environmental change in the course of selection primarily (Hairston & Dillon 1990). The role of direct cues (informing of the state of the environment at the given time) in the initiation of the predictive diapause remains ambiguous (Danks 1987). It seems intuitively likely that the more the decision on diapause initiation precedes the period of appearance of hostile conditions, the less reliable the information on (future) chances for surviving the critical period in terms of its offering direct cues, and hence the less likelihood of their being utilised in the mechanism of diapause induction.

Not all environments deteriorate in a cyclical manner. Different strategies of diapause control are utilised to cope with unpredictable changes in environmental conditions: an extra-long diapause (of duration greater than one environmental cycle) (Cohen 1966), spontaneous production or dormant forms regardless of environmental conditions (Alekseev 1990) or the "responsive diapause" as a third choice. The advantage of the third option is its low costs compared to the other two, since it offers the shortest period spent in dormancy. The responsive diapause is initiated when the effects of an unfavourable factor are in progress, triggered commonly by cues directly associated with a selective factor (e.g., alarm signals from injured conspecific prey). This direct response may be modified according to

other information on the present state of the environment, as indicated above. Therefore responsive diapause may be employed exclusively when real danger appears. However, the responsive strategy also has some serious drawbacks. Mobilisation of dormant forms may take considerable time (on account of the nature of the morphological, physiological and developmental transformations required to enter dormancy), such that dormant forms induced by the onset of hostile conditions may not be ready in time if the environment deteriorates abruptly. This mechanism of diapause induction thus seems to be employed in the face of conditions that deteriorate gradually, by organisms of short generation times (capable of fast mobilisation of the dormant forms). Predation may be such a condition. Unlike some terminal abiotic factors which develop abruptly (e.g., frost, flood or fire), predation pressure may develop gradually and as a selective force that is not fatal to all individuals in a population. After all, visually-orienting planktivorous fish seem to prefer older, bigger, more conspicuous prey. Younger, smaller prey individuals are under less intense pressure and may thus have more time to produce resting eggs, before being finally eaten by fish. In consequence, we may expect expression of the responsive diapause in young individuals primarily.

The responsive diapause may be readily confused with quiescence, since both responses display similar symptoms (retarded development), and both are initiated after the environment has actually begun to deteriorate. The retarded development of organisms in quiescence is a consequence of malfunctioning physiology and commonly disappears once conditions improve. In diapause, however, the metabolic retardation is a consequence of an internal mechanism triggered by environmental signals, and may last longer than the unfavourable conditions do.

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