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TECHNOLOGICAL ASPECTS OF KEEPING *DYTISCUS LATISSIMUS* LINNAEUS, 1758 (COLEOPTERA: DYTISCIDAE) IN LABORATORY CONDITIONS

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During the studies of keeping and breeding issues of *Dytiscus latissimus* Linnaeus, 1758 we have touched a very wide topic, including field studies, as well as applied technical laboratory research. Taking into consideration the topicality of the tasks set within the framework of laboratory research of the species, we have started to elaborate general purpose aquarium system suitable for studying other palearctic freshwater hydrobionts, both invertebrates and small vertebrates (including some species of fish and amphibians) as well.

In order to understand and get acknowledged with the biology of *Dytiscus latissimus* L. part of the research activities were moved to the closed laboratory at Latgale zoo (Daugavpils, Latvia). We have attempted to create a natural microclimate in artificial conditions in order to identify natural behaviour of the species during their lifecycle.

Key words: *Dytiscus latissimus*, Coleoptera, Dytiscidae, larva, pupa, zoo-culture, aquasystem.

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INTRODUCTION

Apparently trials to keep diving beetles in laboratory are practiced since the birth of aquarium science (Зворыкин 1980). A number of authors discuss methodology of keeping diving beetles in order to use them as visual aid during biology lessons at school. However the description of behaviour and life cycles of diving beetles is based on authors' personal observations made in various aquariums. (Герд 1954, Махлин, & Солоницына1984, Павловский & Лепнева 1948, Ribera et al. 1997).

The methods of constructing aquariums and their equipment proposed by some authors several decades ago represented quite simple (affordable at that time) constructive solutions (Герд 1954, Пешков1961, Полканов 1981). However the majority of such models are dealing with systems of warm aquarium. When specialized equipment appeared on the market some authors proposed high-tech laboratories aimed at breeding exotic fish (Гоменюк 2011). Keeping of thermophiles, however, depended on temperature conditions in the premises where aquariums were kept. The room temperature where people live is usually within the range of 18-25˚C, whereas the temperature of the place where aquarium electrical equipment is working, can exceed +30ºC. This means that such environment is not always appropriate for keeping cold-water species. Usually such environment provides a possibility to observe natural behaviour of Palearctic aquatic species only during summer season of their life cycle.

However, there are successful modern "open air" laboratories with artificial aqua systems (Inoda & Kamimura 2004, Mölle 2001). Such equipment allows taking advantage of natural seasonal temperature and light fluctuations.

Palearctic aquatic species demand a specific temperature regime, which in most cases might not correspond to the microclimate provided by poorly equipped laboratory environment. (Vahruševs 2009)

Life activity of *Dytiscus latissimus* in nature passes in a wide temperature range: temperature fluctuations in water reservoir in winter to summer period can vary from 0º to 25ºC. (Fig. 10) Thus the life cycles of these species have a pronounced seasonality. These seasonal changes in the lifetime behaviour of these species create certain obstacles in its research. Besides, construction of habitat model in laboratory conditions demands certain skills and availability of specific equipment. Nevertheless perspective of species' research in simulated conditions encouraged making several revolutionary steps in solving certain constructive issues for reaching desirable results.

The technical issues of implementation of the environmental model for keeping *Dytiscus latissimus* have been divided into three parts:

- The 1st part provides conditions in a laboratory for imitation of imago keeping all-seasons, including wintering conditions, with gradual transition into a spring-summer conditions. This allows provoking and observing mating and breeding behaviour of the species;
- The 2nd part ensures observation of growth, development and behaviour of larvae;
- The 3rd part ensures the process of larva metmorphosis into imago.

In this study we provide a detailed description of technical adjustment of the equipment with a closed water supply, which is adapted for keeping and breeding diving beetles *Dytiscus latissimus*.

MATERIAL AND METHODS

Aqua terrariums for keeping imago

The construction of nurse-cage for keeping imago includes underwater part and above-water part. The above-water part comprises approximately a half of the total size of the cage. It provides the beetles with the possibility to creep out of water during their lifecycle. It also ensures conditions for experimenting with water plants necessary for beetles' reproduction.

During winter such aqua system works as a "freezer box", i.e. during cold season the cage is being aerated by cold air from outside through special ventilation holes. Thus, in a combination with especially cooled water in the aquarium it provides seasonal fluctuation of temperatures.
In order to ensure a proper operation of this aqua system the following activities were done:

- ventilation apertures (120 mm in diameter) were made in a test wall of a building which ensured an inflow of air from outside into the future aqua-terrariums; (Fig. 1: a.)
- central water supply was connected to the future aqua system;
- glass aquariums are constructed on two-storey metal frame of 140 x 75 x 250h (cm). The total size of the aqua-terrarium including above-water part is 131 x 70 x 95h (cm); the underwater part is 131 x 70 x 45h (cm). Each aqua-terrarium is divided into two equal parts by a partition. Each storey of aqua-terrarium has independent filtration and temperature control systems. Thus it is possible to maintain two different keeping environments in one installation (Fig. 1:b,c, 2).
- a protective thermo-insulating layer around aquarium systems and life support systems was made in order to avoid losses of temperature and

Fig. 1. Construction process of aquarium equipment for keeping *D. latissimus*; a - preparing of ventilation apertures in the wall.; b - finished metal frame and central water supply; c - metal frame with styrofoam insulation.

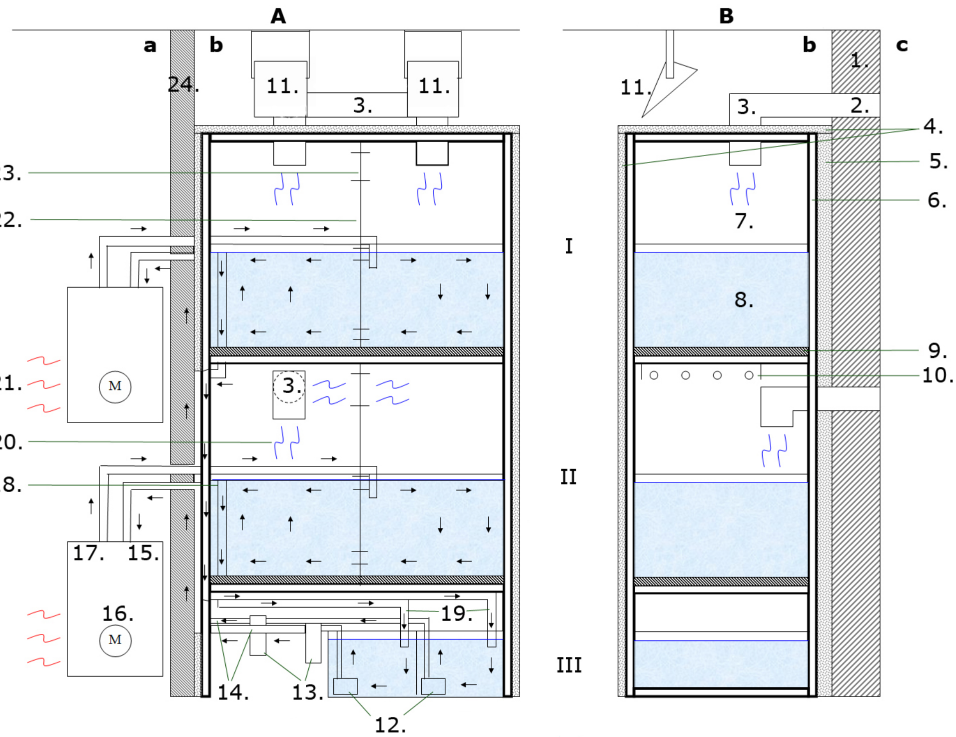

Fig.2. Scheme of aquarium installation with a closed water circulation for keeping *D. latissimus* (in a winter mode of activities). A – front view; B – side view. a – warm laboratory; b – cold laboratory; c – outside environment I – second storey of aqua-terrarium; II – first storey of aqua-terrarium; III – sump and biological filter. 1 – wall of a building (width 550 mm); 2 –ventilation aperture (120mm in diameter); 3 –platstic ventilation pipe (100 mm in diameter); 4 – Seasonal thermo-insulation

(foam plastic, thickness- 3 cm); 5 – Permanent thermo-insulation (foam plastic, thickness -6 cm); 7 – above-water part of aqua-terrarium cooled by outside air; 8 – water level in the aqua-terrarium; 9 –thermo-insulating woodchip shelf for aqua-terrarium, thickness 4 cm; 10 – fluorescent lamps (T8 4 x 18 W); 11 – metal halide lamp (150 W); 12 – reverse pumps of the first and second storeys of aqua-terrarium; 13 – absorbent carbon filters; 14 – reverse water pipes of the first and second storeys; 15 – input of warm water into the cooling equipment; 16 – cooler (480 W); 17 – output of cooled water; 18 – exchange pouring pump (pipe diameter - 32 mm); 19 – pipes of reverse water input of the first and second storeys of the sump; 20 – cold air flow from outside; 21- hot air flow from heat exchange equipment of the cooler; 22 – central glass partition inside aqua-terrarium; 23 – aeration window in the central partition of aqua-terrarium; 24 – interstitial wall of laboratory (thickness 150 mm).

Fig. 3. Preparing the cooler for work; a – making coils of cooling pipes; b – mounting of the cooling equipment; c - cooler ready for use.

appearing of condensate in the constructions; (Fig. 1; c., 2, 4; a)
- cooling equipment was installed and connected; thermo regulating equipment ensuring support of the temperature regime in the system was installed;
- conditioner in the premises of laboratory, which would ensure required temperature during summer season in order to avoid overheating, was installed;
- 2 water filters in the aquarium were installed. Sump parameters are 35 x 70 x 40h;
- radiants with timers allowing to set the required length of daylight were installed.

EQUIPMENT

Cooling equipment

1. It is recommended to use a special cooling equipment for aquariums produced by known manufacturers, although this equipment may sometimes be quite expensive.

2. Due to the lack of funding cooling equipment, which is widely used in food industry for cooling beverages (beer, soft drinks), was used in the experiment. This equipment is much cheaper and was quite suitable for cooling the aqua system, it was accordingly adjusted. (Fig. 3: c)

Preparing the cooler

In its normal condition the equipment for cooling beverages represents a tank with cooling pipes set into it. It also has pipes that are cooling beverages (soft drinks, beer, etc.). The original technology provides a tank filled with special liquid, as it represents a good temperature consuming substance. The basic pipes for cooling beverages were dismantled and instead of them the “spirals” of necessary diameter made of regular hosepipe with thin walls were installed, they route the water out of aquarium. The more coils there are, the better water is cooled. (Fig.3: a,b, Table1). Thus,

Table 1. Details of water cooling path

Inside pipe diameter (mm)	Number of loops	Loops diameter (mm)	Pipe length (mm)
12	15	155	1460

one piece of equipment can be used for several stand-alone aquariums, depending on the size of its cooling tank, which depends on the model of cooler and its power.

Water is supplied to the cooler through a filter with the help of an aquarium pump of a needed throughput. Then the filtered and cooled water is pumped back into the aquarium. It is necessary to remember that any electrical device educes heat. The cooler which is cooling water can also become a strong heating source in laboratory premises. Therefore the cooling equipment was installed outside the laboratory and placed in the adjacent room, where heating was needed. Thus loss of heat was avoided.

Thermo-insulation:

The simplest and cheapest way of insulation – styrofoam and thermo-insulation pipes was used for the aquarium and the connected supportive equipment (Fig. 1:c, 2, 4, 5.). During winter period the facade of the equipment is insulated with styrofoam plates in order to reach maximally low temperatures in it (Fig. 4:a).

While solving similar tasks in warm premises, aquarium scientists often encounter a problem of condensate on aquarium front glass (Karen 2008). There is an assumption that perspective solution to this problem could be application of special glass pack. This material would allow preventing misting up of the front glass in conditions of warm premises.

The aqua system covered with styrofoam does not produce any condensate while working in winter mode.

Temperature control

1. Temperature control of water inside aquariums is ensured by sensors and a thermoregulator (Carel IR), which control cooler work. (Fig. 5). Temperature regulation step of the thermoregulator is ±0,1°C . Temperature setup is always determined in the aquarium water output, i.e. inside the reservoir with exchange pouring pump. The temperature range in the set modes of operation has to correspond to + 1-4°C in winter mode; and +20-25°C in summer mode.

In addition to the cooler work, the aeration apertures in the wall of a building play the most important role in the natural water-cooling process. They provide cold air flow from outside into the above-water part of the aquarium through plastic pipes of 100mm in diameter.

In order to ensure precise control of intensiveness of cold air input from outside, it is possible to install automatic shutters with ventilators onto the aeration pipes regulated by a separate thermoregulator. In this case study such shutters are not required, as during winter season even at the lowest outside temperatures of -30 ºC, the temperature of the flowing water in the aquarium remained within the set range of +1,5-2 ºC.

2. Air temperature regulation in the laboratory premises.

- In summer period.

In order to achieve effective thermoregula-

Fig. 5 Thermoregulator (Carel IR) in semi-season mode.

tion inside the aquarium equipment laboratory premises were equipped with an air conditioner maintaining the inside temperature within the range of +21-22ºC during summer season, when outside air temperature may reach +35ºC. The conditioner makes operational process of aquarium coolers easier and supports comfortable temperature for researchers in the laboratory during hot summer months.

• In winter period.

Conditioner is not required during cold season and comfortable temperature in the laboratory is maintained by the central water heating system of the building. The thermoregulator installed on the heater ensures approximate temperature of + 18ºC in the laboratory.

Filtration

Several rules are to be observed while keeping diving beetles *Dytiscini* in an aquarium:

1. The animals breathe atmosphere oxygen which means that there should not be any bacterial or greasy slicks on the water surface. The presence of greasy slicks hampers gas exchange in water and breathing process of aquatic animals as well, since they get air from water surface. Greasy slicks are particularly dangerous as grease covers tegument of animals and thus air access to spiracles is disturbed; it can cause death;

2. Water flow in aquariums should not be intensive because formation of strong streams adversely impacts the life activity of animals naturally adapted to life in still water;

3. Absorbents (such as absorbent carbon) must be included into the filtration system because they absorb toxic substances appearing as a product of animals' life activity. Especially, in distress the prothoractic (repellent) glands of diving beetles are activating. The secretion excreted by these glands has high steroid concentration and after getting into water it can negatively affect living beings in the aquarium including beetles themselves. (Иванов et al. 1983, Шелег 2010);

4. In certain cases the filtration system may require ultra-violet sterilizer providing water decontamination from bacteria, fungus, viruses and protozoa some of which can become pathogenic for insects (Fig. 6,7).

Operational principle of closed water circulation equipment

Closed water circulation systems are specific because of using the same water amount during operation with only minimal water renewal. For normal life activity of aquatic animals it is necessary to control the presence of toxic nitrogen substances in water. In natural habitats the safe level of toxic nitrogen substances (originated from metabolites and waste of aquatic animals, detritus and meteorological precipitations) is reached by balanced nitrogen circulation in water. In the equipment with closed water circulation purification block should be installed in order to reduce concentration of toxic substances, thus regeneration block of recycled water plays the leading part in such equipment (Тырин & Ковачева 2008).

The best hydro-chemical indications of recycled water are achieved with the equipment with closed water circulation; this equipment contains a bio-filter filled with optimal amount of various materials for biological filtration of water. These materials act as a substrate for the development of colonies of aerobic bacteria that are processing nitrogen organic contamination ("general ammonium"). It appears in the system as a result of accumulation of metabolism products and nutriment remnants and turns into highly toxic (for aquatic animals) nitrites, and nitrites in turn are being further processed into noticeably less toxic nitrates. The latter are being removed from circulating water with the help of partial periodical water renewal. (Тырин & Ковачева 2008).

Filter's bacterial flora requires special water conditions for optimal life conditions. Temperature is an important factor. The best temperature for the development of nitrifying bacteria is from 20 to 30°C. Therefore this requirement is accomplished in the aquarium with warm water. It is important to choose corresponding filtering materials for

Table 2. Productivity of a reverse pump Resun King 4 in connection with the amount of the water circulating in the aqua system and path length

Total amount of water inside the system (L)	Length of water inflow path into the aquarium (m)	Technical parameters of the pump Resun		Actual height level of water supply of the aquarium (m)	Actual pump productivity in the system (L/H)
		output (L/H)	h/max (m)		
512,7	18,60	4800	4,5	2	360

aquariums with cold water in order to provide good filter coverage (Сандер 2004).

We are using Polyamide fibre wastes as load in our biological filter, providing initial rough water filtering, and aquarium SERA Filter wool for further thorough filtering.

The drop of biological filter's productivity in cold water should be taken into consideration. It is related to the fact that harmful substances in filters are removed by bacteria (as it is known, high temperatures accelerate vital functions but low – vice versa). Therefore in cold water there must be bigger purification system and filter than in warm water (Сандер 2004). Filter's cubic capacity for warm water aquarium must be from 3 to 8% from its volume. In our case cubic capacity of biological filter is 19,1%.

Biological filter with reverse pump is being placed into a sump, a special vessel connected to aquarium and always located under it (based on aquawiki.ru material).

Water is going up into the aquarium from sump with the help of reverse pump. When water level in aquarium exceeds certain level, the water gets into the pouring pump from where it flows down back to the sump (Fig. 2: III - 12, Fig. 6, 8).

Advantages of the sump

- It increases the amount of water in the system and thus positively impacts the stability of the system and provides stable performance in extraordinary conditions that could be harmful for the whole system if there wouldn't be the sump.
- With the regard to water supply from the main ("display") aquarium there is no bacterial slick and litter remnants on the water surface.
- It reduces the temperature in the system in summer. Overheating is the major problem in aquarium systems and thanks to the sump the temperature can be reduced by approximately 2°C.
- It supports constant water level in the main aquarium. Water levels' variations due to evaporation or other factors occur inside the sump.
- It improves water circulation inside the system, creates additional stream.
- Due to open water flow the water is being saturated with oxygen (based on aquawiki.ru material).
- It also prevents aquatic animals from possible impact of noises, vibrations and electromagnetic fields resulting from the operating aquarium equipment.

On the setup of the reverse pump water resistance inside the system has to be taken into consideration. The length of the path (pipes), including loops and turns, additional external filters and also the height of the water spout demands setup of a pump with large power reserve (Table 2). The power of water input flow into the aquarium is being regulated by a water tap system. In some cases continuous operating of the reverse pump can be controlled by timers. In this case water filtration in the sump should be done permanently with the help of additional pump and water is filled into the aquarium in batches on defined time interval. (Inoda & Kamimura 2004)

Aeration

In natural water reservoirs there are seasonal and daily fluctuations of oxygen dissolved in water. The process of water enrichment with oxygen is proceeding mainly in two ways: produced by photosynthesis process in plants and from the air. Oxygen is used to maintain life activity of aquatic animals and for oxidation process of organic and mineral substances.

Temperature has considerable impact on the level of water saturation with oxygen since its variations directly influence the oxygen solubility rate. With temperature drop the indicator of dissolved oxygen in water is increasing. However in winter due to the ice cover hampering penetration of oxygen from air the content of dissolved oxygen in water in many reservoirs is falling down to 50–25% of the normal summer level.

The generally assumed rate of the dissolved oxygen in water that providing life activity of aquatic animals is 4-12 mg/lt (Баклашова 1980, Котляр & Мамонтова 2007).

Biological filtration of water in the sump in aerobic environment is related to the oxygen penetrating into “closed” filters only with aquarium water. Intensive water aeration is required in aquariums where such filters are used. This is needed because the oxidation process of organic substance accruing inside filters requires a lot of oxygen and therefore aeration is needed not only for normal breathing process of aquatic animals but also in order to compensate the oxygen content spent on this oxidation process of organic substance (Fig. 6:12,13).

The aeration of the water in aquariums and filters is being provided by the commonly used aquarium air pump (Hagen Elite Air Pump Maxima).

This allows to increase the oxygen level in the water and to avoid appearance of bacterial slick

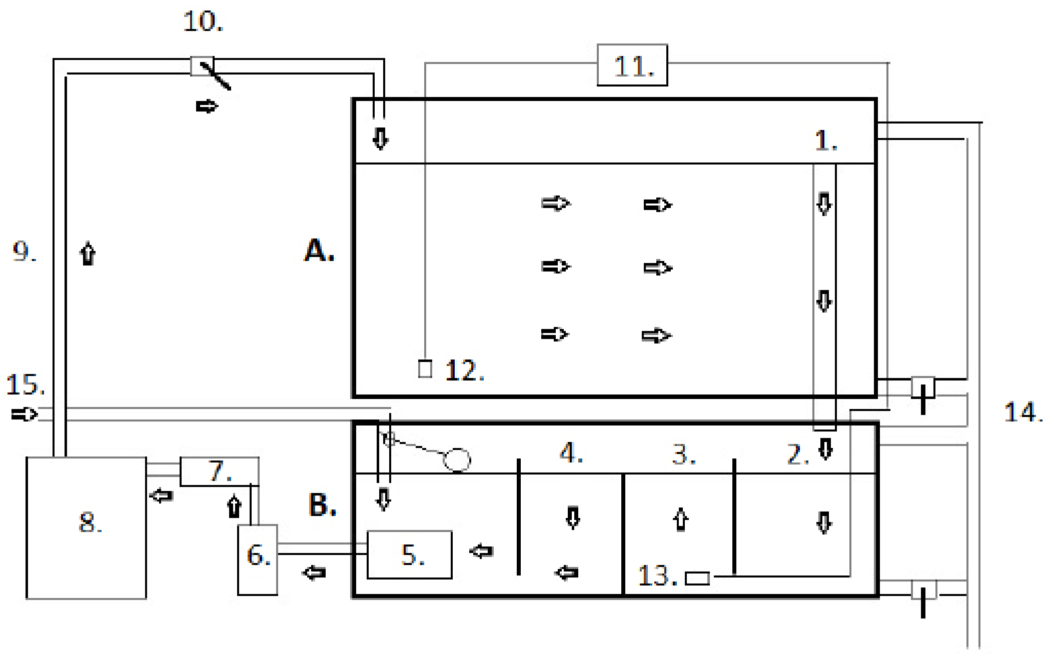

Fig. 6. Principles of water filtration and circulation in an aqua system. A- aquarium; B- sump and biological filter: 1.- Overflow pipes for drainage; 2 – initial rough cleaning filter; 3-4 further thorough cleaning filter; 5- reverse pump; 6- carbon filters; 7 - UV lamp; 8- freezer; 9- PVC pipes of water supply 10 – water rap; 11- Air pump; 12,13 – air supply sprays; 14 – drainage and reserve drainage system; 15- central water supply system regulated by a float valve.

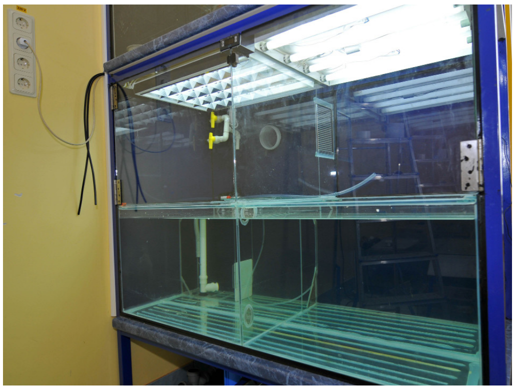

Fig. 7. First level aquarium ready for use.

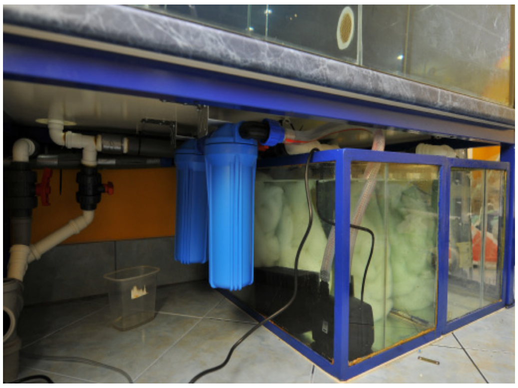

Fig.8. Filtration System of the Aquarium.

on the surface. Besides in winter period of equipment work the ripple on water surface prevents water from freezing.

Lighting

It must be remembered that during exploitation light sources emit heat. Therefore we have defined a way of lights installation by a trial and error method - by placing the heat-educing elements of electrical circuits outside the nurse-cages. The daylight settings are achieved by placing a timer. The upper storey of the aquarium stand is lighted by two lamps Proli Discharge Lamp MHL 02 150W of yellow and white spectrum (Fig. 2: 11., Fig. 9). The lower storey is lighted by luminescent lamps T8, 8 x 18 W (Fig. 2: 10, 7).

In summer period the daytime can last up to 12 hours and the light has to be dispersed. In winter period there is usually no need in lighting (twilight setting). The daylight in the laboratory penetrating through thermo insulation walls gives a natural lighting inside the aquarium.

Density of individuals and substratum

The density of the beetles in the aquarium in summer season can vary up to 60 individuals. During summer season keeping of males and females can be combined. Still, during the transition to the autumn-winter season males and females have to be kept separately except for the mating period. During the period of winter activity the number of individuals can increase. A substratum in the aquarium can represent snags, plastic plants and similar items, which provide perches and shelters for the insects. The more shelters there are the more beetles can be placed into the aquarium.

Hydrochemical water parameters

Data about physical and hydrochemical water parameters in natural water reservoir were collected and analyzed for building of the model of species' keeping conditions.(Fish ponds, Rugeli, Daugavpils, Latvia). Large and stable population of *D. latissimus* is observed for several years there.

Measuring of physical and hydrochemical water parameters was done with sound „Mini Sonde 4 Multiprobe" produced by Hydrolab and equipped with the following sensors for water indicators: pH; t°C, electrical conductivity (mS cm^{-1}), oxygen saturation %, oxygen saturation (mg/l), total quantity of dissolved solid substances (g/l), potential of oxidation and reconstruction(mV) and turbidity. The obtained data was saved with the help of software application Hydrolab "Surveyor 4 Data Display" (Grigorjeva 2011).

The indications of carbonaceous and total solidity, as well as data on ammonia and ammonium, nitrites and nitrates were got with the tests: Sera gH-Test, Sera kH-Test, Sera Ammonium/Ammoniak-Test (NH_4/NH_3), Sera Nitrite-Test (NO_2), Sera Nitrate-Test (NO_3).

Water samples for the analysis of biochemichal oxygen consumption (BOD_5) were taken from the depth of 0.4 m with the help of plastic bottles, which were closed with covers under the water when sample was taken. BOD_5 was determined with the help of YSI 500 Dissolved oxygen meter. Repeated metering of this sample was done after five days. During all this time the sample was kept in the container with a firm cover, which was kept in thermostat in dark conditions at the temperature 20°C.

The samples were also taken from the water in pipes and aquarium system with a moderately polluted filter working in it.

RESULTS AND DISCUSSION

As a result of the work done, we have constructed a multi-functional aquarium system that can be used for studies of cold water aquatic animals and warm water aquatic animals (Fig. 9).

Constructive solutions of this system provide possibility to reproduce seasonal temperature fluctuations in closed premises of laboratory and also to manage other main ecological ratios.

Water temperature and its hydro-chemical parameters in the set operational modes of the aqua system were based on the results of studies of water parameters in nature (Fish ponds, Rugeli Daugavpils, Latvia, 55°52'34.01 N, 26°35'17.41 E).

Water temperature parameters

Water temperature in water reservoir is a result of several simultaneously ongoing processes, such as solar radiation, evaporation and heat exchange with the atmosphere, movement of warm water streams by currents and turbulent water mixing, etc. Usually water is being heated top down. Annual and daily water temperature fluctuations on water surface and in the depth are defined by the amount of heat, that reaches the surface and also by intensiveness and depth of the water mixing process. Daily temperature fluctuations can be within a couple degrees' range and are usually observed in deeper water. In shallow water during warm weather the temperature fluctuations range is close to that of the air temperature fluctuations (Fig. 10).

Fig. 9. General view of the ready aquarium installation with closed water supply for keeping *D. latissimus* (in spring-summer operational mode) ("Latgale Zoo", Daugavpils, Latvia).

These data on temperature levels in a water reservoir and their correlation formed basis of the future aqua system operational principle, i.e. our aim was to create an artificial model reproducing natural temperature fluctuations dynamics and peaks.

Below for comparison purposes we reproduce data on water and air temperature fluctuations inside laboratory premises without using any special equipment (Fig. 11).

An aquarium with water gets warm in the warm space of laboratory in the result of exterior warmth resources influence. The indicators of the temperature in it and it the room have similar dynamics. However taking into consideration the fact that the water in aquarium are influenced by additional sources of warmth such as lamps and other electrical aquarium equipment, water temperature peaks indications can be overstated compared to the temperature of air in laboratory. It means that the water in aquarium is overheated. It critically influences the vital functions of the studied species in artificial conditions.

The above curves are very different from the original model. It is enough to compare the two graphs in order to realize the need for an aqua system. It is obvious that water temperature is directly proportional to the outside air temperature, and the latter figure depicts a temperature range suitable for warm water aquarium science only. Putting a special system for temperature stabilization in an aquarium and in a laboratory has become the solution for the set goals (see above). After putting the aqua system in use we have repeatedly taken the temperature data. Below we show relationship of the three parameters, and not two, (Fig.12) due to the fact that our aquaterrariums have direct access to the outside air through ventilation pipes (Fig. 2:3).

The access to open air had provided us a possibility to adjust the impact of the outside air temperature on the aquarium water temperature. Special freezers, in turn, allow for a reduction in temperature should it be necessary, and together with a conditioner and a heater make the inside air comfortable for work of researchers. In such a way we have got a model with the desired season dynamics of water temperatures in the aquarium, close to natural indications (Fig. 13)

Water temperature is the most important factor influencing physical, biochemical and biological processes that take place inside an aqua system. The oxygen supply mode and self-cleaning processes intensiveness depend on it (Гусева et al. 1999).

Daylight Longevity

Daylight longevity in Latvia is 6-7 hours in winter and 17-18 hours in summer. The main source of daylight in the laboratory is the natural light coming into the room through the window. Together with additional lighting of aquariums it plays an

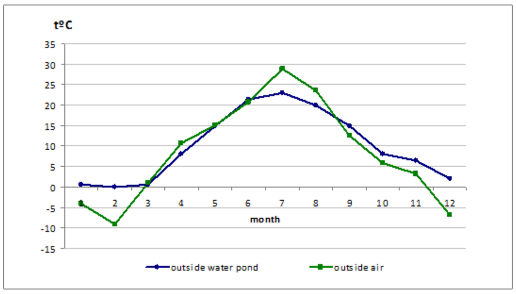

Fig.10. Dynamics of average monthly air and water temperature in nature in 2010-2011 (Fish ponds, Rugeli (Daugavpils, Latvia) (air temperature tºC based on www.gismeteo.ru data).

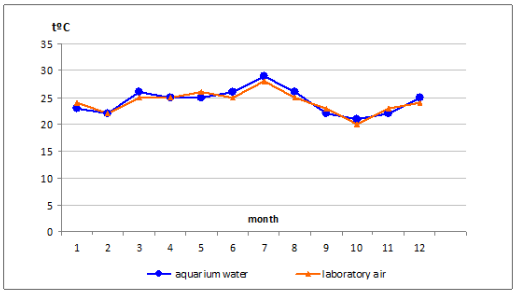

Fig. 11. Dynamics of average monthly air and water temperatures in a non-equipped laboratory in 2009-2010 (“Latgale Zoo”, Daugavpils, Latvia).

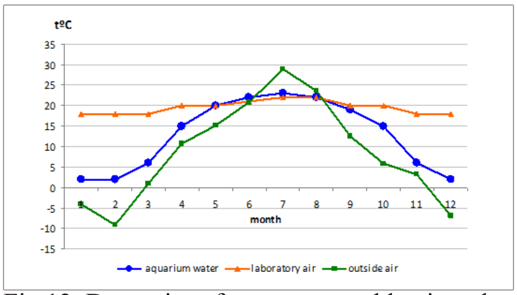

Fig.12. Dynamics of average monthly air and water temperatures in an equipped laboratory relative to the outside air temperature ("Latgale Zoo", Daugavpils, Latvia).

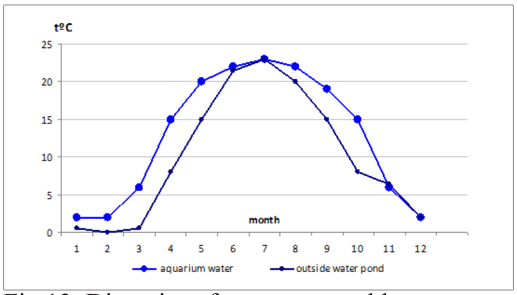

Fig.13. Dinamics of average monthly water temperatures in a natural body of water and in a laboratory aqua system in 2010-2011.

important role in regulating diurnal biorhythms of animals living in laboratories.

We have divided the intensity of daylight into three phases:

• morning twilight;
• day;
• evening twilight.

effect of morning and evening "twilight" in aquarium is achieved with the help of natural lighting coming through the window into the laboratory..

The intensity of the daylight is achieved with the help of the above mentioned equipment.

In such a way in aqua system summer working mode the daylight starts with the dawn . At 8$^{.00}$ the lamps are switched on.. Their work day lasts for 12 hours. At 20^{00} evening twilight starts. The night lasts for about 7 hours (Fig. 14).

The twilight and night are very important periods in *Dytiscus latissimus* life because exactly in these periods their activity is seen to be increased.

Water quality parameters

Quality of the water inside aqua system is a necessary prerequisite for success of keeping the species. We have been able to get to learn on the habitat of the beetles and their larvae based on the results of hydro-chemical analysis of water reservoir water (Table 3,4).

Analysis of Natural Water Hydrochemical Indices

As it can be seen in tables 3,4, Water Hydrochemical Indices are really different that depends upon seasons.

In a natural body of water water with index 9°dkH и 8,5°dgH is considered to be moderately solid and to have weak alkaline reactionимеет with index pH 7,5-8,5. It is characterized by having $Ca(HCO_3)_2$, $Mg(HCO_3)_2$ (Сандер 2004, Гусева 1999).

In summer peak period we observe critical changes in oxygen O_2 content in the body of water. The content of dissolved oxygen is essential for aerobic respiration as well as it is an indicator of biological activity (photosynthesis) in the body of water As it can be seen the most

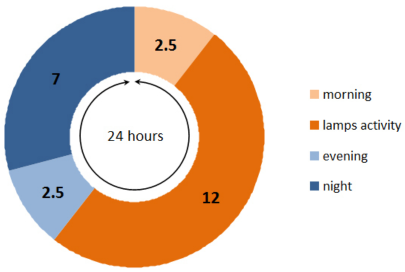

Fig.14. The model of daylight longevity in the aqua system in summer working mode (June-August).

Table 3. Hydro-chemical water parameters in an outdoor water reservoir. 2010 (Fish ponds, Rugeli, Daugavpils) (Matule, 2011)

Month	**t°C**	**pH**	**NH_3/ NH_4** (mg/l)	**NO_2** (mg/l)	**NO_3** (mg/l)	**BOD_5** (mg l^{-1})	**O_2** (mg l^{-1})	**RP** (mV)	**EC** (mS cm^{-1})	**TDS** (g/L)
May	17.7	7,8	0/0	0	0	2,595	10,025	470	0,35975	0,25
June	21,5	7,4	0/0	0	0	1,8475	8,625	445.5	0,351	0,2
November	6,4	7,2	0/0	0	0	2,8575	14,95	492.25	0,348	0,25

Signs in tables:
t°C = water temperature; pH = hydrogen index; kH = carbonaceous solidity ; gH = total solidity; NH_3/NH_4 = ammonia/ammonium; NO_2 = nitrite; NO_3 = nitrate; BOD_5 = Biochemical oxygen demand; O_2 - dissolved oxygen content; RP = Redox potential; EC = Electrical conductivity; TDS = Total Dissolved Solids; Tur = Turbidity

Table 4. Hydro-chemical water parameters in an outdoor water reservoir. 2011 (Fish ponds, Rugeli, Daugavpils)

Month	**t°C**	**pH**	**kH** (°d)	**gH** (°d)	**NH_3/ NH_4** (mg/l)	**NO_2** (mg/l)	**NO_3** (mg/l)	**BOD_5** (mg l-1)	**O_2** (%)	**O_2** (mg l^{-1})	**RP** (mV)	**EC** (mS cm^{-1})	**TDS** (g/L)	**Tur**
March	0,5	7,8	-	-	- -	-	-	5,25	26,63	5,27	437,25	0,385	0,3	1,67
July	20,7	7,12	9	8,5	0/0	0	0	2,885	34	3,023	275	0,440	0,28	22,3

critical temporal fall of oxygen quantity was indicated in July 2011 (3,023 (mg l^{-1})) (tab. 4). During this season the surface of the body of water is covered with water plants, that prevent water from enriching with oxygen and the layers of water from moving. Other indices showing the quantity of oxygen in water during summer period correspond to norms in the categories of pollution and water quality class: very clean, I, clean, II, (8-9 (mg l^{-1})) moderately polluted, III (7-6(mg l^{-1})) (Guseva at al. 1999).

According to universally recognized norms total **Biochemical oxygen demand** for outdoor water reservoirs used for fish farming purposes (of I and II category) must not exceed 3 (mg/l) at temperature 20°C (Guseva et al.1999).

Analyzing **TDS** data we can conclude that water in natural water reservoirs has average mineralization because the quantity of dissolved substances is from 0,2 to 0,5 g /l^{-1}. (Klavins, 1998) The bigger is concentration of the substances having ability to oxidize compared to the concentration of the substances that regenerate, the higher is redo-potential index. However **RP** index in natural bodies of water can vary from -400 to +700 mV (Гусева et al. 1999).

According to the opinion presented by several authors RP index suitable for life is between 25 and 35 rH in natural bodied of fresh water.

For transferring rH indices(reduktion Hydroqenii) into mV (millivolts) we use Nernst's formula (Мельченко1995):

$$rH=(Eh+200)/30+2pH,$$

where Eh – oxidation-regeneration potential, мВ; pH – acid-alkali balance index.

For example having transferred the RP indices of June 2010 (445,5 mV) we get RP 36,31 rH. It means that oxidation-regeneration potential is overstated in regard with indicated data.

From the received data we can conclude that the water in Rugeli pond is poor with salt because electrical conductivity is <1(mS cm^{-1}) (Bidens et al. 1997).

Table 5. Hydro-chemical water parameters in city central water-supply, July 2011 (Latgales zoo, Daugavpils, Latvia)

t°C	pH	kH (°d)	gH (°d)	NH_3/ NH_4 (mg/l)	NO_2 (mg/l)	NO_3 (mg/l)	O_2 (%)	O_2 (mg l^{-1})	RP (mV)	EC (mS cm^{-1})	TDS (g/L)	Tur
21.7	**7,7**	**9**	**8,5**	0/0	0	0	100,4	8,83	419	0,2941	0,19	10,1

Table 6. Hydro-chemical water parameters in an aquarium 2011 ("Latgale zoo", Daugavpils, Latvia)

Month	t°C	pH	kH (°d)	gH (°d)	NH_3/ NH_4 (mg/l)	NO_2 (mg/l)	NO_3 (mg/l)	BOD_5 (mg l^{-1})	O_2 (%)	O_2 (mg l^{-1})	RP (mV)	EC (mS cm^{-1})	TDS (g/L)	Tur
March	6	7,8	9	10	0/0	0	0	1,72	95,2	12,4	441	0,437	0,2	1,5
July	23,6	8,2	10	12	0/0	0	0	2,31	100,1	8,41	404	0,400	0,25	3,2

Water hydrochemical indices in working model.

The water taken from the central city central water-supply has become the starting material for making a model of water in aquarium. Having compared its hydrochemical indices with the indices of the water from the natural water reservoir, we understood that the water from the city water-supply can be appropriate for our experiment practically without preparation. The main criteria for the evaluation of the suitability of the water for the experiment were data comparative characteristics pH, kH/gH (Table 5).

On the basis of received data in nature we have got an imaginative model of water quality in aquarium (Table 6).

One can see a clear dynamics of data depending on seasonal temperatures and contamination extent of the filter. However kH and gH indices data demand paying attention to water-supply mechanism in the aqua system because they influence pH indices. We do not know for present how carbonaceous solidity can influence *Dytiscus latissimus* egg incubation and larvae development, that is why filtering system needs some changes.

The RP results appeared to be controversial. M. Sander states in his work that indications 150-250 mV show normal life conditions for animals in aquariums. But indices 350-450 mV affirm that oxidation-regeneration potential is overstated. It is characterized by the author as "extremely aired water in presence of organic substance". Such indications are reached with the help of intensive ozonizing. It means that the RP index is 350 mV and higher microorganisms are suppressed in the water environment of aquarium, it can be use in process of sterilization (Sander 2004). Data and expert's opinions on RP indices and its influence on aquarium's viability seriously differ. According to the opinions of other leading specialists in the sphere of aquarium science from the company Sia/ "KIBO S" ("Aquarium Doctor") (Riga, Latvia) RP index for normal life in fresh water aquarium with its inhabitants can be between 300 and 400 mV, in some cases up to 450 mV. Everything depends upon demands necessary for an experiment (Ruslan Shalajev (pers. com. 2011), Sia / "KIBO S" ("Aquarium Doctor"), (Riga, Latvia)).

Quality of water depends on good biological filter's functioning. Main criteria of correct biofilter's functioning are nitrogen cycle indices in aquarium water. The most important biofilter's inhabitants are *Nitrosomonas* and *Nitrobacter*. *Nitrosomonas* oxidize toxic ammonia (NH_3) to nitrites (NO_2), while *Nitrobacter* process poisonous nitrites into nitrates (NO_3). Optimal conditions are made for these bacteria thanks to aerobic environment. It is very important that organic nitrogen completely oxidizes to nitrates. Unused fodder, animals' vital activity products are gradually and slowly processed by bacteria. That is why toxic substance concentration in water can


Fig. 14. *D. latissimus* taking food with carp fry as a forage.


Fig. 15. Mating behaviour of *D. latissimus.*

increase. However a correctly functioning biofilter is able to remove by biological oxidizing toxic substances in the quantity in which they come. The final oxidizing level are nitrates which cannot be further processed by aerobic bacteria. Most water inhabitants tolerantly react on relatively high nitrate concentration. Thus concentration about 50 mg/l is acceptable while ammonia and nitrites are poisonous with concentration 1 mg/l (Сандер 2004). In our experiment we considered content of nitrates to be undesirable that is why their level in water is about 0 mg/l (see table 6).

This nitrate level in the aquarium is supported by 10% change of water once a week and by plants *Caltha palustris*. Regular washing of the filter in current water allows making its normal functioning stable. The biological filter has already been working for more than one year.

Aquarium water hydrochemical analysis allows concluding that the water quality model made


Fig. 16. Female of *D. latissimus* laying eggs.

in the laboratory is close to natural. Dissolved oxygen content indices are optimal. It allows providing normal biochemical reaction processing in the aquarium and its filter, as well as improves environmental conditions for animal life in the periods of temperature critical changes during summer and winter months.

The results of operation of the filtration and thermo-regulation systems can be valued as positive, and one can make a conclusion that quality of water obtained during normal mode of operation of the closed water circulation system corresponds to the water quality level (based on its contamination) in a clean water reservoir. (Гусева et al. 1999). The above water quality parameters are quite suitable for the normal life activity of *Dytiscus latissimus* and other aquatic animals from similar eco-systems.

Due to the functionality of the constructed equipment we succeed in our studies of *Dytiscus latissimus* and their behaviour during off-season. Ability to control temperatures, lighting and water quality provide us with option to experiment.

- We have identified that insects are reacting to a forage stimulators and take food at such low temperatures. Males are usually more active in taking food (Fig.14).
- For the first time we received an opportunity to observe *D. latissimus* in winter conditions at low temperatures and discovered that imago are leading active life during whole winter. Males are behaving especially actively and are

constantly moving. Apparently such male activity is related to the search process for females. Females, in turn, mostly are sitting in shelters during winter daytime. One of our discovery was observing mating process of *D. latissimus* at a temperature of +1,5C (Fig. 15).

- 11. Regulating temperature and lighting regimes we for the first time managed to organize for the insects a successful wintering in laboratory and to lead them to spring cycle with preparation for reproduction.
- Female reproductive behaviour also was noted for the first time (Fig. 16). As well as the first progeny was got from them (Fig. 17).

The described system provides for keeping imago of *Dytiscus latissimus* all year round with a smooth transition to an off-season time, as well as observing the beetle's mating behaviour, reproductive behaviour and process of eggs laying. One can also experiment with forage choice and priority mix.

Based on the general filtration and water circulation principles in the system and utilizing experience of the leading experts in the field (Inoda 2003, Inoda & Kamimura 2004), we have started the development of aquarium system for mass keeping and breeding of larvae of *Dytiscus latissimus* with the further transition into the pupa stage and receiving imago.

The examples of aqua systems' constructions and methods of their preparation mentioned by us allow to create a similar laboratory as a part of any heated premises in temperate latitudes, where the study process of objects can be more feasible and comfortable. Some of these developments are only of an experimental nature and certain constructive adjustments might take place at some stages of technocenoses.

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Fig. 17. Laying of *Dytiscus latissimus* in *Carex acuta* and hatchling of larvae.

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