



## On the combined analysis of proximate and ultimate aspects in diel vertical migration (DVM) research

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### Abstract

Although evolutionary ecologists agree that proximate and ultimate aspects are two sides of one coin, they are seldom interested in studies on physiological and behavioural mechanisms at the base of ecological phenomena. Nevertheless, these mechanisms are objects of selection and evolved to realise adaptive significances. This paper is a plea to bring both fields closer together, and, by means of an example of Diel Vertical Migration of *Daphnia*, some proximate and ultimate aspects are discussed. It is argued that light changes, not fish kairomone, is the primary cause for an individual to swim downwards at dawn and upwards at dusk. However, what is called a causal factor might differ when ecosystems or individuals are studied. In addition, causality in ecology is not simple, and has the character of a 'set of necessary conditions'. To illustrate the importance of proximate analyses in DVM, two basic response mechanisms are discussed: Photobehaviour system 1 and 2. The physiological character of these systems leads to a fixed type of migration or to a phenotypically induced DVM, respectively. The adaptive significance of the first might be a reduction of the hazardous effects of UV radiation and of the second a lowering of mortality due to visually hunting predators.

### Introduction

Ecological phenomena should be studied from a proximate and an ultimate point of view. In this paper, we will discuss how these concepts can be integrated in DVM research and why a balanced analysis is needed for real understanding. Specification of the terms is usually given in the simple form of 'how' and 'why' questions. Research on proximate aspects must answer the question of *how* animals migrate; what physiological and behavioural mechanisms animals have at their disposal and how these mechanisms are triggered by causal factors into a DVM. Ultimate aspects have to answer *why* migrations occur: what adaptive significance can be attributed to the phenomenon, what benefits are realised? Confusion of proximate and ultimate aspects seems easily to occur and textbooks caution for this (Krebs, 1972; Alcock, 1989; Krebs & Davies, 1993). There is no problem with the de-

scription of proximate factors and mechanisms and the simple situation of cause and effect in physics is often given as example. It is, however, not that simple in ecology, as we will see. Problems arise from terms like ultimate factors, ultimate mechanisms, causes and reasons. An attempt to clarify thus seems justified.

Contrarily to inanimate nature, processes or behaviour in organisms happen 'for the purpose of' something, they serve a 'function' (Ruse, 1973). In teleological language, behaviour is goal-directed, and in humans (and some higher developed animals), a conscious act might be involved with the (future) goal as cause. Although all animal behaviour might be said to have a goal, this goal is not the cause of the activity. Ethologists, in the first half of the last century, have tried to banish goal-directness from the study of animal behaviour, because anthropomorphisms burdened explanations in animal psychology. Although 'goal' and 'goal-directness' are not com-

monly used in the ecological literature, hidden anthropomorphisms can often be recognised. For instance, when behaviour is ascribed to animals because a supposed adaptive need has to be realised. An example is the statement that ‘organisms balance the risk of predation from various sources against the opportunities of growth and reproduction in order to maximise reproductive output’. This is quite a programme and much proximate research is needed to understand how organisms must realise this.

Since the future cannot act causally on the present, a past or present cause must always be given for an elicited process or behaviour. This is the proximate cause for a physiological or behavioural activity. Nevertheless, reference to the future provides a deeper explanatory understanding of behaviour. A process or a behaviour nearly always serves a function and endows an adaptive advantage on the performer. This function is not a property of the animal, it is not recorded in genes and only indirectly ‘available’. Adaptations are not as easily studied as proximate mechanisms, because results of behaviour must be evaluated in a larger ecological context and on a longer time-scale. Simplification of experimental circumstances and isolation of the object is good strategy for a behavioural analysis, it might be bad strategy for the study of ultimate aspects.

Talking of adaptations is also referring to the evolutionary past. For example, a superior relative fitness of past genotypes with a given trait, might be considered the ultimate cause of the development of a present trait (Ruse, 1973). This sounds like a logical hypothesis, but the problem is that testing is impossible. The ultimate mechanism, responsible for a superior relative fitness, is selection in evolutionary time. We do not really know what happened and must be aware of unduly speculation. Nevertheless, a biology without considerations of the evolutionary history would be very poor. Contemplation becomes less a problem if accompanied by detailed results of quantitative studies of proximate as well as ultimate aspects of a particular phenomenon. This sounds obvious, but proximate and ultimate aspects are almost never studied integrated. It is even worse: in our experience, some editors and referees consider a behavioural analysis the wrong issue to be studied, an anachronism. Attention is completely directed to ultimate aspects, even to such extent that examination of existing knowledge of behaviour seems not to be needed; with assumptions, models can also be made (Iwasa, 1982; Fiksen, 1997). In many studies, behaviour seems to figure, while, in fact, only the result

of behaviour is determined. For example, in a recent *Daphnia* study (Stirling & Roff, 2000), a. o. bimodal depth distributions are used in complicated calculations of behavioural plasticity, genetic variation and trade-offs. We have done many observations on daphnids in experimental cylinders and sometimes observed bimodal distributions. They were often caused by poor experimental conditions (e.g. unsuitable angular light distributions, poor food conditions) and were the result of continuously upwards and downwards swimming of stressed individuals leading to clusters near both ends of the cylinder. Possibly, a similar stress underlies the distributions used for calculations by Stirling & Roff (2000). Since no actual behaviour was studied, the question what caused their distributions remains unanswered.

Proximate, physiological and behavioural mechanisms are products of natural selection and deserve a place in evolutionary ecology. Their interaction with environmental factors determines reaction norms, costs and benefits, thus adaptive values. In future research, a quantitative analysis of the ‘how – why’ interface should be a central theme.

#### **A short outline of diel vertical migration of *Daphnia***

With an example of *Daphnia* migrations in Lake Maarsseveen, our field site for 10 consecutive years, proximate and ultimate aspects will be illustrated. The role of light changes, fish, food and temperature will be discussed.

With the exception of 1990, each year migration started at the end of May and lasted for 6–7 weeks (Ringelberg et al., 1991a). During this period, large shoals of predominantly 0<sup>+</sup> perch (*Perca fluviatilis*), were present in the lake. In daytime, most of them remained in the littoral and sublittoral zone, but after sunset, dispersion over the open water of the epilimnion started. About 90 min before sunrise, *Daphnia* began to move down, and they remained at the daydepth for approximately 17 h. After sunset, the animals ascended. Light intensity changes rapidly during dawn and dusk and correlations exist between the measured descent and ascent velocities and the rate of the relative increases and decreases in light intensity, respectively (Ringelberg & Flik, 1994). In 1990, the beginning of DVM was delayed by nearly a month. Spring was exceptionally cold and the concentration of phytoplankton was low. At the end of June,

temperature suddenly rose and the algae concentration increased rapidly. Within a few days DVM of *Daphnia* started. These field observations suggest that four proximate factors are important for the development of DVM: (1) light changes, (2) fish presence, (3) food concentration, and (4) temperature.

Notwithstanding the coincidence of DVM and large shoals of fish in the lake, the 0<sup>+</sup>perch is not the cause of downwards swimming of daphnids, in our opinion. This might be thought illogical. Especially so, since experiments with planktivorous fish in Tuesday Lake (USA), for example, indicated such role (Dini et al., 1993). After removal of minnows from the lake, *Daphnia* did not migrate anymore. The next year, 6 weeks after the re-introduction of the fish, migration had started again. Therefore, why not call fish presence a proximate, causal factor in this case? To answer this question, we must first discuss causality in ecology, because it is not as downright as it is in classical physics. What we call a causal factor might depend on the biological integration level of the phenomenon which is investigated. At the large scale level of the ecosystem, the arrival of planktivorous fish, naturally, as in Lake Maarsseveen or experimentally, as in Tuesday Lake, can certainly be called the cause for the occurrence of the phenomenon DVM. However, is this also justified at the next lower level in the biological hierarchy, the population of *Daphnia*? Dini et al. (1993) thought of the possible existence of different genotypes of *Daphnia* in the lake. To keep it simple, suppose that two genotypes were present, a migrating and a non-migrating one. Because of the costs involved with DVM, the relative abundance of the migrating genotype was very low in the population before the re-introduction of fish. Therefore, the majority of individuals in the population did not migrate. After the re-introduction of fish, predation rapidly removed non-migrating individuals and the migrating genotype became dominant. The *Daphnia* population now migrated. Planktivorous fish can be held responsible for a shift in genotype abundance, but were not the factor causing DVM at the population level. Let us now turn to the next biological level down, that of the individual. Loose (1993) showed in the Plöner Plankton Towers that in the absence of changes in light intensity no DVM occurred, notwithstanding the presence of fish kairomones. *Daphnia* perceive fish chemicals and are found deeper in the columns of experimental cylinders (Dodson, 1988; Ringelberg, 1991). This response might play a role in depth selection (de Meester & Weider, 1999), but it is not *diel* migration. Because

DVM has a daily rhythm, and is vertically oriented, the causal factor must enable timing and must contain a directional cue. Fish kairomones lack both properties: these chemicals are present for 24 h and distributed over the whole epilimnion as long as fish occur. On the other hand, the light changes of dawn and dusk provide the proper timing cue (Ringelberg et al., 1991) and the typical angular light distribution under water makes vertical plane orientation possible. In the evolution of DVM as a strategy to prevent predation, the properties of light had to be used to make an adequate response possible, because fish signals do not have them. Experiments on the kinetic and orientation aspect of photobehaviour in *Daphnia* have revealed how vertical swimming is realised (Ringelberg, 1999a for review). Fish kairomone is certainly important as a proximate factor, because by its presence, the reaction to changes in light intensity is enhanced. Only then large amplitude upwards and downwards swimming is realised (van Gool, 1998; van Gool & Ringelberg, 1998). For an ecological phenomenon to occur, a 'set of necessary conditions' has to be realised. For migration behaviour, this set consists at least of the environmental factors that were concluded from the field observations. Within the set, changes in light intensity are compulsory, the other factors modify the intensity of the responses. Only few authors recognise this important role of light changes (Forward, 1993; Haney, 1993) and many researchers neglect its role (Dawidowicz et al., 1990; Neill, 1990; Dawidowicz & Loose, 1992; Bollens et al., 1994; Loose & Dawidowicz, 1994). Nevertheless, in laboratory experiments, planned to demonstrate the importance of fish kairomones, periodic light changes were always part of the experimental circumstances. Consequently, vertical displacements also occurred in most controls, in the absence of kairomone. In the presence of fish kairomones, the 'diel vertical migrations' took place deeper in the cylinders and the differences in average depth between the dark and the light periods had increased. Therefore, these experimental results are not in conflict with the statement that light changes cause vertical swimming responses and fish kairomones enhance these reactions. There are, to our knowledge, no experiments that unambiguously demonstrate that fish kairomones alone cause periodical upwards and downwards swimming.

However, considered from an ultimate point of view, predation by visually hunting fish becomes a dominant factor. Leaving the well-lit epilimnion provides the benefit of a diminished mortality but at

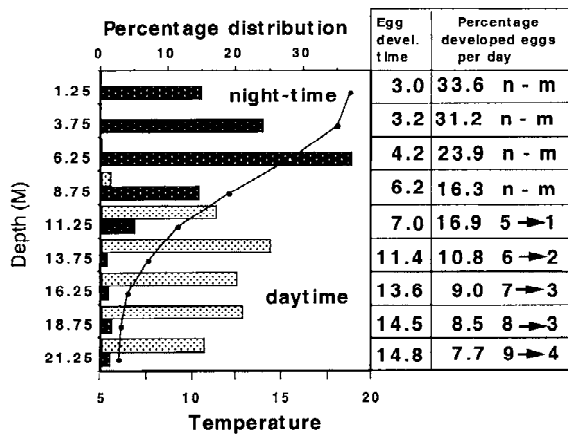


Figure 1. Pattern and consequences of a migrating *Daphnia* population in Lake Maarsseveen on 10 June 1992. The vertical distribution during the day and the night is given in percentages. The curve represents the temperature profile. The first column gives egg development time in days for the temperature at the particular depth. In the second column, the percentage of developed eggs per day is presented for a situation of migration (from a certain depth towards a guessed night-time depth) and for a non-migrating situation (n-m). (Modified from Ringelberg, 1999b.)

the same time introduces costs because of a low food concentration and a low temperature in the hypolimnion. The extent of predation is difficult to measure and good data are absent. Costs, due to a lowered temperature, are easier to calculate and examples are given in Figure 1. For each depth, egg development time was calculated, using the algorithm in Bottrell et al. (1976). Being permanently at a depth of 21.25 m, makes egg development time nearly five times longer than staying near the surface. However, *Daphnia* migrates. In the next column, the fractions of eggs that develop per day are compared, if *Daphnia* remain during 17 h at the daydepth and 7 h at a higher level in the water column. The proposed shifts in night-time depth were guided by the distribution pattern. If we compare the percentage of 7.7% of daily developing eggs for a deep migrating animal (depth 21.25 to 8.75 m) with the 33.6% of a non-migrating animal near the surface, it is obvious that the benefit of DVM must be high enough to compensate for this large difference in costs. Costs are less for individuals migrating from 11.25 to 1.25 m, but benefits might also be less, because they are also exposed to fish predation for a longer period. Nevertheless, it is possible that these individuals are better off. To compare, it would be nice to express both in similar units. The ratio of benefits and costs, calculated for different depths, could serve as a measure of selective advantage. Provided,

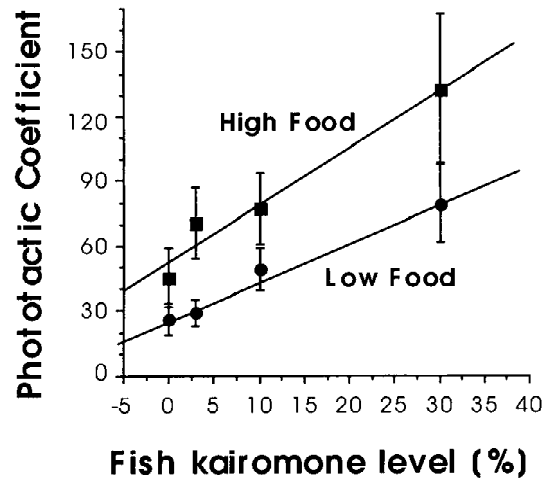


Figure 2. Enhancement of the phototactic reaction, expressed as the Phototactic Coefficient (for explanation, see text), elicited by accelerations in relative increases in light intensity, and influenced by kairomones and food concentration. (Modified from van Gool & Ringelberg, 1998).

of course, that genotypes differ in migration behaviour and segregate over depth. The different depth distribution of allozyme types is an indication for this and the rapid shifts in genotype composition (Spaak & Ringelberg, 1997; Ringelberg, 1999b) indicate that not all types have the same fitness during the short, crucial period. Of course, specific life history analysis must enter the study because genotypes do also differ in age and length at first reproduction (Reede & Ringelberg, 1998) and other characteristics relevant to the problem. Because behaviour of the phenotype is flexible, the possibility to 'choose' an amplitude of migration is available. This also determines whether DVM pays or not at a particular predation pressure and food concentration. Consequently, an individual daphnid must have a proximate 'decision-making' mechanism at its disposal.

### Decision-making

It has been known for a long time, that *Daphnia* reacts to relative increases and decreases in light intensity with phototactic downwards or upwards swimming, respectively (Clarke, 1932). Fish kairomones are not needed and reaction characteristics, like the stimulus strength-duration curve, are not influenced by these kairomones (van Gool & Ringelberg, 1997). If this phototactic swimming, which we might call Photobehaviour system 1 (PBS 1), is used in a model, with

the light increases of dawn as input, a descent of 1.5 – 2 m is the result (Ringelberg, 1999a). It is unlikely that this small-scale DVM is useful for predator avoidance. Several authors have suggested that DVM also prevents damage by UV-B radiation (Ringelberg & Flik, 1984; Siebeck et al., 1994; Rhode et al., 2001), and in lowland lakes, like Lake Maarsseveen, the small-scale migration would suffice. Since UV radiation is nearly always present, and costs of a migration within the limits of the epilimnion are small or absent, we might deal with a constitutive or fixed adaptation. There is another phototactic mechanism, to be called Photobehaviour system 2 (PBS 2), which is stimulated by accelerations in relative changes in light intensity. The intensity of the response is a function of fish kairomone and food concentration (Fig. 2) (van Gool & Ringelberg, 1997, 1998, 2002). Accelerations in light increases are maximum in the beginning of dawn and swimming speeds are high in the presence of fish kairomone. Therefore, high swimming speeds are reached earlier and extend for longer times; thus migration ends much deeper than with PBS 1. Because the behavioural reaction is conditional to kairomone concentration, this large amplitude DVM is phenotypically induced. In Figure 2, the intensity of the response is expressed as the phototactic coefficient (PC), which is defined as the regression coefficient of the function between rates of relative changes in light intensity and the vertical displacement velocity. The faster this velocity increases, the higher is the phototactic coefficient PC. The dependence of PC on kairomone and food concentration has to be analysed further, but it is clear from Figure 2 that with the increase of both environmental factors, also PC increases. At high values, *Daphnia* will swim down fast early in the morning, and the result is a large amplitude migration. On the other hand, if kairomone and food concentration are small, displacement velocity will also be small and not distinguishable from a vertical migration caused by PBS 1 only. The relation in Figure 2 is the expression of a physiological-behavioural ‘decision-making’ mechanism through which an appropriate adaptive circumstance can be obtained. The functionality of the mechanism was selected for in evolutionary time and the criterion for selection was certainly the cost-benefit ratio with fish predation and food concentration as important determining factors. Different genotypes probably have different ‘decision-making’ functions (van Gool & Ringelberg, 1998). If so, shifts in relative frequencies of genotypes with different photobehaviour may provide an additional

source of variation in migration patterns observed in lakes. We are at the beginning of the analysis of proximate ‘decision-making’ mechanisms, not only in *Daphnia* and migration, but in ecology in general (Lima, 1998).

Speculating on the evolutionary origins of behaviour is tricky. Historical narratives are difficult to test and the domain of science is easily left. The development of ecological phenomena, and the evolution of the proximate mechanisms at the base, will have rarely followed a straight course. Evolutionary stories gain credit, the more they are based on knowledge of these proximate mechanisms. The current neglect of physiological and behavioural analysis hampers a balanced development of ecological knowledge.

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