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https://youtu.be/5RC2HhRCQ3s

Step di utilizzo animali in ordine di complessità/permessi

NEL PRIMO STEP USO ZEBRAFISH+
2 STEP USO I RATTI
3 STEP UOMO
PER STUDI SU GENETICA
IN AMERICA è DIVERSO. SI USANO MOLTE SCIMMIE, PRINCIPALMENTE MACACHI
TOPI NUDI USATI PER STUDI SUL CANCRO
CEFALOPODI USATI PER STUDI SULL'AMBIENTE

Direttiva 2010/63/EU: armonizzazione delle legislazioni nazionali in merito all'utilizzo degli animali da laboratorio
Decreto Legge 26/2014: recepimento italiano della direttiva europea

TERMOGRAFIA

there are many potential applications of infra-red thermography or thermal imaging in the area of animal welfare and production. These include the measurement of body temperature and characterisation of thermal exchange in a range of challenging environments and during procedures such as handling, transport and slaughter. The effects of the imposition of various stressors and the welfare outcomes may be assessed by IRT. Thermal status of animals can be determined by IRT at important stages of development and during different production phases. The prediction of heat and cold stress and the definition of thermal comfort zones for each species and age of animals are objectives of research in animal welfare that can be underpinned by thermographic analysis. Thermography is also extremely useful in the detection and diagnosis of disease affecting animal welfare and in the examination of injuries, inflammation, lameness, muscle damage and fatigue and in pathological states altering metabolic rate or metabolic heat production in specific tissues or body regions. IRT can be used to monitor the physiological and welfare outcomes of a large number of routine procedures to which livestock are exposed. Thermography also allows the monitoring the efficacy of treatments and practices aimed at improving animal welfare and health and can be employed to monitor recovery from pathological states and injury and to assess healing. Thermography can also be used to characterise the affective or emotional state of animals in response to stressful, harmful or painful stimuli or procedures and thus to quantify the welfare outcomes.
Thermography may be employed to monitor body temperature responses and thus the degree of heat stress or cold stress experienced and the welfare risks imposed by the challenge or to examine and quantify heat exchange at the animals' surface and to characterise the thermoregulatory responses exhibited
Thermography **can be considered as a refinement method** as it allows to measure the temperature of the animals without resorting to manipulation, minimizing stress.
The detection of a change in temperature can be an indicator of an altered clinical status (for example injuries, inflammation, tumors ecc) or a consequence to a stressor (consequent to a vasodilatation)
Thermography is **also a viable technique to assess the vascularization of superficial (cutaneous or subcutaneous) tumors** in laboratory animals, and to detect the temperature during the transport of animals (environmental and body)
Thermal neutralzone : That range of ambient temperatures within which an endotherm can control its temperature

HANDLING

Mice

1. Wear personal protection equipment (shoe cover, mask, head cover, gloves)
2. DO NOT PUNISH THE MOUSE FOR ITS NATURAL RESPONSE – return the animal to its cage. If bitten wash the wound, disinfect and bandage it

ENVIRONMENT

HIGENIC STATUS: Aims: primary prevention in order to avoid and counteract diseases and promote human well-being and efficiency (in our case: animal)

ANIMAL WEFARE
The welfare and health status of experimental animals are at the core of the science that takes care of them
Health and psycho-physical welfare are the bases for a good science

5 Freedom:

1- Freedom from hunger, thirst and bad nutrition
2- Freedom from environmental problems
3- Freedom fro disease and injury
4- Freedom to be able to express behavioral characteristics of specific species (IMPORTANTE)

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5- Freedom from fear and stress

Be focused on:
- intensity of the light. Have to be low. Consider photoperiod
- sound and vibration. Are very disturbing, sounds can come from people or instruments
- temperature and humidity (50 % circa)

Es:
AMBIENTE PER IL TOPO:
25 lux for albino. Need a low intensity lighting (light light can involved in aggressions. If I have a lot of ightning I have to pun less animals in cage).

LUMINOSITà E COLORI
Mice are sensitive to green and blue
Light is important to regulate circadian rhytms. (photoperiod)
occorre garantire fotoperiodi regolari ed un'intensità luminosa adatta alle varie specie
per il mantenimento di animali albini l'illuminazione tiene conto della sensibilità alla luce

SOUND
Animal are more sensitive to the sound (200 hz-90 hz for animal)
Di solito nei lab non ci sono telefoni ne computer. Eventuale videocamere sono mese a muro e negli angoli

TEMPERATURA
Zona termoneutra (TNZ): calore assorbito = calore perso, in inattività.
Topo: 26-34°C
Ratto: 26-30°C
Coniglio: 15 -20°C Al di sotto, i roditori termoregolano attivamente con adattamenti comportamentali

Thermal neutralzone : That range of ambient temperatures within which an endotherm can control its temperature
Room temperature = generalmente 21 +/-3, dependig from the animals
Ratti e topi: 20-26° / 18-28° /20-24° ←perche sono tutte diverse? Dipende dal tipo di lab. Negli stabilimenti riproduttivi ad es. la t è un po più alta

SLIDE

VENTILAZIONE
Poca ventilazione: accumulo di ammoniaca, anossia
Eccessiva ventilazione: disturbo degli animali, ipotermia, stress
Strutture particolari permettono l'ottimizzazione del microambiente: IVC, AV, isolatori.
La filtrazione dell'aria può essere fisica (filtri HEPA) o chimica (carbone attivo)

Effects of a bad environment can lead to physiological changes (metabolism, cardiovascular, growth, water and feed consumption, reproduction, behavior ecc)

Problema sintomativi vs asintomatici

AIM:we want to have microbiollogically clean animals. An animal can be in a health status meanwile it is infected. Infection is not synoninous of illness. But nevertheless an infected animal cannot show any sympythoms, and stay healty, the infectous can interfere with research. → health monitoring

ZOONOSIS
Can causes by
- Viruses
- Bacteria
- Fungi
- Parasites

_____ slide

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HEALTH MONITORING:

the main oobjective is to identify an infection as soon as possible to be able to take actions to contain and remove it.
When we take animals from outside fist of all we have to evaluate their microbiological status, the take preventive action such as quarantine and identify a destination
QUARATINE:

- For recovery of physical contitions (Gli animali transgenici hanno bisogno di un periodo di quarantena perché rispondono in maniera diversa all'ambiente)
- For health monitoring and clinical observation

Quarantine area can be external or internal to the facility nd the animals can be located in IVC cages or in a isolators.
During this period of time we can observate the animal and starting tests (PCR,foecal tests, blood sample) to have a clinical history, start preventive treatments and evaluate the compatibility with experiment needs.

comportamenti anomali o l'insorgenza di stereotipie tipiche della specie. Le principali sono elencate di seguito:

- bar pacing, ovvero i ripetuti spostamenti a destra e sinistra lungo le griglie del coperchio della gabbia;
- bar chewing, la masticazione ossessiva delle griglie;
- corner digging, lo scavare ossessivamente negli angoli della gabbia;
- cyrcling;
- twirling;
- jumping and back-flipping, una corsa in circolo ruotando su se stesso;
- spinning;
- barbering

GABBIE

Both pigs and rodents are animals that live in groups, and should not be housed alone without a very good reason for it
Alone housing can only be possible if the animal is in immediate danger of being harmed by others, or will harm others. For example, it might be necessary for some post-op animals to be housed in a cage alone for a convalescent period.

CONVENTIONAL CAGE. Una gabba senza filtro
PROTECTION CUP. Una gabbia con un cappuccio di protezione da non usare xò per periodi troppo lunghi
Ventilate cabinets = rack.
IVC CAGE SYSTEM - Individually Ventilated Cages
AV – Armadi ventilati

Si preferisce usare materiali di plastica che non fanno rumore e sono comunque lavabili (noize free)
Come arricchimento ambientale si possono inserire delle zone che permettono all'animale di nascondersi e dei pannelli removibili per permettere la socializzizione quando possibile (transparent panel for mximized visibility and easily removable for cage interlinking and socialization)

I topi vengono stabulati in gabbie aperte tipo 2 in plastica trasparente (eventualmente collocate in isolatori) o in gabbie IVC dotate di filtri HEPA in ingresso e in uscita. In condizioni sperimentali viene garantito uno spazio minimo di almeno 100 cm2 per animale. GLI animali sono sempre stabulati in gruppi o eventualmente in coppie. In allevamento sono invece previsti alloggiamenti di dimensioni minime di 330 cm2 per un trio composto da un maschio e due femmine

ARRICCHIMENTO

gli animali, ad eccezione di quelli solitari, sono alloggiati in gruppi stabili di individui compatibili [...] Si deve sorvegliare attentamente l'inserimento o il reinserimento degli animali in gruppi stabili per evitare problemi di incompatibilità e perturbazione delle relazioni sociali
2) tutti gli animali dispongono di uno spazio sufficientemente complesso che consenta loro di esprimere un ampio repertorio di comportamenti normali. [...] gli stabilimenti mettono in atto tecniche adeguate di arricchimento per ampliare la gamma di attività a disposizione degli animali e aumentare la loro capacità di risposta [...] L'arricchimento ambientale offerto negli alloggiamenti è adattato alle specie e alle esigenze individuali degli animali. Le strategie di arricchimento negli stabilimenti sono riviste e aggiornate periodicamente

Materiali interattivi inerti (giochi, tubi, mouse house)

- arricchimento fisico, sociale e ludico

Materie prime manipolabili (nesting materials)

- piallature di legno, cellulosa, carta, cartone, brick di legno

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Problemi correlati:
- sanitizzazione del materiale immesso, specie tra i cambi di lettiera
- minor osservabilità degli animali
- costi I vantaggi superano i problemi. (inoltre, obbligo di legge)

TESI benessere animali lab

TRASPORTO ANIMALI DA LAB

The transport of laboratory animals takes place under particularly controlled conditions
The conditions imposed by the legislation are very general but the their respect is however a prerequisite to minimize the stress that inevitably transport involves

REGOLAMENTO CE N. 1/2005 – protezione degli animali durante il trasporto e le operazioni

- Idoneità al trasporto
- Means of transport
- Spaces available
- Trasport practice
- Intervals for watering, feeding, period of travel and rest

1. SUITABILITY FOR THE TRANSPORT:

Animals that are not suitable for the trip cannot be transported:

- ARE NOT SUITABLE FOR THE TRANSPORT OF ANIMALS INJURIES OR PHYSIOLOGICAL PROBLEMS OR PATHOLOGIES (exeption if they are transport to reach a vet)
  - They are not able to move independently without suffering or walking without help
  - Animals with open wounds of a severe nature or prolapse
  - Pregnant females who have exceeded 90% of the gestation period or female who gave birth in the previous week
  - Newborn mammals
- No animal shall be transported unless it is fit for planned journey (NON SI POSSONO TRASPORTARE ANIMALI SENZA UN PIANO DI VIAGGIO (percorso durata, pause)

Sick animals may be considered fit for carriage if the transport would not cause additional sufferance (opinion of vet is required)
Are exluded animals that need to be carried to a vet

Non si possono trasportare animali con problemi fisici o patologie. Sono esclusi animali da laboratorio se con motivazione giustificabile e autorizzazione del vet.
Si intendono animali da laboratorio o da reddito che devono essere trasportati. Unica eccezione è se devono essere portati dal vet.

Come riconosco chi sta bene e chi no?

1- In grado di muoversi da solo
2- Senza ferite grai o prolassi

La responsabilità fino al termine del viaggio è del trasportatore e del proprietario/laboratorio
I mezzi devono essere adeguati e ventilati

2. TRANSORT MEANS:

Durante il viaggio, sul veicolo, deve essere sempre presente un accompagnatore (guardian accompanies), unless the animals are transportedi in fixed containers, ventilated, with food and water for a jurney of twice as long planned

- To ensure adequate ventilation is recommended to leave a central corridor between 2 rows of cages and leave a space between the roof and the top level of the cages
- Use vehicles, cages and technical solutions able to guarantee adequate ventilation and therefore suitable environmental conditions (T°, RU%, etc.).
- Equip the vehicles with probes capable of monitoring the microclimate and related alarm systems

3. TRANSPORT PRACTICES

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- Everything have to be designer and maintained and used in order to prevent injury, agitation and discomfort, clean and disinfected
- containers in which animals are transported must be marked to indicate the presence of live animals
- containers have to be placed in a safety way in order to avoid movements during the transport
- if the containers are loaded on multilevel, take precautions to prevent urine an faces from falling on animal placed at lower level. Ensure the stability of the containers an a correct ventilation
- Limit transport of lots of animals coming from different companies

CONTAINERS (IATA) → CONTAINERS WITH OVERSHIPPERS (one or more primary containers with a secondary covering). Overshippers add structurl strength, resistance to microbial contamination, thermal protection and can be add with additional air filtration.
Containers have to be labled, labels must be in English

- SIZE → animals must be able to move freely within the container and have to be able to assume normal posture
- VENTILATION → space for ventilation windows would be minimum 14% of the total area. Primary container windows must have filer that covers them completely (for biosafety)
-

ROAD TRANSPORT → dedicated vehicles, disinfections of means, educated and informed personal, reduce physiological stress (Ambiental conditions and sounds)

AIR TRANSPORT → animals must be transported in containers compatibile with the species following IATA guidelines. Animals can only be transpored in condition in which is possible to maintain air quality, temperature, pressure within appropriate limits.

- PROBLEMS: species cannot be separated. Difficulties in handling quality control. Non flexible shipping and deliverity times

LAB ANIMALS

Rodents (mouse and rats) are very intelligent and social animals
Sensible to changes of RU
Avoid isolation and overcrowding
A good capacity to making a neat (nido) shows good condition of wellness
Grooming is a natural behaviour, but if it is an excess it can be sign of stress EXCESS OF GROOMING CAN BE SIGN OF STRESS
Nocturnal animals so we have to guarantee the photoperiod

HANDLING AND RESTRAINT→ For cage changes, treatments, health monitoring, collect samples

- TUNNEL HANDLING
- CUP HANDLING
- COMBINED TUNNEL+CUP HANDLING
- RESTRAINT – with hands (piking from the tail then from the scruff) or with restrainer

Strumental restraint and sample collecting can be very stressful

When picking up adult mice, grasp them gently but firmly at the base or center of their tail. Do not pick them up by the tip of the tail. Place the animal on a surface such as the wire cage top or lid (Figure 7a). It is best that the surface not be slick or smooth as mice will behave much more calmly if they have firm footing. While still holding the tail near the base, with your other hand firmly grasp the loose skin on the back starting near the ears using your thumb and first two fingers (Figure 7b). The tail can then be held by the last two fingers as shown (Figure 7c). Your grip should be firm enough to keep the mouse from struggling, but gentle enough for it to breathe comfortably.
For quick handling, such as cage to cage transfers, it is acceptable to use forceps (Figure 8). Gently grasp the loose skin on the back and quickly transfer them to the new cage. This technique is useful for fractious or aggressive animals. Be sure to clean gloves or forceps with a disinfectant such as Virkon between cages. Another method of transferring mice is referred to as the "tube" or tunnel method (Figures 9a & 9b). For this method, gently guide the mouse into the tunnel with one hand while holding the tunnel with the other. Once the mouse enters the tunnel, gently tilt it and close your hands over the tunnel. Transport the mouse to the destination cage and release. This method is recommended for more anxious strains of mice.

ADIMISTRATION OF MEDICINES
All operator have ethical responsability. This means an appropriately handling
The operators have to wear protective disposal for both protection, for the animal from the operator and viceversa.
È utile anche che l'operatore sia sempre lo stesso venga riconosciuto dall'animale, stesso odore, stessi vestiti, parlare con voce bassa

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- iNTRAPERITONEAL - into the abdominal cavity, penetrating the peritoneum of the animal. used mainly for injected anestesia. since inadvertent injection of some material into the gut, abdominal fat and subcutaneous tissues is a relatively frequent occurrence. it may be preferable to use other routes such as subcutaneous or oral administration
- SUBUTANEOUS - beneath the skin and above the underlying muscle. Usually along the back or flank
- INTRAMUSCULAR - into the posterior thigh (biceps quadriceps or femoral quadriceps) is potentially more painful than other. in rat and small rodents in due to the small sizes the IM administration could be difficult and painful. If the injection is necessary they can be made into the front or back of the tight
- INTRAVENOUS in a marginal vein of ear pavillion. We can put the animal in a warmer environment in order to stimulate the dilatation of the vein, or keeping the tail in warm water Restrainer is necessary
- ORAL GAVAGE - allows for gastric delivery of substances. The tube length is measured taken the distance from the tip of the nose to the last rib. In vertical position the tube is insert inside the oral cavity. Be careful to not introduce it into the trachea to avoid an AB INGESTIS
  in rats and mice: we can train animals to drink automatically by a siringue or the widley used methods is to use a tube insert dirtectly in the esophagus. The operator have to be trained because he risk is to insert the tube in the trachea causing an ab ingestis. The animal have to be taken in a vertical position during the procedures. Wait few minutes after the procedure to see if there are adverse reaction

COLLECTING BLOOD
Submandibular bleed - puncture the area behind the hinges of the jawbones. Blood is collected with a tube. Be careful to not cut too close to the ears to not perforate the ear drum. If this happens it bust be euthanized

- Saphenous Vein
- Tail artery / vein
- Cardiac punture – conductedONLY under anestesia

The total amount of blood that we can take is the 8% of the weight of the animal
Sedation with ACP is allows easy manipulation and vasodilatation
the withdrawal site should not be used more than twice
Provide adequate rehydration therapy

EUTANASIA
Co2 – using an euthanasia chamber (a compressed gas tank)
Anestesia – injectable or volatile anestesia is performed before a physical eutnasia

Rodent Identification
- color (colorare il pelo x identificare dura 2 sett nn di piu). Colorare la coda idem
- ear punch. Più zootecnico che altro
-distal falange remover (non più usato)
- microchip. Più recente. S può rilevare anche da appena fuori la gabbia.
-tatooing (tail or hands) . a volte si può levare. Dura qualche mese
- ear tags (marca auricolare). Potrebbe essere morsa dagli altri animali

MOUSE

Well adapted to be manipulated if taken in yung age
Well adapted to life In cage
Nocturnal animals
The disappearance of social life and stereotypies are an indication of poore welfare
T. 37-38
T. ambientale 20-24
RU 50/60%
LIFESPAN 2Y
CAGES → 200 squares cm for pair of animal. Avoid overcrowding. Plastic floor.
LITTER → sawdust or wood
TERMOREGULATION → ears tail,foot pads
ENRICHMENT → nest in the shelter with a house
PREGANCY → 21 days. 8-10 newborn hairless and blind

DISEASES→ dermatophytosis, mycoplasma pulmonis, viral or bacterial diarrhoea, nematodes and cestodes
→ Cutaneous, respiratory, gastro enteric

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BEHAVIOUR

Mice are usually mild in temperament and easy to handle. They are not usually aggressive, but can bite if frightened

- Mice are nocturnal animals. Activities such as eating, drinking or mating are typically done at night. 3. Mice groom themselves almost constantly to maintain a smooth, glossy haircoat (Figure 1). A ruffled or dull haircoat is generally a sign of illness or distress
- Dominant mice exhibit a behavior called barbering. Barbering is the dominant mice biting or chewing on the fur of a more subordinate mouse.
- Male mice can be more aggressive and fight more often than females. Aggressive mice should be housed individually to avoid severe injury to cage mates. Generally male littermates may be housed together, but once separated, it is advisable to only house males with females.
- Mice are creatures of habit. Everyday events do not tend to stress or excite the mice. However, handling and restraint can be stressful and result
- Check rodents' teeth frequently. This will insure early detection of malocclusion. If maloccluded, teeth may become overgrown and interfere with eating

Sex Determination

Gender in mice is determined by comparing anogenital distance, or the distance between the urogenital opening and the anus. Male mice typically have a larger anogenital distance when compared with the females. Be aware there are variances in anogenital distance among strains. See Figures 5 and 6 below.

Handling and Restraint

Handling mouse between catching them from their tail, combine by holding from the grass on the back, or catching by taking them on cupped hands, or using a tube positioning in the cage

Restraint will be stressful, even in animals that have become accustomed to handling, so the duration of restraint should be minimised.

When picking up adult mice, grasp them gently but firmly at the base or center of their tail. Do not pick them up by the tip of the tail. Place the animal on a surface such as the wire cage top or lid (Figure 7a). It is best that the surface not be slick or smooth as mice will behave much more calmly if they have firm footing. While still holding the tail near the base, with your other hand firmly grasp the loose skin on the back starting near the ears using your thumb and first two fingers (Figure 7b). The tail can then be held by the last two fingers as shown (Figure 7c). Your grip should be firm enough to keep the mouse from struggling, but gentle enough for it to breathe comfortably.

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RAT

Intelligent and stong adaptability. Very sociable

T 37-39

LIPESPAN 2-4 Y

SEXUAL MATURITY → 2 MONTH

REPRODUCTION → ALL YEAR LONG

PREGNANCY → 20-23 DAYS

CAGE → minimum 40x30x30

LITTER → wood shaving or pellets

ENRICHMENT → GAMES

Apathetic behavior, lack of appetite, excessively arched back, bristly hair, dull eyes, at half-mast or watering, poor whiskers are signs of rat malaise.

Respiratory diseases are very common in rats. To avoid them, it is important to keep the animals in clean environments, feed them in a balanced way and avoid any source of stress.

DISEASE→ respirator diseases and tumors (mammary most of all)

RATS are restrained take the from the gras on the back, and holding between the arms.

RABBIT

T amb 15-20

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Rabbits are used for their dimension. È piu facile prelevare campioni di sangue, monitorare la temperatura e condurre interventi

PAIN ASSESMENT
- anxious and aggressive
-Try to hyde
-vocalize
-lordosi e cifosi
-if the pain is abdominal they grind their teeth
-Respiratory rate increase
-Cannibalize the young

ADMINISTRATION AND COLLETION TEQUNIQUES
All operator have ethical responsability. This means an appropriately handling
The operators have to wear protective disposal for both protection, for the animal from the operator and viceversa.
È utile anche che l'operatore sia sempre lo stesso venga riconosciuto dall'animale, stesso odore, stessi vestiti, parlare con voce bassa

Vie di somministrazione SC, ID(INTRADERMAL) IM, EV

SC: on the back or on the side. Not in the area between shoulder because is the area used to hold them
ID: along the back
IM: bicipes or quadriceps femoris. Be carefoul to sciatic nerve (biceps)
IV: marginal vein of the ear pavilion

PRELIEVO DI SANGUE: marginal vein or central artery (higher volumes of blood) of pavilion auricular. some site require the sacrifices of the animal (left ventricle)
The total amount of blood that we can take is the 8% of the weight of the animal
Sedation with ACP is allows easy manipulation and vasodilatation
the withdrawal site should not be used more than twice
Provide adequate rehydration therapy

CONTENZIONE: hold the rabbits covering eyes and blocking hindlegs, or using rabbit restrainer

HAMSTER
Cannibalism
T.37-38
T ENVIRONMENT 21-25
RU 30-70%

PREGNANCY → 15 GG

GERBIL (Mongolian gerbil is the common one)
T 37-38
T ENVIRONMENT 20-22
RU 30-40%
PREGNANCY → 24-26 GG
Necessary sand container

GUINEA PIG (cavia, porcellino d'india)
Originated from South America (Venezuela, Argentina), in areas with vegetation and bushes to take refuge.
Introduced as lab animals in XIX century. Used for meat production in Peru
Adult weight around 700-1000 gr (male bigger than female), length 20 cm, 20 teeth (incisor, premolars and molars with continuous growth). Artrophied tail. Cecotrophy
T 37-39.5
T ENVIRONMENT 18-24
RU 50%
LONGEVITY 4-8 Y

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REPRODUCTIVE PERIOD all year
PREGNANCY 60-70GG

NEWBORNS : open eyes, without teeth. 2-3 child (up to 6). Birth weight 70-100 gr (sono grandi!). Sel sufficient as early as 1 week

NUTRITION: strictly herbivore.

- Hay ad libitum
- Pellet: 20% protein, 3 %lipids, 10% cellulose
- Vegetable: dandelion, clover, alfalfa, salad, cabbage, cauliflower
- Fruit: apple, pear, cherries, peches, apricots, berries
- Springs to chew
- Cecotrophy
- VIT C must be supplemented (peppers, spinach, citrus fruits, tomatoes, broccoli)

HABITS: Continuous activity, day and night, not good at climbing and jumping, does not bite
groups of 5-6 individuals, even males provided they are grown together
The guinea pig communicates by marking the territory (anal glands), smell, whistling (= calls).
WHISTLES AND SQUEAKS: discomfort or pain
GURGLINGS: comparable to purring

HUSBANDRY:
Cage dimensions: 60x30x40 cm (1800 cm2)
• Litter (soil for rodents, straw, hay, untreated wood shavings, paper). NO grids or nets. Hay holder
• Drop drinker
• Temperature: 18-24°C
• Light: 12-14 ore
• Humidity: 50%

PATHOLOGIES:
• Dental problems and diseases of the oral cavity
• Respiratory infections
• Endotoxemia (later, severe enteritis, abortions, dystocia pregnancies, constipation)
• Skin infections (mycosis, mange, pododermatitis)
• Scurvy (lack of vitamin C)

PRAIRE DOG
T 35-39
T ENVIRONMENT 20-22
RU 30-70 %
PREGNANCY → 30-35 GG
Need thick layer of hay and accessory to set up a burrow

ZEBRAFISH
Because of its fully sequenced genome, easy genetic manipulation, high fecundity, external fertilization and rapid development, and nearly transparent embryo, zebrafish are a unique model animal for biomedical research, including studies of biological processes and human diseases
One reason that zebrafish are an important biomedical model is because zebrafish embryos are transparent and they develop outside of the uterus. This unique developmental process allows scientists to study the details of development starting from fertilization and continuing throughout development
Danio rerio the Latin name for zebrafish formerly called *Brachydanio rerio*
In the natural habitat, zebrafish are usually found near the bottom of the water to minimize attack by predators
zebrafish are classified as omnivores and they eat a variety of foods
The most advantageous features of zebrafish are a fully sequenced genome, easy manipulation of its genome, high fecundity, short generation time (about 3 mo), rapid embryonic development (24 hr), and external fertilization. The translucent zebrafish embryo allows study of the different developmental stages starting from the early stage of embryogenesis. In addition, zebrafish embryos form complete organ systems, including heart, intestine and blood vessels within 48 hr after fujymnhertilization. More than 10,000 mutants in protein-coding genes have been generated (Howe et al., 2013) and several transgenic lines of zebrafish have been made to study human diseases. The availability of multiple strains of zebrafish is another important advantage of this species. In addition, it is also very affordable to maintain a large number of zebrafish in a relatively small amount of laboratory space. Although zebrafish require relatively easy management, special attention must be paid to ensuring a healthy diet and adequate water quality to optimize fish health and growth

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There are several examples of human diseases that have been successfully modeled in zebrafish such as Duchenne muscular dystrophy, human melanoma, acute lymphoblastic leukemia, polycystic kidney disease, nephronophthisis, acute kidney injury, Parkinson's disease, Huntington's disease, Alzheimer disease, myocardial infarction, and some metabolic diseases
The bred of zebrafish is regulated by the Dlsg 26/2014 (non human vertebrates animals)

HUSBANDRY:
Water T 28°
Zebrafish have no apparent heteromorphic chromosomes, which indicates that no clear sex-determining chromosome exists. In addition to genetic factors, sex determination in zebrafi sh is also infl uenced by environmental inputs. Indeed, environmental changes that involve hormones (Hill and Janz 2003; Westerfi eld 1993) and temperature (Uchida et al. 2004) have been shown to affect sex differentiation

Zebrafish adults lack strong sexual dimorphism. Individuals of this species can be sexed by body shape and color. Females have a larger, light silver belly that protrudes from the body in the anterior region. Males typically lack a protruding belly and are therefore more streamlined in shape

Most laboratories have stopped using marbles. Instead, crossing cages have been designed with an additional plastic container that holds the fi sh and is inserted inside the cage. The bottom of this container is perforated, which allows freshly released eggs to fall down into the outer cage and be protected from predation. Imitation plastic plants or green mesh are often placed in the inner container to provide artifi cial spawning sites as well as places of refuge to decrease the effects of aggression related to antagonistic social behavior.
The density of the population also affects female reproductive success. It has been reported that, at higher densities, average egg production per capita decreases

zebrafish is raised in stand alone tanks (where the breeding tanks and the necessary equipment for water treatment are in a single unit) or in solutions consisting of tanks connected to a water circuit that supplies purified water and filters the waste water
The tanks are usually stacked on supports in several rows and columns forming racks. each tank can be extracted individually. the water inlet and outlet pipes are connected to each tank with a valve that allows the flow in each individual tank to be interrupted.
The tanks can have different sizes (from 1 liter for the growth tanks up to 5-10 liters for adults).
The tanks must be illuminated providing 14 hours of light and 10 hours of darkness
the breeding tanks have a particular pyramid-shaped bottom with holes that allow the collection of the eggs after laying.

To reproduce only a selected line, the pairs of reproducers are introduced the day before in a tank that can be divided by a septum permeable to water and the pair divided while maintaining visual contact. on the day of mating the divider is removed and fertilization begins.

Once the eggs have been collected, they are taken by siphoning and placed on a Petri dish for incubation

FEEDING
commercial food is usually supplied with live food supplementation (brine shrimp nauplii), the food is supplied by dispenser in each tank

XENOPUS
is a claw-toed frog from Southern Africa that lays many large eggs, traditionally used for embryology but also for biochemical studies, as extracts can be made from eggs and embryos to provide active cytoplasm in a test tube, that can undergo cell cycles and be used to study many processes, such as protein ubiquitylation and degradation
Aquatic life but they are amphibians, nocturnal
Maintainance of social groups (4-5 females, 5-6 males in standard tanks) IN Standard tank dimensions: 5-10 l
Tadpole are vegetarian, adults are omnivores. They are feeding 3-4 times at week
Breeding in acquaria with controlled water parameters, in smoked transparent tanks with perforate lids
Room t 18 20°, water T 18-22° + 2 for tadpoles
Humidity at least 50-6%
Environmental enrichment: ware lilies and refugies

D.Lgs 26/2014 includes larva forms allow to feed by itself. So we can use xenopus embrio before larval stage and any legal permission is required

Mating can be hormonally induced (FETAX), that provide a lot of eggs(mature and immature) but animals have to be put in rest for 3 month before another induction, or naturally induced by ambiental influence. Temperature, water and humidity have a crucial role, eggs are less but with high efficiency (only mature eggs), animals are less stressed and can be reproduct in 15 days

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NEMATODI

Caenorhabditis elegans is a nematode length around 1 cm. around 60% of its genome is omologue to humans

Caenorhabditis elegans is a small nematode worm, about 1mm long, which we use as a "model organism". **A model organism is a non-human that is used in the lab to help scientists understand biological processes**. In nature, C. elegans is found on compost heaps, where it eats bacteria and fungi.

C. elegans toxicity assays provide data from a whole animal with intact and metabolically active digestive, reproductive, endocrine, sensory and neuromuscular systems. Toxicity ranking screens in C. elegans have repeatedly been shown to be as predictive of rodents LD50 ranking

C. elegans is included in early safety testing and as one component in integrated toxicity testing strategies. Nematodes alone cannot replace data from mammals for hazard evaluation. It's use is avoided of ethical problems and is in line with the "three Rs" principle of Replacement, Reduction, and Refinement on the use of vertebrate animals

HOW TO BREED

- In a solid or in a liquid means
- Can be frozen

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