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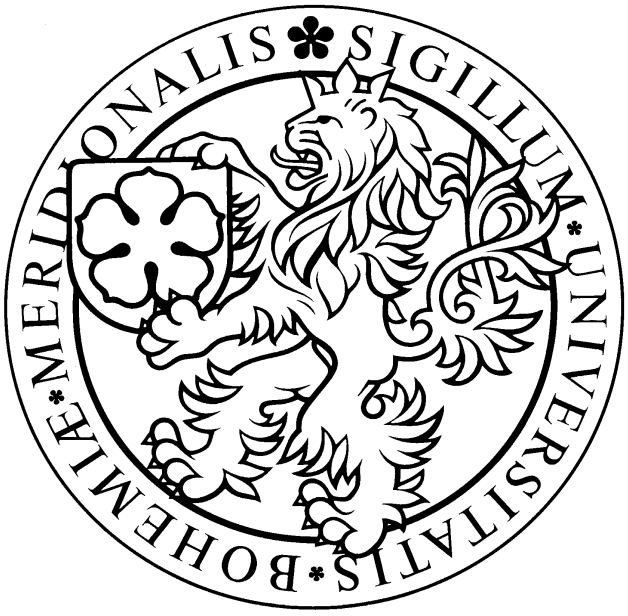


# COLD TOLERANCE OF TERRESTRIAL ISOPOD

MASTER THESIS

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**Annotation**

The woodlice, *Porcellio scaber* (Latreille, 1804), is a terrestrial isopod. Its metabolic reserves and body size are important factors affecting the fitness attributes, such as survival at unfavourable conditions. The larger and heavier individuals did not survive longer than smaller individuals. Amount of glycogen and body weight (fresh and dry) appeared to be an inapplicable parameter in the observed differences among individuals during survival at low temperature. We compared three treatments (long day, short day, natural autumn conditions) of *Porcellio scaber* and found differences in amount of energy reserves and cryoprotectants.

I declare, that I elaborated of my master thesis independently; I used only the adduced literature.

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Kateřina Součková

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# CONTENTS

# I. INTRODUCTION

Crustaceans are a large group of primarily aquatic organisms, consisting of some 35,000 species. Although most crustaceans are marine, many occur in fresh water and a few have become terrestrial. These include pillbug and sowbugs (in Britain woodlice), the terrestrial members of a large order of crustaceans known as isopods (RAVEN & JOHNSON, 2001).

Isopods are perhaps the most intriguing as models of the evolutionary transition from marine to terrestrial habitats. Their ancestors inhabited probably the sea shoreline and had both aquatic and terrestrial characteristics. These characteristics include a primitive water-conducting system and the ability to swim, a mode of locomotion that terrestrial isopods no longer possess (CRAIG *et al*., 2004).

Isopods are poikilothermic, i. e. their body temperature changes, is not controled by internal mechanisms. Their internal metabolic heat production is relatively low (they are bradymetabolic) and as their thermal conductance is high, they are ectothermic. i. e. their internal temperature depends on that of their environment.

## 1. Cold tolerance

Of the many factors that interact to determine the dynamics of arthropod populations, temperature is one of the most fundamental. Low temperature tolerance, by another name cold hardiness, refers to the capacity of an organism to survive exposure to low temperatures. The prolonged periods of low temperatures can pose a threat to the persistence of arthropod populations. During winter, the interaction of an anthropod´s cold tolerance and the temperatures it experiences will be critical in determining its survival and its capacity to develop, reproduce and disperse in the following season.

Cold hardiness in arthropods has been divided into two categories, freezing-tolerant and freezing-susceptible (SALT, 1961):

1. Many freeze-tolerant arthropods synthesize ice-nucleating agents (INAs) in autumn or early winter that initiate freezing at elevated sub-zero temperatures in extracellular areas. Freeze-tolerant insects also synthesize cryoprotectants, including polyols and sugars that have a number of important functions in partially frozen insects and thermal hysteresis (antifreeze) proteins that are thought to inhibit secondary recrystallization when the insects experience higher temperatures prior to the thaw in spring.

2. Freeze-susceptible insects usually remove or mask potential nucleators in the gut in autumn (whether food particles or ingested ´motes´; i.e. dust taken in with food) and then synthesize organic molecules (polyols, sugars) in the haemolymph which further depress the SCP[1] of body fluids, and antifreeze proteins, which stabilize the supercooled state. When insects can surive for prolonged periods at low sub-zero temperatures, the freezing temperature (SCP) can be used as a measure to their cold hardiness.

   In freeze susceptible species, the lower temperature (above SCP) causes chill injury, which can be reversible when the cold exposure did not persist too long (NEDVĚD, 2000).

The terrestrial isopod *Porcellio scaber* is susceptible to subzero temperature: both freezing and chilling were injurious (TANAKA & UDAGAWA, 1993).

The temperature can be dangerous even if it does not fall bellow 0°C. Metabolic reactions slow down and almost stop during adequately low temperature. If it is short-term action it need not be harmful.

Other important factors, which have large effect on cold tolerance, are fluctuation of temperatures during day and during year. The level of cold hardiness of *Porcellio scaber* against chilling and freezing showed different patterns in their seasonal variation. The lower lethal temperature causing 50% mortality, an indicator of the tolerance to chilling, ranged from -1.37°C in August to –4.58°C in December. The whole body supercooling point, the absolute limit of freeze avoidance, was kept about –7°C throughout the year. The winter decrease in lower lethal temperature was concomitant with an accumulation of low molecular weight carbohydrates which are possible compounds protective against chilling injury, whereas the less seasonally variable supercooling point seemed to be associated with the year-round presence of gut content. Food derivates may act as effective ice nucleators. The different trend in seasonal changes between lower lethal temperature and supercooling point may be related to the microclimate of the hibernacula in subnivean environments, where the winter temperature became lower than the lethal temperature in the summer active phase, but remained higher than the summer supercooling point (TANAKA & UDAGAWA, 1993).

Period (duration) of exposure at low temperature may play also important role. The excessive periods of low temperature in winter could lead to threat of population.

We also should take into consideration depletion of metabolic reserves or starvation. Starved arthropods have lower SCPs than fed animals (DAVENPORT, 1992), which was

[1] SCP – supercooling point

confirmed by LAVY *et al.* (1997), but they also determined that starved individuals of *Porcellio scaber* had lower cold tolerance than fed ones.

**2. Body size**

Body size has been considered traditionally a key determinant of organism´s ecological and physiological properties (KLINGENBERG, 1997). Body size together with energy reserves is important factors, which influence condition of crustaceans, success in reproduction and survival. Intraspecific body size variation is largely nongenetically determined (ALCOCK, 1984), being primarily influenced by food intake and temparature during development of the immature stages (JULIANO, 1985).

The relationships among environmental temperature and adult body size have intrigued scientist for over a century, but a rebirth of interest in the last decade has been fueled by the discovery of widespread patterns in diverse taxa. Species distributed over broad geographic ranges often exhibit thermal clines in body size, with the majority of species exhibiting larger adult size in colder environments (PARTRIDGE & FRENCH, 1996). This geographic variation in body size is consistent with the intraspecific version of Bergmann´s rule.

Bergmann´s rule (1847), raising heat maintenance with a bigger body size to surface area ratio, has frequently been used to explain increased body size with latitude (colder environments) in endothermic animals. This hypothesis is not significant only for endothermic animals, but also several authors agree that terrestrial ecthotherms, especially arthropods, also tend to be larger in colder environments (Fox & Czesak, 2000). This thermal plasticity of body size – dubbed *the temperature-size rule* (TSR) – has been observed in many animals, making it one of the most taxonomically widespread "rules" in biology (ANGILLETTA *et al*., 2004).

This principle was for example detected by BOCHDANOVITS & DE JANG (2003), where adults of *Drosophila melanogaster* in the tropics were smaller than individuals in temperate areas. Also, adults of *Coccinella septempunctata* hibernating at higher altitudes on mountains were larger on average than those hibernating at lower altitudes (HONEK, 1989).

When two populations of bug *Pyrrhocoris apterus*, one from České Budějovice (49°N, 380 m a.s.l.), and the other from Bulgaria near Sofia (42° 45´N, 600 m a.s.l.) were compared, differences in weight and in body size were detected. Individuals from Bulgaria population were significantly smaller and more sensitive to low temperature than individuals from the Czech population (KALUSHKOV & NEDVĚD, 2000). An additional example may be the Myrmicinae species *Leptothorax acervorum* (FABRICIUS, 1793) that was among the ants

ranging farthest north in both North America and Euroasia. Workers from 3 populations living in areas with greatly different climates differed in cold hardiness and individual size. Workers from Cape Kindo on the shore of the White Sea (66° 33′ N) survived best at low temperatures and were significantly larger than workers from Nuernberg Reichswald (49.5° N), with workers from the St. Peterburg area (60°N) lying between the two extremes (HEINZE *et al*., 1998).

CRUHMAN *et al*. (1993) found that the body size of worker castes of ant species increased significantly with increasing latitude in Europe. Arctic bumblebees were noted for their large size and hairy appearance, adaptations that reduce radiative loss of heat generated by muscular activity in preparation for flight (STRATHDEE & BALE 1998). Ant colony temperature is often elevated above that of the surrounding environment.

Altitudial cline was also found in the body size of some freshwater crustaceans (FRANCE 1992). The first report of this trend in terrestrial isopods *Porcellio laevis* in northern Chile was detected by LARDIES *et al*. (2004). Indeed, smaller woodlice inhabit low altitudes and larger woodlice inhabit high altitudes.

Similar rule was valid for ectothermic vertebrates. The population of *Rana latastei* from cold foothills in Italy showed higher growth rate at the same laboratory temperature than population from lowland, however, differnce in body size was not discovered (FICETOLA & DE BERNARDI, 2005).

ASHTON & FELDMAN (2003) evaluated Bergmann's rule in two groups of reptiles: chelonians (turtles) and squamates (lizards and snakes). They performed both nonphylogenetic and phylogenetic analyses and showed that chelonians followed the Bergmann's rule (19 of 23 species increase in size with latitude: 14 of 15 species decreased in size with temperature), whereas squamates followed inversed Bergmann's rule (61 of 83 species decreased in size with latitude; 40 of 56 species increased in size with temperature). Another example is the body size of females of *Testudo hermanni* in Greece which grows up with latitude and altitude (WILLEMSEN & HAILEY, 2001).

Conversely, there also exist studies of opposite trend, for example negative correlations between body size and latitude occured in many Lepidoptera (NYLIN & SVARD, 1991).

According to other reviews, Arctic invertebrates are typically smaller and structurally simpler than related temperate species. Specific examples include *Tipula arctica*, whose body length decreases by approximately 4.5% for each 10° increase in latitude in Greenland. In aphids, the same clone has less numer of generations at high latitudes than in temperate regions, which effectively reduces the body size attained (STRATHDEE & BALE, 1998).

In the last decade, both theorists and empiricists have responded to the challenge of identifying the causes, and both nonadaptive and adaptive explanations have been offered (ATKINSON & SIBLY, 1997).

Nonadaptive theories describe how the effects of temperature on biochemical processes can give rise to the observed temperature-size relationship. Such theories, before they can be generalized, must also describe how individual species are able to circumvent these physical constraints on growth and development. Adaptive theories use the costs and benefits of particular life histories to describe why, in most species, natural selection favors genotypes that grow faster but mature at a smaller size when raised at higher temperatures. Because such diverse arrays of organisms are involved, biologists have mainly considered simple, univariate explanations which are seemingly more general than complex, multivariate ones (ATKINSON, 1996).

CUSHMAN and colleagues (1993) concluded that the most plausible explanation of the trend of increasing body size with latitude in ants was the reduced risk of starvation in seasonal and/or unpredictable environments. This is based on the idea that energy reserves increase with size faster than metabolic rate, thus, in a seasonal or unpredictable environment large-bodied species would be able to survive longer periods of unfavorable conditions before succumbing to starvation.

Another hypothesis that could explain reduced size is that with restricted primary productivity at low temperatures: growth is slow, which results in a trade-off between large body size and generation time. Extended generation time increases the risk of mortality, as well as reducing the intrinsic rate of increase. This would be of particular importance when a particular life stage (e.g. eggs or pupae) has to be reached to enable survival of low temperatures in winter or when the risk of mortality is high. With increasing climatic severity, body size and fecundity should decrease until the disadvantage of reduced fecundity balances the increased mortality accompanying a lengthening of development which may take several years to complete one generation (MacLean, 1975). Tentative evidence to support this hypothesis is presented by Nylin & Svard (1991).

We assume that larger individuals within a given species live in colder climate at higher latitudes because larger individuals will be capable of storing larger quantities of energy to withstand the longer periods of adverse weather condition. Small animals can not afford to carry the lipid reserves which allow larger animals to cope with short term shortage and low temperature.

Initial body weight of *Alphitobius diaperinus* was positively corelated with survival at 10°C (RENAULT *et al*., 2003), while mortality was probably a result of exhausted energy reserves. Water mass in the body also played an important role. Also smaller adults of *Coccinella septempunctata* at higher altitude sites are more likely to die during the overwintering period (ZHOU *et al.*, 1995).

**3. Metabolism and energy reserves**

Temperature is one of the most important environmental factors determing life-cycle events in ectotherms, because of its direct influence on metabolism. It has been demonstrated that metabolic rate per unit of body mass is higher in the smallest individuals, and it has been concluded that metabolic rate is related to size (SCHMIDT-NIELSEN, 1984). The largest individuals of *Alphitobius diaperinus* were better able to conserve energy reserves during exposure to cold (RENAULT *et al*., 2003). Conversely, the ability to reduce metabolic rate is a survival strategy of many animal species (STOREY & STOREY, 1988).

Several studies on terrestrial arthropods have shown increased metabolic rate correlated with increased elevation (altitude) (ADDO-BEDIAKO ET AL. 2002). These studies appear to support the metabolic cold adaptation hypothesis, which states that in cold environments, ectotherms compensate for prolonged low temperatures by elevating metabolic rate compared with their counterparts from higher temperatures (HODKINSON, 2003). In contrast, LEE AND BAUST (1982a, 1982b) and NYLUND (1991) found no evidence of such a correlation in two Antarctic terrestrial arthropods.

Accordingly, energy reserves are used as source of energy during periods of disadvantageous conditions, for example during dry summer or cold winter. Heavier insects with large fat reserves have a significant advantage for survival at low temperature but they can die prematurely if their reserves are already exhausted before beginning of cold period (RENAULT *et al*., 2003). Importance of energy reserves was also exhibited by females of *Coccinella septempunctata*, which were larger and heavier, and contained more fat than males. Adults hibernating at higher altitudes and away from their breeding and feeding habitats had significantly more fat than those hibernating at lower altitudes. Fat reserves were reduced by 30% during the hibernation at the top of the Krkonose Mountain, in the Czech Republic, where temperatures were much lower, but more than half of the fat reserves were consumed during the overwintering period at the other sampling sites (ZHOU *et al.*, 1995).

High quantity of energy reserves allows surviving low temperature for long time. DOWNES suggested that exposure to low air temperatures during winter may reduce the requirement for

nutritional reserves, for instance, pupae of *Byrdia groenlandica* can survive for several years before the adult emerge. On the other hand, species as *Anopheles occidentalis*, which can survive the severe prairie winter without any nutritional reserves also exist (STRATHDEE & BALE, 1998).

Lipids and sugars, and sometimes proteins are the most important energy reserves. Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their insolubility in water. Fat and oil are the principal storage forms of energy in living organisms. They are highly reduced compounds, derivates of fatty acids.

The polyols and sugars (low molecular weight ´antifreezes´) can have function other than cryoprotections – they tend to reduce water loss and protect organism against desiccation damage (vital in inactive arthropods which are not eating or drinking); they can also be important energy reserves. Polyols may then help to stabilize proteins and membranes at low temperatures (KALUSHKOV & NEDVĚD, 2000). An accumulation of trehalose and myo-inositol was found in the terrestrial isopod *Porcellio scaber* (TANAKA & UDAGAWA, 1993).

The glycogen is a main storage polysaccharide of animal cells. It is a polymer of (α1→4) – linked subunits of glucose and is especially abundant in the liver, where it may constitute as much as 7% of the wet weight, but it occurs also in skeletal muscles. Among all sugars detected, trehalose was the only one that accumulated in cold-acclimated crustaceans (ISSARTEL *et al*., 2005). The carbohydrate antifreezes from hemolymph of amphipod crustacean (*Gammarus lacustris*) effectively decreased the freezing point of water solutions and diminished the size of ice crystals formed (ANDREEV & PETROPAVLOV, 1996).

Proteins are complex molecules, which are among the most abundant biological macromolecules and are also extremely versatile in their functions. Alanine, arginine, leucin and glycine are the major FAAs [2] found in crustacean hemolymph and, together with other amino acids, are involved in several metabolic processes including protein synthesis/catabolism, gluconeogenesis and oxidative pathways (GRANEY & GIESY, 1986).

## 4. Respiration

The mechanisms that are involved in gas exchange between the blood of an animal and the environment, or the mechanisms that are involved in the transport of gas within body are known as respiration of vertebrates. The blood (hemolymph) of insects doesn´t have a respiratory (oxygen carrying) function. It can be far more viscous than the blood of other animals, thus allowing a more pronounced nutritive role (DAVENPORT, 1992).

[2] FAAs – free amino acids

The temperature determines the respiration rate. The oxygen consumption of crustaceans is influenced by environmental and other factors.

Terrestrial crustaceans have similar respiration organs as arachnids, different from those of insects. In Porcellionidae, two pairs of white, oval structures are apparent on the first two abdominal appendages (pleopods). Desert forms have five pairs. Pleopods contain pseudotracheae, which trap air and give the pleopods their white appearance. In addition to using this source of oxygen, terrestrial isopods breathe by diffusion of gas directly through the cuticule. Hemolyph is involved in oxygen circulation.

In general, similer sized animals were selected for experimental studies but in some case the groups of animals in each flask were of different sizes. This was not regarded as introducing a serious error since it is known that body size within the range used to has rather little effect on the weight-specific respiration rate of *Porcellio scaber* (WIESER & PYE, 1974).

The isopods *Porcellio laevis* from the highland had a higher $VO_2$ [3] than those from lowland habitats for almost all measured temperatures (12, 18 and 25°C), except at 5°C. They concluded that differences in $VO_2$ between the populations contributed to the difference in reproductive output by woodlice from cold and warm habitats (LARDIES *et al*., 2004).

**5. Transpiration**

Water loss by transpiration is one of the most important physiological factors affecting the survival and distribution of woodlice (EDNEY, 1968). Most terrestrial isopods are quite sensitive to desiccation and have to seek out sufficiently moist shelter to survive (WIESER, 1984). The cuticle of land isopod is more permeable than that of insect (WALLWORK, 1970), and it was previously believed that a water-proofing mechanism, an orientated layer of lipid molecule, is lacking in isopods (BEAMENT, 1961).

Measurements of transpiration rates of several species of woodlice at various temperatures have demonstrated a sharp increase at higher temperatures (NAIR *et al*., 2001). The water-proofing barrier ability of cuticle is probably reduced at higher temperatures of 40 and 45 °C in *P.scaber* (NAIR *et al*., 2003).

**6. Hemolymph and osmolality**

Hemolymph is the circulatory fluid of certain invertebrates, analogous to mixture of blood and lymph in vertebrates. Among its general functions are circulation of nutrients, gases and water to and from organs, and stabilizing body temperature and pH to maintain homeostasis.

[3] $VO_2$ – volume of oxygen

Cryoprotectants are substances that are used to protect biological tissues from freezing injury. Traditional cryoprotectants are for example glycols, glycerol, trehalose and proline.

Osmosis is the unmeditated process in which solvent molecules pass through a semipermeable membrane from a solution of lower concentration to a solution of higher concentration. Osmotic pressure is the pressure that stops the net flow of water across the membrane. The force generated by osmosis may be considerable.

The concentration of hemolymph solutes in terrestrial arthropods is important in providing an appropriate environment for cellular and biochemical function (HADLEY, 1994). It also determines vapour pressure deficit, and therefore water loss, and can indicate compounds associated with cold tolerance (LEE, 1991).

Arthropods in polar environment (and winter-acclimated temperate arthropods) typically show significant deviation from these "normal" haemolymph osmolalities (ZACHARIASSEN, 1985). This is often as a result of the accumulation of some cryoprotectants, for example sugars, glycerol and amino acids. In freeze-avoiding species, these cryoprotectants act by colligatively depressing the melting point of the hemolymph, and therefore the freezing point of the whole animal, as well as by stabilising membranes and biomolecules (LEE, 1991). In freeze-tolerant species, they seem to function as molecular stabilisers.

Since hemolymph organic nucleators are products of metabolism, starvation is likely to result in reduced concentrations of nucleating agents (DAVENPORT, 1992). For *P. scaber*, a decreased hemolymph osmolality was found in starved animals compared to fed ones (LAVY *et al.,* 1997).

## 7. Thermal behavior of crustaceans

Organisms capable of locomotion avoid extremes of temperature, although what is 'extreme' depends on the species and its evolutionary thermal history (LAGERSPETZ & VAINIO, 2006).

Many invertebrates such as terrestrial isopods can acclimate to a slow and progressive reduction in temperature (EDNEY, 1968; SUTTON, 1980) but are susceptible to a rapid drop in temperature, which can cause significant mortality (BRONDY *et al*., 1983). There are many ways how to avoid extreme temperatures.

The first type is migration, the movement of animals from one area to another. Survey of *Porcellio scaber* in Europe (LAGERSPETZ & VAINIO, 2006) shows that individuals often travel into trees in the summer and back to the soil in the autumn. This behavior is common to many other terrestrial isopods.

The second type is basking, which is not typical for isopods. *P.scaber* was mostly active at night and early hours of the morning before sunrise, like most other species of woodlice (CLOUDSLEY-THOMPSON, 1977). When temperatures during the day become too high, terrestrial isopods generally move quickly to underground hiding places, where higher humidity can help the animals to avoid desiccation.

The third type is huddling or clustering behavirour which is important in permitting survival in cold conditions. Contact-seeking behaviour (positive thigmotaxis) is common in walking and benthic species like crayfishes and isopods (MUNDAHL & BENTON, 1990; LAGERSPETZ & VAINIO, 2006). Individuals of *Porcellio scaber* have well-developed pseudotracheae to reduce moisture loss from their surfaces, but also aggregate into very tightly packed clumps, which further reduces moisture loss (ALLEE, 1926). When temperatures rise to 20–30°C, woodlice apparently become attracted to the odors of conspecefics and group together. The bunching behavior decreases the exposed surface area of each individual. If necessary, woodlice are able to take up water through abdomen projections and transport it along lateral, exterior grooves (collectively called a water transport system) to the mouth.

The final type is sheltering behavior, which can be defined as actively seeking to avoid adverse environmental conditions (HASSALL, 2007). *Porcellio scaber* consistently used the artificial refugia more than other species of isopods, which can be predicted from its dorsoventrally flattened morphology, enabling it to clamp against flat surfaces as a defense against predators (HASSALL, 2007).

For many animals, the trade-off between time alocated to sheltering and time spent feeding has important fitness consequences. How long an animal can spend sheltering also depends on its digestive strategy (HASSALL, 2007). Isopods have a very straight alimentary canal, but are able to vary gut throughput time widely. They can either feed semi-continuously, filtering soluble components through the proventriculus into the midgut caecae (HASSALL, 1977), or retain food for up to several days in the hindgut, where it is digested more extensively, partly by microorganisms, while the products of digestion are conducted to the midgut via the typhlosole channels (ZIMMER & TOPP, 1998). Isopods are coprophagous (HASSALL & RUSHTON, 1982), reingesting feces that have accumulated in their shelter sites, undergoing futher microbial digestion and thus acting as an "external rumen"(MASON & ODUM, 1969).

This versality in digestive strategies enables all terrestrial isopods to be very flexible in the amount of time they spend sheltering. This may have helped to make them the most successful group of Crustacea to have invaded the terrestrial environment, where they are

subjected not only to higher risks of desiccation but also to a much more widely fluctuating thermal enviroment (HASSALL, 2007).

Crustaceans must rely on behavioural thermoregulation, on thermal acclimation capacity and on the adoption of dormant or resting stages to survive temperature extremes. The resting stages of some crustaceans are extremely resistant: the cysts of *Artemia salina*, which are early embros covered with a chitinous shell, can survive extreme dryness (a vacuum of approximately 1 Pa pressure) and a minimum temperature of approximately -190°C (WHITAKER, 1940).

According to ACHE (1982), no specific thermoreceptors are known in crustaceans, and none have been detected since then. However, Herter (LAGERSPETZ & VAINIO, 2006) mentions some early observations suggesting that the antennae of terrestrial isopods are sensitive to local heat stimuli to air, in contrast to the antennae of crayfish. The sensitivity of the antennae of terrestrial isopods to temperature may depend on local water loss from these thin-walled structures which probably contain mechanosensory neurons and are therefore also humidity sensitive. Another possibility is that the flagellae of antennae, e.g. in *Porcellio scaber*, carry specific temperature sensitive neurons, which respond to evaporative heat loss and therefore also to humidity (SUTTON, 1980).

## II. SPECIFIC AIMS

The aims of my master thesis was to determine whether amount of energy reserves of adult individuals of terrestrial isopod *Porcellio scaber* influences survival duration at low constant temperatures (+3°C, 0°C). My hypothesis was that bigger individuals (expressed as fresh and dry weights) have better cold tolerance than smaller individuals, because they had better input

of nutrients in period of growth and more suitable conditions which allowed growing to bigger size and accumulation of metabolic reserves (proteins, lipids and sugars). Bigger individuals should have considerable nutrient reserves, from which they would take necessary energy and compounds during chill coma, when they can not ingest food. Small animals are not able to accumulate so much reserves which allow larger animals to cope with short term shortage and low temperature.

Other intention was to compare amount of energy reserves (proteins, lipids and sugars) and several ecophysiological parametersof three groups of *Porcellio scaber* from three different treatments: summer conditions (long day, high temperature), intermediate conditions (short day, high temperature), and winter conditions (short day, low temperature). We assumed that glycerol and osmolality may play a key role in adaption of the *Porcellio scaber* to winter cold.

# III. MATERIAL AND METHODS

## 1. Experimental animals

Individuals of population of *Porcellio scaber* (Latreille, 1804) (Crustacea, Isopoda) from České Budějovice, South Bohemia, were reared in the laboratory. We reared and subsequently measured three groups at different conditions. The first treatment, LD (Long Day, 18:6 hours of light:dark) and the second treatment, SD (Short Day, 12:12 hours of light:dark) were reared at 25°C. The third treatment, NP (Natural Population) were individuals (originaly part of LD group) which were transferred in the middle of September to outdoors natural conditions (shortening day and decreasing fluctuating temperature) and in early December they were transferred to cold room at 5°C, continuous darkness (Tab. 1.).

**Table 1:** Treatments of *Porcellio scaber* used in experiments.

| | Code | Place | Temperature | Photoperiod | Humidity |
|---|---|---|---|---|---|
| 1. | **LD** | lab | 25°C | 18:6 | 75% |
| 2. | **SD** | lab | 25°C | 12:12 | 75% |
| 3. | **NP** | outside | autumn (2006) | natural | natural |

All populations were bred in plastic boxes 30×20×15 cm, with moist soil, sand and pieces of wood. Food was prepared from dried ground beech leaves, dry biscuits, fruits and vegetables (for example apple and carrot).

The sexes can be recognized as follows. Male isopods have the first and second pairs of pleopods transformed into mating organs (Fig. 2.). In all experiments, we used only males to avoid variability in females connected with gravidity. The gravidity of females would influence body size and other parameters of individuals including cold tolerance.

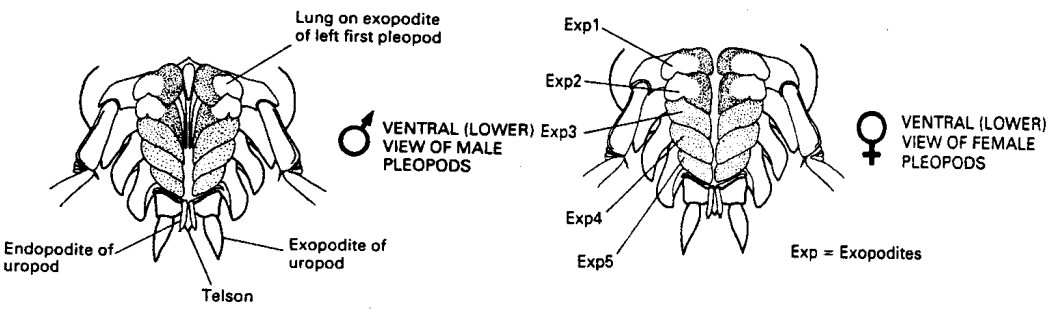


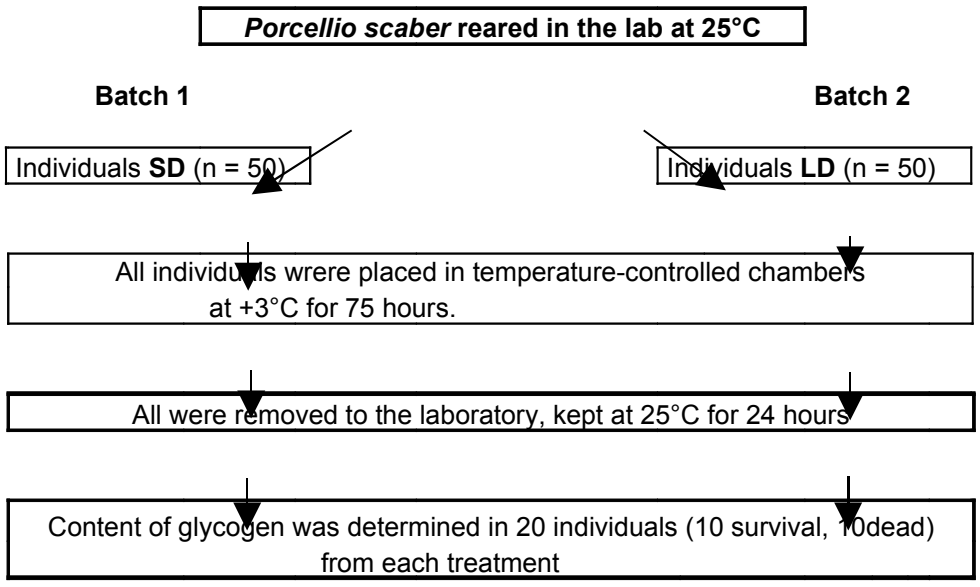

**Figure 2:** Sexually different external features of a woodlouse (*Porcellio scaber*).

## 2. Cold tolerance

To evaluate survival at low temperature, groups of 50 animals from SD and LD treatments were put into small glass Petri dishes with a piece of filter paper, which was wet (moistened with 600µl $H_2O$), because *Porcellio scaber* is sensitive to humidity; and placed in temperature-controlled chambers at +3°C. It was previously detected that the lethal time for 50% ($Lt_{50}$) of both treatments (SD, LD) was about 75 hours at +3°C. After 75 hours, the dishes were removed, and kept in the laboratory at 25°C for 24h. The estimated survival was based on the number of animals able to walk after 24 hours (Tab. 2).

To evaluate survival at even lower temperature, 70 animals from LD treatment were individually put into small glass Petri dishes with a piece of filter paper, which was wet (moistened with 400µl $H_2O$) and placed in temperature-controlled chamber at 0°C. It was previously detected that the lethal time for 50% ($Lt_{50}$) of this group (LD) was about 48 hours at 0°C. After 48 hours, the dishes were removed to the laboratory, and kept at 25°C for 24h. The estimated survival was based on the number of animals able to walk after 24 hours.

**Table 2:** Experimental design of the protocol used to determine the importance of photoperiod on the cold tolerance and reserves depletion of adult of *Porcellio scaber*.



**3. Body size**

Adults were individually weighed before the begginning of any experiment (fresh weight, FW) using a Kern GR-202® micro balances with accuracy of 0.1 mg. Dry weight (DW) was measured after drying dead adults at 50°C for 24 hours in a closed desiccator filled with natrium hydroxide. Water weight (fresh weight–dry weight) and water ratio (Wr = [fresh weight-dry weight]/fresh weight) were calculated for each individual.

To characterize the body size of individuals of *Porcellio scaber* we used several measurements of their weights: fresh weight before exposure at experimental temperatures (initial fresh weight, FW1), the second fresh weight (FW2) after the exposure at experimental low temperatures (+3 or 0°C), which were measured immediately in cold chamber at 5°C, before transfer to 25°C; dry weight (DW), water weight (WW) and water ratios (Wr 1 and 2) based on either initial or final FW. Content of glycogen were determined in 20 individuals (10 survived and 10 dead) from each treatment (SD, LD) randomly took out from the group, determined using method described below.

**4. Measurements of energy reserves**

*Protein determination – BCA method* (STOSCHECK, 1990)

1. measure FW and DW of each individual,
2. homogenize a sample in 400µl of extraction buffer (with 1.74g $K_2PO_4 \cdot 3H_2O$ and 0.66g $KH_2PO_4$) during 1 minute,
3. clean homogenizer (piston) by 100µl of buffer and it add to the sample,
4. pipet 15µl of the sample into Eppendorf tube, place it on ice,
5. add 1100 µl of the reagent (BCA-1, Sigma) and mix well,
6. incubate test tubes 2 h at room temperature,
7. measure the absorbance in spectrophotometer at 562 nm.

This method was also used to measure proteins in the haemolymph. From cut-off antenna of adults, 2 µl of hemolymph were collected on parafin paper. Standard calibration curve was obtained using solution of bovine serum albumin.

*Lipid determination*

1. measure FW and DW of each individual,
2. homogenize the sample with 400 µl HIP (a mixture of hexane:isopropanol = 3:2) during 1 minute,
3. centrifuge the tubes at 13 000 RPM for 5 minutes,
4. pipet the supernatant to second clean tube, dissolve the pellet again in 500 µl HIP, centrifuge again, and pool the supernatants,
5. damp the HIP-samples dry with nitrogen gas at 80°C,
6. add 300 µl $H_2SO_4$ and heat the tubes in the water bath during 10 minutes at 100°C,
7. cool the samples in cold water,
8. add 1000 µl of colouring reagent vanilline (0.198 g vanilline with 66.8 ml. $H_3PO_4$ and 33.2 ml $H_2O$), place the tubes in the stove at 40°C for 15 minutes,
9. measure the absorbance at 540 nm.

*Sugars and polyols determination*

**Extraction:**

1. measure FW and DW of each individual,
2. homogenize sample in 400 µl of 70% ethanol in 1.5 ml tube using homogenizer,
3. shake at 1 200 RPM for 5 min,
4. spin at 13 000 RPM for 10 min., collect supernatant S1,
5. rehomogenize pellet in 400 µl of 70% ethanol,
6. shake at 1 200 RPM for 5 min.,
7. spin at 13 000 RPM for 5 min., collect supernatant S2,
8. pool S1+S2, label sample,
9. store at -20°C.

**Derivatization:**

1. **purification**: add to the sample 200 µl of hexane, mix by Vortexing and leave the phases to separate; remove the hexane phase and put the rest into a 1 ml derivatization vial; add internal standard (xylitol, 10 µg), dry under nitrogen stream at 50°C (during drying, 10µl of dichloromethane could be added to improve the drying process),
2. **oximation**: add 30µl of DMF (dimethylformamide, dried) and 25µ/l µl PY), close tightly, mix by Vortexing, keep in oven at 80°C for 30 min.,

3. **silylation**: cool to room temperature, add 70µl of DMF (dried) and 30µl of 1-(trimethylsilyl)imidazol (TSIM or TMSI, Aldrich), close tightly, mix by Vortexing, keep in oven at 80°C for 30min.,
4. **final extraction**: cool to room temperature, extract in 100µl iC8 (isooctane, dried), use 1µ of the upper (iC8) layer for injection into GC/FID.

**Analysis (GC/FID)**

Column:DB1, 30m, 0.25 mm, 0.25µm

Pressure:18 psi

Flow: 30 cm/s (120°C)

VM: 1.66 min

Temperature program: 110°C/120 min, ↑300°C, DET=320°C, INJ=280°C

Split: 15ml/min

*Glycogen determination*

1. measure FW and DW of each individual,
2. homogenize a sample in 500µl of extraction buffer (with 1.74g $K_2PO_4 \cdot 3H_2O$ and 0.66g $KH_2PO_4$) during 1 minute,
3. clean homogenizer by 100µl of buffer and it add to the sample,
4. remove 200 µl of the sample ana add 300 µl KOH (2 simultaneous measures of one individual),
5. warm up to 100°C, 15 min,
6. shake at 5 000 RPM for 10 min.,
7. add 50 µl saturated solution of $NaSO_4$ and 1 ml ethanol,
8. shake at 2 000 RPM for 15 min.,
9. twice clean pelet by 200 µl $H_2O$,
10. resedimentation with 400 µl ethanol,
11. shake at 2 000 RPM for 15 min.,
12. dissolve the pelet in 100 µl $H_2O$ add 1100 µl anthron-reagent (anthrone 0,14g, deionzed water 26,7 ml and 63,3 ml $H_2SO_4$),
13. warm up to 90°C, 15 min,
14. cool the samples at the room temperature,
15. measure the absorbance at 620 nm.

**5. The respirometric experiments**

In 1884, Van´t Hoff observed that the rate of chemical reactions tends to double for each 10°C increase in temperature. The rates of biochemical reactions are equally affected by temperature, and so we used widely the following $Q_{10}$ relationship to describe the effects of temperature on metabolic processes.

$$Q_{10} = (R_1/R_2)^{[10/(T1 - T2)]}$$

Where $R_1$ = metabolic rate at temperature $T_1$, and $R_2$ = metabolic rate at temperature $T_2$.

The oxygen consumption of two groups (SD, LD) of *Porcellio scaber* acclimated to 25°C was measured by manometric method. The woodlice were taken from the breeding box and placed in the 10 ml respiration vessels to which KOH solution and moinstened cotton had already been added. Respiration vessels were placed into water bath, where they were temperred for 30 min. Than oxygen consumption was measured twice in intervals of 6 hours at either 25°C or 15°C.

Individuals from both treatments (SD, LD) were measured at intervals of 6 hours at 25 °C for 48 hours. During measurements, the same light mode (photoperiod) as in the breeding box was kept; it means, either SD 12:12 or LD 18:6.

Rates of oxygen uptake ($M_{O_2}$) were measured using constant-volume respirometres. This method maintains the gases of the respiration and compensation chambers at a constant temperature and volume. Both the respiration and compensation chambers had a working volume of 10ml and cointained pieces of ashless filter paper soaked in 100µl KOH for the absorption of produced $CO_2$.

**6. Statistics**

Statistical analyses were performed using STATISTICA for Windows, version 7.0 (StatSoft Inc. 2003).Differences between two treatments were tested by t-test, between three or more treatments by ANOVA. Where individual mass could coincide with the analysed variable, we used ANCOVA.

# IV. RESULTS

The total number of individuals used in experiments was 306 adults from LD treatment, 212 adults from SD treatment and 68 adults from NP treatment (Tab. 3).

**Table 3:** The numbers of adults of *Porcellio scaber* in individual experiments.

| | LD | SD | NP |
|---|---|---|---|
| Cold tolerance at +3°C | 48 | 50 | - |
| Body content of glycogen by adults exposed at +3°C | 20 | 20 | - |
| Cold tolerance at 0°C | 70 | - | - |
| Body content of proteins | 50 | 20 | 20 |
| Proteins in the hemolymph | 10 | 10 | 7 |
| Body content of lipids | 20 | 20 | 13 |
| Body content of sugars and polyols | 20 | 20 | 13 |
| Body content of glycogen | 28 | 25 | 15 |
| Oxygen consumption at +25°C | 15 | 15 | - |
| Oxygen consumption at +15°C | 22 | 25 | - |
| Oxygen consumption at +25°C (influence of photoperiod) | 3 | 7 | - |
| Total number of adults used in the experiments | **306** | **212** | **68** |

### 1. Fresh and dry weights

Fresh weight of all individuals measured significantly differed among the three treatments (one-way ANOVA, F=6.528, p=0.002). $FW_{(LD)}$ was lower than $FW_{(SD)}$ (Tukey test, p=0.00096).

Dry weight of all individuals measured significantly differed among the three treatments (F=9.574, p<0.00001). $DW_{(LD)}$ was lower than $DW_{(SD)}$ (p=0.000058). (see Tab. 4)

### 2. Water weight and water ratio

Water weight of all individuals measured significantly differed among the three treatments (F=4.366, p=0.013). $WW_{(LD)}$ was significantly lower than $WW_{(SD)}$ (p=0.0095).

Water ratio of all individuals measured was not significantly different (F=2.538, p=0.08). (Tab. 4)

**Table 4:** Average values ±. S.D. of physiological and morphological traits of all individuals of *Porcellio scaber* used in the experiments.
Letters indicate significant differences on 0.05% level.

| Treatment | Fresh weight [mg] | Dry weight [mg] | Water weight [mg] | Water ratio [%] |
|---|---|---|---|---|
| **LD** | 33.27 ± 17.99a | 11.73 ± 6.36a | 21.54 ± 12.44a | 64.27 ± 4.95a |
| **SD** | 40.40 ± 20.50b | 14.98 ± 8.15b | 25.43 ± 13.17b | 62.98 ± 6.21a |
| **NP** | 35.51 ± 10.11ab | 12.86 ± 3.52ab | 22.65 ± 6.91ab | 63.56 ± 3.36a |

### 3. Cold tolerance

*Cold tolerance at +3°C*

The $Lt_{50}$ of adults *Porcellio scaber* at constant temperature +3°C was about 75 hours for both treatments (LD, SD).

The individuals from LD treatment used in the survival experiment were not significantly different in any of the description parametres (FW, DW and Wr) from the individuals from SD treatment.

T-tests showed that there were not any differences between dead and survived individuals from LD treatment except in Wr 2. The water ratio of dead individuals was significantly higher than that of survived individuals (Tab. 5). The t-tests also showed the same difference between dead and survived individuals from SD treatment (Tab. 6).

**Table 5:** Statistical description of physiological and morphological parameters ± S.D. (initial fresh weight FW 1, final fresh weight FW 2, dry weight DW, initial and final water ratios Wr) in individuals of *Porcellio scaber* LD treatment exposed to temperature +3°C ( ☺ survived n=30, †dead n=18) and t-test.

| LD | ☺ survived | †dead | t | p |
|---|---|---|---|---|
| **FW 1** | 34.50±22.43 | 28.12±22.9 | 0.913 | 0.366 |
| **FW2** | 36.35±23.36 | 33.30±24.11 | 0.417 | 0.679 |
| **DW** | 12.07±7.81 | 9.38±7.79 | 1.114 | 0.277 |
| **Wr 1** | 65.05±4.16 | 66.33±5.64 | 0.876 | 0.385 |
| **Wr 2** | 66.96±4.26 | 72.40±5.58 | 3.701 | 0.000595 |

**Table 6:** Statistical description of physiological and morphological parameters ± S.D. (initial fresh weight FW 1, final fresh weight FW 2, dry weight DW, initial and final water ratio Wr) in individuals of *Porcellio scaber* SD treatment exposed to temperature +3°C ( ☺ survive n=31, †dead n=19) and t-test.

| SD | ☺ survived | †dead | t | p |
|---|---|---|---|---|
| **FW 1** | 37.20±18.23 | 33.59±19.33 | 0.664 | 0.510 |
| **FW2** | 39.13±21.19 | 47.42±30.24 | 1.139 | 0.260 |
| **DW** | 13.16±6.72 | 11.53±6.74 | 0.831 | 0.410 |
| **Wr 1** | 64.75±2.94 | 65.31±3.71 | 0.589 | 0.558 |
| **Wr 2** | 65.97±2.91 | 73.92±6.33 | 6.056 | <0.00001 |

The fresh weight of *Porcellio scaber* in both treatments increased during the exposure at low temperature. The water ratio increased during the exposure at low temperature in both treatments (LD, SD) (Fig. 2). Wr $2_{(LD)}$ and Wr $2_{(SD)}$ was significantly higher in dead individuals than in survived ones.(Tab. 5).

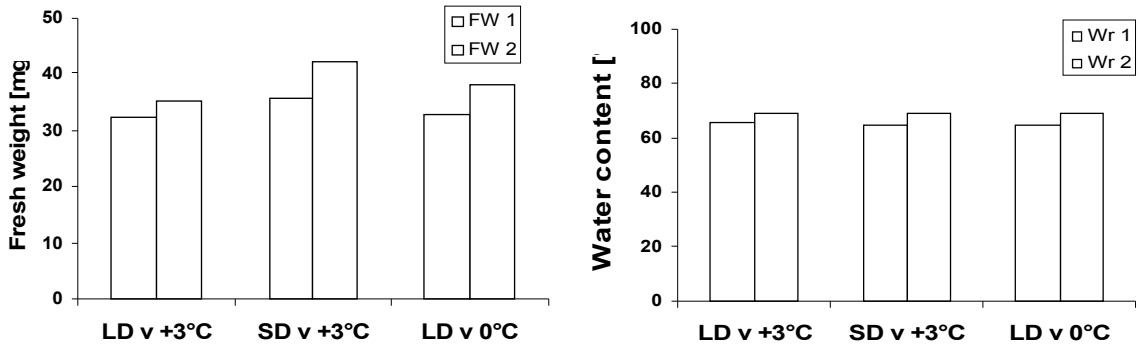



**Figure 2:** Changes during survival experiments (+3°C, 0°C) in fresh weight (FW) and water content (Wr) in *Porcellio scaber*. LD=long day treatment, SD=short day treatment. 1 = initial value, 2 = value after exposure;

We did not manage to prove that the body size of adults of *Porcellio scaber* from LD and SD treatments had influence on survival at +3°C. (Fig. 3, 4).

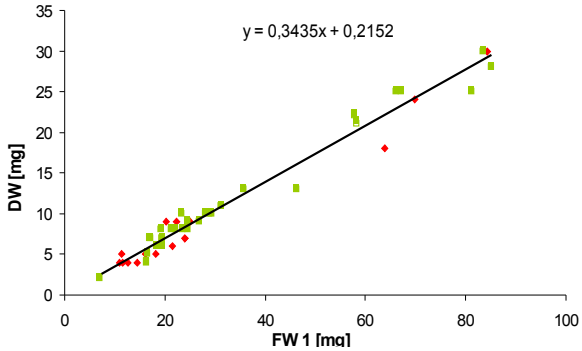



**Figure 3:** Relationship between the fresh weight (FW) and the dry weight (DW) of males of *P.scaber* from LD treatment (n = 48) and survival during exposure at +3°C.

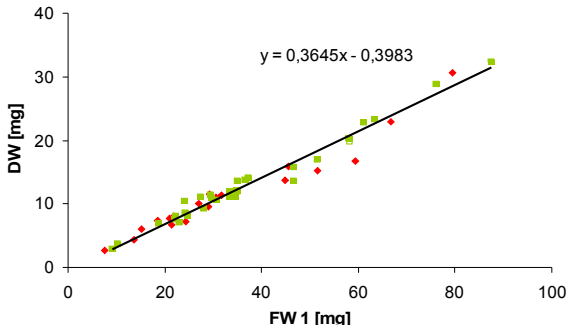
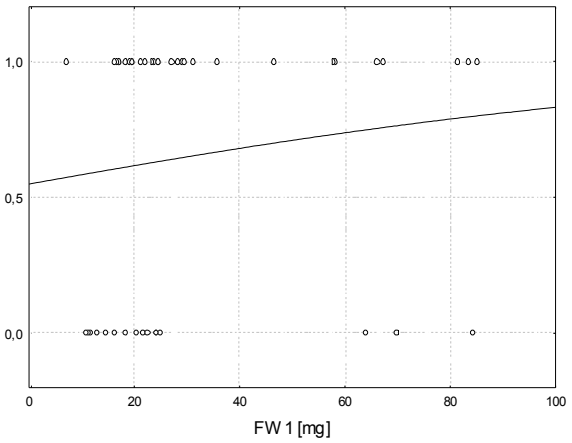



**Figure 4:** Relationship between the fresh weight (FW) and the dry weight (DW) of males of *P.scaber* from SD treatment (n = 50) and survival during exposure at +3°C.

The survival of adults of *Porcellio scaber* from LD treatment was analysed by logit regression. The relationships between survival and FW 1, FW 2 and DW were weakly possitive (Fig. 5A, B, C), between survival and Wr 1 there was weakly negative correlation (Fig. 5D). The relationship between survival and Wr 2 was clearly negative (Fig. 5E).

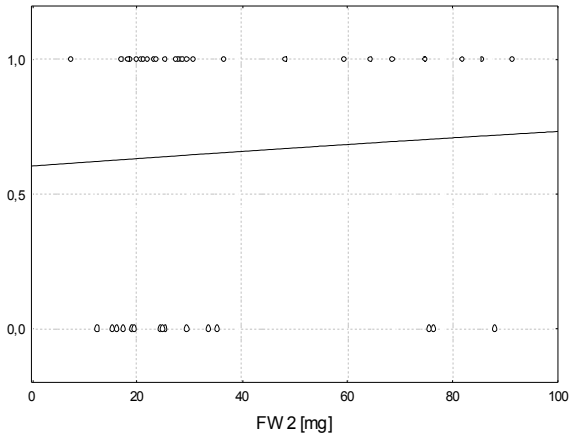
The survival of adults of *Porcellio scaber* from SD treatment was also analysed by logit regression. The relationships of survival with FW 1 and DW were weakly possitive (Fig. 6A, C) and with FW 2 and Wr weakly negative (Fig. 6B, D). The relationship of Wr 2 to survival was clearly negative as in LD treatment (Fig. 6E).

**A.**



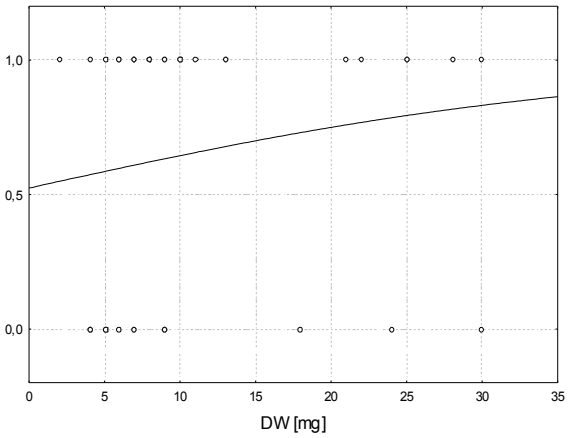

y=exp(0.195+0.0139*x)/(1+exp(0.195+0.0139*x))

**B.**



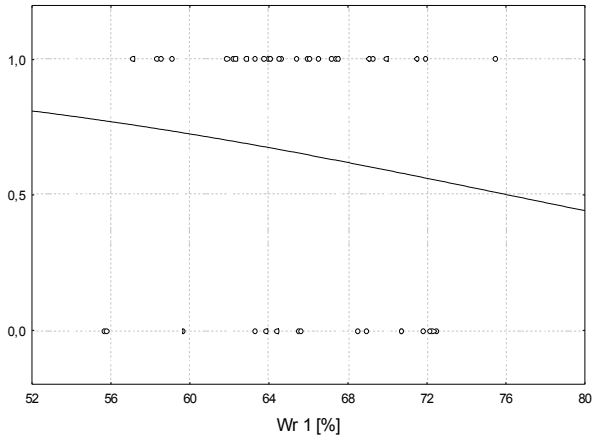

y=exp(0.425+0.00586*x)/(1+exp(0.425+0.00586*x))

**C.**



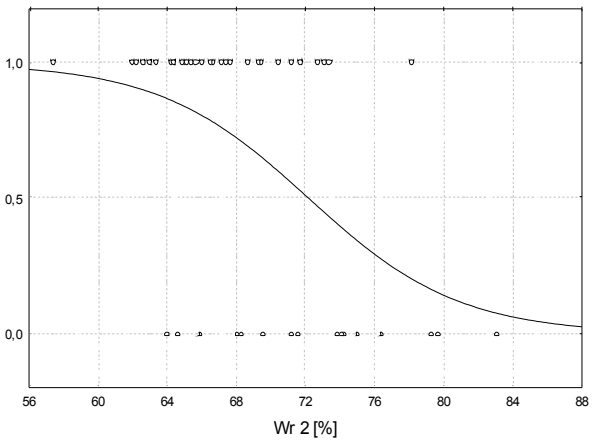

y=exp(0.0973+0.0501*x)/(1+exp(0.0973+(0.0501*x))

**D.**




y=exp(4.59+0.0603*x)/(1+exp(4.59+0.0603*x))

**E.**



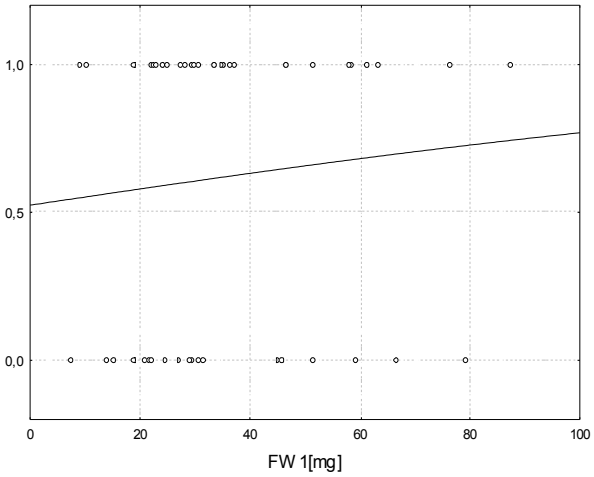

y=exp(16.635+0.230*x)/(1+exp(16.635+0.230*x))

**Figure 5:** Logit regression of dependence of survival (0-dead, 1-survived) on physiological and morphological parameters (initial fresh weight FW 1, final fresh weight FW 2, dry weight DW and initial and final water ratios) in adults of LD treatment of *Porcellio scaber* (n=48).



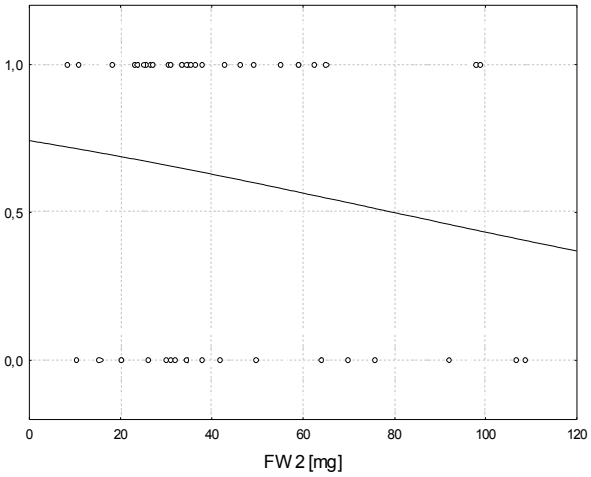
dead survive

**A.**



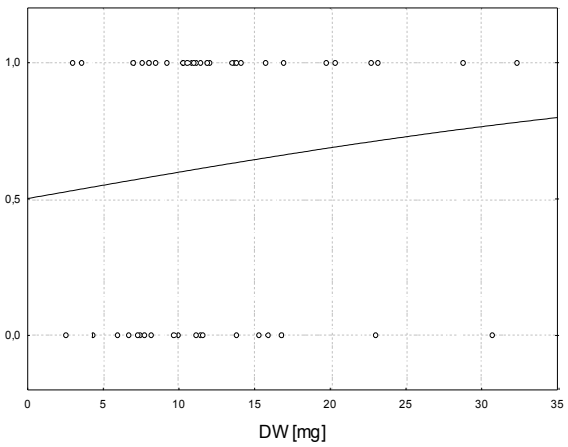

y=exp(0.0987+0.0111*x)/(1+exp(0.0987+0.0111*x))

**B.**



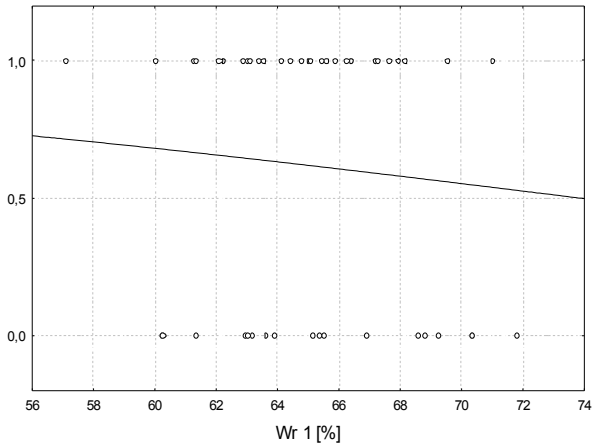

y=exp(1.0608+0.0133*x)/(1+exp(1.0608+0.0133*x))

**C.**



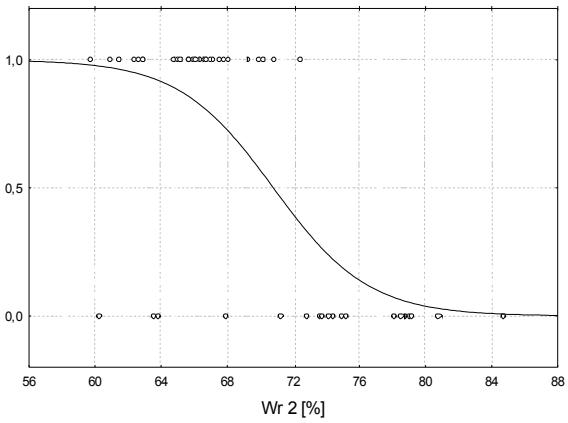

y=exp(0.0081+0.0391*x)/(1+exp(0.0081+0.0391*x))

**D.**




y=exp(4.059+0.0549*x)/(1+exp(4.059+0.0549*x))

**E.**




y=exp(24.71+0.349*x)/(1+exp(24.71+0.349*x))

**Figure 6:** Logit regression of dependence of survival (0-dead, 1-survived) on physiological and morphological parameters (initial fresh weight FW 1, final fresh weight FW 2, dry weight DW and initial and final water ratios) in adults of SD treatment of *Porcellio scaber* (n=50).

*The content of glycogen in individuals exposed at +3°C*

**Table 7:** Physiological and morphological parameters (average ± S.D): initial fresh weight FW 1, final fresh weight FW 2, dry weight DW and the content of glycogen measured in adults of *Porcellio scaber* exposed at +3°C ( ☺ survived n=31, †dead n=19, glycogen and weight measured only in 10 individuals from each combination).

| Treatment | † | FW 1 [mg] | FW 2 [mg] | DW [mg] | Glycogen [µg/mg DW] |
|---|---|---|---|---|---|
| LD | 10 | 28.29±25.06 | 32.19±26.79 | 9.4±8.44 | 7.75± 4.74 |
| SD | 10 | 20.21± 6.91 | 26.74±10.17 | 7.4±3.09 | 18.43±15.91 |
| | ☺ | | | | |
| LD | 10 | 40.57±27.87 | 41.88±29.00 | 13.30±9.36 | 10.04±7.30 |
| SD | 10 | 49.56±20.93 | 53.88±26.38 | 18.27±7.71 | 18.08±8.32 |

The mean body content of glycogen of adults from LD treatment was different between adults exposed and not exposed at +3°C (corrected for different weight by ANCOVA, $F_{(DW)}$=7.42, p=0.009, $F_{(exposure)}$=27.96, p<0.00001). The adults not exposed had significantly higher content of glycogen than adults after exposure at +3°C (Tukey test, p=0.00013) (Fig. 7).

The glycogen concentration of adults from SD treatment was different between adults exposed and not exposed at +3°C (weight corrected ANCOVA, $F_{(DW)}$=23.32, p<0.00001, $F_{(exposure)}$=15.22, p<0.00001). The adults not exposed to cold had significantly higher content of glycogen than adults after exposure at +3°C (Tukey test, p=0.02892) (Fig. 7).

The glycogen concentration of all individuals from LD treatment after exposure at +3°C was 8.82±5.99 µg/mg DW and from SD treatment after exposure at +3°C was 18.26±12.36 µg/mg DW. The adults of LD treatment had significantly lower content of glycogen than adults of SD treatment (ANCOVA corrected for different dry weight, $F_{(DW)}$=2.791, p=0.104, $F_{(exposure)}$=9.853, p=0.003).

Significant difference between dead and survived individuals from both treatments (LD, SD) in glycogen concentration was not detected (Fig. 8).

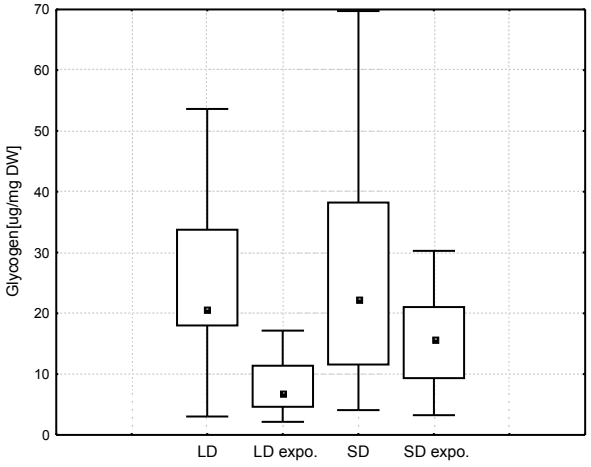



**Figure 7:** Glycogen content of adults of *Porcellio scaber* LD and SD treatments exposed at +3°C and not exposed.

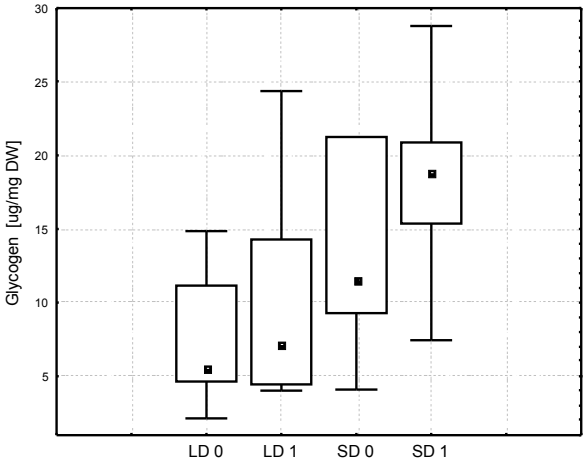



**Figure 8:** Relationship between the glycogen content of adults of *Porcellio scaber* LD and SD treatments and survival (0-dead individuals, 1-survived individuals.

*Cold tolerance at 0°C*

**Table 8:** Statistical description of physiological and morphological parameters: initial fresh weight FW 1, final fresh weight FW 2, dry weight DW, initial and final water ratios Wr after exposure at 0°C in LD treatment of *Porcellio scaber*, ( ☺ survive n=21, †dead n=49), averages ± S.D. and t-test.

| | ☺ survive | †dead | t | p |
|---|---|---|---|---|
| **FW 1** | 35.44±14.37 | 31.70±13.76 | -1.020 | 0.311 |
| **FW2** | 40.78±19.15 | 37.16±17.14 | -0.776 | 0.441 |
| **DW** | 12.54±4.67 | 10.94±4.41 | -1.353 | 0.181 |
| **Wr 1** | 63.75±2.70 | 64.93±3.08 | 1.456 | 0.150 |
| **Wr 2** | 68.15±4.94 | 69.42±4.86 | 0.955 | 0.343 |

The $Lt_{50}$ at constant temperature 0°C was about 48 hours for both treatments of *Porcellio scaber* (LD, SD).

We did not manage to prove that the body size of adults of *Porcellio scaber* from LD and SD treatments had influence on the survival at 0°C. (Fig. 9).

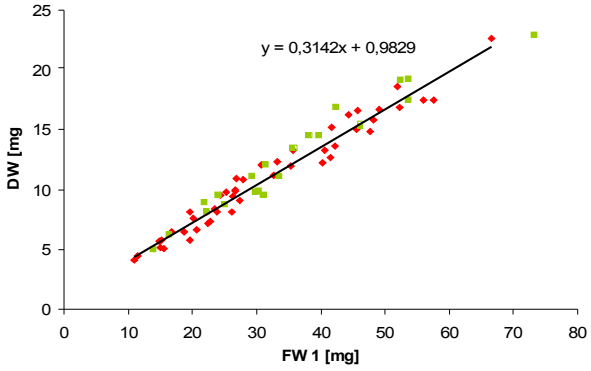
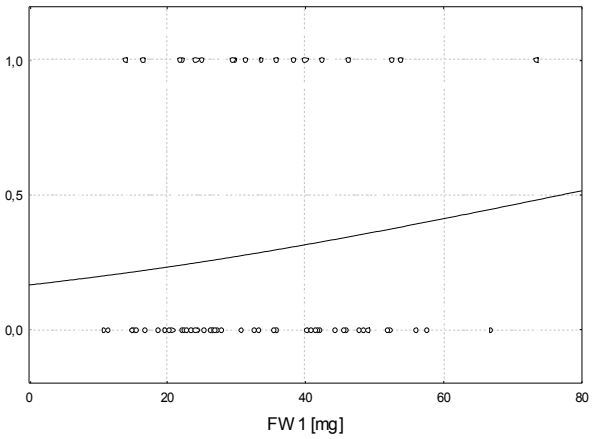



**Figure 9:** Relationship between the fresh weight (FW) and the dry weight (DW) of males of *P.scaber* from LD population (n = 70) and survival during exposure at 0°C.

dead survive

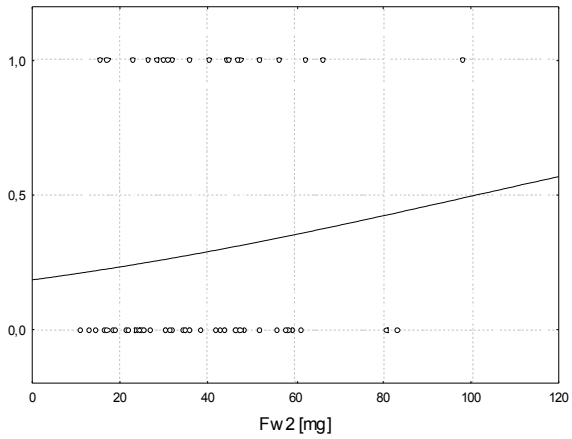
The survival of adults of *Porcellio scaber* from LD treatment was analysed by logit regression. The relationships between survival and FW 1, FW 2 and DW were weakly possitive (Fig. 10A, B, C), and relationships to Wr 1 and Wr 2 were weakly negative (Fig. 10 D, E).

**A.**



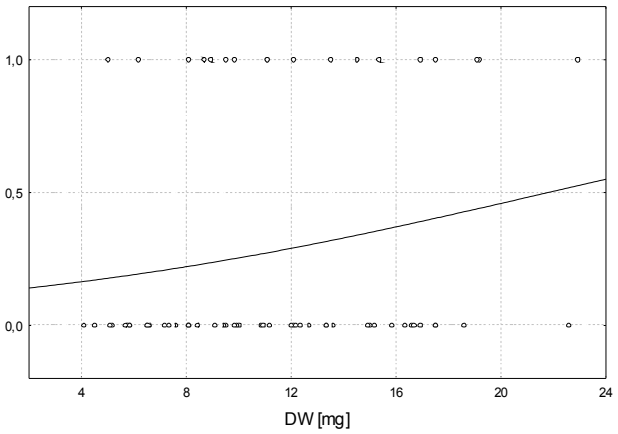

y=exp(-1.616+0.021*x)/(1+exp(-1.616+0.021*x))

**B.**



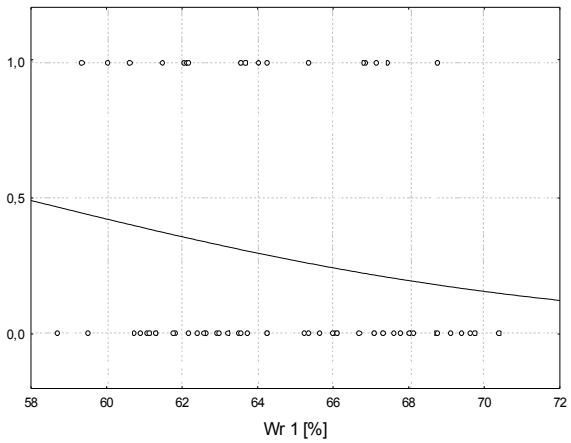

y=exp(-1.4854+(0.014689)*x)/(1+exp(-1.4854+(0.014689)*x))

**C.**



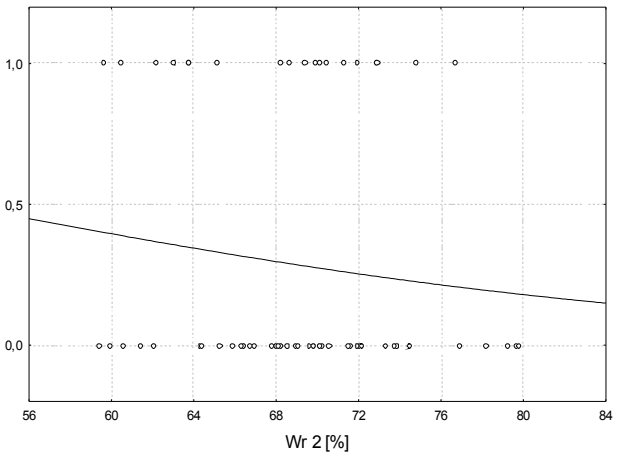

y=exp(-1.99+0.0914*x)/(1+exp(-1.99+0.0914*x))

**D.**




y=exp(7.919+-0.137*x)/(1+exp(7.919+-0.137*x))

**E.**




y=exp(2.866-0.0548*x)/(1+exp(2.866-0.0548*x))

**Figure 10:** Logit regression of dependence of survival (0-dead, 1-survived) on physiological and morphological parameters (initial fresh weight FW 1, final fresh weight FW 2, dry weight DW and initial and final water ratios) in adults of LD treatment of *Porcelli scaber* exposed at 0°C (n=70).

## 4. Energy reserves

*Proteins*

**Table 9** Protein content and size (average values ±. S.D.) of males in the three treatments

| Treatment | n | FW [mg] | DW [mg] | Body content of proteins [µg/mg DW] |
|---|---|---|---|---|
| **LD** | 50 | 31.36±10.57 | 11.44± 3.81 | 101.19± 21.15 |
| **SD** | 20 | 31.73± 8.56 | 11.62± 3.61 | 103.5 ± 39.83 |
| **NP** | 20 | 35.14±13.99 | 12.98± 4.48 | 62.82± 16.13 |

The mean body content of proteins in LD treatment was 101µg/mg of dry weight, in SD it was 104µg/mg DW and in NP it was 63µg/mg DW (Tab. 9). The treatments differed in protein content even when the values were corrected for different weight (see Fig. 12) of analysed individuals (ANCOVA, $F_{(DW)}<0.01$, $p=0.96$, $F_{(treatment)}=11.45$, $p<0.00001$). The protein concentration in NP treatment was significantly lower than in both SD treatment (Tukey test, $p=0.000531$) and LD treatment ($p=0.000143$) (Fig. 11).

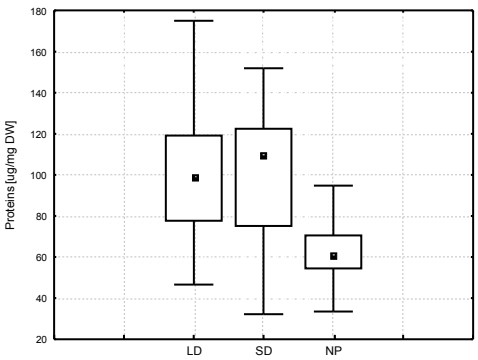
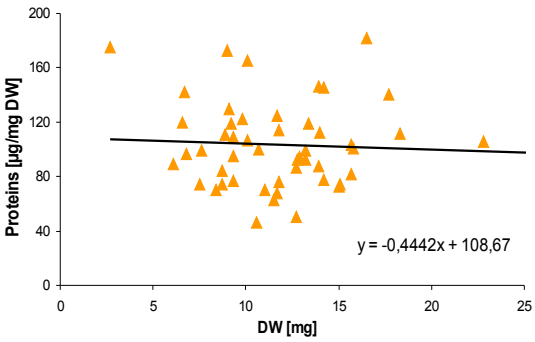
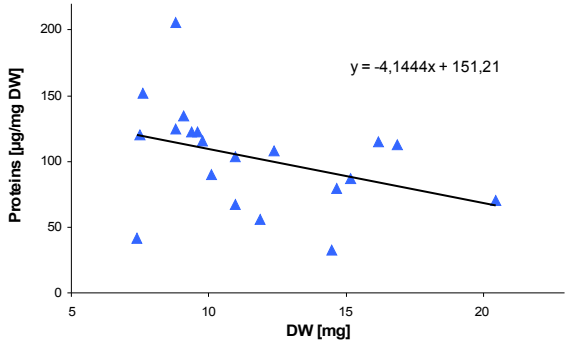



**Figure 11:** The relationship between the body proteins content and type of treatment.

Median 25%-75% Non-Outlier Range



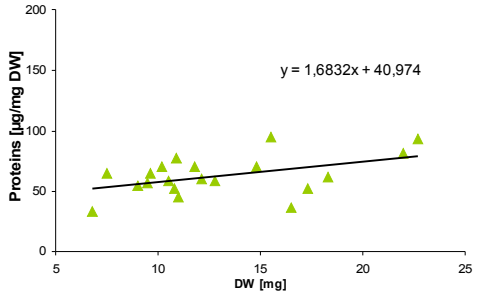



**Figure 12:** Relationship between the dry weight (DW) of *Porcellio scaber* and the body content of proteins.

▲ = **LD** (n=50)
▲ = **SD** (n=20)
▲ = **NP** (n=20)

*Proteins in the hemolymph*

**Table 10:** Concentration of proteins (average values ± S.D.) and size of individuals of the three treatments.

| Treatment | n | FW [mg] | DW [mg] | Content of proteins in the hemolymph [µg/µl] |
|---|---|---|---|---|
| **LD** | 10 | 61.2 ±20.03 | 15.09± 5.57 | 8.59± 2.72a |
| **SD** | 10 | 76.1 ±12.93 | 28.87± 6.04 | 9.76± 1.99a |
| **NP** | 7 | 42.29± 9.23 | 14.77± 2.99 | 13.37± 4.58b |

The protein concentration in the hemolymph in LD treatment was 8.59µg/µl, in SD it was 9.76µg/µl and in NP it was 13.37µg/µl (Tab.10). The treatments differed in protein content even when the values were corrected for different weight (see Fig. 13) of analysed individuals (ANCOVA, $F_{(DW)}$=1.933, p=0.178, $F_{(treatment)}$=6.3, p=0.007) (Tab. 10).

The protein concentration in the hemolymph of NP treatment was significantly higher than that of LD treatment (p=0.0106) (Fig.13). Relationship between the dry weight (DW) of and the concentration of proteins in the hemolymph was generally positive (Fig. 14).

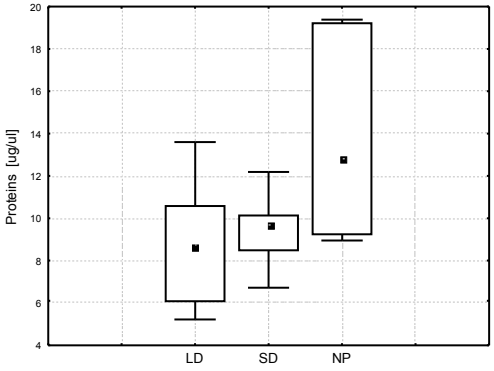



**Figure 13:** The relationship between the concentration of proteins in the hemolymph and type of treatment.

Median 25%-75% Non-Outlier Range

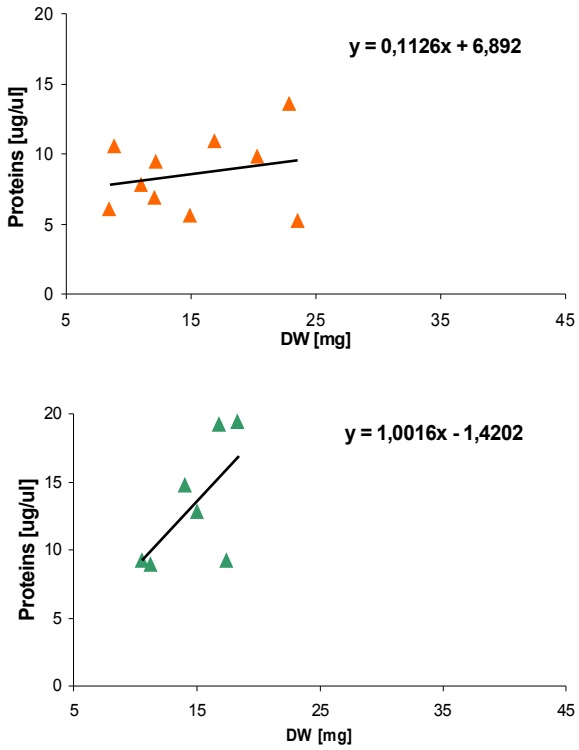



**Figure 14:** Relationship between the dry weight (DW) of *Porcellio scaber* and the concentration of proteins in the hemolymph.

▲ = **LD** (n=10)
▲ = **SD** (n=10)
▲ = **NP** (n=7)

*Lipids*

**Table 11:** Body content of lipids (average values ± S.D.) and size of individuals in the three treatments.

| Treatment | n | FW [mg] | DW [mg] | Lipids [µg/mg DW] |
|---|---|---|---|---|
| **LD** | 20 | 24.54±15.54 | 8.60±4.08 | 103.56± 9.38a |
| **SD** | 20 | 32.94±12.20 | 11.85±4.29 | 107.60±14.19b |
| **NP** | 13 | 34.66± 7.84 | 11.68±2.99 | 98.19±11.89ab |

The mean body content of lipids in LD treatment was 103.6µg/mg of DW, in SD it was 107.6µg/mg and in NP it was 98.2µg/mg. The treatments differed in lipid content even when the values were corrected for different weight (see Fig. 15) of analysed individuals (ANCOVA, $F_{(DW)}$=45.50, p<0,00001, $F_{(treatment)}$=7.15, p=0.002) (Tab. 11).

The lipid concentration of NP treatment was significantly lower than that in SD treatment (Tukey test, p=0.0109) (Fig. 15). Bigger individuals were generally fattier in all treatments (Fig. 16).

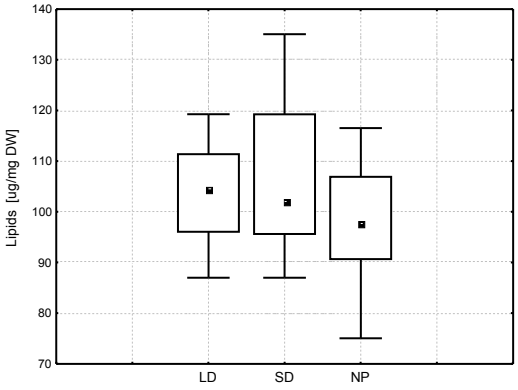
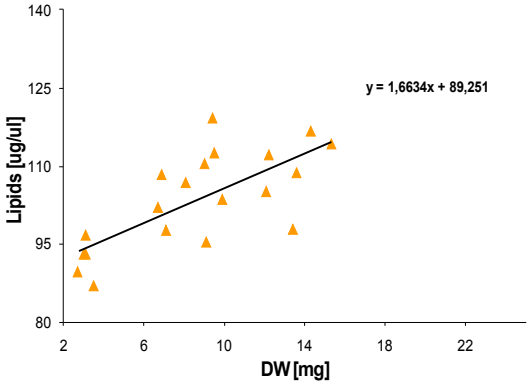
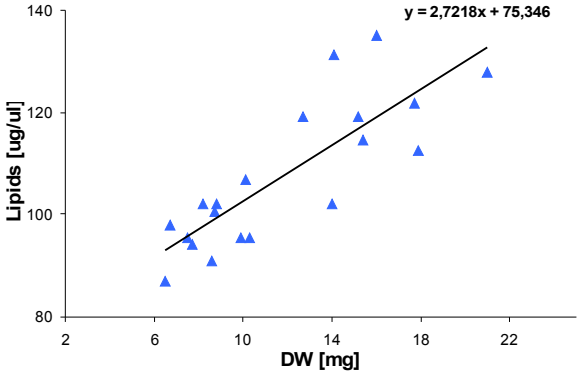



**Figure 15:** The relationship between the concentration of lipids and type of treatment.

Median 25%-75% Non-Outlier Range



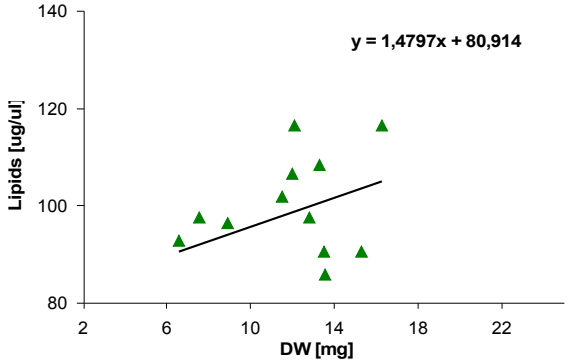




**Figure 16:** Relationship between the dry weight (DW) of *Porcellio scaber* and the concentration of lipids in the whole body.

▲ = **LD** (n=20)
▲ = **SD** (n=20)
▲ = **NP** (n=13)

## *Glycogen*

**Table 12:** Morphological parameters and glycogen concentration (average ±. S.D.) of individuals of *Porcellio scaber*.

| Treatment | n | FW [mg] | DW [mg] | Glycogen [µg/mg DW] |
|---|---|---|---|---|
| **LD** | 28 | 33.81±11.98 | 12.20± 3.98 | 26.18±14.60a |
| **SD** | 25 | 49.13±24.59 | 20.18±10.20 | 28.21±21.52a |
| **NP** | 15 | 35.30± 8.58 | 13.57± 3.37 | 8.67± 4.56b |

The mean body content of glycogen in LD treatment was 26 µg/mg DW, in SD treatment it was 28 µg/mg DW and in NP treatment it was 8.7 µg/mg DW (Tab. 12). The glycogen content significantly differed between treatments even when body dry weight was added as a covariate to the analysis of variance (ANCOVA, $F_{(DW)}$=48.55, p<0.00001, $F_{(treatment)}$=24.07, p<0.00001). The glycogen concentration in NP treatment was significantly lower than in SD treatment (Tukey test, p=0.00014) and in LD treatment (p=0.00021) (Fig. 17). Higher glycogen content was found in smaller individuals (Fig. 18).

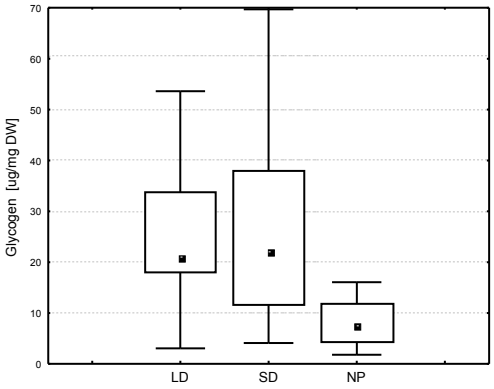
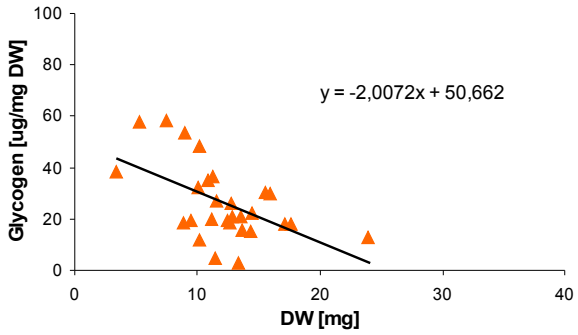
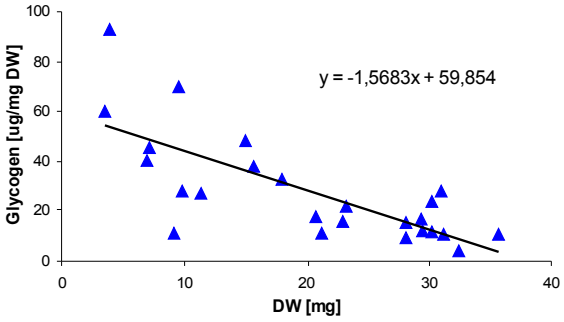



**Figure 17:** The relationship between the concentration of glycogen and type of treatment.

Median 25%-75% Non-Outlier Range



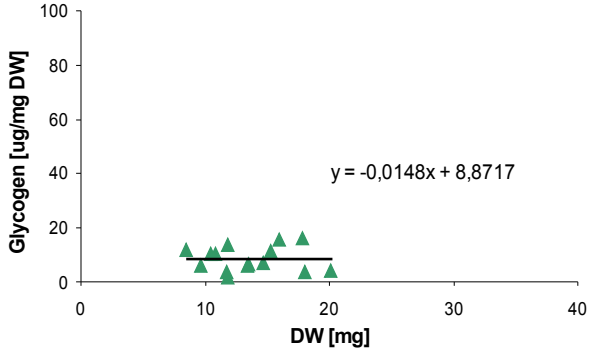




**Figure 18:** Relationship between the dry weight (DW) of *Porcellio scaber* and the concentration of glycogen in the whole body.

▲ = **LD** (n=28)
▲ = **SD** (n=25)
▲ = **NP** (n=15)

*Sugars and polyols*

| Treatment | n | FW [mg] | DW [mg] |
|---|---|---|---|
| **LD** | 20 | 59.46±10.28 | 23.43± 6.92 |
| **SD** | 20 | 39.23±16.34 | 14.11± 5.63 |
| **NP** | 13 | 33.50± 6.26 | 12.02± 2.37 |

**Table 13:** Morphological parameters (average ±. S.D.) of individuals of *Porcellio scaber* used in sugars and polyols measurements.

The glycerol content significantly differed between treatments even when body dry weight was filtered out as a covariate (ANCOVA, $F_{(DW)}$=1.19, p=0.28, $F_{(treatment)}$=16.60, p<0.00001). LD was higher than SD (Tukey test, p=0.00013) and higher than NP (p=0.00017) (Fig. 19).

The arabinitol content significantly differed between treatments even when body dry weight was filtered out as a covariate (ANCOVA, $F_{(DW)}$=3.132, p=0.083, $F_{(treatment)}$=6.735, p=0.003). NP was higher than LD (p=0.02213) and higher than SD (p=0.01177).

Ribitol was not detected in any of the three treatments.

The fructose content significantly did not differ between treatments even when body dry weight was filtered out as a covariate (ANCOVA, $F_{(DW)}$=0.025, p=0.875, $F_{(treatment)}$=1.144, p=0.327).

The glucose content significantly differed between treatments even when body dry weight was filtered out as a covariate (ANCOVA, $F_{(DW)}$=0.483, p=0.49, $F_{(treatment)}$=8.488, p=0.001). SD was higher than LD (p=0.00014) and also than NP (p=0.02438).

The manitol content significantly differed between treatments even when body dry weight was filtered out as a covariate (ANCOVA, $F_{(DW)}$=4.142, p=0.047, $F_{(treatment)}$=5.369, p=0.008). SD was lower than NP (p=0.01349).

The sorbitol content significantly did not differ between treatments even when body dry weight was filtered out as a covariate (ANCOVA, $F_{(DW)}$=2.306, p=0.135, $F_{(treatment)}$=0.203, p=0.817).

The myo-inisitol content significantly did not differ between treatments even when body dry weight was filtered out as a covariate (ANCOVA, $F_{(DW)}$=4.746, p=0.034, $F_{(treatment)}$=0.106, p=0.9).

The sacharose content significantly did not differ between treatments even when body dry weight was filtered out as a covariate (ANCOVA, $F_{(DW)}$=3.00, p=0.09, $F_{(treatment)}$=0.743, p=0.481).

The trehalose content significantly did not differ between treatments even when body dry weight was filtered out as a covariate (ANCOVA, $F_{(DW)}$=2.266, p=0.139, $F_{(treatment)}$=1.124, p=0.333).

**Table 14:** Sugars and polyols (average values ± S.D.) detected in individuals of the three treatments.

| | **LD** (n = 20) | **SD** (n = 20) | **NP**(n = 13) |
|---|---|---|---|
| | [µg/mg DW] | [µg/mg DW] | [µg/mg DW] |
| **Glycerol** | 2.40±0.91 | 0.94±0.70 | 1.16±0.39 |
| **Arabinitol** | 0.06±0.28 | 0.03± 0.15 | 0.38± 0.55 |
| **Ribitol** | 0.00±0.00 | 0.00±0.00 | 0.00± 0.00 |
| **Fructose** | 0.01±0.02 | 0.03±0.04 | 0.45±1.60 |
| **Glucose** | 0.24±0.19 | 3.24±2.82 | 1.44±1.33 |
| **Manitol** | 0.04±0.03 | 0.02±0.04 | 0.06±0.05 |
| **Sorbitol** | 0.02±0.02 | 0.02±0.04 | 0.02±0.03 |
| **Myo-inositol** | 0.15±0.06 | 0.20±0.11 | 0.20±0.07 |
| **Sacharose** | 0.04±0.05 | 0.23±0.51 | 0.13±0.15 |
| **Trehalose** | 0.04±0.06 | 0.09±0.06 | 0.09±0.05 |

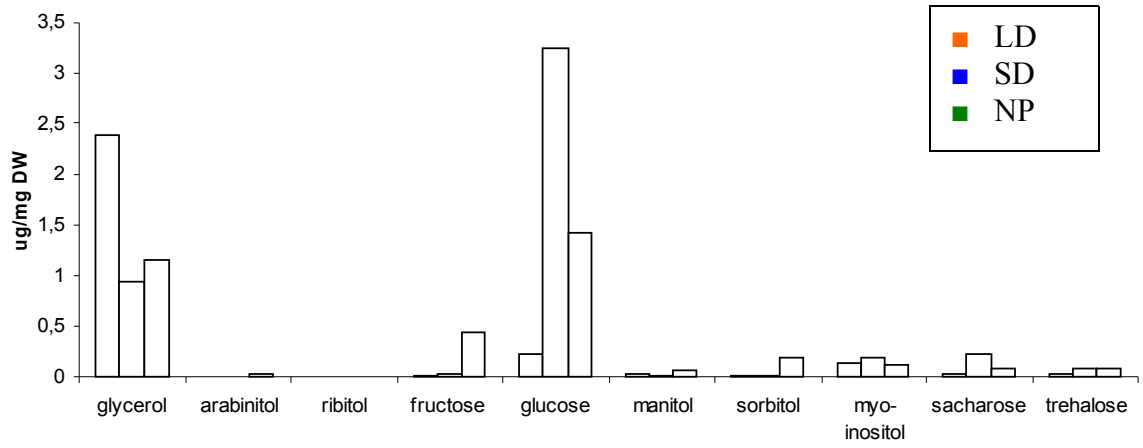



**Figure 19:** Mean concentrations [µg/mg DW] of sugars and polyols in all three treatments.

## 5. Respiration

*Respiration at +25°C for 2 days (influence of photoperiod)*

| Treatment | n | FW [mg] | µl $O_2.g^{-1}.hr^{-1}$ (dark) | µl $O_2.g^{-1}.hr^{-1}$ (light) |
|---|---|---|---|---|
| **LD** | 3 | 45.00±7.55 | 190.31±63.44 | 123.28±37.50 |
| **SD** | 7 | 41.29±7.57 | 155.18±50.24 | 92.50±19.83 |

**Table 15:** Body weight and respiration during photophase and scotophase (average values ±. S.D.) of *P. scaber* from LD and SD treatments.

The oxygen consumption of two treatments (LD, SD) of *Porcellio scaber* was measured during 48 hours at 25°C with the same photoperiod as at breeding boxes. The oxygen consumption of adults from LD population was not significantly different between exposure at light and dark (F=2.862, p=0.158). Also the oxygen consumption of adults from SD population was not significantly different between exposure at light and dark (F=6.420,

p=0.059). However, in both treatments, some periodicity appeared: during exposure at dark or periods near scotophase, there was higher oxygen consumption than at light (Fig. 20).

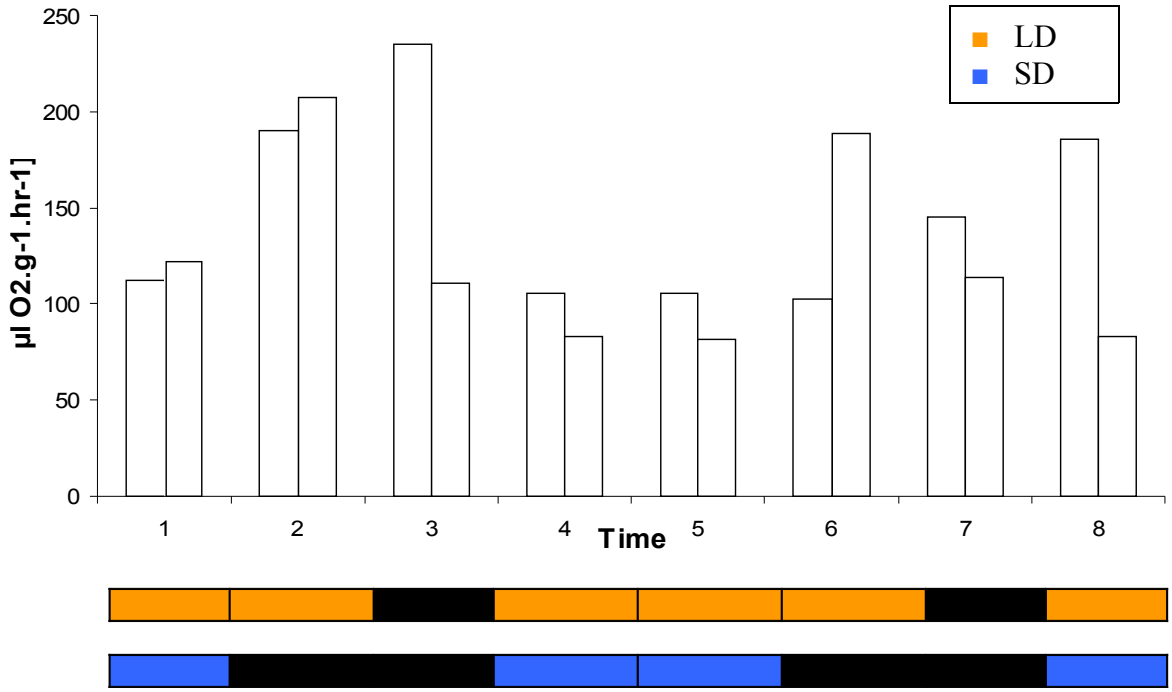



**Figure 20:** The oxygen consumption of two treatments (LD, SD) of *Porcellio scaber* was measured during 48 hours at 25°C with the same photoperiod as at breeding boxes. (■ LD, photophase, ■ SD, photophase, ■ scotophase)

*Respiration at +25°C*

| Population | n | FW [mg] | µl $O_2.g^{-1}.hr^{-1}$ |
|---|---|---|---|
| **LD** | 14 | 32.2±12.48 | 174.94±62.49 |
| **SD** | 26 | 54.82±10.87 | 116±56.55 |

**Table 16:** Body weight and respiration during at 25°C (average values ±. S.D.) of *P. scaber* from LD and SD treatments.

The oxygen consumption of adults of *Porcellio scaber* at +25°C was not significantly different between LD and SD treatment (ANCOVA for fresh weight correction, $F_{(FW)}$=0.396, p=0.533, $F_{(treatment)}$=3.528, p=0.068) (Tab.16).

The relationship between the oxygen consumption of adults from LD treatment and the body size was negative, as expected. Conversely, the bigger adults from SD treatment had higher relative oxygen consumption than smaller individuals (Fig. 21).

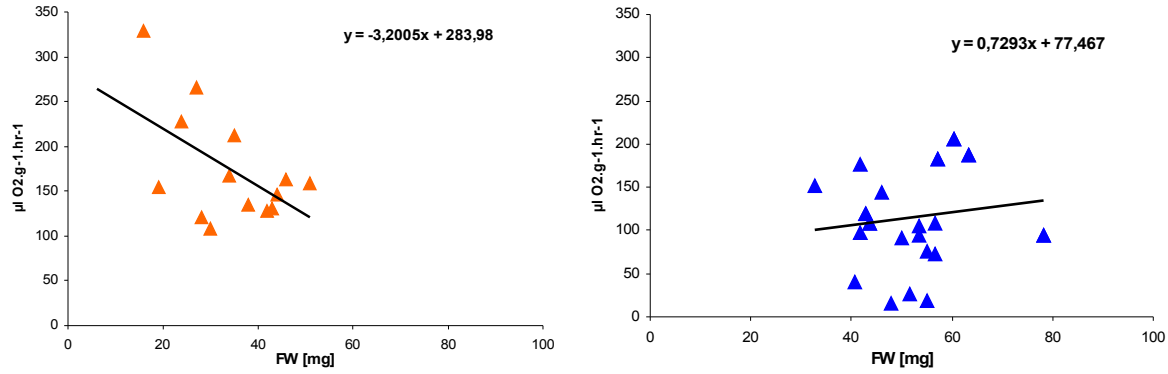



**Figure 21:** Relationship between the fresh weight (FW) of *Porcellio scaber* and the oxygen consumption at +25°C. **(▲ = LD, ▲ = SD)**

*Respiration at +15°C*

| Population | n | FW [mg] | µl $O_2.g^{-1}.hr^{-1}$ |
|---|---|---|---|
| **LD** | 22 | 43.55±8.13 | 54.70±22.78 |
| **SD** | 25 | 45.00±17.58 | 64.08±14.38 |

**Table 17:** Body weight and respiration during at 15°C (average values ±. S.D.) of *P. scaber* from LD and SD treatments.

The oxygen consumption of adults of *Porcellio scaber* at +15°C was not significantly different between LD and SD treatment (ANCOVA for fresh weight correction, $F_{(FW)}$=0.061, p=0.806, $F_{(treatment)}$=2.897, p=0.096).

The relationship between the relative oxygen consumption of adults from LD treatment and the body size was surprisingly positive. Conversely, the smaller adults from SD treatment had higher oxygen consumption than bigger individuals (Fig. 22).

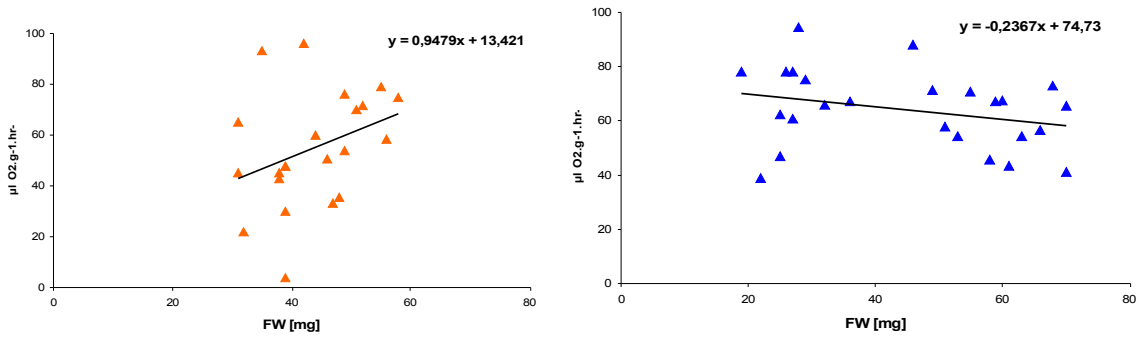



**Figure 22:** Relationship between the fresh weight (FW) of *Porcellio scaber* and the oxygen consumption at +15°C. **(▲ = LD, ▲ = SD)**

*Temperature Coefficient ($Q_{10}$)*

The temperature coefficient ($Q_{10}$) represents the factor by which the rate ($R$) of a reaction increases for every 10-degree rise in the temperature ($T$). The rate ($R$) represent the oxygen consumption [**µl $O_2$.$g^{-1}$.$hr^{-1}$]** adults of *Porcellio scaber* from both treatments (LD, SD) at two different temperatues, *+15* and *+25°C*. $Q_{10}$ for adults of LD treatment was 3.2 and for adults of SD treatment was 1.8.

# V. DISCUSSION

## 1. Cold tolerance

Survival experiment with males of *Porcellio scaber* from two treatments different in acclimation photoperiod (long day LD, short day SD) was performed at constant temperature +3°C. Isopods are freezing susceptible organisms (TANAKA & UDAGAWA, 1993), thus we did not use subzero temperatures. Lethal time ($Lt_{50}$) was found approximately the same for both treatments (75 hours) in preliminary measurements, and thus survival percentage was not significantly different between both treatments in subsequent experiments.

We showed that the survival at low temperature (+3°C) in both treatments (LD, SD) was not correlated to isopod body size expressed as fresh and dry weights, and water ratio in individuals prior to the exposure.

Laboratory and field studies have already demonstrated a correlation between large adult size and fitness components such as fecundity and survival in adults (PARTIDGE & FOWLER, 1993), large size being often associated with high survivorship and fecundity. It has been shown in adult *Stenotarsus rotundus*, a tropical beetle, that relative fat content was higher in larger insects (NEDVED & WINDSOR, 1994). Moreover, PULLIN (1987) showed significant correlation between lipid content and adult weight in *Aglais urticae* and *Inachis io* (Lepidoptera: Nymphalidae) and demonstrated a significant linear relationship between survival time and initial fresh weight. Also RENAULT *et al*. (2003) found that the duration of survival at low temperature (+10°C) was positively correlated to insect body size of *Alphitobius diaperinus* in both sexes, whereas body size had no significant impact on adult survival during exposure at +6°C to chilling temperatures (RENAULT *et al*., 2003).

This low temperature (+3°C) probably caused cumulative chill injury and a prolonged chilling might finally cause metabolic disorder to such an extent that it was impossible to be repaired after the end of cold exposure or it might cause injury to the repair mechanisms themselves, ending in individual death (NEDVED *et al*., 1998). Prolonged exposures may critically affect energy reserves and it can not be excluded that individuals rather die from the depletion of energy reserves.

During survival experiments, isopod activity was reduced, but not all individuals kept at +3°C entered chill-coma. Paralyzing (chill coma) temperatures were reported from 1.3 to 1.9 °C in *A. nasatum*, *P. scaber* and *O. asellus* (ŠUSTR *et al*., 2005). Their activity became normal when isopods were placed at +25°C. Some individuals kept at +3°C were active (able to walk) during entire exposure and after exposure without visible unusual display. However,

during exposure at low temperature they did not intake food. Thus, a factor important for survival was relative saving to be made in terms of energy consumption or resource allocation (FIELDS, 1992). During starvation, energy is derived solely from metabolic stores (lipids, proteins and eventually glycogen), and, therefore, body tissues are lost due to catabolic activities (HERVANT *et al*., 2000). Similarly, we found a loss of glycogen during cold exposure (Fig.7).

**2. Body size and water content**

Our results revealed highly significant differences between fresh weight of individuals before exposure at +3°C and fresh weight after exposure (Fig.2). The body weight gain of adult *Porcellio scaber* over the course of the experiment probably indicates water reception. Over the duration of exposure, the reserves consumption had to be much smaller than the amount of water gain. This was found also in subterranean *N. rhenorhodanensis* acclimated to 3°C and -2°C that showed a significant increase in its bound water content (ISSARTEL *et al.,* 2006). Adaptations that increase the amount of bound water are used to ensure that the lethal limit is not exceeded (STOREY & STOREY, 1989).

An increase of the water ratio (percentage) during exposure at +3°C may cause decrease of the concentration of solutes in the body fluid, which might be a reason of death in part of individuals. While decrease of water ratio may trigger physiological mechanisms for the production of cryoprotective substances (STOREY & STOREY, 1992), it can still be a critical factor for survival and a potential cause of insect mortality during low temperature exposures (ZACHARIASSEN, 1985). On the other hand, for instance, LAVY *et al*. (1997) agree that decreased cold tolerance in starved animals of two species (*Orchesella cincta* and *Porcellio scaber*) may be caused by increased water content or, more probably, by the decrease in reserves needed to produce cryoprotective substances.

Adults of aphidophagous ladybirds (Coleoptera: Coccinellidae) were able to maintain their water level by drinking and/or oxidation of metabolic reserves (EL-HARIRI, 1966). Therefore, fresh weight measurements of isopods cannot be used as an indicator of their fat content. For these isopods living in moist conditions, it seems better to use the close box with constant high humidity not to enable them to drink but also not to dry out.

In our experiments, the increase in water content was small in survived animals, while very large in dead ones. This difference suggests small passive or active water gain during chilling

in living but not active individuals (in chill coma), while large water gain appeared after death when the organism lost homeostasis mechanisms.

Similar survival rates throughout different body size classes in adults indicate that the amount of fat reserves in the hibernating adults at beginning of hibernation is more important for survival than body size itself at low altitudes where larger amount of fat reserve is depleted after hibernation (ZHOU *et al*., 1995).

Another survival experiment with males of *Porcellio scaber* from long day photoperiod (LD) was performed at constant temperature 0°C. Lethal time ($Lt_{50}$) was approximately 48 hours for adults of this treatment.

Our results showed that the survival at low temperature (0°C) in LD treatment was not correlated to isopod body size expressed as fresh and dry weights, and water ratio in individuals prior to the exposure.

During survival experiment, isopod activity was much reduced, all individuals kept at 0°C entered chill-coma. Their activity became normal when isopods were placed at +25°C.

Our results revealed high differences between fresh weight of individuals before exposure at 0°C and fresh weight after exposure. The body weight gain of adult *Porcellio scaber* over the course of the experiment probably indicates water reception. Contrary to the exposure at +3°C, this increase was similar in both survived and dead individuals.

The body water content of normal *P. scaber* is between 65 and 72 % (LINDQVIST *et al.,* 1971). Also, high cuticular water content may be advantageous in view of the fact that the terrestrial isopods seem to excrete most of their nitrogen as ammonia in gaseous form through body surfaces (WIESER & SCHWEIZER, 1970).

The water content of adults *Porcellio scaber* in our experiments was lower (63-64% FW) compared to those found by LAVY *et al*. (1996), who reported water content of starved (79-81%) and fed individuals (75-79%).

Some authors have studied the changes in the free water/bound water ratio during low temperature acclimation in cold-hardy ectotherms. The bound water is the water that is so closely associated with cellular or other components in an organism that it is not available to participate in the freezing processes (HAZELWOOD, 1977). The bound water content of the freeze-tolerant larvae of *Eurosta solidaginis* increased with cold acclimation (STOREY *et al.,* 1981), and this was due to changes in water binding by cryoprotectants and macromolecules (mainly glycogen and proteins). In freeze-tolerant species, bound water will not participate in ice formation and this results in non-freezable shells of water surrounding cellular

components, protecting them from the denaturation due to freezing dehydration (STOREY *et al*., 1981).

The work of STOREY (1981) showed that both low-molecular weight compounds (LMWs; mainly polyols and sugars) and high-molecular weight compounds (mainly glycogen and proteins) decreased with increasing bound water.

### 3. Energy reserves

The amount and quality of the reserves in the adult body significantly affect survival during the winter and fecundity in the breeding season (STEWART *et al*., 1991).

The mean body proteins concentrations in our experiment with males of *Porcellio scaber* were somewhat lower (63–104 μg/mg DW) compared to those found by LAVY *et al*. (2001), who reported protein concentrations in adult *P. scaber* from 173 to 264 μg/mg DW. Our results revealed a significant difference between treatments, where individuals from natural autumn conditions (NP) had lower protein content. The cause of this difference is not clear. On the other hand, the mean protein concentration in the hemolymph was higher in NP than in both laboratory treatments (LD, SD). NP individuals might have bigger amount of soluble storage proteins or rather other specific hemolymph proteins (heat shock etc.).

The mean lipid concentrations in our experiment with *Porcellio scaber* were somewhat lower (98–108 μg/mg DW) compared to those found by LAVY *et al*. (2001), who reported lipid concentrations in males *P.scaber* from 113 to 115 μg/mg DW, and equal to those found by VAN BRUMMELEN & STUIJFZAND (1993), who reported lipid concentrations in adult *P. scaber* of 98.8 μg/mg DW. The lipid concentration in SD treatment was slightly but significantly higher than in NP treatment. Even if NP individuals stored bigger energy reserves for prospective unfavourable conditions during autumn, they might have depleted part of those reserves during winter conditions before measurements were done.

There was difference in average glycogen reserves before and after exposure at the low constant temperature (+3°C, Fig. 7). Similarly, glycogen content of the pupae of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) gradually decreased during cold acclimation (IZUMI *et al.,* 2005). This could be explained by the fact that animals took energy to keep metabolic functions primarily from glycogen reserves. Dead individuals had only non-significantly lower glycogen content than survived ones. Thus strong depletion of this type of energetic reserves was not likely the direct cause of mortality.

The glycogen concentration in NP treatment was significantly lower than in both SD and LD treatments before cold exposure (Fig. 17) but similar to the levels in LD and SD treatments after cold exposure (Fig.7). It suggests that the natural conditions during acclimation of NP treatment were similar to those of cold laboratory exposure, causing reserves decrease. The higher glycogen content found in smaller individuals (Fig. 18) was probably compensated by higher lipid content in big ones (Fig. 16).

Cold-acclimated *N. rhenorhodanensis* accumulate both glycogen and amino-acids and trehalose (ISSARTEL *et al.*, 2005b). An increase in glycogen during cold exposure is rather paradoxical as numerous studies have reported a decrease in glycogen during cold acclimation of ectotherms: glycogen being generally used as a fuel for synthesis of polyols and sugars. Furthermore, glycogen levels are twice as high in *N. rhenorhodanensis* than in *G. fossarum*, which may partly explain the larger bound water content found in the former. However, even if the increased glycogen in cold-acclimated *N. rhenorhodanensis* may be partly responsible for the increased bound water (together with increase of amino acids and trehalose), its function in the freeze tolerance adaptation still remains unclear and needs further investigations.

## 4. Cryoprotectants

Overwintering insects can overcome severe winters by increasing concentrations of various polyols and sugars. Glucose, trehalose and fructose, as low molecular sugars, and glycerol, sorbitol and manitol, as polyols, have been detected in several overwintering insect species (SOMME, 1982). Furthermore, changes of trehalose content correlate with survival, suggesting that trehalose plays a cryoprotectant role in diapausing pupae, as reported in other overwintering insects (STOREY & STOREY, 1991; LI *et al.,* 2002).

The males of *Porcellio scaber* from LD treatment (simulating summer conditions) had higher content of glycerol than those from SD and NP treatments. Thus it seems likely that glycerol does not have cryoprotective function in *P. scaber*, but protects the isopods against desiccation.

The glucose concentration was significantly higher in males from SD treatment (simulating autumn conditions) than from LD and NP treatments. This glucose may represent transient activated energy and material for synthesis of winter energy reserves and/or other cryoprotectants.

Significant differences between treatments were not found in the content of trehalose (Tab. 14), and the individuals from NP treatment had not somewhat higher amounts than those from the other two treatments (Fig.17), as predicted.

Among all sugars detected, trehalose was the only one that accumulated in cold-acclimated crustaceans (ISSARTEL *et al*., 2005). Trehalose is widely recognized as a compatible solute: it has been identified as a membrane and protein protectant under desiccating conditions and thermal stress in a variety of organisms (CARPENTER & CROWE, 1988).

Also in *N. rhenorhodanensis*, large accumulations of trehalose and amino acids were found during low-temperature acclimation (ISSARTEL *et al*., 2005b).

## 5. Respiration

The ability to reduce metabolic rate in harsh conditions is a survival strategy of many animal species (Storey & Storey, 1988). Our results are in agreement with prediction that the oxygen consumption is lower at lower temperature. The males of *Porcellio scaber* from both treatments (LD, SD) had lower oxygen consumption at 15°C than at 25°C (Tab. 16, 17).

It has already been demonstrated that metabolic rate per unit of body mass is higher in smaller individuals, and it has been concluded that metabolic rate is related to size (Schmidt-Nielsen, 1984). However, we found just an opposite tendency in individuals from SD treatment at 25°C and individuals from LD treatment at 15°C (Fig.21, 22).

Fluctuations in oxygen consumption at 25°C during two days measurements under characteristic photoperiod followed my assumptions (Fig. 20). The species *Porcellio scaber* is an isopod which prefers dark and moist places, and has often nocturnal activity, so their oxygen consumption during dark part of photoperiod (scotophase) was higher because they were more active. Conversely, their oxygen consumption during light part of photoperiod (photophase) was lower because they were less active. The oxygen consumption probably approximated the level of basal metabolism.

The effect of temperature on the metabolic rate of terrestrial isopods is non-linear. The van ´t Hoff rule is one way to express this: within the biological temperature range the rate of chemical reactions and biological functions increases approximately twofold for every 10°C increase in temperature (the temperature coefficient $Q_{10}$ is approximately 2) (LAGERSPETZ & VAINIO, 2006). The $Q_{10}$ values for heterotherms are usually close to 2, through there is an effect of body size, with small animals having higher $Q_{10}$ values than bigger animals (DAVENPORT, 1992). Our results showed that the temperature coefficient ($Q_{10}$) for males of

*Porcellio scaber* from LD treatment was 3.2 and for SD treatment 1.8. $Q_{10}$ values measured in *Mesoniscus.graniger* ranged from 1.01 to 3.56 and did not differ markedly from those reported for other isopod species (ŠUSTR *et al*., 2005) For example, $Q_{10}$ values from 1.0 to 3.86 have been reported for *Armadillidium nasatum* and *Porcellio scaber* (ALIKHAN, 1983).

Metabolic rate of *Porcellio laevis* showed low dependence on ambient temperature in the range from 5 to 15°C. Then it increased exponentially between 15 and 25°C. Above 25°C, the increase in metabolism with temperature was slower. $Q_{10}$ values were 1.01 and 3.56 in the ranges 5–15 and 15–30°C, respectively (LARDIES *et al*. 2004). Anyway, differences in Q10 values between animals acclimated to different photoperiods were not previously reported and no explanation exists why animals in summer conditions are more sensitive to temperature.

Mass-specific oxygen consumption in *Mesoniscus.graniger* at the lower end of the range values reported for terrestrial isopods. About 53 µl O2/gh was measured in *Mesoniscus.graniger* at 10°C (ŠUSTR *et al*., 2005). This is comparable to values of 51–131, 144, 148–240 and 164 µl O2/gh reported for *A. vulgare*, *A. nasatum*, *Porcellionides pruinosus* and *Porcellio scaber* (WARBURG, 1983).

Low metabolic rate may be an imposed physiological consequence of the restricted energy sources in caves. ŠUSTR *et al*.(2005) reported minimal temperature mimit at 1.7°C, mean preferred temperature 23°C, and upper limit 36.6°C for *A.nasatum*, –2,2, 21.3 and 36.5°C for *P.scaber*, 0.1, 22.0 and 33°C for *Oniscus asellus*. The most apparent difference seems to be in the upper temperature limit (18.5°C for *M. graniger*). The corresponding upper limit lies at about 29°C in more hygrophilic species from thermally stable conditions such as *Ligidium hypnorum*, *Hyloniscus transsilvanicus* and *Porcellium conspersum* and at 40°C in more xenophilous species from exposed habitats (*A. versicolor*) (Šustr et al., 2005).

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