also be the case that a reduction of the antioxidant defence systems would render the older animals more susceptible to free radical-related oxidative stress and, consequently, to cellular peroxidative damage, as indicated by the net increase in the content of toxic aldehydes (MDA) observed as a function of age.

These results are consistent with the general definition of aging as the progressive accumulation of changes which are responsible for the decreased ability of the organism to maintain the homeostatic equilibrium and to adapt to various environmental stimuli[25]. In this view, the increased susceptibility to peroxidative stress observed in aging mussels would affect their capacity to respond to the natural fluctuations of oxygen levels. Moreover, since mussels are able to accumulate in their tissues high levels of environmental contaminants[26], such as metal cations or organic compounds, which are known to stimulate the production of free radicals[10-12,27], the age-related decrease of the antioxidant defence systems would impair the natural protection against the potential toxicity of pollutants.

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## Biophoton emission from *Daphnia magna*: A possible factor in the self-regulation of swarming

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*Summary.* The formation of swarms by planktonic organisms was first described almost 100 years ago, but the mechanisms governing the development of patterns in population size and density are still not understood. In this study, we investigated one biophysical factor that may play an important role in swarm-formation. Spontaneous ultraweak photon emission in the visible range has been well documented for living cells, tissues and individuals in the plant and animal kingdom, including humans. We demonstrate here that the intensity of light emitted by the planktonic crustacean *Daphnia magna* is a function of population density in relation to body size. The effects are discussed on the basis of the theory of Dicke[1,2], and it is suggested that biophoton emission may be a basic factor in the self-regulation of swarm density.
*Key words.* Swarm density; animal distance; body size; photons; self-regulation; *Daphnia magna*.

Pattern formation in time and space represents a fundamental principle in the differentiation and evolution of living systems, and probably of all matter[3,4]. Many physical, chemical and biological factors which may control this process have been studied in the broad range from the molecular to the ecological levels, and many hypotheses have been put forward. One of the phenomena which is least understood, although it has often been

described, is patching and swarming of planktonic organisms. No biological advantage or importance could be suggested so far for swarming behavior [5, 6], but it can be assumed that there is a common interactive force or communicative mechanism. As swarms are formed by a single species, are constant in form, are dense, and behave like a 'super-organism', some cooperativity must underlie swarm formation. A long-distance interaction mechanism is known from biophysical analyses. Spontaneous ultraweak, and evidently coherent, photon emission in the visible range has been documented for a variety of living cells, tissues and individuals in the plant and animal kingdoms, including humans [7]. The intensities of this radiation, ranging from a few up to some hundreds of photons per second and $cm^2$ of surface area, are too weak to be classified as bioluminescence as it is found, for example, in fireflies and in some bacteria.

In our opinion (see also Tembrock [8]) the basic nature of communication between animals includes the regulation of distance. And as some theoretical and experimental work supports the suggestion of a regulatory role of biophotons in living systems [9], we investigated the relationship between light emission and population density in the planktonic crustacean, *Daphnia magna*. Earlier studies had already indicated some long-range interaction between these animals by means of photons [10].

*Materials and methods*

For reasons of biological simplicity and clarity, we chose individuals of the same sex, body size, and developmental stage for the present experiments, a so-called partnership of similarity. Juvenile parthenogenetic females, about 1.5 mm in size, were cultured in 2-l beakers in carbon-filtered and oxygen-enriched tap water at a density of 30 individuals per vial at 20 °C, under a day/night rhythm of bright/dim light. The animals were fed with the unicellular alga, *Scenedesmus subspicatus*, up to the time when they had developed eggs but had not released the neonates. The animals were then transferred to another 2-l beaker without food, at a density of 300 individuals per cup, for 2 h. The subsequent measurements, which took place during the daytime, i.e. from 10.00 to 16.00 h, in September and October, were performed according to the following schedule. Increasing numbers of *Daphnia* females from one to about 250, were put into a 15-ml photomultiplier cuvette made of quartz glass. The population in the cuvette was increased by addition of new animals to those already assayed. After each addition, the photon emission decreased, and it reached a stable value after 5 – 7.5 min. Measurements of the light which was emitted from the animals were made in the dark with an EMI 9558QA photomultiplier operating in the single photon counting mode [11]. During the 6 h of measurements, the animals did not release neonates. The water was changed several times. Control measurements were made using stock water without animals. A full-length description of the experimental conditions and procedures will be published later, but is already available on request from the first author.

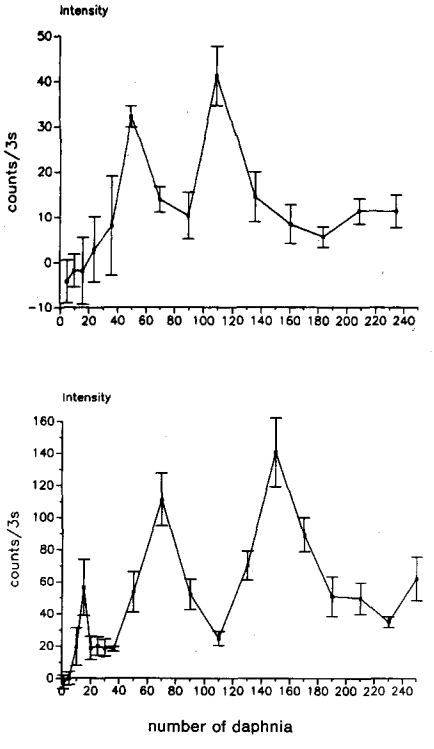
*Results*

The figure illustrates the results of two typical experiments, and table 1 shows the complete set of data obtained in six series of measurements. It can be seen that well-defined maxima and minima of photon emission occur, depending on the population size. The following calculations give, in addition, evidence for a clear connection of this relationship with body size. If we take the average number (56.7) of animals at the 2nd maximum ($N_{max2}$), for instance, the mean distance (d) between the individuals in volume V (15 ml) is, assuming a regularly-spaced distribution and that the animals are spherical:

$$d = \frac{1}{2} d_{max2} = \frac{1}{2} \sqrt[3]{\frac{V}{N_{max2}}} = \frac{1}{2} \sqrt[3]{\frac{15\,cm^3}{56.7}}$$

$$= 3.21 \pm 0.23\,mm$$

where $d_{max2}$ is the mean distance between the centers of the spheres (animals) at the 2nd maximum, and the error given is calculated from the volume error and the SD in table 1. The result corresponds approximately to the body size of the Daphniae (3.25 ± 0.27 mm; see SD in table 1). The relationship between the number of animals




The intensity of photon emission as a function of the number of individuals (results of experiments Nos 2 and 3). The number of *Daphnia magna* in a 15-ml quartz cuvette was increased after each measurement. The diagrams show the mean values and standard deviation of 100 readings 7.5 min after transfer of animals into the cuvette.

Table 1. Relationship between the minima and maxima of intensity of photon emission (I; counts per 3 s) and the number (N; per cuvette and 15 ml) and body size (in mm) of Daphniae as monitored in six experiments. – M = mean of 100 values taken 7.5 min after transfer of the animals into the cuvette; SD = standard deviation. The first maximum and minimum were not identifiable in all experiments.

| Exp. No. | Body size | 1st maximum | | 1st minimum | | 2nd maximum | | 2nd minimum | | 3rd maximum | | 3rd minimum | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | I | N | I | N | I | N | I | N | I | N | I |
| 1 | 3.5 | – | – | – | – | 50 | 118.0 | 93 | 52.7 | 123 | 132.0 | 153 | 72.0 |
| 2 | 3.5 | – | – | – | – | 50 | 32.1 | 90 | 10.3 | 110 | 41.1 | 184 | 5.6 |
| 3 | 3.0 | 15 | 56.4 | 36 | 18.0 | 70 | 111.3 | 110 | 24.9 | 150 | 141.1 | 230 | 35.1 |
| 4 | 3.0 | 15 | 16.3 | 20 | 6.0 | 70 | 48.7 | 90 | 38.7 | 130 | 75.5 | 210 | 42.6 |
| 5 | 3.5 | – | – | – | – | 50 | 41.7 | 110 | 27.4 | 130 | 82.1 | 150 | 47.8 |
| 6 | 3.0 | 15 | 8.5 | 20 | 4.8 | 50 | 30.7 | 70 | 20.9 | 130 | 55.0 | 170 | 30.1 |
| M | 3.25 | 15 | 27.1 | 25.3 | 9.6 | 56.7 | 63.8 | 93.8 | 29.2 | 128.8 | 87.8 | 182.8 | 38.9 |
| SD | 0.27 | 0 | 25.7 | 9.2 | 7.3 | 10.3 | 40.0 | 15.0 | 14.8 | 13.0 | 40.6 | 31.9 | 21.9 |

Table 2. Comparison of the theoretically expected values (T) for the ratio of the mean distances of animals from each other within the minimae and maximae of photon emission, expressed in terms of integer ratios of body size, and the corresponding ratio of the mean distances as calculated from the experimental data (E) according to the formula

$$E = \frac{d_x}{d_{max\,2}} = \sqrt[3]{\frac{\varrho_{max\,2}}{\varrho_x}} = \sqrt[3]{\frac{N_{max\,2}}{N_x}}$$

$N_x$ represents the mean number of animals in the cuvette underlying the minima and maxima of intensity except the (mean) number underlying the peak given in the numerator (SD taken from table 1). $\varrho$ is the density ($d_x$ = mean distances of the centers of the spheres [animals] at the minima and maxima of intensity). M indicates the theoretical value of the mean distance in units of body length between the animals. The minima and maxima are ordered according to their empirical appearance.

| Photon emission | E | T | M |
|---|---|---|---|
| 3rd minimum | 0.667 ± 0.058 | 0.625 = 5/8 | 1/4 |
| 3rd maximum | 0.761 ± 0.072 | 0.750 = 6/8 | 1/2 |
| 2nd minimum | 0.845 ± 0.095 | 0.875 = 7/8 | 3/4 |
| 2nd maximum | Reference | 1.000 = 8/8 | 1 |
| 1st minimum | 1.308 ± 0.238 | 1.125 = 9/8 | 5/4 |
| 1st maximum | 1.557 ± 0.094 | 1.250 = 10/8 | 3/2 |
| 0 Minimum | – | 1.375 = 11/8 | 7/4 |
| 0 Maximum | – | 1.500 = 12/8 | 2 |

and the resulting mean distances of maxima and minima of photon emission from the Daphniae indicates integer ratios of these parameters with the body size (table 2). More details, and data from additional experiments, are given in the Ph. D. Thesis of M. Galle[19].

*Discussion*

The present results indicate a correlation between biophoton emission and pattern formation in swarm populations of *Daphnia*. The physical basis underlying that correlation can be seen in the theory of Dicke[1,2]. In brief, with increasing density and decreasing distance, spontaneously-emitting photon sources undergo couplings, and both coherence and interference phenomena arise. The resulting emission pattern (which is rather complex, and only qualitatively measurable at the moment) is very sensitive to the distance apart of the photon sources and may result in either attractive or repulsive forces. Hence, the Dicke theory allows us to conclude that such forces are involved in the regulation of swarm formation, and even to explain the integer ratios as given in table 2.

We can exclude self-absorption of photons as a cause for the appearance of maxima and minima, because even the highest possible value of self-absorption in the system is too low to account for the differences between the maxima and minima measured (see fig. 1 in Popp et al.[10]). The development of patterns as reported here has also been observed in studies of microaggregations of Daphniae in different laboratories, when the animals involved were of the same sex and of similar size and developmental stage, and at about the same range of densities[12,13]. Even in nature the densities of swarms are in the same range as the density in the cuvette. For example, Colebrook[14] observed swarms of *Daphnia hyalina* in a juvenile stage at a density of 9.1 individuals per ml (the body size of adult females in this species is about 2 mm). Ratzlaff[15] observed dense swarms of females of *Moina affinis* with a density of 284 individuals per ml (the body size of adult females is about 1 mm).

In addition to influencing pattern formation and cooperativity in swarms of planktonic animals and other organisms, biophotons apparently exhibit some regulatory role in cell populations also, as was recently shown for normal and malignant human amnion cells[16,17]. We suggest, therefore, that biophotons might be much more fundamentally and frequently involved in the control of pattern formation in living systems, from cell communities to animal populations, than has been realized so far. There is an increasing number of papers giving evidence for this suggestion, and most recently Tsong[18] and our group reviewed the ways by which long-distance cell-to-cell and organism-to-organism communications might be accomplished by transmission and reception of electromagnetic signals[7]. The work described here suggests a way in which swarming phenomena can be seen in a unified way, in terms of a simple physical principle.

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## Mastoparan induces hypothermia in mice

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*Summary.* The polypeptide mastoparan, isolated from the venom of the Oriental Hornet, *Vespa orientalis*, induces hypothermia in white mice 15 minutes after its intraperitoneal injection. The hypothermic effect is induced by mastoparan obtained from different hornet and wasp venoms. The normal murine core temperature is lowered by mastoparan from 38 °C to as far as 33 °C. This lowering lasts for one hour and is reversible.
*Key words.* Mastoparan; hypothermia; hornet venom.

The Oriental Hornet *Vespa orientalis*, Vespinae, Hymenoptera, is prevalent in the Mediterranean basin as well as in Southeast Asia[1–3]. During the last three decades the contents and pharmacological activities of Oriental Hornet venom sacs have been investigated by several authors[4–10]. The low molecular-weight substances thus far identified in the venom are: 1) volatile compounds, especially ketones, which act as alarm substances[11]; 2) sugars that probably help to fasten the venom droplets to the victim's body[10]; 3) biogenic amines like histamine, 5-hydroxytryptamine, dopamine, adrenaline, noradrenaline or acetylcholine[5,12]; and 4) kinins, which are polypeptides that cause pain and slow contractions of various isolated smooth muscle organs and also raise the vascular permeability[12]. Among the various wasp and hornet kinins isolated to date, one group has lately been identified as mastoparan(s)[13]. Although the various mastoparans present in different hornet and wasp species differ slightly in their amino acid composition, they all induce mast cell degranulation as well as other pharmacological and toxicological effects[13]. From some of the wasp and hornet venoms, several protein components have been fractionated and subsequently identified as enzymes such as hyaluronidase(s), phospholipase(s), trehalase, mono-, di- and polysaccharidases, DNAases, and proteases[7,8,10,12,14–16]. It is an interesting property of hornet venom that shortly after being stung, the victims of a hornet attack usually complain of feeling cold, even if the ambient temperature is as high as 28 °C. The recorded drop in human body temperature in such cases has been of the order of 0.5–1.0 °C. Ishay et al.[17], who studied this hypothermic effect, found that intraperitoneal (i.p.) injection of mice with Oriental Hornet venom sac aqueous extract (VSE) induced in less than an hour a drop in body temperature which, within 3 h, amounted to 8 °C or even 10 °C and was accompanied by a high rate of mortality[17]. Similar results were subsequently obtained in rats, rabbits, cats and dogs following i.p., i.m. or i.v. injection of VSE (Ishay, personal communication). The fall in temperature in all these instances was dose-dependent[17]. However, in mice previously immunized against whole venom, the temperature drop was only by 3 °C to 4 °C and the mice proved to be resistent to $5 \times LD_{50}$ doses of VSE. In the present report we provide evidence that the mastoparan isolated from the venom of *V. orientalis* rapidly reduces body temperature when injected into mice and thus comprises one, if not the only, factor responsible for the observed murine hypothermia. The mode of action whether central or peripheral is discussed.

### *Materials and methods*

Venom of *V. orientalis* was collected from the sting tip by 'milking' the insects. The 'milking' procedure entails extruding the sting with forceps and then applying pressure to the dorsum of the abdomen, whereupon the small drop of venom that appears at the top of the sting is