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Scientific and technical assistance on welfare aspects related to housing and health of cats and dogs in commercial breeding establishments

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Abstract

This Scientific Report addresses a mandate from the European Commission according to Article 31 of Regulation (EC) No 178/2002 on the welfare of cats and dogs in commercial breeding establishments kept for sport, hunting and companion purposes. The aim was to scrutinise recent recommendations made by the EU Platform on Animal Welfare Voluntary Initiative on measures to assist the preparation of policy options for the legal framework of commercial breeding of cats and dogs. Specifically, the main question addressed was if there is scientific evidence to support the measures for protection of cats and dogs in commercial breeding related to housing, health considerations and painful procedures. Three judgements were carried out based on scientific literature reviews and, where possible a review of national regulations. The first judgement addressed housing and included: type of accommodation, outdoor access, exercise, social behaviour, housing temperature and light requirements. The second judgement addressed health and included: age at first and last breeding, and breeding frequency. Judgement 3 addressed painful procedures (mutilations or convenience surgeries) and included: ear cropping, tail docking and vocal cord resections in dogs and declawing in cats. For each of these judgements, considerations were provided indicating where scientific literature is available to support recommendations on providing or avoiding specific housing, health or painful surgical interventions. Areas where evidence is lacking are indicated.

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Keywords: animal welfare, cats, dogs, commercial breeding, housing, health, painful procedures

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Summary

The European Commission requested that EFSA produce a Scientific Report according to Article 31 of Regulation (EC) No 178/2002 on the welfare of cats and dogs in commercial breeding premises kept for sport, hunting and companion purposes. EFSA was requested to review recommendations, prepared by the EU platform on animal welfare and its voluntary initiative, on housing, health and painful procedures of commercial breeding of cats and dogs. Specifically, the main question to address was if there is scientific evidence to support (so called, judgement) the recommended measures for protection of cats and dogs in commercial breeding establishments.

A scoping literature search was carried out as basis to address the question. The available scientific literature was complemented by consultation of independent experts in an EFSA *ad hoc* working group on the welfare of dogs and cats. Where possible, examples of national legislative provisions on the different topics (housing, health and painful procedures) were highlighted to support the judgement of the mandate questions.

This Scientific Report details the approach and results of this judgement.

Three judgements were carried out based on scientific literature reviews and, where possible, grey literature and examples of national provisions. For each of these judgements, considerations were provided indicating where scientific literature is available to support recommendations on providing or avoiding specific housing, health or painful surgical interventions.

Judgement 1: Housing of cats and dogs in commercial breeding establishments

The first judgement addressed housing and included: type of housing (i.e. type of accommodation), outdoor access, exercise, social behaviour, housing temperature and light requirements.

In relation to the **type of housing**, boxes, crates and cages are considered small enclosures, and a permanent stay in these impairs welfare of cats and dogs. On the basis of the retrieved evidence, breeding cats and breeding dogs should not be kept permanently in boxes, crates or cages, regardless of whether or not these are tiered.

The confinement of both cats and dogs in tiered boxes and crates leads to welfare consequences such as abnormal behaviour and distress; a permanent confinement in tiered enclosures may exacerbates these welfare consequences.

Regarding the **need for outdoor access, exercise and social behaviour** of dogs no publications were found on the need for breeding dogs to have access to an outdoor area on a daily basis. Publications, available mainly in shelter dogs, studied the need for exercise combined with outdoor access and the need to socialise with either humans or dogs, but not necessarily on a daily basis. From those, it is recommended that breeding dogs should have an area to socialise and exercise regularly. It is preferable that this area for exercising and socialising is outdoor because it provides more stimuli for the dogs. It is also preferable that dogs have access to the outdoor area on a daily basis. Overall, more evidence is needed to support that breeding dogs need an outdoor area for exercising and socialising on a daily basis.

In relation to **housing temperature**, to maintain stable internal body temperature, homeothermic animals must balance their total heat production with heat losses or gains. In this Scientific report specific issues related to the thermal comfort zone (TCZ) and the thermal neutral zone (TNZ) of cats and dogs were described.

For the majority of adult cats, a temperature between 15 and 26°C prevents negative welfare consequences linked to temperature (thermal stress). However, TNZ of cats is probably wider than the above reported range, and it may need adjustment to fit certain breeds; therefore, further studies are needed to support this statement. As it may be more relevant to establish thresholds for ABMs measuring heat and/or cold stress in cats, rather than to establish breed specific TNZ, further research on animal-based measures (ABMs) for assessing thermal stress in cats is also recommended.

Young kittens are unable to regulate temperature on their own and an exact range of TNZ cannot be provided as it depends highly on the insulating properties of the kittening area and on the presence or absence of the mother. Further research is needed to establish the optimal temperature and conditions for keeping kittens in kittening areas in the first 3 weeks of kittens' lives. However, when kittens are kept without the mother during the first 21 days of life, the temperature of the kittening area should be higher than when the mother is with the kittens.

In dogs, insufficient scientific evidence could be retrieved in order to support the statement that a TNZ between 10 and 26°C prevents thermal stress in adult breeding dogs. As large differences and variation exist among diverse dog breeds and types, a common and general range defining the TNZ

for all dog breeds that prevents thermal stress cannot be provided. Further studies are needed regarding the quantification of TNZ in the different dog breeds. As it may be more relevant to establish thresholds for ABMs measuring heat and/or cold stress in dogs, rather than to establish breed specific TNZ, further research on ABMs for assessing thermal stress in dogs is recommended.

No scientific evidence was retrieved to support that, from an animal welfare perspective, a TNZ in the whelping areas between 22 and 28°C during the first 10 days of life prevents thermal stress in puppies. Further research is needed to establish the optimal condition in the whelping boxes and to establish the optimal temperature for keeping puppies during the first 10 days of life. As young puppies are unable to regulate temperature on their own, the TNZ depends on the presence or absence of the mother; therefore, for puppies kept without the mother during the first 10 days of life the temperature of the whelping area should be higher than when the mother is present.

Scientific evidence was retrieved to support that aspects related to certain breeds or types of dogs (e.g. size, coat, fur, level of activity) play a role on how the thermal conditions are perceived by dogs, and dog's ability to regulate temperature. However, due to the large variations of dog breeds, further research is needed to clarify susceptibility to thermal stress among dogs of different breeds and types. Regarding cats, although no scientific evidence was found on these aspects for certain breeds or types of cats, the findings reported in dogs are considered relevant, to some extent, also to cats. Further research is needed to clarify susceptibility to thermal stress among cats of different breeds and types.

Although no scientific publications specific for cats and dogs were retrieved, by use of analogy with other mammalian species, it is highly likely that cats and dogs of different categories and life stages will have different TCZ and TNZ. Further research is needed to set the optimal thermal conditions for indoor accommodation and breeding cats and dogs according to specifics of different categories and life stages. As it may be more relevant to establish thresholds for ABMs measuring heat and/or cold stress in cats and dogs, rather than to establish specific TCZ and TNZ, further research on ABMs for assessing thermal stress in cats and dogs is recommended.

Regarding **light requirements**, light (and related parameters like intensity, wavelength and duration of exposure) is an important factor as it regulates many animals physiological, hormonal and behavioural parameters including the sleep–wake cycle and behavioural homeostasis.

Insufficient scientific evidence was retrieved to support the statement that access to natural daylight is important to ensure welfare of breeding cats and dogs. Therefore, further research is needed and recommended on access to natural daylight by cats and dogs.

For breeding cats and dogs, a period of darkness every day is recommended to maintain the circadian rhythm. Further research is recommended to indicate the optimal dark and light duration. Further research is also recommended to indicate the optimal light intensity.

Defining the parameters for artificial light (i.e. the illuminance (lux), the spectrum and the light-time schedule) would be important for breeding cats' and dogs' welfare; however, more research is recommended to define these parameters quantitatively and qualitatively.

Judgement 2: Health of cats and dogs in commercial breeding establishments

The second judgement addressed the health and focused mainly on welfare issues related to reproduction in particular on skeletal maturity for first and last breeding as well as breeding frequency.

From the retrieved evidence, queens should not be bred before skeletal maturity. Puberty (sexual maturity) may come before skeletal maturity but animals should not be bred before they are fully grown (the energetic demand for a pregnancy would impact the growth of the mother). Skeletal maturity is breed-specific and should be checked before breeding is initiated. Further research is needed to suggest a minimum breeding age for queens.

Frequency of pregnancy should be controlled. To prevent exhaustion, the physical state of the queens should be checked before breeding through assessment of general health status and body condition score. Further research is needed to suggest a minimum period of time between two kittenings.

Queens older than 6 years old should be checked by a veterinarian for general health status and body condition score.

Bitches, alike queens, should not be bred before skeletal maturity. The mature age is a relevant element to ensure good welfare in bitches, but no unequivocal minimum age for breeding bitches can be suggested. From the scientific evidence retrieved, the size of the dogs the size of the dogs was considered an important element: for small breeds an age of 18 months is suggested as they will be fully grown by that age while for larger breeds the skeletal maturity is the pre-requisite for breeding

and should be checked beforehand. In addition, it was recommended to skip the first oestrus in bitches and therefore bitches should preferentially be bred from the second oestrus onwards.

In relation to the frequency of pregnancy it was recommended that to prevent exhaustion, the physical state of the bitch should be checked before breeding through assessment of body condition score and general health status. Further research is needed to suggest a minimum period of time between two whelpings. Bitches older than 8 years old should be checked by a veterinarian for general health status and body condition score before being used as breeding animals.

Judgement 3: Painful surgical interventions

In the third judgement, convenience surgeries (also called cosmetic surgeries) are addressed. These surgeries are painful procedures that are not justified from a veterinary medical perspective and included: declawing in cats and ear cropping, tail docking and vocal cord resections in dogs.

Declawing (onychectomy) in cats consists of the removal of the distal phalanx and is performed in cats to prevent unwanted scratching. It was concluded that scratching is a natural behaviour of cats and the practice of declawing impairs this natural behaviour and has detrimental effects on the welfare of cats. Therefore, declawing in cats should not be performed, unless necessary for the health of the animals.

Overall, there is evidence to support that **tail docking** in dogs is a painful procedure that should not be permitted (unless necessary for the health of the animals) as it causes acute and chronic detrimental effects on dog welfare. Dogs that have been tail docked may have problems in balancing and in communicating since the tail is a means of balance and communication. Based on experience with other animal species, and evidence in dogs, it is likely that tail docking has welfare impact in dogs also later in their lives (e.g. caused by the development of traumatic neuromas). Although some breeds have a slightly higher risk to injure their tails, the number of adult dogs that would have injured tails does not justify applying tail docking to all puppies of these breeds.

From an animal welfare perspective, scientific evidence showed that also **ear cropping** in dogs has detrimental effects on their welfare. This procedure, generally performed in puppies, is associated with pain that may last for weeks, with the risk for puppies to be removed from the group, thus experience isolation stress. The same is true for surgical **debarking**, which is an invasive procedure that can have complications and affect dog communication with humans and other dogs. Both ear cropping and surgical debarking should not be performed, unless necessary for the health of the animals.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

In accordance with The Farm to Fork Strategy,¹ published on 20 May 2020, the Commission is working on revision of the EU animal welfare legislation. An important novelty in this exercise is *inter alia* inclusion of specific provisions on protection of dogs and cats when kept as companion animals and for sports in the context of commercial breeding. Protection of cats and dogs has so far not been regulated in detail in the EU legislation. Providing a scientific base for new regulatory framework is proving challenging for these two species. The Commission has been assisted by the EU Platform on Animal Welfare and its Voluntary Initiative on health and welfare of pets in trade. Members of this group are representatives of competent authorities, non-governmental organisations, as well as veterinary practitioners and come from 10 Member States. The Voluntary Initiative has prepared recommendations on measures to be considered in preparation of policy options for the legal framework of commercial breeding of cats and dogs. In doing so, the group followed the Guidelines on responsible cat and dog breeding, endorsed by the EU Platform on Animal Welfare, as well as available scientific findings.

In accordance with Article 31 of Regulation (EC) No 178/2002² the Commission has requested EFSA to provide scientific assistance by responding to the question on measures considered for the revision of EU animal welfare legislation related to commercial breeding of cats and dogs on the basis of specific documentation^{3,4,5} and other relevant sources and to conclude on the following:

Is there sufficient scientific evidence to support the measures for protection of cats and dogs in commercial breeding related to the housing, health considerations and painful procedures?

1.1.2. Terms of Reference

The Commission has requested EFSA to analyse the scientific evidence which may support the following documents:

- 1) Recommendations on possible elements for EU legislation on Breeding (of dogs and cats). 3 May 2023. Ref Ares (2023)3092486
- 2) Responsible Cat breeding guidelines. Endorsed by EU platform on animal welfare on 3 November 2020-Doc/2020/11982. Rev1
- 3) Responsible dog breeding guidelines. Endorsed by EU platform on animal welfare on 3 November 2020-Doc/2020/11972. Rev1

In particular, the above-mentioned documents should be assessed with view to the question presented in the Background for the following specific Terms of reference (ToRs), with a list of questions on measures for protection of cats and dogs in commercial breeding:

ToR 1: On housing of cats and dogs

- 1) Should the accommodation of cats and dogs permanently in tiered boxes and crates be avoided for welfare of these animals?
- 2) Do dogs have the need to exercise and socialise outdoors on a daily basis?
- 3) Is there scientific evidence in the literature to suggest the following thermal comfort zones to prevent negative welfare consequences linked to temperature?

¹ COM/2020/38 1 final.

² Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety OJ L 31, 1.2.2002, p. 1–24.

³ Report by the EC Ref. Ares (2023)3092486 – 3/5/2023: Recommendations on possible elements for EU legislation on Breeding (of dogs and cats).

⁴ Report by EC endorsed by EU Platform on Animal Welfare 3 November 2020 – Doc/2020/11982-Rev1: Responsible cat breeding guidelines (available at: https://food.ec.europa.eu/animals/animal-welfare/eu-platform-animal-welfare/platformconclusions_en).

⁵ Report by EC endorsed by EU Platform on Animal Welfare 3 November 2020 – Doc/2020/11972-Rev1: Responsible dog breeding guidelines (available at: https://food.ec.europa.eu/animals/animal-welfare/eu-platform-animal-welfare/platformconclusions_en).

- adult dogs between: 10 and 26°C;
- adult cats between: 15 and 26°C;
- whelping areas between 22 and 28°C and this during the first 10 days of pups' lives;
- kittening areas between 22 and 28°C and this during the first 21 days of kittens' lives;
- 4) Are there other considerations in that regard in relation to certain breeds or types of cats and dogs?
- 5) Is setting a thermal comfort zone for indoor accommodation of cats and dogs according to specifics of every category of animals relevant for welfare of these animals?
- 6) Is access to natural daylight important to ensure welfare of cats and dogs?

Sub questions: Is there scientific evidence in the literature to suggest a minimum intensity of 50 k for at least 16 h per day? Is setting parameters for artificial lighting (illuminance (lux), spectrum, time schedule) where needed, a key element to ensure the welfare of these animals?

ToR 2: On health of cats and dogs

- 1) Should breeding of bitches and queens before they reach skeletal and sexual maturity be avoided? Sub question: Is there scientific evidence in the literature to suggest a minimum breeding age of 18 months for bitches and 12 months for queens?
- 2) Can controlling the frequency of pregnancies have a beneficial impact on health and welfare of bitches and queens by preventing physical exhaustion? Is there scientific evidence in the literature to suggest a minimum of 12 months between two whelpings or kittenings?
- 3) Is the mature age a relevant element of welfare in older bitches and queens?

Topic 3: On painful procedures

Do surgical interventions, such as ear cropping, tail docking, partial or complete digit amputation, and resection of vocal cords or folds, have a detrimental effect on the welfare of cats and dogs?

1.2. Interpretation of the Terms of Reference

This scientific report is meant to provide technical assistance to the European Commission in relation to guideline documents produced by a voluntary initiative of the EU platform on animal welfare on cats and dogs in commercial breeding (see the list of documents in Section 1.1.2). This scientific report only focuses on the recommendations provided in these documents and analyses those in function of the available literature. The technical assistance provided here is not considered as a full risk assessment that would rather consider a wide range of factors and a more holistic understanding of the risks related to these factors. In addition, a full risk assessment should have considered a multidisciplinary approach with different stakeholders, experts and/or a peer review process. Therefore, a full risk assessment is a broader process that examines risks comprehensively across various dimensions, whereas the approach presented in this scientific report is more narrowly focused on evaluating the specific questions (statements) at stake.

The scope of this report was the protection of cats and dogs in commercial breeding establishments, mainly cats and dogs kept as companion animals and for sports, including hunting. Consequently, cats and dogs in non-commercial breeding settings (e.g. private ownership, stray animals, military dogs) were not considered. In addition, only welfare related to specific aspects mentioned in the ToRs were discussed; welfare aspects related to transport and other aspects in the breeding establishment, such as feeding, the prevention of health and behavioural problems, the competence of the breeders and appropriate handling and training of companion animals, were considered out of scope.

The documents provided by the voluntary initiative of the EU platform on animal welfare (listed in Section 1.1.2) contain provisions for breeding of cats and dogs for which EFSA was requested to provide scientific background and evaluate whether, based on the scientific background, the provisions are supported.

The risk question '*is there sufficient scientific evidence to support the measures for protection of cats and dogs in commercial breeding related to housing, health and painful procedures*' should be interpreted as 'is there available scientific literature to support the statements mentioned in these documents?'. The word 'sufficient' is deleted in all risk questions below (Sections 3, 4 and 5 on the Judgements) because the scoping literature searches performed as basis for this scientific report could only be limited and no full extensive literature searches could be executed. The retrieved scientific

literature was complemented by consultation of independent experts in an EFSA *ad hoc* working group on the welfare of dogs and cats. Where possible, examples of national legislative provisions on the different topics (housing, health and painful procedures) were highlighted to support the judgement of the mandate statements.

In relation to **question 1** on housing (ToR1, Section 1.1.2), it was clarified that the focus of this question was mainly on the kind of accommodation for cats and dogs kept in the context of commercial breeding establishments. The judgement should consider in particular permanent confinement in cages, crates and boxes and confinement in tiered crates and boxes. Thus, the two aspects (permanent confinement and housing in tiered enclosures) should not be considered separately. It was also clarified that this mandate question refers mainly to adult animals.

In relation to **question 2** on housing (ToR1, Section 1.1.2), it was clarified that the focus of this question was principally related to the need for outdoor access, and in a second place related to the need to socialise and exercise.

In relation to **questions 3 and 5** on housing (ToR1, Section 1.1.2) it was clarified that these questions were intended with reference to the thermoneutral zone (TNZ) and not to the thermal comfort zone (TCZ). Factors such as relative humidity and wind speed strongly affect the response of the animals to a given temperature. However, these factors were not considered in the context of this report.

Question 6 on housing (ToR1, Section 1.1.2) should actually be rephrased and it was clarified that the time period for the light administration should rather mean 'for a maximum of 16 hours' and not for 'at least 16 hours' per day. This was confirmed by the European Commission as in the responsible dog breeding guidelines the recommendation on this issue stated to provide a period of darkness for a minimum of 8 h per day (EC, EU platform on animal welfare 3 November 2020 – Doc/2020/11972-Rev1).

Regarding ToR2, specific focus has been put on the health and welfare of the reproductive systems of intact female cats and dogs (called queens and bitches, respectively), in particular in relation to the start of breeding, the management (e.g. frequency of whelping and kittening) and the end of their reproductive life. Other health and behavioural problems, such as respiratory and gastroenteric disorders or aggressivity and unsuitable temperament, were considered out of scope. Other topics related to reproduction, such as artificial insemination, inbreeding and extreme selective breeding, after discussion and clarification, were also considered out of scope.

Regarding ToR3, only convenience surgeries (i.e. surgeries not intended to treat clinical situations; sometimes referred to as mutilations) were of interest, and other surgeries, such as neutering and castration or other surgery applied to rectify breeding deficiencies, were out of scope. There are several painful procedures not intended to treat clinical situations that cats and dogs might be subjected to; after further information on the mandate provided by the European Commission it was clarified that the current assessment will focus on: partial or complete digit amputation (declawing) in cats and ear cropping, tail docking and removal or resection of vocal cords (debarking) in dogs. It needs to be noted that: (i) debarking and declawing will be part of the current report although, generally, they are not performed in puppies or in commercial breeding establishments; (ii) the removal of dew claws in dogs is not included in the current report, because the variety of consequences associated with the different types of dew claws would require a specific mandate.

2. Data and Methodologies

2.1. Data

Peer-reviewed scientific articles, in particular those considered as primary research articles and reviews, were the main source of data used in this report; in addition information from legislative provisions was also considered when deemed useful to complement the judgement.

2.2. Methodologies

2.2.1. Protocol

According to the EFSA guidance on protocol development (EFSA, 2020) and in line with the methodological guidance that was developed by the AHAW Panel for the Farm to Fork mandates (EFSA AHAW Panel, 2022a), EFSA translated the mandate questions (i.e. the protection measures for breeding cats and dogs included in the mandate Terms of References for which EFSA is asked to

assess whether they are supported by scientific evidence) into more specific statements that better specify and clarify the question.

The translation of the mandate questions into statements is mapped in Appendix A, and precisely in Table A.1 (housing of cats and dogs), Table A.2 (health of cats and dogs) and Table A.3 (painful surgical procedures in cats and dogs).

The approach to assess the statements is a simple two-steps method:

- Step 1: retrieval of scientific evidence through specific scoping bibliographic searches on the topics included in the statements. The literature search was outsourced, and the outcomes were taken in consideration by an *ad-hoc* EFSA working group. The scientific evidence is presented in this scientific report divided by sections corresponding to the statements requested in the mandate.
- Step 2: based on the retrieved evidence in Step 1 and after consulting with *ad-hoc* experts, EFSA compiled a table with a final consideration on whether the statement is or is not supported by the evidence (called from here onwards 'EFSA Judgement table').

Judgement tables are presented at the end of each section, and report (i) a clear statement whether the statement is supported or not by the evidence (ii) some examples of the evidence in support of the statement or (iii) in absence of literature supporting the statement, the reasoning for supporting the judgement (e.g. extrapolation from other sources or additional studies from the experts) and (iv) overall recommendations.

An example of an EFSA Judgement table is shown in Table 1.

#	Statement	<u>EFSA Judgement</u> Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	There is scientific evidence to support that:		
	 dogs have the need to access to outdoor for exercising and socialising 		

Table 1:	Example of table reporting EFSA Judgement

2.2.2. Literature searches

A rapid scoping review was used to address the research questions and is described in detail in Appendix B. A rapid scoping review is defined as a knowledge synthesis in which components of the systematic and scoping review process (Tricco et al., 2018) are simplified or omitted to speed up the process (Khangura et al., 2012). A rapid review was conducted for the three different topics (see ToRs Section 1.1.2) and the literature search was limited to the scientific database 'Scopus'. Literature search, selection and abstraction were done by three persons with experience in animal welfare topics.

Initially, a 'topic search' strategy (searching in abstract, title, author keywords) by using a specific key word combination (cat OR dog AND welfare) was defined. Subsequently, the search was refined based on the year of publication (from 1980 to 2023), scientific area (Veterinary and Agricultural and Biological Sciences topics), article type (review and scientific article) and language (English). During the screening, a part of the relevance (inclusion/exclusion), to each publication, a main topic (Housing, Health, Surgery), a subtopic (Space, Thermoneutral zone, Light, Reproduction, Skeletal maturity, Sexual maturity and Cosmetic surgery) and a ranking (see Tables B.1–B.8 in Appendix B) were attributed.

Out of 2,532 search results, 127 articles were retained for further evaluation.

The main reason for the exclusion of the 2,405 articles was because their topics concerned: other animal species; behaviour and behavioural problems; missing abstracts; diseases; human perception of pets; the management of stray dogs and cats or the management of shelters; animal-assisted interventions and human-pet relationship; ethics and ethical issues related to pets; training methods; COVID-19 pandemics and its effect on pets; nutrition, genetics, medical treatments, pain assessment, reproduction techniques, animal trade and transport, effects of exercise and work on working/military dogs, laboratory animals, breed preferences and pet microchipping.

At the end of this first stage, the 127 articles retained were categorised into topics and subtopics (Table 2).

Торіс	Number of articles	Subtopic	Number of articles
Housing	79	Space, exercise, social behaviour	65
		TNZ	13
		Light	1
Health	30	Reproduction	30
		Skeletal maturity	0
		Sexual maturity	0
Surgical	18	Convenience surgery	18

Table 2: Topics and subtopics attributed to the 127 articles retained in the first step of the literature search

For the topics and subtopics where fewer than 20 articles were identified, more specific searches were performed. The publications obtained from the more specific searches were screened with the same criteria. All search strings used for the literature search as well as the inclusion and exclusion criteria for the assessment of welfare of cats and dogs for commercial breeding are reported in Appendix B (Tables B.1-B.8).

After this screening, scientific articles cited in the reference list reported in the Report with the Recommendations of the European Platform and in Responsible cat breeding guidelines and Responsible dog breeding guidelines were searched and retrieved. Additional searches were conducted on Google to retrieve further grey literature, and a total of 21 files were considered. After checking for duplicates with previous searches only six unique items were added for a total of 215 records.

After the selection, the full text of publications was retrieved. Not all selected articles were available in full on Scopus (e.g. papers published before 2,000 and journals not included in the University platform), so they were requested directly from the corresponding authors using other platforms (e.g. ResearchGate) or by email. At the end of the process, 206 full text publications were retrieved.

All retrieved documents were read carefully, and citation chaining was used to find other possible relevant literature while some other articles were excluded as considered not pertinent. When relevant, other articles were included from the reference lists of the selected articles.

2.2.3. Uncertainty assessment

The uncertainty assessment was performed in a qualitative manner following the procedure detailed in the EFSA guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018). The sources of uncertainty associated with the methodology used are listed in Appendix C (Table C.1).

3. Judgement 1: Housing

3.1. Type of housing (type of accommodation)

3.1.1. Question to address and guidelines from the EU AW platform

The question to be addressed is:

Should accommodating of cats and dogs permanently in tiered boxes and crates be avoided for welfare of these animals?

The recommendations from the EU AW Platform in relation to type of housing were:

- 'Cats must not be housed in pens that are tiered or stacked on top of one another'.
- 'Dogs must not be housed in cages that are tiered or stacked on top of one another'.

3.1.2. Introduction

Dogs and cats are kept in a variety of settings around the world, in homes, veterinary hospitals, shelters, research centres and laboratories (Rochlitz, 1999; Prescott et al., 2004; Wagner et al., 2018a,b). It is commonly acknowledged that sub-optimal environments and the emergence of stereotypical behaviours in animals like cats and dogs are related (Nogueira et al., 2021). Providing dogs and cats with housing that satisfies their physiological and behavioural needs is crucial to protect their welfare

particularly when companion animals are kept in conditions like research facilities, laboratories, breeding centres and shelters (Prescott et al., 2004; Wagner et al., 2018a,b; Arena et al., 2019). Common risk factors related to housing that can contribute to the development of behavioural problems and poor level of welfare in dogs and cats include prolonged confinement, insufficient social stimulation in sensitive as well as less sensitive periods of their lives (Sonntag and Overall, 2014).

3.1.2.1. Types of enclosures

For companion animals, the term enclosure is used to describe confinement that should provide a comfortable and safe place for the animals (Prescott et al., 2004). There is no standardised way of describing enclosures for companion animals; for the purpose of this report some clarifications are here provided. There are different types of enclosures, usually referred to as cages, crates or pens (see Table 3 for a short description). A crate is usually a plastic or collapsible metal enclosure that is large enough for the animal to stand up and turn around.⁶ The cage has more perforation than the crate and it is designed as a safe, secure area where the animal can be kept into for short periods of time. When the crate is used as a portable carrier or container, it should offer a secure spot for a pet to rest or hide safely (for cats). A pen is generally larger than a crate and often does not have a roof. For dogs, there are also kennels, which can be placed indoors or outdoors, of different materials, and they are also usually larger than a crate. During their life, dogs and cats may need to be confined for different reasons and situations (e.g. during transportation, during the night in home environments and during veterinary hospitalisations). Crating a cat for example at night can help with injury recovery, a secure night's sleep for the household and the prevention of movements of the cats to avoid any household disturbance (Overall and Dyer, 2005).

Types of enclosures	Description
Box	An enclosure, of different materials, with full walls, not necessarily open but with perforations or windows, the vast majority is fully closed.
	It can be tiered.
Crate	A plastic or collapsible metal enclosure that is large enough for the animal to stand up and turn around but not much more, with more perforation than the box.
	It can be tiered.
Cage	A plastic or metal enclosure that is large enough for the animal to stand up and turn around allowing it more freedom of movement than the crate, it also has more perforation than the crate, typically has a roof.
Kennel	There are different types of kennels; in some cases, it is defined as a 'dog-house', that, in other cases can be combined with a run. A 'dog-house' is a plastic or metal enclosure (of various dimensions) which the animal can enter or leave freely, which can be placed indoors or outdoors, of different materials, often placed in a pen.
Pen	Larger than a crate (box or cage), may be several square meters, and often does not have a roof. Indoors, outdoors or a combination of the two.

Table 3:	Types of enclosures and short description
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Animals can be confined also in tiered boxes and crates up to four levels. They are named duplex if they have only two levels (Overall and Dyer, 2005). They are used to keep dogs and cats housed for short or long periods in different environments, such as laboratories, shelters, veterinary hospitals, breeding centres and more rarely in households where more animals are kept together (e.g. amateur breeding and in cases of hoarding).

On the market, it is possible to find a variety of cages, containers, kennel, multi-level cages, tiered cages or boxes, of different dimensions and shapes, depending on the size of the animals and the purpose of the crating. As examples, a cage used in veterinary clinics for 'day cat patients' (up to 24 h' stay),⁷ usually has a minimum internal floor area of 3,600 cm² (e.g. 60×60 cm) and a height of 55 cm, while if cats stay longer than 24 h, and the minimum internal floor area is 6,300 cm² (e.g. 70×90 cm) with a height of 55 cm.

Another environment where crates or cages are commonly used is shelters. The common dimensions of cages in shelters for short-term (no longer than 2 weeks) single-cat housing are $0.75-1 \text{ m}^2$, and the

⁶ https://www.paws.org

⁷ www.catfriendlyclinic.org

dimensions of the standard cage unit are around 152 cm wide \times 70 cm deep \times 66–76 cm high (Wagner et al., 2018a,b).

Depending on the size of the dogs, the dimensions of crates typically used in veterinary clinics are the following: for small dogs, crate dimensions are 60 cm (Length = L) \times 44 cm (Width = W); for medium size dogs they are 76 cm (L) \times 47 cm (W); for larger dogs, they are 106 cm (L) \times 70 cm (W). In veterinary clinics, dogs are typically confined for a short period, and limited movements are preferred to help in recovery or administration of the treatments or rehabilitation after orthopaedical surgery (Dorn, 2017). On the contrary to vet clinics, dogs can be confined for a long period in kennels. The kennel environment, even if larger than a typical crate, is still constrained in size and may also include limited contact with other dogs, humans and the outside world for reasons of practicality, expense and standardisation (Taylor and Mills, 2007). In their review, Taylor and Mills (2007) reported that several studies investigated the effects of different cage and kennel sizes (from 1 to 8 m^2) on dog behaviour and welfare, and all had similar results, concluding that the dogs were mainly inactive when confined in enclosures within this size range. Dogs housed in confinement cages or kennels have negative welfare consequences mainly due to the inability to control their environments, and the lack of social contact with conspecifics and humans (Nogueira et al., 2021). Overall, all types of boxes, crates, cages and kennels, when used for a long duration or permanently, can affect the welfare of dogs and cats because they can lead to welfare consequences, such as, restriction of movements, resting problems and inability to perform natural behaviours. Possible hazards to the welfare of these animals are limited space allowance, size of the group (individual or group housing), exposure to artificial light, exposure to loud or aversive sounds, arousing odours, uncomfortable temperature and floors, whereas the reported welfare consequences are restriction of movement, resting problems, sensorial overstimulation, isolation stress and inability to perform play behaviour (Morgan and Tromborg, 2007).

3.1.3. Specific aspects related to types of housing in cats

The type of housing has an important effect on the behaviour, health and welfare of cats, and several studies have been conducted trying to identify their optimal housing system (Finka et al., 2014; Wagner et al., 2018a,b; Finka and Foreman-Worsley, 2022).

3.1.3.1. Literature findings about the permanent confinement in cages, crates and boxes

No studies were found specific to the mandate statement i.e. the permanent keeping of breeding cats in cages, crates and boxes, and its effect on cat welfare consequences. However, elements for judging this scenario may be derived by publications with diverse focus (e.g. on the space allowance) that report information about the impact of confinement on cats welfare.

Studies reported in the next sections, focus on the effect of either space allowance in the enclosure, enrichment provision or group size, and are summarised here to provide an overview of the welfare consequences occurring to cats when confined. The studies on group housing of cats were mainly done on neutered cats and the results should therefore be interpreted with caution when used for breeding colonies.

Studies on space allowance

Loberg and Lundmark (2016) investigated the social and spatial behaviour of cats in large groups, taking into account different space allowances, and assessing stress-related behaviours using the Cat-Stress-Score. In the study, 89 cats from two different shelters were split into six groups with 15 cats in each group (one group had 14 cats) and were kept in different sized areas; $15 \text{ m}^2 (1 \text{ m}^2/\text{cat})$, $30 \text{ m}^2 (2 \text{ m}^2/\text{cat})$, $60 \text{ m}^2 (4 \text{ m}^2/\text{cat})$. Each group of cats underwent a two-week acclimatisation period, followed by a 3-day observation period in each designated floor area. In the room where the cats were housed during the experiment, they had access to seven shelves mounted on the walls at different heights, one shelf standing on the floor with nine different compartments, three climbing trees with several levels of shelves and hiding places, four benches and two travel cages placed on the floor. Two observations were conducted: one in the morning after feeding and another in the afternoon before feeding. After the first and second observation period, each cat group was transported back to their shelter. Resources including shelves, climbing trees, food and water were either relocated farther apart at each change in treatment area (if the room size was increased), or closer together (if the room size was decreased). When the available space area for the cats increased, solitary play (one cat

playing with a toy, without any other cat playing with the same toy within 3 s) also increased and cats performed more solitary play when in the largest area. Social interactions between the cats (e.g. licking each other) also increased when cats were held in a space of 4 m²/cat than in 2 m²/cat (p < 0.05); but there was no difference between the other areas. This suggests that with a larger space area, cats have more opportunities to choose whom they interact with and for how long. During observations, the median Cat-Stress-Score was overall low in all available areas, ranging from 1 to 4, with a median of 2 (weakly relaxed). Overall, the observed play behaviour and increased activity are indicative of positive welfare, indicating that larger areas (specifically the recommended size of 4 m²/cat as suggested by the authors), can have beneficial effects on the welfare of the cats.

Studies on enrichment

Besides the space allowance, environmental aspects may affect the welfare of cats when confined. Particularly important to cats are the physical components called 'macro-environment' (i.e. cat's housing space and its surroundings and includes factors such as the thermoregulatory environment, lighting, odours and sounds), 'micro-environment' (e.g. usable floor space, place for food presentation, place for elimination facilities), 'outlets for the expression of species-typical behaviours' and the 'social environment', which includes human-animal interactions (Stella and Croney, 2016).

Cats kept in well-managed environments (including enriched cages) acclimated faster than cats kept in poorly managed enclosures and barren cages and showed less abnormal behaviour, less time spent in the hide box, and more affiliative and maintenance behaviour (Stella et al., 2017).

Stella et al. (2014) compared the effects of macro (room) and micro (cage) environments on the behaviour and welfare of cats. The cats were randomly assigned to one of four treatment groups. In each group there was a different combination of a managed (M+) or unmanaged (M-) macro environment and an enriched (m+) or unenriched (m-) micro-environment.

The study showed that cats housed in the M+ environment had a significant lower level in the number of sickness behaviours and hiding behaviours and significantly more cats exhibited affiliative and maintenance behaviours compared to cages.

This is in agreement with the study of Loberg and Lundmark (2016) who recommended a high number of hiding places rather than few large hiding places as an enriched environment minimising the stress due to the cat's opportunities to hide. These studies' findings indicate that besides the provision of enough space allowance, the complexity of the environment plays an important role on cats' health and welfare. This was confirmed by a study of Gourkow and Fraser (2006) comparing four different housing variations in 165 cats.

The results showed that the cats that were housed in the basic single stainless-steel cages had the highest score in the Cats-Stress-Score (fearful behaviour) and that housing cats in groups and in larger spaces safeguards cat welfare.

Studies on group size

Group housing for cats may also cause welfare problems, such as fighting and development of urinary diseases induced by stress; therefore, precautions should be put in place before grouping cats. A systematic review was conducted by Finka and Foreman-Worsley (2022) to identify peer-reviewed literature comparing single and multi-cat housing in confined environments (i.e. rehoming centre, shelters). Out of a total of 959 papers initially identified, only six articles met the inclusion criteria. The authors did not find significant differences between the two types of housing on stress levels measured by physiological and behavioural indicators. However, it is important to note that the authors reported that there were many limitations in the studies included in their analysis. Among the limitations identified, there was the different handling of the cats (enrichment), different numbers of cats, previous social interactions and environments and duration of the observation/experiments as well as the different definitions of stress in each study. Also cats in shelters and rehoming centres are typically neutered, as opposed to the ones in breeding colonies, and at least for males this affects their ability to live in groups.

Wagner et al. (2018a,b) concluded that that inappropriate cat housing is one of the most significant factors that impair the health and welfare of cats. Regardless of the design, a cat's enclosure needs to be big enough for the cat to stretch out and play naturally, as well as big enough to keep feed, water and the resting area distant from the place designated for eliminative behaviour. A raised viewing area, a hiding spot, a firm, cool surface to stretch out on and a soft, comfortable lying area should all be included in every cage.

3.1.3.2. Literature findings on the use of tiered boxes and crates

Cats can be also housed in a duplex, where one cat is housed above the other, sometimes with a connecting door. Given the feline propensity to seek hiding spots and to use elevated spaces, duplex housing likely impacts welfare in cats (Overall and Dyer, 2005), leading to both stress and distress for cats. This is also related to the fact that tiered enclosures are small (crates and boxes, see Table 3). Crowding effects may be intensified in laboratory environments (and farms), where cats are forced to use less area available to them than what they would choose. In addition, in their review of sources of stress in captivity, Morgan and Tromborg (2007) across animal species, but without specific information on cats, pointed out that animals placed in cages at different heights might be exposed differently to ambient parameters. Lower-tier cages are less illuminated and could predispose some animal species to abnormal behaviours or reduced overall activity (Morgan and Tromborg, 2007).

3.1.4. EFSA judgement of mandate statements related to type of housing in cats

The outcomes of the judgement of the mandate statements are reported in Table 4.

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	There is scientific evidence to support that, from an animal welfare perspective: - breeding cats should not be permanently confined in boxes, cages and crates	Overall statement if supported or not from literature From the scoping literature search, no publications on permanent confinement in boxes, cages and crates were found. Reasoning Papers referring to the effects of space allowance, group size, indoor confinement or lack of enrichment were found, which report for example the following effects:	Breeding cats should not be kept permanently in boxes, cages and crates
		 inability to perform social interactions in small spaces (fear of humans and other dogs, Gourkow and Fraser, 2006) and in non-enriched enclosures (Stella and Croney, 2016) group stress in small space allowances (Stella et al., 2014, 2017; Wagner et al., 2018a,b) and when kept permanently indoor (Sandøe et al., 2017) inability to perform exploratory behaviour in not well-managed and non-enriched enclosures (Loberg and Lundmark, 2016), inability to perform play behaviour (Loberg and Lundmark, 2016, Wagner et al., 2018a,b). 	
		There are examples of national provisions on permanent keeping of cats, e.g. in Switzerland, the 2008 Animal Protection Ordinance ^(a) states that: ' <i>Cats kept in single-</i> <i>occupancy cages must be able to move</i> <i>outside the cage for part of the time or at</i> <i>least five days per week</i> '	

Table 4: EFSA Judgement of mandate statements related to type of housing in cats with examples of the retrieved evidence



#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
		<u>Judgement</u> EFSA considers that cages, crates and boxes are small enclosures, and therefore a permanent stay in these enclosures impairs welfare of cats.	
		A permanent stay in these enclosures leads to the same welfare consequences observed in the studies about space allowance and group size such as, fear, anxiety and boredom.	
2	There is scientific evidence to support that: – breeding cats should not be kept in tiered boxes and crates	Overall statement if supported or not from literature From the scoping literature search, no publications were retrieved on the keeping of breeding cats in tiered crates or boxes.	Breeding cats should not be kept permanently in tiered boxes and crates.
		Reasoning Two publications were found from the scoping literature search about the use of tiers: (i) one review on captive animals (not specific on cats; Morgan and Tromborg, 2007) and (ii) another one on laboratory cats and dogs (Overall and Dyer, 2005), suggesting the following welfare consequences:	
		 abnormal behaviours and reduced overall activity in some animal species in lower cages as they are less illuminated (Morgan and Tromborg, 2007). stress and distress in laboratory cats housed in tiers given the feline propensity to seek hiding spots and use elevated spaces (Overall and Dyer, 2005). 	
		Judgement EFSA considers that confinement in tiered boxes and crates leads to welfare consequences in cats, such as abnormal behaviours and distress; a permanent confinement in tiered enclosures may exacerbates these welfare consequences.	

(a): Available online: https://www.blv.admin.ch/blv/en/home/tiere/tierschutz/heim-und-wildtierhaltung.html

3.1.5. Specific aspects related to type of housing in dogs

Similarly to cats, the behaviour, health and welfare of dogs are affected by the type of housing, space allowance, group size and enrichment. These represent crucial risk factors for dogs' welfare as they directly influence the ability to perform species- specific behaviours. Sufficient space and company allow dogs to move, run, play and explore, which are essential for their physical and mental welfare (Prescott et al., 2004). Moreover, adequate space allows for environmental enrichment, which is also deemed vital for the dog's welfare.

McMillan et al. (2011) performed a study on breeding dogs in canine commercial breeding establishments (CBEs) to examine whether the anecdotal reporting of behavioural and psychological abnormalities in these dogs, compared to the general pet dog population, could be confirmed. A questionnaire was used to collect information from owners on the behaviour of adult dogs bought at or rescued from CBEs. The average age of the dogs originating from the CBEs was 5 years and they had been living in their new homes for an average of 24 months. The owners of dogs reported that

dogs which used to live in CBEs showed persistent behavioural problems when compared with typical pet dogs. The authors examined various causes of the behavioural differences, among lack of socialisation. They refer to several publications to support their findings: in summary, multiple factors may, by themselves or in combination, play a contributory role in the psycho-behavioural abnormalities seen in the former breeding dogs from CBEs. However, the data are unable to determine specific causative relationships. Extreme fear, compulsive or stereotypic behaviours, reduced trainability, excitability (reactivity), increased touch sensitivity, fear of stairs and house-soiling were reported at significantly higher rates among owners of former CBE dogs than among owners of the matched controls. In the opinion of the same authors, this is because on commercial breeding farms, dogs are frequently kept in small size cages and crates, sometimes also tiered cages, for the entirety of their reproductive lives.

To understand how much space dogs kept in pairs need, a study was conducted by Normando et al. (2014), comparing the effect of two types of space allowances (4.5 m²/dog vs 9 m²/dog). Twelve mixed-breed, medium-sized neutered (eight males, five females; aged 3–8 years, permanence in the shelter from 1 to 8 years) dogs were used. The dogs were housed for the entire length of the study in six pairs in modular enclosures consisting of an indoor and an outdoor area. Dogs were housed in the experimental pens on day 1: three pairs were assigned to Treatment A (4.5 m²/dog) and three pairs to Treatment B (9 m²/dog) and then swopped to repeat the experiment. The data collection started after 10 days of adaptation, and during the experimental period, each pair of dogs was observed once a week using an instantaneous scan sampling recording method. The findings of the study indicate that an increase in space allowance increased the general level of activity, standing, positive social interactions, visual exploration of the environment and vocalisations. Results suggest that when dogs are housed in pairs with a space allowance of 9.0 m²/dog show more positive welfare indicators than when kept in 4.5 m².

Since dogs may need to be housed for long period in restricted environments for different reasons (shelters, quarantine, laboratory, breeding), Hubrecht et al. (1992) compared the behaviour of single-housed and group-housed dogs kept in shelters and laboratory facilities. The study proved that group housing with adequate space and enrichment was associated with higher activity, social behaviour and investigation (expression of natural behaviour) compared with the single housing in barren enclosures where repetitive behaviours (circling, pacing, social pacing, jumping, tail chasing, wall bounce, flank sucking) were more frequent. The authors highlighted there were no differences in behaviour in the laboratory single-housing dogs housed in 4.13 m² pens compared with those in 6.83 m² pens, apart from the type of stereotypy (pacing when in the smaller cage and cycling in the larger cage). The authors concluded that individual housing is deleterious for dog welfare, but even when dogs are group-housed, they still benefit from larger space, enrichments, outdoor space and exercise, along with opportunities to interact with humans.

Barnard et al. (2016) developed a welfare assessment protocol for shelter dogs and applied it to assess the welfare of dogs housed in 29 shelters in Europe. In the protocol, space allowance was considered adequate, if the minimum space for one or two animals was 4 m² (up to 20 kg) and 8 m² (over 20 kg) with a minimum height of 2 m (Barnard et al., 2014). The average space allowance in the examined pens was $7.06 \pm 7.38 \text{ m}^2/\text{dog}$ (minimum 0.5 m^2 – maximum 48 m^2), so, according to the protocol definition, 69.6% of pens offered an adequate amount of space, while 30.4% did not. Results showed that there was a significantly higher probability of observing an animal with a dirty/wet coat when the space allowance or the bedding (kennel/basket/other/absent) was recorded as inadequate ($p \le 0.01$). Space allowance inadequacy (p = 0.004), bedding inadequacy (p < 0.001) and the presence of sharp and harmful edges (p < 0.001) were predictive variables of poor skin conditions (e.g. presence of skin lesions). Authors suggested that space allowance should be considered a high risk factor for example for skin conditions and cleanliness.

Jongman et al. (2018) studied behaviour and physiological parameters in greyhound dogs that were individually housed in kennels of either 3 m² or 10 m². The initial aim was to answer the research question of whether greyhounds housed in individual kennels would benefit from a larger kennel size. Since greyhounds are generally housed individually when in training for racing, the effects of kennel size with and without routine exercise were evaluated since both factors may influence the dog's welfare. The study consisted of 36 healthy male and female adult greyhounds which were individually housed in adjacent kennels with limited contact between the neighbouring dogs. In large kennels dogs spent more time in the front of the kennel. In the interpretation of the authors, this was a way to alleviate the social isolation experienced in individual housing, which was possible only in the large kennels where a more effective use of the space was possible. Other behaviours, including stereotypies and abnormal behaviours (such as pacing, licking, excessive grooming and jumping) were rarely observed and did not differ between treatments. There was no significant (p > 0.1) effect of kennel size on saliva cortisol concentrations (6.5 vs. 6.1 nmol/L for young dogs and 5.5 vs. 5.3 nmol/L for adult dogs in the 10 m² and 3 m² kennels, respectively). No effects of exercise were found on any studied parameters. The study did not observe any indications of increased stress levels (as measured by basal cortisol concentrations and cortisol response to ACTH), abnormal behaviour (no stereotypies or abnormal behaviours were observed), changes in body weight or injuries associated with the kennel sizes. The authors concluded that the larger kennels may enable better welfare by providing additional social contact.

3.1.6. EFSA judgement of mandate statements related to type of housing in dogs

The outcomes of the judgement of the mandate statements are reported in Table 5.

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	There is scientific evidence to support that, from an animal welfare perspective: - breeding dogs should not be permanently confined in cages, boxes and crates.	Overall statement if supported or not from <u>literature</u> From the scoping literature search it resulted that in most studies space is confounded by either enrichment or group size. <u>Reasoning</u> Scientific publications were found on the welfare implications of prolonged confinement (also in small enclosures) of dogs. For example, in breeding dogs from canine commercial breeding establishments, compared to typical pet dog populations, McMillan et al. (2011) reported abnormal physiological and behavioural parameters (e.g. higher level of hair cortisol stereotypical behaviour), which persisted even after adoption/rescue. There are studies that compare relatively small enclosures (e.g. Hubrecht et al., 1992; Normando	Breeding dogs should not be permanently confined in cages, boxes and crates
		et al., 2014; Jongman et al., 2018) and indicate that larger enclosures may enable better welfare e.g. by providing the possibility to have separated functional areas (Barnard et al., 2016) and thereby allowing dogs the freedom to choose and providing control.	
		There are examples of national provisions establishing that dogs cannot be permanently crated/boxed (e.g. in Sweden and Finland). Judgement	
		EFSA considers that cages, crates and boxes are small enclosures, and therefore a permanent stay in these enclosures impairs welfare of dogs.	
2	There is scientific evidence to support that: - breeding dogs should not be kept in tiered boxes and crates	Overall statement if supported or not from literature From the scoping literature search, no publications were retrieved on the keeping of breeding dogs in tiered crates or boxes.	Breeding dogs should not be kept permanently in tiered boxes and crates

Table 5: EFSA Judgement of mandate statements related to type of housing in dogs with examples of the retrieved evidence

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
		<u>Reasoning</u> Although no scientific literature was retrieved to support this statement, the considerations reported for cats could be relevant, to some extent, also to dogs (see Sections 3.1.3.2 and 3.1.4).	
		Judgement EFSA considers that confinement in tiered boxes and crates leads to welfare consequences in dogs, such as abnormal behaviours and distress; a permanent confinement in tiered enclosures may exacerbate these welfare consequences.	

3.2. Outdoor access, exercise and social behaviour in dogs

3.2.1. Question to address and guidelines from the EU AW platform

The question to be addressed is:

Do breeding dogs have the need have access to an outdoor area on a daily basis for exercising and socialising?

The recommendations from the EU AW Platform in relation to the need to exercise were:

`Provide dogs with large and complex housing spaces that allow them to choose where and when they spend their time' and 'Dogs kept in a home must have free access to more than one room, plus access to an outside area for exercise. Dogs kept in a kennel environment must have an adjoining run or secure outside space in addition to the minimum space allowance above. Part or all the adjoining area should be outdoors; dogs must have constant access to shade and shelter to avoid extremes of weather'.

3.2.2. Specific aspects on need to exercise, outdoor access and social behaviour in dogs

Most of the studies retrieved related to the need to exercise, to socialise and to the need to have access to outdoor were performed in shelters, comparing e.g. shelter dogs with daily outdoor access with shelter dogs permanently confined, or shelter dogs housed individually with shelter dogs housed in pairs etc. No information on these aspects of welfare was retrieved for breeding dogs. The information is summarised below in the next sections.

Studies on exercise

Being sociable animals, dogs require social connection as well as exercise on a regular basis (Takáčová et al., 2021). Enrichments provided to dogs can be animate (enrichment through the provision of social contacts with conspecifics and humans) or inanimate (enrichment through the provision of toys, cage furniture, auditory and olfactory stimulation).

A welfare assessment was conducted on 64 long-term dog shelters in Italy by Arena et al. (2019). Three levels of assessment (shelter, pen, individual) were carried out and a significant sample size of pens and dogs was selected for each level. The findings of the study revealed that, the body condition score (BCS) was significantly better in dogs that were given access to an external fenced area compared to dogs that were not lead-walked or walked irregularly by shelter personnel. The level of the exercise was also a predictor for abnormal reactions towards humans: when dogs were denied access to outdoor fenced areas or afforded access only sporadically, there was a greater probability of observing aggressive responses.

A study conducted by Menor-Campos et al. (2011) investigated the effects of 25-min sessions of exercise and human contact on dog welfare. The study contained 50 shelter dogs that were admitted to a shelter and were housed in individual cages measuring 9 m^2 , which allowed the animals to see, smell and touch only their adjacent neighbours. The dogs were classified randomly into two groups: an

experimental group (EG) and a control group (CG). Treatment given to the EG dogs consisted of a walk on a leash through the facilities to an outdoor fenced area of $\sim 1,000 \text{ m}^2$, where the dogs were let loose for 5 min over two sessions on days 7 and 9. The dogs were also encouraged to play with a ball or run alongside the person accompanying them. During these sessions, the dogs were also given basic commands such as 'come', 'sit' or 'lie down'. Finally, after leashing the dogs, they were taken for a short walk around the perimeter of the outdoor area, and then brought back to their cages. These exercise sessions (ESs) lasted ~ 25 min during which a person interacted with the animal in a friendly and affectionate manner. All the animals in the study were given two behaviour tests, and also saliva samples from each dog were collected. Salivary cortisol levels in CG (4.22 [1.97] nmol/L and 4.3 [1.98] nmol/L in the 7th and 9th day, respectively), which were consistently higher than those of EG (cortisol level decreased from 3.53 [1.59] nmol/L to 2.84 [1.48] nmol/L over two sessions from 7th to 9th), provided evidence that the human contact and ESs conducted in this study decreased cortisol levels in dogs living in shelters. These changes were shown to be substantial after just two ESs, with cortisol levels remaining constant in the dogs who did not participate and significantly falling for those who did.

Studies on outdoor access

Similar results were obtained in a study done by Gunter et al. (2021) reporting an increase in cortisol after short-term outings. The study was performed on 164 shelter dogs living in 4 animal shelters and experiencing approximately two-and-a-half-hour-long outings with a person between 11:00 a.m. and 3:00 p.m., off the property of the animal shelter. All these shelters had existing short-term outing programs before the study (Less than 2% of outings occurred outside of these times). The study lasted for 3 days and data were collected five times per dog. Stress and physical activity were measured with urinary cortisol and accelerometers before, during and after the short-term outings. It was determined that the increase in cortisol levels during the afternoon of the field trip cannot be solely attributed to increased activity. This suggests that the difference in cortisol levels may be linked to the psychological stress experienced during the outing. Unlike being in a calm environment where dogs could seek refuge from the stressors of the shelter or spend time resting in a home, the field trips involved active participation from the dogs. They were exposed to various unfamiliar sights and sounds, which could have contributed to their heightened cortisol levels. Increases in cortisol, as reported here with short-term outings, do not necessarily indicate poorer welfare for dogs. Cortisol tends to increase indeed also due to exercise and positive excitement, as more likely happened in the tested dogs.

A study by Spangenberg et al. (2006) on eight kennelled laboratory Beagle dogs kept in pairs revealed that giving the dogs access to outdoor kennels significantly increased activity, increased movement frequency and decreased passive behaviour. This suggests that giving dogs access to the outdoors will increase their ability to express their natural behaviour, reducing boredom. Since it boosted the dogs' movement and activity-related behaviours, an outside setting is valuable to the dogs and advantageous to their welfare. This confirms the study by Hubrecht et al. (1992) which suggested that group housing in a kennel with daily outdoor access was associated with high activity, social behaviour and investigation (expressions of natural behaviour) compared with single housing where repetitive behaviours were more frequent.

Nogueira et al. (2021) conducted a study on kennelled police dogs during their rest periods to determine the impact of two interventions consisting of a 350 m² space (lawn) and a toy suspended from the kennel's roof. The intervention was available for 15 min each day for four consecutive days. Behavioural data by filming and faecal samples for cortisol metabolites evaluation were collected before, during and after interventions. The results indicated that faecal cortisol metabolites decreased on the second day of intervention regardless of the type of intervention. Additionally, in the same study using cluster analysis it was shown that high-stereotyping dogs tended to reduce their stereotyping behaviours when exposed to the lawn intervention, while low-stereotyping dogs did not present significant behavioural changes during the experiment. A tendency to reduce these behaviours in high-stereotyping individuals was observed after dogs were able to go to the lawn area, indicating the beneficial effects of this intervention, emphasising that the provision of daily access outdoor may be beneficial to dog welfare.

Studies on social interactions

Housing dogs individually or in groups can affect their welfare and social behaviour.

In terms of the welfare of the dogs, group housing is better than individual housing. To investigate the effect of group- *versus* single- housing, Mertens and Unshelms (1996) performed a study on 211 dogs previously kept in single and group housing in two animal shelters. After observation and testing of each dog and interviews with 197 dog owners who adopted the dogs, the study found that a high percentage

of dogs previously kept individually were affected by behavioural problems and showed stereotypies (e.g. walking in cycle). These problems were quite rare in the group-housed dogs, which did not exhibit stereotypies. Grigg et al. (2017) conducted a study to measure the impact of switching dogs housed in a long-term kennel facility from solitary to pair housing. The dogs (n = 12) were initially housed individually in kennels (2.36 m long, 1.07 m width, 2.24 m height) with grates on both sides and the door allowing visual contact and limited (non-contact) interaction with neighbouring dogs and passing dogs, kennel staff and students. For the needs of the experiment, eight of the dogs were pair-housed and the remaining four were kept in the individual kennels. All the dogs were videotaped for 30 min twice per week for 2 weeks before the transition. Video data collection was resumed for 8 weeks on all 12 dogs after the transition. From the video footage, the authors recorded the duration of stress-related behaviours (i.e. repetitive vocalisation (barking), circling, spinning, pacing, barrier jumping and conflictrelated behaviours), as well as the time spent resting (lying down and/or sleeping). Hair samples to determine hair cortisol were collected from the shoulder of all dogs, at two times: prior to the start of the study (for baseline cortisol levels) and after the dogs had been in the pair housing for 8 weeks. Results showed that dogs in paired housing decreased in vigilance behaviour, repetitive behaviours (pacing and jumping) and barking, suggesting that pair housing is better than individual housing.

Baqueiro-Espinoza et al. (2023) tested the impact of positive human interaction on the welfare of breeding dams from licensed UK CBEs. All dams were housed in kennels in groups of two to four dogs. Kennels were adjacent to each other and constructed of two rectangular corrugated metal sheets used as wall panels (4.40 m length \times 1.10 m height). The front of the kennel (1.10 m) consisted of a metal gate (1.10 m width \times 1.20 m height). At the back of each kennel, dogs were provided with a wooden crate (1.10 m width \times 0.60 m height \times 0.60 m depth) for sheltering and sleeping. After receiving 4 weeks of either baseline levels (control, N = 16) or additional positive human interaction (enriched, N = 15), an attention bias test (ABT) was conducted to assess dogs' affective states. Afterwards, dams' human sociability level was evaluated using a stranger approach test. The study found that giving dogs human contact as an enrichment lowers their anxiety levels and makes them more sociable with people, including strangers (Baqueiro-Espinosa et al., 2023). Gfrerer et al. (2018) also found that dogs with prior experience with conspecifics and humans (one section of 3 h once a week) showed reduced offensive and defensive behaviours towards both fake and real dogs even though the previous social exposure period was short. Breeding dams and puppies should be regularly exposed to conspecific and humans to increase their social behaviour and human-dog relationships.

Using behavioural and physiological parameters, Dalla Villa et al. (2013) conducted a study to compare the impact of two different types of housing (group- and pair-housing) on 17 spayed or neutered long-term shelter dogs. During a preliminary phase that lasted for 4 months, dogs were organised into groups as part of an initial experiment. The purpose of this phase was to facilitate daily sessions of group socialisation, aimed at determining which dogs were compatible with each other. Once this process was completed, four experimental groups were established, and the dogs were placed in their respective designated pens for the duration of the experiment. After a period of 1 month to allow the dogs to become familiar with their new living environment, data collection for the experiment began. This initial data collection, referred to as Time 1 (T1), took place in a specific experimental setup. The dogs were housed together under the same confinement conditions, forming groups of four to five animals consisting of both males and females. They were accommodated in four outdoor enclosures, each measuring $\sim 35 \text{ m}^2$. When the first data collection was completed, eight dogs (four male, four female), from two of the four confinements, were pair-housed in smaller enclosures (6 m²). These dogs were defined as the experimental group and the remaining was the control group. These enclosures had access to a common fenced area (120 m²) where dogs were allowed to exercise in pairs for 2 h/day, usually in the morning during cleaning routines. Dogs were allowed to habituate to the new confinement condition for 1 month before the commencement of the second data collection (Time 2, T2). Video recordings of the behaviour (active, inactive, repetitive, social, tail, exploratory, alimentary, grooming, barking etc.) and saliva samples for cortisol levels were collected. Dogs in groups showed more locomotor activities such as walking, trotting and standing and more social play and exploration activity than the dogs in pair groups who spent more time lying and significantly less time standing and showing visual exploration compared to group-housing. Cortisol levels decreased from the first test to the second test in all groups. A general decrease in most activities (e.g. locomotor, social and exploratory) was recorded when dogs were transferred from the group to pair housing condition, confirming that spatial restriction and partial social deprivation can decrease the activity of adult long-term shelter dogs. Nevertheless, it should be noted that, in the present study, pair-housed dogs had still daily access to outdoor runs, but the behaviour expressed during that time was not recorded.

3.2.3. EFSA judgement of mandate statements related to access to outdoor in dogs

The outcomes of the judgement of the mandate statements are reported in Table 6.

Table 6:	EFSA judgement of mandate statements related to access to outdoor in dogs w	vith
	examples of the retrieved evidence	

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
	There is scientific evidence to support that, from an animal welfare perspective: - breeding dogs have the need	Overall statement if supported or not from literature From the scoping literature search, no publications were found on the need for breeding dogs to have access to an outdoor area on a daily basis.	Breeding dogs should have an area to socialise and exercise regularly. It is preferable that this area for exercising and socialising is outdoor because it provides more stimuli for the dogs.
	have access to an outdoor area on a daily basis for exercising and socialising	Reasoning However, publications were found mainly in shelter dogs on the need to exercise combined with outdoor access (Hubrecht et al., 1992; Menor-Campos et al., 2011; Arena et al., 2019; Nogueira et al., 2021), and on the need to socialise with either humans or dogs, but not necessarily on a daily basis (Gfrerer et al., 2018; Baqueiro- Espinosa et al., 2023). In one study (Hubrecht et al., 1992) shelters with and without access to outdoor were compared, and positive behavioural parameters were observed in dogs that had access to outdoor. Another study (Arena et al., 2019) observed better body condition score and social behaviour in dogs with outdoor access. A study (Menor-Campos et al., 2011) reported lower cortisol in 'police' dogs with outdoor access.	It is also preferable that dogs have access to the outdoor area on a daily basis. More evidence is needed to support that breeding dogs need an outdoor area for exercising and socialising on a daily basis.
		Judgement EFSA considers that an area is needed, where breeding dogs can socialise and exercise regularly. Although no scientific literature was retrieved, an outdoor area would be preferable because it would provide more stimuli for the dogs. An indoor space could serve the same function but there are no scientific studies supporting this.	

3.3. Housing temperature

3.3.1. Questions to address and guidelines from the EU AW platform

The questions to be addressed are:

- a) Is there scientific evidence in literature to suggest the following thermoneutral zone to prevent negative welfare consequences linked to temperature?
 - i) adult dogs between: 10 and 26°C;
 - ii) adult cats between: 15 and 26°C;

- iii) whelping areas between 22 and 28°C and this during the first 10 days of pups' lives;
- iv) kittening areas between 22 and 28°C and this during the first 21 days of kittens' lives;
- b) Are there other considerations in that regard in relation to certain breeds or types of cats and dogs?
- c) Is setting thermoneutral zone for indoor accommodation of cats and dogs according to specifics of every category of animals relevant for welfare of these animals?

The recommendations from the EU AW Platform in relation to the need to exercise were:

— For cats:

`Ensure indoor accommodation for adult cats is kept between 15 and 26°C. An optimal range lies between 18 and 21°C.

Brachycephalic cats and those with extreme coat types require careful management as they have markedly different thermal-tolerances.

Ensure the kittening box is kept between 18 and 27°C'.

— For dogs:

`Ensure indoor accommodation for adult dogs is kept between 10 and 26°C. An optimal range lies between 15 and 21°C.

Brachycephalic dogs and those with extreme coat types require careful management as they have markedly different thermal-tolerances.

Ensure the whelping area is kept between 22 and 28°C'.

3.3.2. Introduction

3.3.2.1. Concepts of thermoregulation in homoeothermic mammals

In order to maintain stable internal body temperature, homoeothermic animals must balance their total heat production with heat losses or gains. Heat is the major form of energy produced and exchanged in the mammalian body. Around 60% of the energy released from ATP production processes by cells is in the form of heat. The rules of physics apply to heat exchange in animals, and dissipation mechanisms rely on the property of heat to flow from a higher concentration to a lower concentration. Mammals use four mechanisms of heat exchange to maintain homeostasis: conduction, convection, radiation and evaporation (Bakken, 1976).

<u>Convection</u>: In the context of heat regulation in mammals, convection occurs when the surrounding air carries away heat from the body. For example, if a mammal is in a cool breeze, the air passing over the skin absorbs heat, aiding in cooling. Air velocity has marked effects on the amount of heat lost and is an important component of thermal balance (Berman, 2006).

<u>Conduction</u>: When a mammal comes into contact with a surface that is either warmer or cooler than the body temperature, heat is transferred between the two. For instance, if a mammal lies on a cold surface, such as a cool floor, heat is conducted away from the body of the animal. Conversely, if a mammal lies on a warm surface, such as a sunny floor, heat will be conducted in the opposite direction.

<u>Evaporation</u> is the process by which a liquid changes into a gas, taking away heat in the process. Mammals regulate body temperature through evaporation primarily through respiration and sweating. When a mammal sweats, moisture on the surface of the skin evaporates, carrying away heat from the body. Increased respiration rate with closed or open month (i.e. panting) involves rapid, shallow breathing, which increases the airflow across the respiratory system and facilitates the evaporation of moisture from the respiratory surfaces. High relative humidity hinders evaporative heat dissipation, thus increasing the effect of a hot environment.

<u>Radiation</u> is the transfer of heat through electromagnetic waves. In the context of heat balance in mammals, radiation refers to the emission of heat in the form of infrared radiation. Mammals constantly radiate heat from their bodies into the surrounding environment. The rate of radiation depends on the temperature difference between the mammal and the surroundings. If the surroundings are colder than the body temperature, more heat will be radiated, and vice versa.

Such mechanisms of heat exchange are necessary for animals to balance the amount of heat obtained from external sources with the amount produced by organs and cellular functions within their bodies. <u>Basal metabolic rate</u> refers to the minimum sustained rate of energy turnover of an endotherm. Such a state is described as an 'inactive, postabsorptive, adult, nonreproductive and thermoregulating nontorpid animal in its inactive circadian phase' (White and Seymour, 2003; Porter and Kearney, 2009). Basal metabolic rate is measured under standardised conditions (typically in a laboratory), by exposing the animal to a range of air temperatures (e.g. using calorimetric/metabolic chambers). The amount of heat produced by the animal varies during its life and depends on various endocrinological and biochemical factors. For example, animals' bodies produce more heat during muscle exercise and digestion of food. There are also differences related to specific breeds, morphological characteristics and categories of cats and dogs; further information on this is provided in Section 3.3.8.

3.3.2.2. Concepts of thermal zones

The concept of basal metabolic rate is essential for the definition of thermal zones, as the measurement of basal metabolic rate originally was used in laboratory studies to identify thermal comfort and thermoneutral zones.

Thermal comfort zone (TCZ) is a temperature range in which homoeothermic mammals are in thermal homeokinesis, express satisfaction with the thermal environment, and thus do not change their behaviour to cope with the thermal environment (Schlader et al., 2011; Kingma et al., 2014; EFSA AHAW Panel, 2022b). Thermal homeokinesis is defined as a steady state where the internal body temperature of a homoeothermic animal is kept constant at the normal internal body temperature level with little additional energy expenditure. Thermal homeokinesis is kept when ambient temperatures are within the range of TCZ. TCZ was first hypothesised based on human perception of the thermal environment (Kingma et al., 2014) but is now applied in non-human animals as well. As described by EFSA AHAW Panel (2022c), translated into animal welfare, Silanikove (2000) described the TCZ as the environmental temperature interval, where the energetic and physiological efforts of thermoregulation are minimal, and the animal is in the preferred or chosen thermal environment. This zone of thermal well-being is the most suitable way to describe the relation between an animal and its thermal environment from the viewpoint of animal welfare. Outside the TCZ, animals will activate physiological thermoregulation processes, and may start to display thermoregulatory behaviour. Both may be associated with negative affective states and thus be signs of heat stress (or cold stress) as defined by EFSA AHAW Panel (2022a) as a situation where an animal experiences stress and/or negative affective state(s) such as discomfort and/or distress when exposed to a high (or low for cold stress) effective temperature'. Thus, if a negative impact on animal welfare from the thermal conditions is to be fully prevented, animals should be kept in their TCZ. The TCZ can be influenced by various factors, including species-specific adaptations (including different breeds, different types of animals and different animal categories), acclimation to certain temperatures and individual variations. Factors like humidity, wind speed and the presence of shade or shelter can also impact the TCZ, as they affect heat exchange between the animal and the surroundings. The TCZ is comprised in a wider range of ambient temperatures, namely the Thermoneutral Zone (TNZ).

Recently, EFSA defined the TNZ as the range of environmental temperatures within which metabolic rate and heat production are constant and independent of the ambient temperature (EFSA AHAW Panel, 2022c). TNZ varies among species, breeds, animal types and categories and is highly influenced by basal metabolic rate. Other factors that influence TNZ are body size, sex, body insulation and behavioural adaptations. For example, small mammals typically have higher metabolic rates and larger surface-to-volume ratios, making them more susceptible to heat loss. Therefore, the lower boundary of their TNZ is often higher than that of larger animals. Additionally, some species (and breeds) have specialised adaptations, such as thick fur, which expand their TNZ and allow them to inhabit environments with extreme temperatures (Mota-Rojas et al., 2021).

The concepts of TNZ and basal metabolic rate are closely related (Mitchell et al., 2018); inside the TNZ the increase in basal metabolic rate is modest, and almost negligible. As temperatures approach the extremes of the TNZ, the basal metabolic rate increases. At the temperatures limiting TNZ, the basal metabolic rate starts to increase exponentially. TNZ boundaries are represented by upper critical temperature (UCT) and lower critical temperature (LCT), as illustrated in Figure 1. Thus, as described by EFSA AHAW Panel (2022d) even though heat stress and cold stress, respectively, may start when animals are no longer in their thermal comfort zone, the risk and severity of heat stress and cold stress is high when the thermal conditions reach the UCT and LCT, respectively. A schematic

representation of the thermal zones and the animal thermal stress as a function of environmental temperature is reported in Figure 1.



LCT: Lower critical temperature (LCT), UCT: Upper critical temperature (UCT); B: Lower limit of thermal comfort zone; C: Upper limit of thermal comfort zone.

Figure 1: Schematic representation of thermal zones as a function of the environmental temperature (adapted from EFSA, 2004)

Above UCT, even if physiological, endocrinological and behavioural responses are present (and observed using animal-based measures (ABMs)), the risk of and the severity of heat stress increases as these thermal-driven responses do not counteract a further increased temperature. Subsequently, energy expenditure and basal metabolic rate increase, resulting in increased heat production. As a first response to the imbalance in heat generation and dissipation, internal body temperature starts to increase (hyperthermia). Evaporative cooling may occur through sweating or salivation and panting, but here great differences exist among animal species (Folk and Semken, 1991). For example, sweating is the main evaporative cooling mechanism in humans and horses but is almost absent in cats and dogs (that have few sweat glands and are located primarily in paw pads). In dogs and cats the primary cooling mechanisms are instead represented by increased respiratory rate, salivation and panting. Three types of panting patterns are found in heat stressed animals: (i) increased respiratory rate but the animal inhales and exhales through the nose; (ii) the animal inhales through the nose and exhales through the nose and mouth; and (iii) the animal inhales and exhales through the nose and mouth. The least amount of cooling is accomplished by the first pattern of panting, whereas the second and third patterns are the most effective for dissipating heat through evaporative cooling (Goldberg et al., 1981). Panting is commonly expressed in dogs. Dogs often express panting with mouth open; however, this behaviour is also observed in other domestic animals, such as cats (Reece and Rowe, 2017).

When an animal is exposed for an extended period at the UCT or at slightly higher temperatures, chronic heat stress develops, leading to shifts in energy metabolism, increased glucocorticoid levels, enhanced inflammatory processes, infertility, decreased growth and production, decreased immune system efficiency, cellular and mitochondrial oxidative damage (Belhadj Slimen et al., 2016). In some cases, respiratory distress may also appear as an effect of prolonged exposure to temperatures above UCT: high temperatures indeed increase respiratory rates and decrease respiratory efficiency, particularly in animals with limited sweating or panting capacity. In case of severe hyperthermia (Elsasser et al., 2012), even for a short time the risk of heatstroke increases (Johnson et al., 2006). Heatstroke is a life-threatening condition particularly common in dogs, and rarely reported in cats (Johnson et al., 2006), characterised by organ failure, brain damage and death (Osilla et al., 2018).

Below LCT, metabolic heat production increases exponentially, due to responses such as shivering (irregular frequent muscle contractions), vasoconstriction and in some animal species also by activating brown adipose tissue catabolism, all functioning to prevent body temperature from decreasing below the normal range (Grigg et al., 2004). Animals exposed to freezing temperatures can also develop frostbite, where tissues are damaged due to freezing. Extremities such as ears, tails and limbs are particularly vulnerable. When surrounding thermal conditions are below the LCT and the physiological

and behavioural responses activated by the body are not able to maintain or restore body temperature, the animal enters hypothermia. Hypothermia is associated with several organ failures and cardiovascular dysrhythmias such as ventricular fibrillation and pulmonary oedema. Information on specific national legislation on thermoneutral zones in cats and dogs.

The thermal conditions such as ambient temperatures and relative humidity, are recognised as being important for the welfare of dogs and cats (Kurz, 2008; Bruchim et al., 2017; Gogolski et al., 2020; Caldas et al., 2022) however, there are few examples where precise requirements on temperature and humidity for the buildings where dogs and cats are kept are provided by national legislative provisions (e.g. in Ireland, UK and USA, see Tables 7 and 9).

3.3.3. Specific aspects related to thermal comfort zone and thermoneutral zone in cats

From the scoping literature search, it resulted that only few papers investigated aspects of thermal biology of cats and could be used for addressing the mandate questions. Most of the publications retrieved on adult cats were performed in metabolism chambers and focused on physiological responses, making them less useful to interpret in terms of the EFSA definitions of heat and/or cold stress, focusing on stress and/or negative affective states (EFSA AHAW Panel, 2022a). In addition, in several cases the temperature ranges that were examined in the available studies were not covering the ranges indicated in the mandate questions. Nevertheless, the information could be used to derive considerations to reply to the mandate ToR, as detailed in the Judgement table (see Table 7, Section 3.3.5).

3.3.3.1. Specific aspects related to thermal comfort and thermoneutral zones for adult cats

Cats are homoeothermic mammals and are thus able to thermoregulate to maintain a stable body temperature within a certain range despite fluctuations in thermal environmental conditions.

Heat loss occurs in cats through convection, conduction and evaporation. As previously described, a cat in TCZ has minimal energetic and physical efforts to thermoregulate body temperature and does not change behaviour in response to the thermal environment (Schlader et al., 2011; Kingma et al., 2014; EFSA AHAW Panel, 2022b). Outside the TCZ, thermal-related behaviours functioning to modulate heat dissipation through conduction and convection will be initiated. For example, during the winter months cats search for heat sources, preferring to sleep near sources of heat (see Figure 2a) (including solar radiation), to rest on insulating material or in contact with their conspecifics or owners, and to assume curled up positions.

In warmer seasons, cats tend to seek out cooler surfaces made of material that convey heat away from their bodies (such as metallic surfaces), avoiding spots exposed to direct sunlight, and to sleep in lying positions that promote heat dissipation through conduction and convection (Figure 2b).



Figure 2: Images of cats expressing thermal behaviours: (a) Cat seeking heat source during cold season; and at dissipating heat through conduction with cold floors during hot seasons (b) (© M. Zappaterra & M. Felici).

However, when such behaviours are not sufficient to maintain thermal balance, the heat dissipation rate is controlled through the autonomic responses of vasoconstriction or vasodilation. The more temperatures deviate from the TCZ, the more vasomotor activity becomes evident, and the energy used in thermoregulation increases.

As explained above (see Section 3.3.2.2), when temperatures approach or exceed UCT, heat loss mechanisms are activated. Like dogs, cats have minimal sweating capacity (cats have few sweat glands located primarily in paw pads), and thus thermoregulation when temperatures exceed UCT relies primarily on evaporative mechanisms through panting or increased respiratory rates. When temperatures exceed UCT, the risk of and the severity of heat stress increases, rectal temperature gradually increases above 41°C if ambient temperatures continue to be high or increase further, and if no cooling mechanisms are put in place, heatstroke occurs. Heatstroke is less common in cats, though, as compared to dogs (Johnson et al., 2006).

Several authors reported that the TNZ in cats is between 30 and 38°C (Stella and Croney, 2016; Anggraeni et al., 2023), and quoted an earlier study by Adams et al. (1970) for this information. However, the range between 30 and 38°C might have been misinterpreted and should be reconsidered for adult cats.

The results from the experiments presented in Adams et al. (1970) derived from studies on thermoregulation in adult cats exposed to different ambient temperatures between 20 and 41°C when kept in metabolic chambers. It is important to keep in mind, that this does not mean that the LCT of adult cats is 20° C, as it is considered to be lower than 20° C.

Jacobson and Squires (1970), studied the respiratory rate, evaporative heat loss and rectal temperature of cats and suggested that at temperatures between 32 and 35°C, the cats were no longer in their TNZ. Similar conclusions can be drawn from the results presented in Adams et al. (1970).

Besides a few older studies focusing on physiological responses and metabolic rate are performed in metabolism chambers (e.g. Adams et al., 1970) and thus difficult to interpret in terms of TNZ and animal welfare. Thermoregulation in cats has not been addressed by other published studies. However, a recent study has dealt with the effects of different environmental temperature and humidity parameters on feline sperm quality (Nuñez Favre et al., 2022). A total of 512 ejaculates were gathered from 20 male cats (toms), belonging to mixed short hair breeds, aged 2 to 7 years and weighing 4–5 kg. The animals were housed in individual cages and underwent similar photoperiods. The authors concluded that the best semen quality was obtained from cats kept at temperatures between 17 and 22°C. The authors suggest that this temperature range could be considered the TNZ for cats.

3.3.4. Specific aspects related to thermal comfort and thermoneutral zones in the kittening areas during the first 21 days of kittens' lives

Cats are altricial mammals. During their first days of life, kittens are essentially poikilotherms (Little, 2011), they are not able to autonomously thermoregulate, and have not yet developed the motor skills and/or vasomotion mechanisms suitable for controlling heat convection. Furthermore, kitten coat is likely to be less thick than that of adults and subcutaneous fat is lacking or still underdeveloped (Hill, 1961). In addition, kittens are born wet, causing a substantial loss of heat through evaporation during the first ~ 1 h after birth. As a consequence, kittens have a relatively high thermal conductance, which decreases as they develop thicker fur and fat layer, and gains the ability to shiver, crawl and walk autonomously (Hill, 1961). During the motor skill development stages, kittens reach crawling ability between 7 and 14 days of age and are able to walk between 14 and 21 days. These developmental stages can influence the efficiency of thermoregulation, as some thermal behaviours can be expressed when cats reach the motor skills necessary to shiver and move to seek warmer or colder spots. The growth of kitten fur provides additional insulation, enhancing their ability to regulate body temperature independently. Therefore, as kittens approach the end of the 3-week period, they become more independent in their thermoregulatory abilities.

Based on the above newborn kittens rely on their mother, littermates and/or external factors for thermoregulation during their first weeks of life. Three scientific papers investigated the thermoregulatory ability of kittens during the first days of life (Olmstead et al., 1979; Hull, 1965; Hill, 1961). Hull et al. (1965) investigated the rate of oxygen consumed by kittens during the first 10 days of life. One possible interpretation for the oxygen consumption is to consider it an ABM for basal metabolic rate, indicating whether the animals are using more energy to cope with the thermal environment. On the day of birth, the rate of oxygen consumption increased when the ambient temperature fell below 35°C. On the fourth and fifth day after birth, kittens showed an increase in

oxygen consumption when ambient temperatures fell below 32°C. By the ninth and tenth day, basal metabolic rate increased as ambient temperatures fell below 30°C. Similarly, Hill (1961) evaluated the basal metabolic rate and the rectal temperature of kittens exposed to environmental temperatures ranging from 20 to 40°C. Three hours after birth, kittens showed a rectal temperature of 34°C (hypothermia) when they were exposed to environmental temperatures of 32°C. The decrease in body temperature when exposed to low environmental temperatures became less abrupt with the advancement of age. At 7 days of age, the body temperature started decreasing as they were exposed to environmental temperatures lower than 28°C and their mother was not present.

A study by Olmstead et al. (1979) assessed the physiological and behavioural responses of 37 kittens, males and females, from birth until 60 days of life. Behavioural responses and rectal and ear temperatures were recorded as kittens were exposed to five different conditions: when kittens were huddling in the nesting box with littermates and the mother at an ambient temperature of 22-24°C; when kittens were exposed to ambient temperatures of (i) 22-24°C (with the mother), (ii) from -15 to -9° C (without the mother), (iii) above 50°C (without the mother). The rectal temperatures remained stable in the kittens kept with their littermates and their mother at an ambient temperature of 22-24°C. The changes noticed in their body temperature followed their maturation, and minimum and maximum values of rectal temperatures were in the range of normality. When exposed to environmental temperatures between -15°C and -9°C, kittens showed a different response in the decrease of rectal temperature. During an exposure period of 10 min, kittens less than 20 days old showed a decrease of 2°C in their rectal temperature, whereas kittens from 21 days of age upwards decreased their rectal temperature by less than 1.5°C. Olmstead et al. (1979) noted that at 21-25 days of age, almost 90% of kittens expressed shivering when exposed to -15° C. By contrast, the percentage of kittens exhibiting such behaviour was considerably lower in the age range of 16–20 days old, with only 50% of kittens shivering when exposed to -15° C (Olmstead et al., 1979). Regardless of the age of kittens, when they were kept at ambient temperatures of 22-24°C for 15 min, their rectal temperature showed to be fairly stable. However, it should be noted that this exposure time was rather limited, never exceeding 15 min, but the authors did not evaluate the prolonged effects of exposure to such temperatures, and thus it cannot be excluded that such temperatures, if maintained for periods longer than 15 min, could affect the health and welfare of the kittens. Without presenting any evidence, Peterson (2011) suggested that nesting areas should have an environmental temperature of 26.5°C until 28 days of life.

The results presented above suggest that kittens, huddling with their mother and littermates in kittening areas at environmental temperatures of 23–24°C, are in thermal balance. However, as only few other temperature intervals were examined, the boundaries of the TNZ of kittens are not known. In case of orphaned kittens or when the queen has been taken away, the results suggest that ambient temperature should be 30–35°C during the first 10 days of kittens' life to prevent hypothermia.

3.3.5. EFSA judgement of mandate statements related to temperature in adult breeding cats and kittening areas

The outcomes of the judgement of the mandate statements are reported in Table 7.

Table 7: EFSA Judgement of mandate statements related to thermoneutral zone in adult cats and kittening areas (during the first 21 days of kittens' lives), with examples of the retrieved evidence

#	Statement	EFSA Judgement Is this statement supported by the studies	Overall recommendations on		
		from the scoping literature search and/or other sources?	this statement		
1	There is scientific evidence to support that, from an animal welfare perspective: - a thermoneutral zone between 15 and 26°C prevents negative welfare consequences linked to temperature in adult breeding cats	Overall statement if supported or not from literature From the scoping literature, no scientific publications were found suggesting 15–26°C as the TNZ for adult breeding cats. Reasoning Few scientific publications that investigated aspects of thermoregulation in cats were retrieved, but only few were relevant for addressing this mandate statement. However, the temperature ranges that were tested in these papers were not covering the range indicated in the statement. For example: - Nuñez Favre et al. (2022) reported that feline sperm quality is best between 17 and 22°C. - According to data from Adams et al. (1970) and Jacobson and Squires (1970), at temperatures between 32 and 35 °C, cats were no longer in their TNZ.	A temperature range between 15 and 26°C prevents negative welfare consequences linked to temperature in adult breeding cats. This range may need adjustment to fit certain breeds. Further studies are needed to support this statement. Further research on ABMs for assessing thermal stress in cats is recommended.		
		There are few examples of national provisions establishing ranges of temperatures for cats, for example, the UK Code of Practice for the Housing and care of animals bred, supplied or used for scientific purposes ^(a) indicates that ' <i>cats may be</i> <i>maintained within a wide temperature range</i> <i>provided that their welfare is not compromised. A</i> <i>temperature range between 15 and 21°C is</i> <i>suitable when precise control is required for cats</i> <i>under procedure (as determined by the</i> <i>investigator and justified as necessary)'.</i> The US Animal Welfare Act and Animal Welfare Regulations ^(b) indicates that: ' <i>When cats are</i> <i>present, the ambient temperature in the facility</i> <i>must not fall below 50°F (10°C) for cats not</i> <i>acclimated to lower temperatures, for those</i> <i>breeds that cannot tolerate lower temperatures</i> <i>without stress or discomfort (such as short-haired</i> <i>breeds), and for sick, aged, young or infirm cats</i> <i>and cats, except as approved by the attending</i> <i>veterinarian (). The ambient temperature must</i> <i>not fall below 45 °F (7.2 °C) for more than 4</i> <i>consecutive hours when cats are present, and</i> <i>must not rise above 85 °F (29.5 °C) for more</i> <i>than 4 consecutive hours when cats are present'.</i> Judgement			
		<u>Judgement</u> In general EFSA considers that a range temperature of 15–26 °C falls within the TNZ of the majority of adult cats and that this range prevents negative welfare consequences linked to			



#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement		
		temperature. The TNZ of cats is, though, probably wider than this range.			
		It may be more relevant to establish thresholds for ABMs measuring heat and/or cold stress in cats, rather than to establish breed specific TNZ.			
2	There is scientific evidence to support that, from an animal welfare perspective: - a thermoneutral zone in the	Overall statement if supported or not from literature From the scoping literature, no scientific publications were found suggesting 22–28°C as the TNZ in the kittening areas (during the first 21 days of kittens' lives).	Further research is needed to establish the optimal temperature and condition for keeping kittens in kittening areas during the first 21 days of life.		
	kittening areas between 22 and 28°C during the first 21 days of kittens' lives prevents negative welfare consequences	Reasoning Some papers investigating aspects of thermoregulation in kittens were found, but few papers were relevant for addressing this mandate question. However, temperature ranges that were tested were not covering the range indicated in the question. Examples of the findings:	For kittens kept without the mother during the first 21 days of life the temperature of the kittening area should be higher than when kittens are kept with the mother.		
	linked to temperature in kittens	 When kept with their mother at 23–24°C, kittens can maintain body temperature (Olmstead et al., 1979). Hill, 1961: kittens without the mother should be kept at ambient temperatures > 32°C in the few days after birth. After the 1st week of life, the temperature can be decreased to 27°C. Without the presentation of scientific evidence, Peterson et al. (1991) and Little (2011) suggested that kittens without their mother, in the first few days of life, are kept at 32°C. The temperature can be gradually decreased to 27°C during the following weeks. 			
		There are few examples of countries with national provisions, reporting specific of temperatures for kittens, and these are broader compared to the range indicated in the mandate. For example, the Irish Code of Practice for Sellers and suppliers of pet animals (2019) ^(c) recognising that kittens are relatively sensitive to low temperatures, sets a minimum ambient temperature of 18°C for kennels where kittens are kept. The UK Code of Practice for the Housing and care of animals bred, supplied or used for scientific purposes ^(a) indicates that: 'as kittens have limited thermoregulatory control for around the first ten days of life, it is recommended to provide additional local heating during this period. Where experimental protocols allow, a 'basking area' provided by a heat lamp or radiator would provide environmental enrichment and allow animals some control over their environmental			

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
		Judgement EFSA considers that a range of 22–28°C as the TNZ for preventing negative welfare consequences linked to temperature in the kittening areas (during the first 21 days of kittens' lives) is not supported by the scientific literature retrieved. Young kittens are unable to regulate temperature on their own and an exact range of TNZ cannot be provided as it depends highly on the insulating properties of the kittening area and on the presence or absence of the mother.	

(a): https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388895/ COPAnimalsFullPrint.pdf

(b): https://www.aphis.usda.gov/animal_welfare/downloads/AC_BlueBook_AWA_508_comp_version.pdf

(c): https://www.gov.ie/en/publication/fe3427-code-of-practice-for-sellers-of-pet-animals/#

3.3.6. Specific aspects related to thermal comfort zone and thermoneutral zone in dogs

From the scoping literature search, only few papers investigated aspects of thermoregulation in dogs and were relevant for addressing the mandate question. As reported above for the adult cats, most of the publications retrieved on adult dogs were performed in metabolism chambers and focused on physiological responses, making them less useful to interpret in terms of the EFSA definitions of heat and/or cold stress, focusing on stress and/or negative affective states (EFSA AHAW Panel, 2022a). In addition, in several cases the temperature ranges that were examined in the available studies were not covering the ranges indicated in the mandate questions. Furthermore, for dogs, considerably larger differences than for cats exist among breeds (for example in size) and it would be difficult to identify a range of TNZ to generalise to all dogs. Nevertheless, the information could be used to derive considerations, as detailed in the Judgement table (see Table 9, Section 3.3.7).

3.3.6.1. Specific aspects related to thermal comfort and thermoneutral zones for adult dogs

Thermoregulation in dogs involves multiple body systems, combining behavioural and physiological mechanisms. When dogs are exposed to temperatures below the lower threshold of TCZ, they may seek sheltered areas or burrow into blankets, pillows or seek warm spaces. Dogs may also curl up or tuck their paws and tail close to their body to reduce heat loss. Furthermore, dogs may engage in physical activity to generate heat through muscle contractions. Autonomic activation of vasoconstriction is also established and enhanced as temperatures deviate from the lower threshold of TCZ. Vasoconstriction allows a greater blood flow to the internal organs, dissipating less heat. When temperatures drop below the LCT, dogs shiver to generate body heat (Mota-Rojas et al., 2021). Shivering is a muscle response characterised by rapid, repetitive muscle contractions that generate heat by increasing metabolic activity, which helps dogs raise their body temperature and maintain warmth in colder environments (Mota-Rojas et al., 2021). If these thermal responses are not sufficient to sustain body temperature, the body temperature will gradually decrease, and the animal will enter hypothermia. Dogs are identified to be in hypothermia when their rectal temperature falls below 37.2°C (96°F) (Konietschke et al., 2014). A hypothermic dog may shiver intensively, seek warmth, develop pale or bluish gums, become lethargic and be unable to move.

On the other hand, dogs exposed to temperatures above their comfort zone will perform behaviours such as seeking shade, lying on cool surfaces, digging holes to rest in cooler soil or finding a water source to swim or soak in. These behavioural responses function to increase heat loss through conduction. Dogs may also reduce physical activity and move at a slower pace thereby reducing heat production. When even such behaviours do not allow the re-establishment of thermal comfort, thermoregulation is enhanced. Physiologically, when the perceived temperatures are above UCT, dogs rely mainly on evaporative cooling through thermal panting (Bruchim et al., 2017; see also Figure 3).

Panting increases the rate of airflow over the moist surfaces of the respiratory tract, allowing for evaporation and heat loss. This helps dogs to release excess body heat and regulate their temperature.



Figure 3: Image of a dog expressing thermal behaviour: dissipating heat through conduction with cold floor and panting (© M. Herskin)

Additionally to panting, dogs have sweat glands on their paw pads, which provide a minor degree of cooling through evaporation. When the dog is no longer able to dissipate enough heat to maintain a normal body temperature, the animal enters into a state of hyperthermia. Hyperthermia is considered mild when the rectal temperature rises above 39.5°C and severe when it reaches 41.5°C. When the rectal temperature rises above 40°C, the animal may enter heatstroke. Heatstroke is a heat disorder with a high level of severity, described as an acute emergency characterised by a systemic inflammatory response, neurological dysfunction (e.g. delirium, convulsions or coma), and multiple organ dysfunction syndrome (Caldas et al., 2022). Rectal temperatures around 42°C are usually fatal (Stanley et al., 1980). Heatstroke-related diseases are quite common in dogs belonging to brachycephalic breeds, due to the morpho-functional alterations in their airways, or in military, rescue, or work dogs, which are subject to physical exercise in hot and adverse ambient conditions (Otto et al., 2017; Gogolski et al., 2020; McGraw and Thomas, 2021).

Whether and how much dogs will be affected by the thermal conditions of the surroundings depends on many factors. Among these are the degree of insulation of the coat (long/short hair, with and without undercoat) and size of the dog, but also characteristics connected to specific breeds such as brachycephalia. Below, we present findings from available studies, where most of them have used one breed or one type of dog. This information is presented to give overview. In Section 3.3.8, possible effects of factors such as breed and type of dogs are discussed. A comprehensive evaluation of the influence of these factors are not provided, though, as this would require a full risk assessment.

The first studies that sought to determine the basal metabolic rate and changes in physiological responses of dogs exposed to different environmental temperatures were conducted in metabolic chambers. In one of these studies (Hammel et al., 1958), three adult dogs of about 10 kg body weight (thus, small dogs) were kept for 4 h in metabolic chambers and several ABMs were tested at different environmental temperatures (between 20 and 40°C, see Table 8). Hales and Dampney (1975) performed similar experiments on Greyhounds (dog breed with very short fur, known to be sensitive to cold), but testing ABMs at 16, 24 and 40°C only (see Table 8).

On the basis of the changes in the ABMs reported in these studies, the environmental temperatures can be categorised into three groups: 'cold' temperatures between 8 and 12°C; 'hot' temperatures above 33°C; and the range of temperatures considered 'neutral' (i.e. when the ABMs remained stable) was between 16 and 27°C. The upper limit of this interval could be higher, but in the case of the experiments on the Greyhounds (Hales and Dampney, 1975) ABMs were not measured at temperatures between 25 and 39°C.

Table 8 provides an overview of the findings about the physiological and behavioural ABMs measured in dogs kept in metabolic chambers and reported in the studies of Hammel et al. (1958) and Hales and Dampney (1975). Green cells indicate temperatures at which ABMs remained stable; yellow

was used to indicate temperatures at which physiological AMBs showed perturbations; and red colour was used to indicate temperatures at which physiological ABMs changed markedly or, in the case of the behavioural ABMs to indicate the temperatures at which these were observed.

Table 8: Patterns of ABMs from the results obtained from experiments performed in metabolic chambers by Hammel et al. (1958) in adult dogs of 10 kg of body weight (small dog, and thus sensitive to cold) (indicated as Reference #(a)) and Hales and Dampney (1975) in Greyhounds (dog breed with very short fur and known to be sensitive to cold) (indicated as Reference #(b)). Green colour cells indicate temperatures at which ABMs remained stable; yellow cells indicate temperatures at which physiological ABMs showed perturbations; and red colour is used to indicate temperatures at which physiological ABMs changed markedly. In the case of the behavioural ABMs, red cells indicate the temperatures at which these ABMs were observed. Empty cells mean that the temperature was not tested

	Reference #	Environmental temperatures									
ABMs		8°C	16°C	21°C	24°C	25°C	27°C	30°C	33°C	35°C	40°C
Physiological ABN	ſs										
Rectal temperature	(a)										
	(b)										
Average skin	(a)										
temperature	(b)										
Leg skin temperature	(b)										
Ear skin temperature	(b)										
Vasomotor activity	(b)										
Respiratory frequency	(b)										
Heart rate	(b)										
Oxygen consumption	(b)										
Basal metabolic rate	(a)										
Respiratory heat loss	(a)										
Tissue conductance	(a)										
Skin cooling constant	(a)										
Behavioural ABM	S										
Curling up	(a)										
Lying with spread legs	(a)										
Panting	(b)										
Shivering	(a)										

(a): Study of Hammel et al. (1958) in three adult dogs of 10 kg body weight.

(b): Study of Hales and Dampney (1975) in six Greyhounds, males and females, weighing about 30 kg.

However, in addition to the limitations from studying only one breed or only one size of dogs, as was done in the studies so far presented, limitations exist for studies that use metabolic chambers. Firstly, basal metabolic rates measured in metabolic chambers does not fully reflect metabolic rates of the same animals in normal conditions (White and Seymour, 2003; Burggren et al., 2017). This is mainly because animals are not free to move or have the additional heat production determined by digestion and social interactions. Secondly, studies involving experiments in metabolic chambers typically consider limited numbers of individuals. In addition, as mentioned above these studies used Greyhounds and small dogs of 10 kg of body weight, which are both more sensitive to cold temperatures than larger breeds or breeds with for example a fur that contains undercoat.

More recently, field studies have been done, also contributing with information concerning dogs' perception of ambient temperature. Again, however, these studies involve only one breed and/or type of dog and, thus, do not allow for comparison between breeds. Berteselli et al. (2019) tested a protocol to assess the welfare and health of dogs kept in shelters located in central Italy. The welfare and behaviour of dogs were assessed over different seasons, and panting and shivering behaviours were used to assess thermal comfort. No dog was noticed shivering at 9°C with a relative humidity of about 70%. About 20% of dogs were noticed panting during summer, at temperatures of 24°C, 27°C and 28°C and RH of 61%, 42% and 50% respectively. These results seem to indicate that dogs can already show signs of heat stress at an ambient temperature of 25°C with relative humidity values up to 50%. No information is given on either the size or the coat type of these dogs. However, it is possible to assume that they were mainly medium to large mixed-breed animals, as dogs in kennels are predominantly of this type (Raudies et al., 2021).

Al-Shammarai et al. (2019) tested the effects of long periods of exposure to different ambient temperatures on police dogs deployed in Iraq, by assessing a number of physiological ABMs (e.g. rectal temperature, respiratory rate and heart rate, levels of the growth hormone and thyroid hormones). Although several information on the experimental conditions were not reported in this publication, and conclusions thus need to be considered carefully, the results suggested that no changes in the ABMs were found at temperature up to 35°C. ABMs peaked in the dogs subjected to 45°C. At 25°C, however, the level of blood sugar was significantly increased. Considering that blood sugar increases during heat stress as an effect of increased glucocorticoids (Belhadj Slimen et al., 2016), this result may suggest that the metabolism of the dogs kept at 25°C had already undergone an initial adaptation as part of their coping with the ambient temperatures.

Gogolski et al. (2020) performed a retrospective analysis of heatstroke cases in 103 military working dogs to find the risk factors associated with the occurrence of severe heat stress and heat-induced diseases. The considered dogs were adults of both sexes belonging to three breeds (Malinois, German Shepherd and Labrador Retriever; on average 30 dogs per breed). They were working in a military base in Texas (USA) performing physical activity daily. Heat-induced problems increased significantly when the ambient temperature was $> 26.7^{\circ}$ C with a relative humidity > 60%. At temperatures $> 21^{\circ}$ C with a relative humidity > 80% the risk of having dogs with heat-induced pathology events increased exponentially, suggesting that in addition to temperature, the relative humidity should always be considered when recommendations about thermal zones in dogs are made. The results identified by Gogolski et al. (2020) indicating a significant increase in the risk of health issues in working dogs subjected to ambient temperatures $> 26^{\circ}$ C seem to be supported by a retrospective study conducted by Schwartz et al. (2018).

Exertion at extreme temperatures is also common in sled dogs, which must perform physical exercise at low ambient temperatures. The effects of exertion, season, environmental temperatures and food regimes were investigated by Gerth et al. (2010) on the basal metabolic rate of 12 male Inuit sled dogs kept at different food regimes and trained at different intensities in Greenland. The authors report based on observations and experience that thermoneutrality intervals for these dogs ranged between -25° C and $+10^{\circ}$ C. The UCT in those dogs was slightly above $+10^{\circ}$ C as at those temperatures the dogs were noticed panting.

In 2006, a publication of the U.S. National Research Council (NRC, 2006) reported that dogs have a TNZ ranging from 20 to 30°C, except for Huskies, which were supposed to have LCT lower than 0°C. Later, Sjaastad et al. (2010) reported that short-haired dogs had LCT of 15°C. However, these indications of TNZ do not seem to be supported by scientific evidence.

Overall, the available studies highlight the importance of environmental temperature on the occurrence of heat stress and to a lesser extent cold stressing dogs. In addition, despite not being mentioned in the current mandate, relative humidity parameters are also of a key importance (Berteselli et al., 2019; Gogolski et al., 2020).

In summary, due to the limited number of studies, and the huge diversity among dog breeds and types in factors such as fur length and type, body weight etc., it is not possible to establish one common TNZ in across all dogs. The review of the literature above shows that factors such as dog breed, type and activity level are important for the establishment of thermal thresholds. Differences between breeds exist and are discussed in Section 3.3.8.

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3.3.6.2. Specific aspects related to thermal comfort and thermoneutral zones in the whelping areas during the first 10 days of pups' lives

Dogs are altricial animals, which give birth to newborns that are immature, relatively immobile, are not able to obtain food on their own. During the first days of life, puppies are highly dependent on their mother and their environment for thermoregulation (Reyes-Sotelo et al., 2020). They are born with underdeveloped thermoregulatory abilities and limited possibility to maintain body temperature. Their ability to maintain the internal temperature is so limited during the first weeks that pupples are reported to be to some extent poikilothermic at birth. Puppies have indeed underdeveloped thermoregulatory systems, including poorly functioning sweat glands and limited fat reserves for insulation (Reyes-Sotelo et al., 2020). As a result, they are less able to regulate body temperature than adult dogs. In addition, puppies have limited mobility and may not be able to actively seek appropriate thermal environments. Therefore, puppies rely heavily on their mother and littermates for warmth, and they seek contact with their mother's body and often huddle together, thereby sharing body heat. Furthermore, newborn puppies have a relatively high surface area to body mass ratio, which makes them susceptible to heat loss. Due to their immaturity in motor and muscle development, they also have a limited ability to generate metabolic heat through shivering or increased muscle activity, unlike adult dogs. Because of all these factors, the first 72 h of life are associated with a risk of hypothermia in puppies. Particularly, at birth, puppies make the transition from the intra-uterine environment, and this sudden change may cause a significant decrease in body temperature. Another element that influences body temperature at birth is heat lost due to evaporation through the wet dermal surface, as puppies are born wet. This thermal change during the first days of life has been found to impact the health of the newborn (Reyes-Sotelo et al., 2020). In altricial newborn mammals, environmental temperatures in the nesting area should therefore be kept as close as possible to the body temperatures of the newborns. In puppies, the rectal temperature during the first days of life ranges between 35 and 36°C, and it's higher (up to 38°C) in the following weeks (Fitzgerald and Newquist, 2011). When puppies are kept with their mother, warmth from her body maintains a suitable microclimate for them. Lezama-García et al. (2022) showed that puppies' thermal stability and superficial temperatures are dependent on their mother basal metabolic rate and weight. During the first days of life, the thermal stability of puppies can be kept if the nesting area is provided with insulating materials (e.g. blankets, textiles, straw) and the healthy, well-functioning bitch rarely leaves the nesting area. In cases, where maternal care cannot be provided, puppies should be kept at temperatures near their body temperature of 35–36°C. Over time, their thermoregulatory capacity mature. However, the mechanisms generating metabolic heat, like the ability to shiver and the vasoconstriction mechanisms, are not observed in dogs until 6-8 days of life, and are not fully developed until day 18 (Reyes-Sotelo et al., 2020). Hence, mortality due to hypothermia can be a high risk during the first 3 weeks of life. As puppies grow, their ability to maintain body temperature strengthens: they develop better control over their body temperature regulation, increased fat stores for insulation, and improved ability to generate metabolic heat.

From the scoping literature search (Section 2.2.2), the evidence that specifically relates to the identification of thermal zones in puppies is scarce. The only scientific study retrieved was published by Hull (1973), who indicated a strong similarity in the development of puppies and kittens and suggested a temperature range of 30–35°C during the first 10 days of life for both species when the mother is not present. No publications were found on TNZ for puppies with the mother present.

3.3.7. EFSA judgement of mandate statements related to temperature in adult breeding dogs and in whelping areas

The outcomes of the judgement of the mandate statements are reported in Table 9.
Table 9: EFSA Judgement of mandate statements related to thermoneutral zone in adult dogs and in whelping areas (during the first 10 days of life of the puppies), with examples of the retrieved evidence

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	There is scientific evidence to support that, from an animal welfare perspective: - a thermoneutral zone between 10 and 26°C prevents negative welfare consequences linked to temperature in adult breeding dogs	Overall statement if supported or not from literature From the scoping literature, no scientific publications were found suggesting 10–26°C as the TNZ for adult breeding dogs. Reasoning Few publications investigated aspects of thermoregulation in dogs, and among them only few were relevant for addressing this mandate question. There is huge variety among dogs in factors that are important for thermoregulation, such as body size and fur; large differences are documented among different dog breeds (e.g. Hammel et al., 1958; Berteselli et al., 2019).	Further research is needed to establish the optimal temperature for keeping adult breeding dogs of different breeds and types. Further research on ABMs for assessing thermal stress in dogs is recommended.
		 There are few examples of national provisions establishing ranges of temperatures for adult dogs, for example: in the UK Code of Practice for the Housing and care of animals bred, supplied or used for scientific purposes^(a) it is indicated that: 'dogs may be maintained within a wide temperature range provided that their welfare is not compromised (). A temperature range between 15°C and 24°C (as determined by the investigator and justified as necessary)'is appropriate when precise control is required for cats under procedure'. the US Animal Welfare Act and Animal Welfare Regulations^(b) indicates that: 'When dogs are present, the ambient temperature in the facility must not fall below 50 °F (10°C) for dogs not acclimated to lower temperatures, for those breeds that cannot tolerate lower temperatures without stress or discomfort (such as short-haired breeds), and for sick, aged, young or infirm dogs and cats, except as approved by the attending veterinarian (). The ambient temperature must not fall below 45°F (7.2°C) for more than 4 consecutive hours when dogs are present, and must not rise above 85 °F (29.5°C) for more than 4 consecutive hours when dogs are present. 	



#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources? in adult breeding dogs is not supported by	Overall recommendations on this statement
		scientific evidence. Breed and type of dog (and dog activities) are important for the occurrence of heat and/ or cold stress in dogs and therefore for defining the TNZ. As large differences exist among different dog breeds, a TNZ exact range common for all breeds cannot be provided.	
		It may be more relevant to establish thresholds for ABMs measuring heat and/or cold stress in dogs, rather than to establish breed specific TNZ.	
2	There is scientific evidence to support that, from an animal welfare perspective: - a thermoneutral zone in the whelping areas between 22 and 28°C during the first 10 days of life prevents negative welfare consequences linked to temperature in puppies	 Overall statement if supported or not from literature From the scoping literature, no scientific publications were found suggesting 22–28°C as the TNZ in the whelping areas (during the first 10 days of life of puppies). Reasoning From the scoping literature search, few papers were found investigating aspects of thermoregulation in puppies and few were relevant for addressing this mandate question. Hull (1973), suggested a temperature range of 30–35°C during the first 10 days of life for puppies when the mother is not present. No publications were found on TNZ for puppies with the mother present. There are few examples of national provisions establishing ranges of temperatures for puppies, but these are broader compared to the range indicated in the mandate. For example, the Irish Code of Practice for Sellers and suppliers of pet animals (2019)^(c) sets a minimum temperature of 18°C for kennels where puppies are kept. the UK Code of Practice for the Housing and care of animals bred, supplied or used for scientific purposes^(a) indicates that: 'as puppies have limited 	Further research is needed to establish the optimal temperature for keeping puppies in the whelping areas during the first 10 days of life. For puppies kept without the mother during the first 10 days of life the temperature of the whelping area should be higher than when puppies are kept with the mother.
		thermoregulatory control in the first ten days of life, it is advisable to provide additional local heating within the whelping enclosure. New-born puppies require a local environmental temperature of 26–28°C for at least the first five to ten days of life'.	

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement	
		Judgement EFSA considers that a range of 22–28°C as the TNZ for preventing negative welfare consequences linked to temperature in the whelping areas (during the first 10 days of pups' lives) is not supported by scientific evidence.		
	Young puppies are unable to regulate temperature on their own and the TNZ depends also on the presence or absence of the mother.			

(a): https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388895/ COPAnimalsFullPrint.pdf

(b): https://www.aphis.usda.gov/animal_welfare/downloads/AC_BlueBook_AWA_508_comp_version.pdf

(c): https://www.gov.ie/en/publication/fe3427-code-of-practice-for-sellers-of-pet-animals/#

3.3.8. Specific breeds or categories of cats and dogs: further considerations

3.3.8.1. Considerations on certain breeds and morphological characteristics of cats and dogs

Differences in breed characteristics have been identified as an important factor leading to variations in the perception of thermal conditions in livestock species (Carvalho et al., 1995).

Domestic dogs are characterised by a large range of body sizes, with breeds being the most phenotypically and physiologically diverse among the domesticated animals. Body mass, body surface area and metabolic rate are tightly associated. Although the scientific literature about dog thermal zones is still scant, a recent study by Jimenez et al. (2023) suggested that there are differences in how dog breeds of different sizes produce heat and dissipate it after physical exercise. These results are in agreement with previous studies. For example, Zanghi (2016) reported that Retrievers had a higher body temperature than Beagles. Also, a positive correlation between body weight and rectal temperature was found in greyhounds (McNicholl et al., 2016).

As mentioned above, dogs and cats have limited sweating capacity; thus heat dissipation in warm environments is primarily driven by panting. Skull and nose shapes are major factors influencing the efficiency of panting. Brachycephalic dog and cat breeds are characterised by their shortened noses and flat facial structures. The distinctive anatomical features of these breeds significantly impact their thermoregulation capabilities. The shortened nasal passages result in limited airway space, that affect the animal's ability to breathe efficiently and cool itself. The flatter face and compressed upper respiratory tract has been reported to lead to a variety of breathing difficulties, collectively known as brachycephalic obstructive airway syndrome (BOAS) (Gallman et al., 2023). BOAS can further exacerbate thermoregulation challenges. Brachycephalic breeds, therefore, are less able to regulate their body temperature due to compromised heat dissipation. Their shortened noses reduce the surface area available for heat exchange, potentially leading to heat retention and increased susceptibility to heat. The restricted airflow and reduced cooling capacity make it challenging for brachycephalic breeds to engage in vigorous exercise or exert themselves for extended periods, particularly in hot weather (Bruchim et al., 2017; Davis et al., 2017; Caldas et al., 2022; Gallman et al., 2023). Brachycephalic breeds are at an increased risk of heat stress and heatstroke, as their ability to use panting to thermoregulate is adversely affected. A limited ability to cool down efficiently can lead to rapid overheating, especially when exposed to high temperatures or humidity. Brachycephalic breed was found to be a risk factor for heatstroke in a retrospective study conducted to associate the heat-related illness events in UK with the dogs' characteristics and the environmental variables (Hall et al., 2022). The risk of a fatal outcome in dogs was indeed 12 times higher in dogs aged more than 12 years, and three-times higher if these dogs were brachycephalic when compared to mesocephalic dogs aged less than 2 years old. Another study by Lilja-Maula et al. (2017) found that English Bulldogs, a brachycephalic breed, show signs of heat stress at an ambient temperature as low as 21–22°C when walked. No scientific literature is available on the effect of high temperatures on

brachycephalic cat breeds. However, the compressed upper respiratory tract and shorter nasal passages characterising these feline breeds are expected to hinder the capacity of these animals to dissipate heat efficiently through their airways. In line with this, the International Air Transport Association (IATA) Live Animal Regulations also establish more specific and differentiated guidelines for the air transport of brachycephalic breeds of dogs and cats (IATA, 2023).

Coat is another factor that influences thermoregulation as it is a major component of body insulation (Turnpenny et al., 2000). Understanding the relationship between coat and the thermoregulatory abilities is important for ensuring welfare and safety of cats and dogs. Dogs possess various coat types, broadly classified into three categories: single-coated, double-coated and hairless. Single-coated dogs have a single layer of fur, often consisting of shorter hair. Double-coated dogs possess two layers of fur: a dense undercoat and a longer, protective topcoat. Hairless dogs lack significant fur and may have patches of hair or be completely hairless. In addition, coat length varies significantly among dog breeds, ranging from short to medium or long. Short-coated breeds have fur that is positioned close to the body and is not providing a lot of insulation. Dogs with short, darker hair are also more prone to develop dorsal thermal necrosis when exposed to temperatures $> 26^{\circ}$ C and when engaged in sports or physical activities in direct sunlight (Schwartz et al., 2018). On the other hand, medium-coated breeds and longcoated breeds, have fur that is longer and thicker with sufficient undercoat, providing enhanced insulation from direct sunlight and cold temperatures. Denser and thicker fur coat increase the capacity for trapping of heat near the skin and is one of the thermal adaptations occurring during colder months in cats and dogs, and other animal species. Effects of coat type have been investigated in some studies. The length of coat was found to be associated with heat dissipation after exercise in the study by Jimenez et al. (2023). In agreement with this finding, other studies have found that changes in superficial skin temperatures in dogs are negatively correlated with coat length (Kwon and Brundage, 2019; Cugmas et al., 2020). This finding may be explained by a lower insulating capacity exerted by short coats when compared with long coats. The effects of coat type and colour are, however, unclear, as results in the scientific literature are contrasting (Jimenez et al., 2023). Coat colour affected the efficiency of thermoregulation in racing dogs, for example, McNicholl et al. (2016) reported that dark coated greyhounds had an increased post-race rectal temperature when compared with light coloured greyhounds. Similarly, differences were found between medium- and dark-coloured coats in dogs post-racing, with lower tympanic temperatures found in dogs with a medium-coloured coat (Carter and Hall, 2018). However, others have found no effect of coat colour or length. For example, after a walk in the sun, black and yellow Labrador dogs did not have different body temperatures (Neander et al., 2021). Further studies are needed to clarify this.

Overall, the scientific literature strongly supports the recommendation that different breeds and types of dogs have different TNZ. These considerations, although probably of a smaller magnitude than dogs, could be extended to cats, although no studies were retrieved for this animal species. Further research on the effect of temperature and other environmental parameters on ABMs and welfare consequences (thermal stress) across breeds should be developed.

3.3.8.2. Considerations on specific categories of cats and dogs

Age is a major factor affecting thermoregulation capacity in mammals including dogs and cats.

The first few weeks of life in cats and dogs are a risk factor for cold stress and hypothermia, as kittens and puppies are born immature and unable to thermoregulate (Hill, 1961; Hull, 1965; Olmstead et al., 1979). Conversely, geriatric cats and dogs may be subjected to heat stress, similarly to many other physiological performance traits, the capacity of homoeothermic mammals to thermoregulate declines with age. Ageing compromises the capacity to maintain or dissipate heat in several animal species (DeGroot and Kenney, 2007; Hanna and Tait, 2015; Grosiak et al., 2020). A retrospective study of heatstroke in dogs in the UK showed that animals over 12 years of age, especially those belonging to brachycephalic breeds, are at greater risk of heat-related diseases and heatstroke and have a reduced capacity to dissipate heat (Hall et al., 2022). This result therefore seems to support that older dogs have an impaired capacity to thermoregulate, in agreement with what has been observed in other animal species.

There are also differences related to **sex**, **stage of gestation** and **lactation**. Sex, pregnancy and lactation are factors that change thermoregulation in other mammalian species that gives birth to altricial young, such as for example humans and mice (Kaciuba-Uscilko and Grucza, 2001; Jackson et al., 2014). Body heat and body temperature are indeed highly dependent on sexual hormones in mammals (Fernández-Peña et al., 2023). Progesterone causes changes in the hypothalamus that

determine shifts in the body temperature towards higher temperatures. This results in a higher body temperature in females during progesterone peaks in ovarian cycles and pregnancy. No publications were found from the scoping literature search investigating TNZ and TCZ in queens and bitches. However, it is highly likely that the same observations made in other animal species also apply in pregnant and lactating dogs and cats. In several mammalian species, during pregnancy and lactation, fluctuations of approximately three to four times the normal basal metabolic rate in non-gestating females have been shown (Speakman and McQueenie, 1996). These differences are caused by a combination of endocrinological, morphological and metabolic changes; for example, during these reproductive stages food intake peaks and the internal organs increase their mass to respond to the enhanced energy and work demand due to fetus development and milk production (Speakman and McQueenie, 1996; Kazma et al., 2020). This causes an increase in body heat production.

Overall, mainly based on knowledge from other mammalian species, it is considered highly likely that TNZ differ depending on animal categories of dogs and cats (puppies, kitten, adult animals, pregnant and lactating animals).

3.3.9. EFSA judgement of mandate statements related to temperature in certain breeds or specific categories of cats and dogs

The outcomes of the judgement of the mandate statements are reported in Table 10.

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	 There is scientific evidence to support that, from an animal welfare perspective: regarding temperature, other considerations exist in relation to certain breeds or types of cats 	Overall statement if supported or not from literature From the scoping literature, no scientific publications specific for cats were found reporting, how specific aspects related to certain breeds or types of cats can influence how the thermal conditions are perceived by cats, and their abilities to regulate temperature.	Further research is needed to clarify susceptibility to thermal stress among cats of different breeds and types.
		Reasoning Some publications specific for dogs on these aspects were retrieved (see #3 of this Table) and they could be taken into consideration for addressing this question in cats.	
		<u>Judgement</u> Although no scientific literature was retrieved to support that regarding temperature other considerations exist in relation to certain breeds or types of cats, there are findings reported in dogs (e.g. on size, fur, coat, activity) that could be relevant, to some extent, also to cats (see #3 below in this Table).	
2	 There is scientific evidence to support that, from an animal welfare perspective: setting thermoneutral zone for indoor accommodation of cats according to specifics of every category of animals is relevant for their welfare 	Overall statement if supported or not from <u>literature</u> From the scoping literature, no scientific publications specific for cats were retrieved on the aspects reported in the statement. <u>Reasoning</u> Publications were found to support that age and reproductive status are important for how mammals perceive heat and cold stress. Some of these factors, e.g. gestational status and	Further research is needed to set the optimal thermal conditions for indoor accommodation of breeding cats according to specifics of different categories and life stages of animals.

Table 10:EFSA Judgement of mandate statements related to temperature in certain breeds or
specific categories of cats and dogs, with examples of the retrieved evidence



		EFSA Judgement	Overall
#	Statement	Is this statement supported by the studies from the scoping literature search and/or other sources?	recommendations on this statement
	l a F r A t	lactation are particularly relevant for breeding animals. For example, Fernández-Peña et al. (2023) is related to pregnancy and lactation. Although this has not been examined in cats, the knowledge from other mammals can be used.	Further research on ABMs for assessing thermal stress in cats is recommended.
		Judgement By use of analogy with other mammalian species, EFSA considers that it is highly likely that different cat categories and life stages will have different TCZ and TNZ.	
		It may be more relevant to establish thresholds for ABMs measuring heat and/or cold stress in cats, rather than to establish specific TCZ and TNZ for different categories and life stages.	
3	There is scientific evidence to support that, from an animal welfare perspective: - regarding temperature, other considerations exist in relation to certain breeds or types of dogs	Overall statement if supported or not from literature From the scoping literature search, scientific publications were found to support that specific aspects related to certain breeds or types of dogs can influence how the thermal conditions are perceived by cats, and their abilities to regulate temperature.	Due to the large variations of dog breeds, further research is needed to clarify susceptibility to thermal stress among dogs of different breeds.
		 <u>Reasoning</u> For example: depending on the breed, type (e.g. brachycephalic) and also use (e.g. work), there is evidence for a large variation in exposure and sensitivity to heat stress (Otto et al., 2017; Gogolski et al., 2020; McGraw and Thomas, 2021). Hales and Dampney (1975), investigated vulnerability to cold stress in Greyhounds. Other examples are studies on brachycephalic-type breeds (Bruchim et al., 2017; Davis et al., 2017; Caldas et al., 2022; Gallman et al., 2023), on coat and fur (Turnpenny et al., 2000; McNicholl et al., 2016) and on different sizes (Jimenez et al., 2023). Judgement EFSA considers that regarding temperature, other considerations exist in relation to certain breeds or types of dogs. Aspects related to e.g. size, coat, fur, level of activity, influence how the thermal conditions are perceived by dogs, and their abilities to regulate temperature. 	



#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
4	There is scientific evidence to support that, from an animal welfare perspective: - setting thermoneutral zone for indoor accommodation of dogs according to specifics of every category of animals is relevant for their welfare	Overall statement if supported or not from literature From the scoping literature, no scientific publications specific for dogs were retrieved on the aspects reported in the statement. Reasoning Publications were found to support that age and reproductive status are important for how mammals perceive heat and cold stress. Some of these factors, e.g. gestational status and lactation are particularly relevant for breeding animals. For example, Fernández-Peña et al. (2023) is in particular related to pregnancy and lactation. Although this has not been examined in dogs, the knowledge from other mammalian species, EFSA considers that it is highly likely that different dog categories will have different TCZ and TNZ. It may be more relevant to establish thresholds for ABMs measuring heat and/or cold stress in dogs, rather than to establish specific TCZ and TNZ for different categories and life stages.	Further research is needed to set the optimal thermal conditions for indoor accommodation and breeding dogs according to specifics of different categories and life stages of animals. Further research on ABMs for assessing thermal stress in dogs is recommended.

3.4. Light requirements

3.4.1. Question to address and guidelines from the EU AW platform

The question to be addressed is:

Is access to natural daylight important to ensure welfare of cats and dogs?

- Is there scientific evidence in literature to suggest a minimum intensity of 50 lx during a maximum of 16 h per day?
- Is setting parameters for artificial lighting (illuminance (lux), spectrum, time schedule) where needed, a key element to ensure the welfare of these animals?

The recommendations from the EU AW Platform in relation to light were:

'Dogs require sufficient periods of daylight and darkness to follow their natural day/night activity patterns

- Keep dogs under natural lighting conditions
- Natural daylight should be provided. Where additional artificial lighting sources are used, they
 must follow a light period equal to the natural day length providing at least 10–12 h of light.
 Artificial lights must be switched off overnight to provide a period of darkness for a minimum
 of 8 h. White artificial lighting, preferably broad or full spectrum (including UV), must provide at
 least 50 lx at the height of the animals'.

`Cats require sufficient periods of daylight and darkness to follow their natural day/night activity patterns.

- Keep cats under natural lighting conditions.
- Natural daylight should be provided. Where artificial lighting sources are used, they must follow
 a light period equal to the natural day length providing at least 10–12 h of light and be of
 the same periodicity and intensity to natural daylight. Artificial lights must be switched off
 overnight to provide a period of darkness for a minimum of 8 h'.

3.4.2. Light and circadian rhythms

Light is one of the factors, named also 'time-givers', regulating many animal physiological, hormonal and behavioural parameters, including the sleep-wake cycle and behavioural homeostasis (Dauchy and Blask, 2022). The intensity, wavelength and duration of light exposure influence the circadian rhythms of the aforementioned parameters (Reppert and Weaver, 2002). A behavioural or physiological parameter is considered to follow a 'circadian rhythm' when it has a self-sustained cycle of \sim 24 h which can persist in constant environmental conditions (e.g. constant light or permanent darkness) (Cerutti et al., 2022). Circadian rhythms are therefore an inherent property of animal physiology (Refinetti and Piccione, 2005; Piccione et al., 2008; Cerutti et al., 2022) and are fundamental for the living systems to ensure their functioning and coping mechanisms in an environment (Cerutti et al., 2022). Different physiological (e.g. cortisol, body temperature) and behavioural (e.g. time budget of active and inactive behaviour during light and dark phases) parameters have shown circadian rhythmicity (Zanghi et al., 2012; Giannetto et al., 2014; Cerutti et al., 2019; Cerutti et al., 2022), driven by an internal 'clock' located in the suprachiasmatic nucleus of the hypothalamus (Cerutti et al., 2022). This mechanism is an autonomous internal daily timekeeper that is influenced by environmental factors (Bertolucci et al., 2008; Cerutti et al., 2022; Schork et al., 2022). Among these environmental factors, light and light-dark cycles are the most important (Dauchy and Blask, 2022). The circadian rhythm of a physiological or behavioural parameter is usually described by a series of characteristics, namely mesor (mean level), amplitude (half of the range of oscillation), acrophase (time of peak) and robustness (a stationary rhythm). These characteristics are studied in relation to the photoperiod, which is composed of a photophase (i.e. light phase) and a scotophase (i.e. darkness phase) (Giannetto et al., 2014). For physiological and behavioural parameters which still have a cycle of ~24 h, but are not endogenously self-sustained, the term 'daily rhythm' is used (Cerutti et al., 2022).

Depending on the species-specific responses to the light, the circadian or daily rhythms of several physiological parameters and behavioural patterns differ, for instance cortisol (Cerutti et al., 2022).

In dogs, cortisol production has its peak (i.e. acrophase) in the middle of the light phase (i.e. photophase) (Giannetto et al., 2014). Therefore, dogs show fluctuations in daily rhythms compatible with the characteristics of a diurnal animal.

Cats are instead nocturnal animals, cortisol blood levels and activity reach their peak during the night (Leyva et al., 1984). Light inhibits melatonin production (Dijk and Archer, 2009) and since melatonin level affects the release of reproductive hormones (Gonadotropin release hormone, GnRH) in cats, light indirectly affects reproductivity. Cats are long-day seasonal breeders, as melatonin has negative feedback on GnRH whereas light has positive feedback on GnRH (Leyva et al., 1984; Johnson, 2022). In cats, ovarian activity, therefore, ceases under decreasing photoperiod (i.e. autumnwinter) and resumes with increasing photoperiod (spring–summer) (Leyva et al., 1984). Cat's reproductive activity can be indeed controlled by applying light manipulation with artificial illumination (Johnson, 2022).

In conclusion, light appears to be a fundamental environmental cue, which impacts daily rhythms in dogs and daily rhythms and reproduction in cats. Changes in the light setting could therefore impair the welfare of dogs and cats.

3.4.3. Specific aspects related to light in cats

The length of the photoperiod has multiple effects on cats' physiology, behaviour and reproduction (Di Pietro et al., 2022; Johnson, 2022; Parker et al., 2022). Queens are long-day, seasonal polyoestrous breeders (i.e. they have multiple cycles within a season when the hours of light are increasing). The breeding season consequently varies depending on latitude and photoperiod. For example, above the Cancer Tropic, in the USA, queen cycle when the light duration increases (i.e. from January to June–July) and cyclicity ceases when the light duration decreases (i.e. from August–September to December). In addition to reproduction, light affects the daily rhythms of several physiological and behavioural parameters (Dauchy and Blask, 2022).

Studies on light spectrum (natural light, artificial light, artificial daylight)

Di Pietro et al. (2022) investigated the influence of constant artificial lighting vs. natural lighting on daily rhythms of tear production in cats. The study was conducted on 20 European short hair cats (10 males and 10 females; mean age: 12 ± 2 months) divided into two groups (n = 10), a control and an experimental one. The control group consisted of staff-owned cats living indoors under natural lighting (CG). The experimental group was composed of cats hospitalised under constant artificial lighting (HG). The CG was exposed to a natural 12:12 light:dark cycle (sunrise 06:10, sunset 18:10), while the HG cats were exposed to a constant lighting scheme (i.e. 24:0 light:dark). A tear test was performed every 4 h over a 48-h period. Robust daily rhythmicity in tear production was found in cats exposed to natural daylight, while this was disrupted in the cats kept under constant artificial light. This result agrees with the scientific literature, as constant lighting exposition may impact on welfare in different animal species (e.g. mice, chickens) as animals may lose the circadian rhythms of many physiological and behavioural parameters (Morgan and Tromborg, 2007). Parker et al. (2022) investigated the effects of seasonal daylight duration on food intake and locomotor activity of indoor-managed cats. A group of six cats (five spayed females and one castrated male, aged 7 months) were housed in an environmentally controlled cattery (i.e. temperature: 21-23°C) and exposed only to natural daylight through two large bay windows. Cat behaviour was observed continuously and simultaneously during 21 subsequent days each season. Tags were attached to cats' collars to track their locomotor activity (i.e. distance covered) and feeding habits (i.e. time and weight of food consumption). As rhythm parameters, the period (duration of a full cycle) and amplitude (rhythmicity of the measured parameters, e.g. activity or food intake) were measured. Locomotor activity and feeding maintained daily rhythms during all seasons. Cats showed the maximum value of daily distance covered during spring and autumn, and the minimum value during winter. Moreover, in spring and summer, cats were more active during the day than at night. Conversely, during autumn and winter, the cats were similarly active between day and night. Cats resulted to be less active in the middle of the night in all seasons and in the middle of the day during summer and winter. Daily feeding pattern was less affected by seasonal variations. The light was not reported to impact the hourly/daily feeding consumption in the studied cats. However, feeding occurrence was higher during sunset and sunrise during autumn and winter, due to the crepuscular nature of the cats. From the results of Parker et al. (2022), it is clear that natural daylight and its variations during the different seasons are important for cats to keep their behavioural homeostasis. This could suggest that cats need light-dark cycles to regulate their rhythms and when exposed to natural daylight are able to keep their seasonality. Alternating light and dark and different durations of daylight (from 10 to 15) with gradual increase may therefore be necessary for the cat to synchronise its rhythms and appears to respect the cats' welfare. This is in line with the literature on other mammalian rhythms (Murphy et al., 2019). The effects of the setting of artificial lighting (i.e. illuminance, spectrum, time schedule) are reported in different scientific studies investigating cats' physiology (i.e. intraocular pressure, hormonal release, reproduction) and behaviour (i.e. locomotor patterns). Del Sole et al. (2007) investigated the rhythm of intraocular pressure (IOP) on 30 adult cats of different sex and with or without ocular pathologies. The cats were kept under a 12:12 light-dark ratio or under 48 h of constant darkness. The animals were adapted to a 12:12 light:dark cycle (no details about lux or spectrum) for 2 weeks before IOP values were assessed for 24 h. Of the total number of cats, eight were randomly selected from the group, kept under constant darkness for 48 h and then IOP was assessed every 3 h over a period of 24 h. In both groups, IOP maintained the circadian rhythms and from the results, it was possible to conclude that the light:dark cycle affected it, while no effects of sex and pathologies were statistically found. A possible influence of the light on IOP was reported also by Piccione et al. (2010) in dogs, where constant lighting (i.e. 24:0 light:dark) resulted in disruption of IOP rhythms. Further studies are needed, to examine if light exposure over 12 h in cats would disrupt the IOP rhythms in cats, as already reported in dogs (Piccione et al., 2010).

Studies on light duration and light/dark schedules

As aforementioned, in cats, the light influences the release of hormones (Dauchy and Blask, 2022). Leyva et al. (1984) investigated the effects of different photoperiods on melatonin, prolactin (PRL) and cortisol plasma levels. A total of 12 adult female cats housed in environmentally controlled rooms (i.e. 22 C° and controlled lighting) were used. The cats were subjected to a 2-year 14:10 light:dark photoperiod (lights on between 06:00 and 20:00) before the experiments. Each room had an illuminance of 323 lx at floor level (i.e. four 40-W fluorescent strip lights) during the light period and 3 lx (i.e. 25-W red light

bulb) was constantly provided during the dark period. In the first experiment, 12 cats were divided into three groups of four, and exposed to different lighting regimens, namely (i) 8:16 light:dark (i.e. short photoperiod (SP); lights on between 06:00 and 14:00); (ii) 14:10 light:dark (i.e. normal photoperiod (NP); lights on between 06:00 and 20:00); and (iii) 24:0 light:dark (i.e. long photoperiod [LP]). In addition to this first experiment, the four cats under the SP regimen underwent a second experiment, being exposed to light for 12 h after 6 h of dark (from 20:00 to 08:00). After 140-day exposure to the three photoperiods, blood was collected via indwelling catheters at 2-h intervals for 24 h, and for the second experiment, samples were taken every 2 h from 14:00 through to 08:00 the following day. In the first experiment, melatonin levels varied among the three photoperiods, with the highest values in the SP group compared to the LP group. A positive relation was found between duration of darkness and the magnitude of the peak of melatonin concentration. Fluctuations of melatonin concentrations were found in SP cats (increasing in the beginning of the dark period and then declined during the last 2-h of this phase), while similar concentrations were found in the NP group. During a long photoperiod (LP) the melatonin pattern was dampened. PRL concentrations were significantly higher in dark periods in the SP versus the NP and in the NP versus the LP. The variations of the PRL pattern during the dark period did not differ from those of melatonin. PRL concentrations peaked during the dark phase both in SP and NP groups, and then declined during the last 2 h of darkness. A 24-h cycle for PRL secretion in SP and NP cats was detected, but not in LP cats. Conversely, cortisol concentrations were not affected by photoperiods and did not differ between groups. In the second experiment, light exposition during the dark phase caused a significant decline in PRL and melatonin concentration. This decline was maintained for the entire experimental period. Melatonin and prolactin secretion thus seem to be driven by the light: dark cycles and their rhythms seem to change in relation to different photoperiods. A continuous light regime (24:0 light:dark) had a disruptive effect on melatonin and prolactin production in cats, and longer light exposition at night decreased the normal secretion of these hormones during the dark phase. Conversely, darkness favoured the secretion of both melatonin and prolactin. Therefore, both constant light and light exposition during nocturnal hours seem to compromise the physiological rhythms of melatonin and prolactin in cats. The disruptive effects of both constant lighting and exposure to light at night have also been reported in other studies (Morgan and Tromborg, 2007; Dauchy and Blask, 2022). Exposure to extended photoperiods is reported to alter the melatonin-to-serotonin ratio and compromise the synthesis of important enzymes for the correct functioning of the central nervous system (Morgan and Tromborg, 2007). Moreover, exposition to light at night increases the risk of physiological and behavioural circadian rhythms disruption (Dauchy and Blask, 2022). In contract to constant lighting, in the study by Levya et al. (1984) the cats kept under 8:16 and 14:10 light:dark schemes were able to maintain their hormonal secretion rhythms. Light schemes ranging from 8 to 14 h of light seem to respect cats' hormonal physiology.

Light influence reproduction activity in cats and artificial light schemes (light on or off) can be used to manipulate their breeding patterns. Johnson (2022) reported that exposure to a 14:10 light:dark scheme of artificial lighting for at least 2 months induced and maintained cyclicity year-round in queens. Conversely, the same author (Johnson, 2022) said that reducing light administration to 8 h or fewer may affect queen cyclicity, inducing an anoestrus period. Exposure to continuous lighting maintains cyclicity in cats, the same author said, but the number of heat cycles obtained with this light regimen decreased from two per month (in a 14:10 light:dark scheme) to one per month, with a prolonged interestrous period (30 vs 15 days) and possible reduction in reproductive parameters (Johnson, 2022). Hurni (1981) investigated the effects of different photoperiods on the mating rate and litter production in cats. Cat-breeding units (composed of 15-30 females and one male) were housed in controlled environmental rooms (room temperature $23 \pm 2^{\circ}$ C, floor temperature in pens 26°C, relative humidity 55 \pm 10%) lit by white fluorescent tubes (no full spectrum). The illuminance ranged from 90 to 250 lx (average 150 lx) at 1 m from the floor. Breeding was performed in two segregated units in separate buildings and the number of litters each week was expressed as a percentage of the number of brood cats at that time. Over the tested photoperiods, the number of litters produced per year is largest when a regular 12 h light/dark cycle is maintained.

The latter study (Hurni, 1981) was included in a recent review by Eaton et al. (2022). The review investigated the effects of different artificial light photoperiods, spectrum (e.g. type of artificial light) and illuminances on cats' reproduction, but not on other welfare parameters.

Dawson (1941) investigated the effects of increased light duration on the induction of oestrus in female cats. Administering increasing light schedules, from a 7:17 light:dark scheme to a 13:11 light dark scheme and then adding artificial light to natural light in a 12:12 light:dark scheme induced the oestrus in 100% of the studied queens. Similarly, Scott and Lloyd-Jacob (1959) studied the effect of

increased illumination (12:12 light:dark scheme) in a group of cats from a local colony compared to cats from the same colony not exposed to light. Cats exposed to light showed earlier oestruses compared to other cats. Robinson and Cox (1970) investigated the effects of 14:10 light:dark scheme (natural + artificial light) on the queens' yearly litter production. Litter production peaked in winter and summer, with a more clearly defined peak in winter. An internal rhythm in cats' reproduction has therefore been suggested by the authors. Both the studies by Leyva et al. (1989, 1984) sought the effects of different photoperiods on reproductive hormone (i.e. oestradiol) concentrations. In both studies, under 8:16 light; dark schemes, queens showed ceased cycle activity. Levva et al. (1989) reported also that cats exposed to 24:0 dark: light schemes showed decreased oestrus cycle per month than those exposed to 14:10 light:dark cycle (i.e. one per month vs. two per month). Also in the study by Chantal (Michel, 1993) cats exposed to an 8:16 light:dark scheme showed anoestrous, while oestrus occurred both in cats exposed to 14:10 light:dark (after a month under 12:12 light:dark scheme) and cats that received 1-hour light flash during the night (i.e. 12:1:1:10 light:dark:light:dark or 12:4:1:7 light:dark:light:dark schemes). Finally, Caransa et al. (2011) investigated the effect of a 14:10 light:dark scheme (natural daylight + artificial lighting) on the number of days on heat per month in cats. Although the cats under a 14:10 light:dark schedule cycled non-stop for the whole year, an increase in the days of heat per month was found in the long days being accompanied by an ascending photoperiod.

Overall, it seems that the administration of different light schemes of artificial or combined light (i.e. natural + artificial) in an illuminance range between 90 and 323 lx impacts on the reproductive cycles in cats. Shorts period of light lead to anoestrous, while long periods induce oestrus in queens. However, continuous exposition of light (24:0 light:dark) was disruptive for the reproductive rhythms in cats, since the queens' reproductive cycle and hormone production were impaired. This agrees with the principle that reproduction in animals can be altered by an aberrant administration of light (Dauchy and Blask, 2022).

The effects of artificial lighting and different photoperiod durations have been studied on cats' behavioural patterns (i.e. locomotor activity) (Cerutti et al., 2019; Cerutti et al., 2022).

The study by Cerutti et al. (2019) aimed to investigate the influence of four different photoperiods on daily total locomotor activity in cats. Four adult male cats were individually caged in an airconditioned room under controlled temperature ($20 + 0.2^{\circ}$ C). Each cage was equipped with a fluorescent lamp of 200 lx of illuminance and each cat was subjected to four different artificial lighting regimes, namely (i) 12:12 light:dark (light on at 08:00, light off at 20:00), (ii) 12:12 dark:light (light on at 20:00, light off at 08:00), (iii) 24:0 light:dark (continuous light), (iv) 0:24 light:dark (constant darkness). Cats' locomotor activity was recorded for 24 h by an accelerometer. Cosinor analysis detected a significant daily rhythm in the locomotor activity of cats. In 12:12 light:dark conditions, the cats were more active during the dark phase compared to the light phase. Conversely, during the 12:12 dark:light cycle, cats quickly resynchronised their locomotor activity rhythm and two peaks of activity continued to occur during the new dark phase (8:23 \pm 1:47 h and 20:01 \pm 1:45 h). Under 24:0 light:dark scheme, cats showed endogenous rhythms for the locomotor activity. Conversely, when the cats were exposed to constant darkness, endogenous periodicity was not observed. Therefore, both environmental cues and an endogenous circadian oscillator result in influencing locomotor activity rhythms in the cat. Cerutti et al. (2019) defined cats as crepuscular animals with a great capability of adapting to changes in the photoperiod. When the photoperiod shifted to the 12:12 light: dark scheme cats had to adapt to the new conditions, or were endogenously driven in the case of constant light. However, constant darkness seemed to be more disruptive compared to the other light schemes.

In a similar study, Cerutti et al. (2022) investigated how different light:dark cycles impact the daily locomotor rhythms in wild and domestic felids. Two groups of felids (10 wild cats: 5 Geoffroy's cats and 5 Jaguarundi; 5 domestic cats: 4 male, 1 female) were subjected to different photoperiods, namely (i) 12:12 light:dark (L/D1), (ii) constant lighting (24:0 light:dark; L/L) and (iii) 12:12 light:dark (L/D2) schemes. All the felids were housed in cages, under controlled environmental conditions (i.e. temperature: $20^{\circ}C \pm 0.2^{\circ}C$; lighting: 200 lx). The whole experimental period was 37 days: 22 days of L/D1, 6 of L/L and 9 days of L/D2 schemes. During the experimental period, an actimeter with a sampling frequency of 15 min was used to collect information about the locomotor activity. Four rhythmic parameters (mesor: mean level; amplitude: half of the range of oscillation; acrophase: time of peak; and robustness: a stationary rhythm) were assessed and compared among the 3 species and the light schemes. All the rhythmic parameters were statistically different among light:dark schemes and the different species. Mesor value was statistically lower in domestic cats compared to L/D2 and L/D1 and was lower compared to other species in all experimental light:dark schemes. Acrophase

was observed during the scotophase (i.e. dark phase) in domestic cats. Robustness was lower in L/L than L/D2 and L/D1 in all species examined. A lower robustness value was observed in domestic cats compared to wild cats in all L/D schedules. Most rhythmic parameters (mesor, amplitude and robustness) had lower values in domestic cats than in wild felids, probably due to the formers' adaptation to the domestic environment. However, the acrophase of locomotor activity in domestic cats was found during the scotophase, confirming the crepuscular nature of cats. Amplitude and robustness of locomotor activity decreased in both domestic and wild cats under continuous lighting administration compared to the 12:12 light:dark schemes. This confirms the disruptive effect of continuous artificial lighting on the daily rhythms of cats. Therefore, both constant darkness (Cerutti et al., 2019) and constant lighting (Cerutti et al., 2022) seem to lead to an alteration of their active behaviour patterns (i.e. locomotor activities).

Studies on light intensity

Eaton et al. (2022) listed different light sources (i.e. clear bulbs, fluorescent lamps, fluorescent tubes, fluorescent strips, neon tubes and incandescent bulb lamps) but their specific effects have not been investigated yet. The literature about the effects of specific light spectrum on female cat reproduction is scant (Dauchy and Blask, 2022).

3.4.4. EFSA judgement of mandate statements related to light in cats

The outcomes of the judgement of the mandate statements are reported in Table 11.

Table 11:	EFSA judgement of m	nandate statements	related to	light in	cats with	examples of the
	retrieved evidence					

Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
There is scientific evidence to support that: access to natural daylight is important to ensure welfare of breeding cats	Overall statement if supported or not from literature From the scoping literature search, no publications were found on the need for breeding cats to access to natural daylight. Reasoning There are publications to state that cats need access to light for being able to perform locomotor behaviour and feeding behaviour (Parker et al., 2022). However, no studies demonstrated that light should be direct natural light from the sun. Most publications studied the effects of different daylight duration on behavioural or reproductive parameters in outdoor settings, but the aim of these studies was not to compare with indoor settings. Judgement EFSA considers that the need for cats to have access to light is evident. However, this does not necessarily mean that it should be natural light.	Further research is needed to indicate if cats need access to natural daylight.
There is scientific evidence to support that, from an animal welfare perspective: a minimum intensity of 50 lx for maximum 16 h per day is needed for breeding cats	Overall statement if supported or not from literature From the scoping literature search, no publications were found to suggest a maximum duration of light per day. Reasoning It was agreed that the part of the question stating if evidence can support 'maximum 16 h	A period of darkness within a day is recommended for keeping the circadian rhythm. Further research is recommended to



#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
		of light' was not meaningful (it would include a too large range: 0–16 h). It was rather considered more relevant to assess the question in relation to the minimum duration of darkness which is set to 8 h in the AW Platform guidelines.	indicate the optimal dark and light duration. Further research is recommended to indicate the optimal
		The publications retrieved report the effects of different daylengths and night-length in relation to reproduction and locomotion and not to other aspects of animal welfare (Cerutti et al., 2019). Continuous light is harmful for welfare because cats lose the circadian rhythm (Leyva et al., 1984; Di Pietro et al., 2022). Similarly, continuous darkness is harmful because they lose the reproduction cyclicity (Johnson, 2022).	light intensity.
		However, for welfare reasons it is important that there is a natural and gradual dark/light switch (Dauchy and Blask, 2022). In several publications the provision of 8 h and mostly 12 h of darkness was tested versus 0 h of darkness (continuous light) (e.g. Cerutti et al., 2019; Di Pietro et al., 2022) but no information for shorter darkness duration was obtained. Also, there is no information in the publications supporting 50 lx for the light intensity.	
		<u>Judgement</u> EFSA considers that there is no evidence to suggest the minimum 8 h of darkness (as suggested in the document of the Platform). A period of darkness within a day is needed for keeping the circadian rhythm, although the optimal duration is unknown.	
	There is scientific evidence to support that: setting parameters for artificial lighting (illuminance (lux), spectrum, time schedule) where needed, is a key element to ensure the welfare of these	Overall statement if supported or not from literature From the scoping literature search, evidence was retrieved supporting that setting parameters for artificial light is important for breeding cat welfare. Reasoning	Parameters for artificial light should be set, however more research is recommended to define light parameters quantitatively and qualitatively.
	animals	The review paper from Eaton et al. (2022) reported that setting parameters for artificial lighting is important for breeding cats but no studies were retrieved to suggest a specific setting.	The frequency of artificial light should never be lower than 80 hertz.
		Judgement EFSA considers that setting parameters for artificial light is important for breeding cat welfare. The optimal specific setting is unknown. Artificial light at a higher frequency than 80 hertz produces flickering which can be disturbing to the animal.	

3.4.5. Specific aspects related to light in dogs

Studies on light spectrum (natural light, artificial light, artificial daylight)

In dogs, natural daylight regulates the circadian clock and the fluctuations of some behavioural (e.g. sleeping) and physiological parameters (Siwak et al., 2003; Schork et al., 2022).

Schork et al. (2022) investigated the associations between light and sleep structure in dogs. The sleep patterns of 13 mixed-breed adult dogs (six females and seven males, aged from 1.5 to 7 years) housed in an outdoor kennel were documented. Dogs' behaviour was assessed during the day (07:00–17:59) and night (18:00–06:59), from October to May. The lighting followed natural light/dark cycles; in particular, the light length was on average 12 h 46 min \pm 00 h 36 min (sunrise: 05:18, sunset: 18:05), with an increase of 02:14:16 h from the start to the end of the study (+16.7% in daylight duration). Increased day length was associated with increasing number of sleeping bouts in the dog at night (sleep bouts = ~ 50 min of sleep before waking and sleeping again, typical of a dog sleeping pattern). Later sunset led instead to a decrease in the number of sleep bouts during night, but it is important to highlight that the authors indicated that later sunsets were not related to increased day length, because days with the latest sunsets had also latest sunrises. So, the authors concluded that an exposition of more than 12 h of natural daylight could affect sleeping behaviour as previously shown in other animals (Morgan and Tromborg, 2007).

Natural light also affects locomotor activity in dogs. Siwak et al. (2003) compared the effect of natural versus artificial light on activity rhythms in dogs of different ages. The activity pattern of 12 elderly dogs (aged 10-13 years) housed indoors with access to outdoor space (INT/OUT) were compared with the activity patterns of other eight elderly dogs (aged 9–13 years) housed only indoors. The INT/OUT dogs could be exposed to natural light (\sim 10,000 k) for a maximum of 14 h, while the dogs housed exclusively indoors, were under a 12:12 light:dark scheme (no lux reported). The dogs housed INT/OUT were active earlier in the day and exhibited a higher frequency and longer duration of activity than the others. Therefore, the authors suggested that the natural light, probably because of its higher intensity and more gradual reduction from light to darkness, seemed to be more effective in synchronising and consolidating the activity cycle. Therefore, dogs' activity pattern seems to be influenced not only by natural light duration and intensity but also by the graduality in the transition between light and darkness. This is in line with the literature, as gradual photoperiod transition was reported to reduce stress in rodents (Dauchy and Blask, 2022). Similarly to Siwak et al. (2003), Giannetto et al. (2014) compared the effect of natural versus artificial photoperiods on rectal and vaginal daily temperature rhythms in dogs. Rectal and vaginal temperatures were recorded in six dogs (aged 2–3 years old) every 3 h for 48 h under three different photoperiods: natural light (11:13 light: dark scheme), constant artificial lit (24:0 light:dark; no lux specified; fluorescent tubes located 3 m from the floor) and constant darkness (0:24 light:dark). A daily rhythmicity was found for rectal temperature, under all three different lighting regimes. Vaginal temperature instead maintained daily rhythmicity only under natural light and the constant darkness regime. When the dogs were kept under constant artificial light, the daily rhythms of vaginal temperature were indeed disrupted. Prolonged artificial constant lighting seems therefore to hurt the circadian rhythms of dogs compared to natural light.

Studies on light duration and light/dark schedules

Bertolucci et al. (2008) investigated the effects of light and light shift (i.e. 6 h shift) on daily variations of serum lipids (i.e. non-esterified fatty acids (NEFA), triglycerides, phospholipids, total cholesterol and total lipids) in five healthy male Beagles. The light was administered in a 12:12 light: dark scheme (from 09:00 to 21:00 or from 15:00 to 03:00 when shifted), with 800 lx of illuminance, using cool fluorescent tubes located 2 mt from the floor. Moreover, a dim red light (< 3 lx) was used to manage dogs in the dark phase of the experiment. All the serum lipids, except for NEFA, showed daily rhythmicity and rhythms were correlated to the light scheme. No significant effects of the shift in the light scheme were detected, except for triglycerides. In a standard 12:12 light:dark scheme triglycerides peaked in the middle of the day, while in the 6-h shifted light scheme they peaked during the night. In conclusion, the authors reported physiological daily rhythmicity in serum lipids of dogs when exposed to 12:12 light:dark and 800 lx artificial light setting. Alternating light and dark in a 12-to-12 ratio seems to preserve the rhythmicity of serum lipids production even when the light–dark times were shifted. This might suggest the greater importance of the light–dark ratio of 12:12 on lipids circadian fluctuation. Moreover, the low-intensity dim red light used during the dark phase does not

seem to alter melatonin production in dogs. This is in line with what was reported in horses (i.e. diurnal animals) (Murphy et al., 2019). Low-intensity dim red light can be, therefore, a useful tool for managing animals in the dark without impacting their welfare. Similarly to Bertolucci et al. (2008), Piccione et al. (2008) investigated the effects of the light cycle and two different feeding schedules on the daily rhythm of serum lipase and α -amylase in 14 purebred Beagles dogs. The dogs were housed in individual pens under the 12:12 light:dark schedule (light on at 08:00 am), with an illuminance of 600 lx (no further details on light type). Dogs were divided into two groups (fed once vs fasting) consisting of seven animals each. Blood was collected every 4 h for 48 h. The results showed daily variations in the serum levels of lipase and α -amylase in dogs regardless of being fed once or kept fasting. Results also showed a nocturnal acrophase and robustness of rhythm for both lipase and α amylase with no statistical difference between the two groups (feed once vs fasting). Therefore, the authors reported that 12:12 light:dark scheme with an illuminance of 600 lx respected the daily rhythm in serum lipase and α -amylase secretion in dogs. Moreover, these rhythms were found to be more entrained by light than by feeding patterns. Light can, therefore, be considered essential for the homeostasis of some physiological parameters in dogs. In other studies from Piccione et al. (2009, 2010), the same scheme of 12:12 light:dark with an illuminance of 600 lx was applied. Piccione et al. (2009) investigated tears production in eight female Beagles subjected to three different light conditions: (i) 12:12 light:dark scheme with an illuminance of 600 lx (light on at 8:00), (ii) 24:0 light: dark and (iii) 0:24 light:dark cycles. All the light schemes were administered using cool daylight fluorescent tubes. A dim red light (< 3 lx) was used to manage dogs in the dark phase of their experiment. A tear test (Schirmer tear test) was performed every 4 h over 24 h. Acrophase (i.e. the peak) in tear production was shifted and rhythmicity was lost under constant light condition (i.e. 24:0 light:dark) compared to 12:12 light:dark and 0:24 light:dark schemes. In conclusion, an artificial lighting setting of 12:12 light:dark and 600 lx protected the physiological tears production in dogs. Conversely, constant lighting resulted in the disruption of tear production. This is similar to what was reported by Giannetto et al. (2014) and confirms the negative impact of constant lighting on the dogs' physiological homeostasis. Therefore, it is possible to conclude that a balanced ratio between light and darkness period (i.e. 12:12) safeguards the rhythmicity of physiological parameters in dogs. In a similar experiment, Piccione et al. (2010) investigated the fluctuations in intraocular pressure (IOP) in 10 Beagles (five female and five male) exposed to four different artificial light regimes. The light regimes were administered using fluorescent tubes as follows: (i) 12:12 light:dark period at 600 lx of illuminance (light on at 07:00); (ii) 12:12 dark: light period (light on at 19:00 and off at 7:00); (iii) constant light (24:0 light:dark); and (iv) constant darkness (0:24 light:dark). IOP detection was performed every 4 h over 24 h (starting at 8:00 AM on day 1 and finishing at 8:00 AM on day 2). A dim red light (< 3 k) was used to collect data during the dark phases of the experiment. Robust daily rhythmicity was detected in IOP under all light schedules except for the constant light condition, during which IOP values were decreased. Like in the other studies, constant light administration results in a suppression of circadian rhythms. Conversely, a balanced exposition (12:12 light:darkness) to light results in the maintenance of the physiological rhythms in dogs. As for natural light, also artificial light seems to affect body temperature rhythms in dogs. Refinetti and Piccione (2005) investigated the daily rhythm of rectal temperature under controlled laboratory conditions in dogs, horses, rats and squirrels. Seven female Beagle dogs were involved and kept in individual pens with an artificial light schedule of 12:12 light:dark (no lux or other lighting details specified). Rectal temperature was collected every 2 h for 7 days, using an electronic thermometer inserted 3 cm into the rectum. In dogs, rectal temperature ascended during the lights-on phase and the acrophase occurred earlier than in the other nocturnal species (e.g. rats and squirrels). However, rectal temperature showed robust rhythmicity. Overall, when dogs are exposed to a light:darkness ratio of 12:12, independently whether the light is natural (Giannetto et al., 2014) or artificial (Refinetti and Piccione, 2005), they can keep the rhythmicity of the rectal temperature.

Studies on light intensity

The effects of artificial light and its characteristics, such as intensity (illuminance (lux)), spectrum, time and schedule have been investigated. The manipulation of artificial light characteristics has often been used to control food intake, reproduction efficiency and social communication in domestic animals (i.e. chicks, pigs, goats, sheep) (Adamczyk et al., 2015). Standard parameters for artificial lighting (i.e. illuminance and time schedule) are reported in different scientific studies investigating dogs' physiology (Siwak et al., 2003; Refinetti and Piccione, 2005; Bertolucci et al., 2008; Piccione

et al., 2008; Piccione et al., 2009; Piccione et al., 2010; Giannetto et al., 2014) and behaviour (i.e. sleep patterns) (Fukuzawa and Nakazato, 2015; Houpt et al., 2019).

In the literature, artificial lighting 12:12 light:dark scheme, under 600 lx of illuminance, has been often used as a control situation assuming that this setting would not affect dog behaviour, health and welfare (Zanghi et al., 2012; Zanghi et al., 2013).

Other settings for light intensity seem indeed to have an impact. However, most studies investigate light intensity in relation to light:dark schemes and not on the direct effect of different light intensities on welfare.

Fukuzawa and Nakazato (2015) investigated the sleeping patterns of dogs exposed to different luminous emittances before lights were turned off. Eye and head position, posture and behaviour (e.g. vocalising, yawning, panting, grooming, scratching, stretching, drinking and excretion) were compared in six dogs (three females and three males, aged from 1 to 4 years), all exposed to three different luminous emittances before sleep, namely (i) 50 lx (i.e. poorly lit condition), (ii) 600 lx (i.e. control condition) and (iii) 1,600 lx (i.e. brightly lit condition). The light was administered for 2 days in succession before the dark period (from 16:00 to 21:00), over a total 6-day period. The total light duration in a day followed a 14:10 light:dark scheme (from 21:00 to 07:00). The dogs' behaviour was observed from 16:00 to 07:00 each day. The results reported that eye-closed and head contact with the floor was more manifested after the administration of poor or bright light compared to control. Conversely, body position during sleeping and, in general, dog behaviour seemed not to be affected by luminous emittances. The authors concluded that the tested artificial light administration before the light was turned off did not affect sleeping patterns in dogs (Fukuzawa and Nakazato, 2015). This could suggest that neither 50 lx nor 1,600 lx is disruptive for the sleeping rhythmicity in dogs when administered within a 14:10 light dark scheme. Compared to the 10,000 lx of daylight intensity reported by Siwak et al. (2003) in fact, Fukuzawa & Nakazato illuminance values were much lower.

Similarly, Houpt et al. (2019) investigated the behaviour (i.e. sleep behaviour, bout length and bout number and the number of turns and walking) of 10 sheltered dogs (six females and four males, aged from 6 months to 7 years) exposed or not to light during the night, aiming to describe the night-time behaviour of dogs and the influence of artificial light. Five dogs housed individually in rooms of 30 m² (4.32 m \times 6.96 m) were exposed to artificial light during the night (from 19:00 to 07:00) using a standing lamp with a 200-watt bulb (3,000 lm; 1 lx = 1 lm/m²; 100 lx = 3,000 lm/30 m²) or fluorescent light (2,000 lm; 66 lx = 2,000 lm/30 m²). The behaviour of dogs exposed to light during the night was compared with that of dogs not exposed to light. No difference was found in the sleeping behaviour between the group in the dark and the group in the light (Houpt et al., 2019); however, the authors acknowledged the limitations of the small sample size and the lack of intra-observer accuracy tests.

Overall, light intensity seems to have an impact on dog welfare, in particular on the patterns of inactive behaviours, but no studies directly investigated the effect of light parameters (e.g. type of light, intensity, etc.) on animal welfare.

In Byosiere et al. (2018) the vision of dogs is reviewed. They indicate that compared to human eyes the retina in the dogs eye contains more rod photoreceptors and the centre contains less concentrated con receptors, which makes them more adapted to function in dim light, but less able to distinguish colours. Also brightness discrimination is less developed in dogs compared to humans. The authors also suggest that there are breed differences, where larger breeds most likely have a higher light sensitivity in dim light (due to morphological differences in the eyes). On the contrast, visual aquity (clarity of vision) is worse in dogs than in humans. Although dogs have no colour vision in the red area, they can see well in the yellow and blue areas, intensity, etc) on animal welfare. In Byosiere et al. (2018) the vision of dogs is reviewed. They indicate that compared to human eyes the retina in the dogs eye contains more rod photoreceptors and the centre contains less concentrated con receptors, which makes them more adapted to function in dim light, but less able to distinguish colours. Also brightness discrimination is less developed in dogs compared to humans. The authors also suggest that there are breed differences, where larger breeds most likely have a higher light sensitivity in dim light (due to morphological differences in the eyes). On the contrast, visual aquity (clarity of vision) is worse in dogs than in humans. Although dogs have no colour vision in the red area, they can see well in the yellow and blue areas.

There is evidence (Coile et al., 1989) that dogs can see flickering in low-frequency lights (this is the case e.g. of LED with certain dimming devices). As this flickering is generally seen as adverse for animal (and human) welfare, LED lighting with proper dimmers should be used (e.g. dimmers that do not produce a frequency or produce a high frequency, that cannot be perceived by dogs).

3.4.6. EFSA judgement of mandate statements related to light in dogs

The outcomes of the judgement of the mandate statements are reported in Table 12.

Table 12:	EFSA judgement of mandate statements related to access to light in dogs with examples
	of the retrieved evidence

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	There is scientific evidence to support that: access to natural daylight is important to ensure welfare of breeding dogs	Overall statement if supported or not from <u>literature</u> From the scoping literature search, no publications were found on the need for breeding dogs to access to natural daylight. <u>Reasoning</u> There are publications to state that dogs need access to light (for being able to perform locomotor behaviour and sleeping behaviour (Parker et al., 2022). However, no studies demonstrated that light should be direct natural light from the sun. Most publications studied the effects of different daylight duration on behavioural or reproductive parameters in outdoor settings, but the aim of these studies was not to compare with indoor settings. <u>Judgement</u> EFSA considers that the need for dogs to have access to light is evident. However, this does not necessarily mean that it should be natural light.	Further research is needed to indicate if dogs need access to natural daylight.
	There is scientific evidence to support that, from an animal welfare perspective: a minimum intensity of 50 lx for at least 16 h per day is needed for breeding dogs	Ingrit.Overall statement if supported or not fromliteratureFrom the scoping literature search, nopublications were found to suggest amaximum duration of light per day.ReasoningIt was agreed that the part of the questionstating if evidence can support 'maximum 16 hof light' was not meaningful (it would include atoo large range: 0–16 h). It was ratherconsidered more relevant to assess thequestion in relation to the minimum durationof darkness which is set to 8 h in the AWPlatform guidelines.The publications retrieved report the effects ofdifferent daylengths and night-length inrelation to reproduction and not explicitly onanimal welfare.Continuous light is harmful for welfare becausedogs lose the circadian rhythm (Leyvaet al., 1984; Di Pietro et al., 2022). Similarly,continuous darkness is harmful because theylose the reproduction cyclicity (Ceruttiet al., 2019).	dark and light duration. Further research is recommended to indicate the optimal light intensity.



#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
		However, for welfare reasons it is important that there is a natural and gradual dark/light switch (Dauchy and Blask, 2022). In several publications the provision of 8 h and mostly 12 h of darkness was tested versus 0 h of darkness (continuous light) (e.g. Piccione et al., 2010) but no information for shorter darkness duration was obtained. Also, there is no information in the publications supporting 50 lx for the light intensity.	
		<u>Judgement</u> From the retrieved literature, EFSA considers that there is no evidence to suggest the minimum 8 h of darkness (as suggested in the document of the Platform). A period of darkness within a day is needed for keeping the circadian rhythm, although the optimal duration is unknown.	
	There is scientific evidence to support that: setting parameters for artificial lighting (illuminance (lux), spectrum, time schedule) where needed, is a key element to ensure the welfare of these	Overall statement if supported or not from literature From the scoping literature search, there is available evidence that setting parameters for artificial light is important for breeding dog welfare.	Parameters for artificial light should be set, however more research is recommended to define light parameters quantitatively and qualitatively.
	animals	Dauchy and Blask (2022) reported that setting parameters for artificial lighting is important for dogs but no studies were retrieved to suggest a specific setting. Schork et al. (2022): relation between sleep and daylength (more than 12 h of natural daylight could affect sleeping behaviour). There is evidence that dogs can see flickering in low-frequency lights (Coile et al., 1989).	The frequency of artificial light should never be lower than 80 hertz.
		Judgement EFSA considers that setting parameters for artificial light is important for breeding cat welfare, although the optimal specific setting is unknown. Artificial light at a higher frequency than 80 hertz produces flickering which can be disturbing to the animal.	

4. Judgement 2: health

4.1. Question to address and guidelines from the EU AW platform

The questions from the European Commission are:

- 1) Should breeding of bitches and queens before they reach skeletal and sexual maturity be avoided?
- 2) Sub-question: Is there scientific evidence in literature to suggest a minimum breeding age of 18 months for bitches and 12 months for queens?
- 3) Can controlling the frequency of pregnancies have beneficial impact on health and welfare of bitches and queens by preventing physical exhaustion?

- 4) Is there scientific evidence in literature to suggest a minimum of 12 months between two whelpings or kittenings?
- 5) Is the mature age a relevant element for the welfare in older bitches and queens?

The recommendations from the EU AW Platform in relation to reproduction were:

For cats: 'Queens and stud cats must not be used for breeding until they are fully grown (have reached sexual and skeletal maturity) - this age is breed-specific; some larger breeds mature much later. Ideally breeding should be delayed at least until cats reach 18 months of age and they should not be mated before 12 months of age.

Queens over the age of 7 should not be bred unless certified as healthy by a veterinarian. Queens must not have more than one litter a year or more than four litters in her lifetime.

Although stud cats can have a longer breeding life than queens, given they are frequently kept alone and in cattery-style pens, they should not be used after 6 years of age.

Once retired they should be neutered and found a permanent home'.

For dogs:

'The effects of age interact with other factors such as breed (physical size) and overall health in determining the reproductive fitness of dogs, and the subsequent welfare of their puppies. Breeding should be delayed until dogs are physically mature and should not extend into old-age. Bitches are more likely to experience gestational complications from middle-age; whilst sperm quality declines with age and changes in health status in stud dogs.

The ages listed below are given as a guide. It is recommended to let the dogs be examined regularly by a veterinarian, to ensure no objections are found against using the dogs for breeding.

- Bitches and stud dogs must not be used for breeding until they are fully grown (have reached sexual and skeletal maturity) this age is breed-specific; some larger breeds mature much later. Bitches younger than 18 months of age should not be bred.
- Bitches over the age of 7 years should not be bred unless examined by a veterinarian and when the veterinarian found no objections against further breeding with the bitch. Veterinary advice must be sought before breeding bitches from larger breeds if they are 6 years of age or older.
- Breeders should avoid breeding bitches for the first time if they are aged 6 years or older. Bitches must not have a litter within 12 months of the previous litter and must not give birth to more than four litters in her lifetime.
- Stud dogs over the age of 7 years should be examined by a veterinarian to see whether the veterinarian has no objections against further using the stud dogs for breeding. Veterinary advice must be sought before breeding stud dogs from larger breeds if they are 6 years of age or older'.

4.2. Reproduction in cats

4.2.1. Introduction part 1: reproductive physiology in cats

Female cats are long-day seasonally polyoestrous breeders meaning that they exhibit multiple oestrous cycles within a season, followed by a period of non-cyclicity (Johnson, 2022). The start of their oestrous cycles are generally influenced by day length and latitude (da Silva et al., 2006; Ng et al., 2023). Under natural light conditions, cat cycle when the days are longer than 12 h, but they can also be induced to cycle all year around by providing 14 h of continuous artificial lighting (Johnson, 2022). The oestrous cycle of cats is unique among domestic species, as domestic cats are induced ovulators and both copulatory and non-copulatory stimulation can cause ovulation (Griffin, 2001; Goericke-Pesch, 2010). Therefore, the cycles of cats consist of five instead of four phases, and these phases are proestrus, oestrus, inter-oestrus, dioestrus and anoestrus. During the different phases of the oestrus cycle, there are changes in sexual behaviours, hormone concentrations and structural changes in vaginal epithelial cells (Ng et al., 2023). Procestrus is the first phase during which queens attract toms but are not sexually receptive to them. During this stage, ovarian follicular development and oestradiol synthesis are rapid. Oestrus (follicular phase) is the period of sexual receptivity that lasts 4–7 days on average, with a range of 1-21 days. During this time, serum oestradiol concentrations increase sharply (Griffin, 2001; Little, 2011) and the queen accepts to be mated. A distinct vulvar discharge may occur during oestrus; however, this may not be detected due to the cat's sanitary behaviour (da Silva et al., 2006). As cats are polyoestrous and do not ovulate after every oestrous phase, when mating and ovulation do not occur, inter-oestrus follows oestrus. In cycling queens, inter-oestrus is the period of sexual inactivity between waves of follicular function. During this time, serum oestrogen levels rapidly revert to baseline, and all mating behaviours cease (Griffin, 2001). Oestrous cycles occur at varying intervals, most commonly every 14–21 days (Little, 2011), but it is well-known that queens have a wide range of individual variances in cycle duration (Johnson, 2022).

A diagram of cat oestrus is represented in Figure 4.





Dioestrus happens if ovulation occurs after mating or after spontaneous ovulation. Within 24–48 h after ovulation, corpora lutea develop and begin secreting progesterone. If pregnancy occurs, corpora lutea last until kittening, if not corpora lutea regress within 30–50 days (on average 35 days) after ovulation. After kittening or regression of corpora lutea proestrus/oestrus will resume if daylength allows it. Anoestrus is the time of sexual inactivity in queens. During these periods the queens are not sexually attractive and are not receptive (Griffin, 2001).

4.2.2. Introduction part 2: skeletal and sexual maturity in queens

The rate at which a cat reaches reproductive maturity varies greatly between breeds and individuals (Kutzler, 2022). Puberty is the commencement of reproductive function, and it was suggested to coincide with the female's first oestrus and the male's creation of functional spermatozoa (Joyce and Yates, 2011). In other female domestic animals, the onset of puberty is usually associated with the onset of the first oestrus and the first ovulation. In cats, the first oestrus would be more appropriate to define the onset of puberty since they are induced ovulators (Kutzler, 2022). Puberty in queens, with acceptable physical and nutritional health, occurs between 4 and 12 months of age, at a weight that is $\sim 80\%$ of adult weight (2–3 kg or more depending on the breed) (Griffin, 2001; Johnson, 2022). In general, smaller breeds are more precocious than larger breeds, as they reach a weight that is $\sim 80\%$ of the adult weight earlier. Shorthair breeds, such as the Siamese and Burmese, mature faster and can reach puberty at lower body weight (Little, 2011). Overall, the age at which female cats reach puberty is influenced by several factors, including season (which impacts the length of daylight), and the queen's body conditions (Little, 2011).

A survey-based study (Jemmett and Evans, 1977) analysed data from 168 breeding queens and showed that puberty was reached on average at 9–10 months of age. Romagnoli et al. (2019) conducted a survey in a breeding establishment of Norwegian Forest Cats, Maine Coon, Persian and Bengal cats in Italy. They showed that the mean age at puberty was 12.3 \pm 7.4 months when all four breeds were combined and about 10 months in Bengal, Maine Coon and Norwegian Forest Cat queens, and 16.8 months in Persians. The authors concluded that there is a significant difference in the onset of puberty depending on the breed. These findings confirmed those previously obtained by Povey (1978) that reported an average age of queens at first oestrus of 10.3 months with significant differences between and within breeds: Persians at 12.1 [range: 6–12] months, Himalayans at 9.6 [4–16] months, Siameses at 9.3 [4–20] months, Burmeses at 9.2 [4–19] months and others short-haired queens at 9.3 [5–14] months.

A large study by Fournier et al. (2016) including 45 breeds and responses of 1,521 breeders investigated the age of first mating and reported a mean age at mating (\pm SD) of 2.7 \pm 1.6 years with

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a range from 4 months to 11.5 years (n = 7,699). For large-breed cats, the age at first litter reported was 24 months or more, depending on the season (Goericke-Peschand Packeiser, 2022). Overall, based on the literature, there is evidence supporting the fact that the onset of puberty depends strongly on the breed.

Other factors, such as the management and birth season, influence the onset of puberty (Griffin, 2001). Kittens born in the spring and experiencing 6–8 months of short daylength may not reach puberty until the following breeding season compared to kittens born in winter that may enter puberty at 4–5 months during late spring/summer (Johnson, 2022). Tsutsui et al. (2004) undertook a study to analyse the breeding season of cats (n = 125) kept at a constant room temperature and exposed to natural light and natural light:dark periods. They determined the interval between birth and the initial oestrus cycle. The time from birth to first oestrus ranged from 6 (180 days) to 18 months (540 days), with a mean of 345 ± 9 days. Kittens born between March and June (n = 67) entered oestrus between January and August the following year, whereas 58 kittens born between July and October exhibited oestrus the year after birth or 2 years after birth. The gap between entering oestrus in 33 kittens between January and August the year after birth ranged from 6 to 10 months, with a mean of 242.0 ± 6.3 days. The onset of the first oestrus was significantly shorter in cats born between March and June. This study confirmed the season of birth (spring versus autumn) strongly influence the age at which puberty was initiated.

However, it is important to highlight that there is a difference between puberty and sexual maturity, which is the age at which queens are mature and exhibit normal maternal behaviour (Ng et al., 2023). This will be discussed in the next section.

4.2.3. Introduction part 3: methods to assess sexual and skeletal maturity in queens

Normal queen fertility needs ovulation of normal ova into a patent, healthy reproductive tract, mating at an ideal time during heat and pregnancy maintenance for 2 months (Fontbonne, 2022).

Different studies identified the reproductive aspects of queens including ovarian morphology and blood flow in prepubertal and pubertal cats (Vercellini et al., 2018), the normal reproductive tract (Davidson and Baker, 2009), pregnancy diagnosis and characterisation (Davidson et al., 1986), follicular development and oocyte quality in pre-pubertal cats (Uchikura et al., 2010), changes that occur in ovary and uterus during oestrus and the early post-oestrus period (Vercellini et al., 2022). Identifying and understanding the pre-pubertal and pubertal changes that occur in the reproductive tracts may be helpful for breeders and owners of cats to decide when to start breeding their cats. Vercellini et al. (2018), performed ultrasonographic characterisation of domestic cat ovaries and concluded that cats achieved puberty at 31 ± 3.1 weeks of age weighing 2.4 \pm 0.1 kg. The mean ovarian longitudinal diameter significantly increased gradually towards puberty but no significantly towards puberty and the largest follicles were found at day of puberty of size 2.6 \pm 0.5 mm compared to size below 2 mm for earlier time points. This study suggests that ultrasonographic detection of developing follicles in female cats is possible.

A recent study by Lapuente et al. (2023) suggested that anti-Mullerian hormone (AMH) can provide an indirect, reliable marker for the assessment of ovarian follicle size and functionality. Overall, the mean AMH level for the whole female population in this study was 6.31 ± 0.54 ng/mL, but there was a significant variability in AHM levels in relation to pubertal development. Prepubertal females (< 12 m) had higher levels than in neonatal and post-pubertal animals. Furthermore, the age of pre and postpubertal females was negatively correlated with their own serum AMH concentrations (r = -0.5).

To be considered sexually mature, there is the need not only to have mature follicles on the ovary but also that the oocytes are mature enough to be fecundated. Therefore, Uchikura et al. (2010) assessed the follicular development and availability of sound ovarian oocytes for *in vitro* production (IVP) of embryos in pre-pubertal cats. The authors followed and examined the relationship between body weight and ovarian growth in 93 cats. The results revealed that ovarian weight rapidly increased until 100 days of estimated age.

According to this study, the oocytes derived from pre-pubertal cats with 100–120 days of age have in vitro developmental competence to progress to blastocysts however this does not indicate that the cats are mature for breeding, but gives important information on the fact that cats can be fertile from the first oestrus, as reported in the literature (Welsh et al., 2014). The study by Uchikura et al. (2010) confirmed the positive correlation between body weight and the weight of ovaries; female cats with higher body weight have significantly higher ovarian dimensions which present a higher percentage of oocytes in secondary follicles. Monitoring regularly the daily body weight gain and the growth curve of queens and measuring the dimensions of the ovary using ultrasound could therefore be used as tools to identify sexual maturity, although it is not very practical and would be stressful to the cats.

To identify skeletal maturity X-rays can be used. Miranda et al. (2020) conducted a study aimed at assessing the skeletal development of the pelvis and the femorotibial joints of the domestic cat from the first week of life until the closing of the growth plates. Results indicated that the greater trochanter and proximal fibular epiphysis growth plates close at 9.4 and 10.3 months, respectively. The distal femoral and proximal tibial growth plates fuse at 13.6 and 18.0 months, respectively. Females had shorter closure times for the iliopubic and ilio-ischial growth plates, as well as the distal femoral growth plate than toms. In this study, the closing time of the growth plates was shorter in females than in males and 18 months was suggested as the average age for reaching skeletal maturity. Based on the literature, it seems clear that queens can reach puberty and be fertile far before the onset of skeletal maturity.

4.2.4. Minimum breeding age and effects of breeding of queens before they reach skeletal and sexual maturity

Cats are considered prolific breeders and may give birth early in life. There is, however, poor knowledge of feline reproduction among owners, and this has led to several unwanted and accidental litters born when the queens were as young as 6 months (Welsh et al., 2014). In a study by Nutter et al. (2004), a group of 10 female cats born into controlled feral cat colonies were rigorously monitored to ascertain age at first parity. Nine of them gave birth at 1 year of age and one cat gave birth at 6 months of age. The breeding of cats should be done when the cat is fully grown (Goericke-Peschand Packeiser, 2022) as reproductive efficiency, health and welfare of the queens and the litters are closely interconnected. Therefore, measures linked to reproductive performance are often used to assess the overall health and welfare of a breeding queen (Nowak et al., 2020).

No studies were found investigating the direct relation between minimum breeding age and welfare parameters. The studies described here below, focussing on reproductive performance, are reported for information.

Reproductive performance measures include litter size and litter characteristics (i.e. the total number of animals born per litter, the number of born alive per litter, the number of stillborn per litter and the number of weaned animals per litter). In cats, reproductive performances were documented in three epidemiological studies (Johnstone, 1987; Ström Holst and Frössling, 2009; Fournier et al., 2016) and in two of them, possible associations between queens' age and reproductive performance were investigated.

Fournier et al. (2016) documented common reproductive management and average performance in cat breeding in France. Information was recorded voluntarily by 1,521 French breeders between 2011 and 2014 with a study population of 5,303 queens (belonging to 45 breeds) which gave birth to 28,065 kittens. Data coming from 9063 oestrous periods were recorded. Catteries were either small or did not practise intensive reproduction, as 71% (n = 1,076) of them registered only 1–5 oestrus cycles and 70% of breeders (n = 1,062) recorded five or fewer litters over the 4 years. Eighty percent of queens had less than two matings over this period and the mean age at mating was 2.7 \pm 1.6 years (ranging from 4 months to 11.5 years). Most matings (70.2%, n = 5,434/7,738) occurred during increasing day-length periods (winter and spring). Seventy-five percent (n = 4,779/6,321) of matings happened during the first 3 days of heat. The overall kitten mortality rate was 15.7%, with peaks reported in the Burmilla, Singapura and Highland Fold breeds. However, it is to be noted that this study focuses on management of breeding in breeding facilities, therefore not necessarily reflecting cat physiology.

Similarly, Ström Holst and Frössling (2009) performed a retrospective epidemiological study on the reproductive performances and diseases observed in cats from 265 Swedish cat breeders. The breeders had on average 6.1 ± 3.4 cats, of which 3.4 were intact female cats. The average age of the queen was 3.3 years, varying from 8 months of age to 10 years old. The mean number of litters per breeder was 2.8, ranging from 0 to 7, with a total of 694 litters considered. Most litters were born to queens aged between 1 and 3 years, and births were distributed all over the year, with the highest number of litters born from March to July. Very few litters were born from queens aged less than 1 year. Despite this, postnatal kitten mortality was significantly higher in these litters due to a significantly higher risk of post-partum dead kittens when compared to older females (incidence risk rate of 5.8, ranging from a lower limit of 1.7 to an upper limit of 20.2).

These results are an indication that having the first litter before the first year of age may lead to higher kitten mortality rates.

Together with litter mortality rate, also litter size showed to be associated with queens' age. Johnstone (1987) reported that litter size was smaller when queens were aged 1 year for all cat breeds, and it increased from 2 years of age onwards, up to 6 years when it started decreasing again.

4.2.5. Effects of frequent pregnancies on the health and welfare of queens

No studies were found on the direct effect of frequency of pregnancies on queen welfare parameters. Studies here below report the effects of frequency of pregnancy on reproductive parameters and are reported for information.

The reproductive capacity of free-roaming cats has been studied (Nutter et al., 2004), as well as the reproductive capability of cats in catteries (Jemmett and Evans, 1977; Povey, 1978). Nutter et al. (2004) investigated the reproductive capacity of naturally free-roaming cats and kitten survival rate. On average, the examined cats gave birth to 1.4 L/year, although two cats had three litters in a single year. In the two females who produced three L in a single year, at least one of the litters did have 100% mortality rate. The authors suggested that the production of multiple litters per year can be negatively associated with the survival of kittens and should be avoided.

Jemmett and Evans (1977) reported on reproductive activity in catteries obtained from a survey of 168 breeding queens. The interval between the birth of a litter and the next oestrus in 108 queens ranged from 1 to 21 weeks, but the average first oestrus followed by mating was 8 weeks after kittening. The authors reported that this was in line with the literature since queens tend to return to heat after kittening usually after 4 weeks. However, if queens would breed 4 weeks after kittening, pregnancy would occur while the cats were still lactating. The breeders examined by Jemmett and Evans (1977) tended to breed their cats 8 weeks after kittening, after having weaned the previous litter.

When considering frequency of pregnancies, a note should be made about lactating cats. Lactation and suckling can suppress fertility in most mammals, but some queens may show spontaneous oestrus during lactation. This is why the prevalence of lactational oestrus and its consequences for kittens has been studied and should be taken into account to decide the intrapartum period. Schmidt et al. (1983) studied the sexual behaviour of eight adults (3–5 years of age) gueens (2.5–3.7 kg) to investigate the oestrous-pregnancy interval, parturition interval, lactation-weaning interval and post-partum oestrous interval. The duration of pregnancy was 64 days, on average three kittens were born per litter and no queen demonstrated oestrous behaviour during the 6-week lactation period. The interval from weaning to the post-partum oestrous was 2 weeks in seven out of eight cats. The latter results are in disagreement with a recent study performed by Furthner (2023), showing that almost half of gueens (n = 108 representing 195 births) came into oestrus during lactation. The first onset of oestrus in queens with lactational oestrus, in queens without lactational oestrus and in queens altogether was on average 40 \pm 20 days (range 2–86), 122 \pm 60 days (range 62–271) and 71 \pm 56 days (range 2–271) after birth, respectively. Four queens showed lactational oestrus as early as the first week after parturition, 12 queens during the first 2 weeks and 20 in the first 3 weeks. In this study, queen age and breed were not associated with the onset of lactational oestrus. Small litter size and births between February and April were associated with the onset of oestrus during lactation. In 38% of cases of lactational oestrous, a negative outcome was reported, including variations in milk quality, loss of maternal motivation, reduced general condition of kittens, diarrhoea, early weaning and mortality. This study suggests that kitten welfare benefits from the prevention of mating during lactation.

The Cat breeding associations usually suggest a maximum of three litters per queen over a 2-year period (Goericke-Pesch and Packeiser, 2022). A common recommendation given by the Governing Council of the Cat Fancy (GCCF) is to respect at least 26 weeks between two kittenings for young breeding queens and 12 months for multiparous queens (more than three litters) (Goericke-Pesch and Packeiser, 2022).

When determining the timing for the next mating, previous litter size, body condition and the health status of the reproduction systems must also be checked (Goericke-Pesch and Packeiser, 2022). In a review by Fontbonne (2022), it is recommended that before deciding whether a queen should be mated again a thorough examination should be performed by examining the vaginal discharge. The technique indeed helps in identifying whether the queen is cycling or not, and if there is an inflammation of the reproductive tract. It is also very helpful to determine the stage of the cycle (oestrus, inter-oestrus, anoestrus).

Several postpartum complications could occur in queens after delivery, including, mastitis, metritis, retained placenta, eclampsia or post parturient hypocalcaemia. The latter is most typically recorded in queens who have had prior litters and have to nurse a big litter (Little, 2011). Moreover, changes in ovarian and uterine morphology, such as ovarian cysts and postpartum metritis can also occur, and this is why detailed evaluations of the female cat reproductive tract after delivery and onset of a new breeding cycle have been recommended (Davidson and Baker, 2009; Fulton, 2021).

4.2.6. Mature age in queens

Queens' age is a major factor affecting litter size and kittens' survival rate. After the age of 6 years, the queens included in the Johnstone's study (1987) showed indeed a deterioration in reproductive performance, with litter sizes decreasing. This was confirmed by the study of Ström Holst and Frössling (2009) where the average overall mortality rate ranged from 15 to 30% depending on the breed, the mean number of litters per breeder was 2.8, and the average age of the queen was 3.3 years, varying from 0.7 (around 8 months of age) to 10 years old. Even if most litters were born from queens aged between 1 and 3 years, the queen's age was significantly associated with the rate of stillborn kittens, especially for queens aged more than 5 years. Not only the rate of stillborn kittens increases after 5 years of age, but the average litter size also decreases. The most drastic decrease in liveborn kittens was, however, observed after the age of 8 years, when the rate of stillborn kittens reached almost 30%.

The association between litter size reduction and older age could be linked to variation in the conformation of the uterus in older queens, as it happens in bitches older than 5–6 years as suggested by Borge et al. (2011) and Thomassen et al. (2006).

Increased age is also associated with greater risks of health problems in queens. Among those, pyometra occurrence is more common in middle-aged and older cats. Pyometra is a uterine infection caused by either hormonal or bacterial factors which leads to loss of breeding potential and, in the most severe cases, can be a life-threatening condition (Hollinshead and Krekeler, 2016; Holst, 2022). The first clinical signs of pyometra are present at 4 weeks after oestrus, during the luteal phase. In cats, pyometra is less common than in dogs, as queens are induced ovulators. However, some cases of spontaneous ovulation have also been reported and may be more common in queens than originally thought. Pyometra was found to be more common in queen of 5 to 7 years of age (queens aged 7.6 years on average), and in particular, some breeds seem to be more prone to develop this potentially life-threatening condition (i.e. Siberian, Ocicat, Korat, Siamese, Ragdoll, Maine Coon, Burmese, Birman and Bengal) and this is why it is suggested to stop breeding at that age (Hollinshead and Krekeler, 2016).

Pyometra and other disorders of the reproductive tract can be easily identified using ultrasonography (Gatel et al., 2020). Ultrasound examination is even more recommended in case of elderly queens before deciding whether to retire or breeding again.

4.2.7. EFSA judgement of mandate statements related to reproduction in cats

The outcomes of the judgement of the mandate statements are reported in Table 13.

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	 There is scientific evidence to support that, from an animal welfare perspective: breeding of queens before they reach skeletal and sexual maturity should be avoided 	Overall statement if supported or not from literature From the scoping literature search, there is evidence that breeding of queens before they reach maturity should be avoided. <u>Reasoning</u> Some papers support that for cats it is important to wait for sexual and skeletal maturity for initiating breeding (Goericke-Pesch	Queens should not be bred before skeletal maturity. Skeletal maturity is breed-specific and should be checked before breeding is initiated.

Table 13: EFSA judgement of mandate statements related to access to reproduction in cats with examples of the retrieved evidence



#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature	Overall recommendations
		search and/or other sources?	on this statement
		and Packeiser, 2022; Johnstone, 1987; Ström and Frössling, 2009).	
		Sexual maturity is not only the physical maturity (puberty) but also the mental maturity (the ability of cat to perform maternal behaviour). Puberty is the moment of the onset of the first oestrus.	
		Studies were found in stray cats demonstrating that they are not mature enough to take care of their litter if they breed when still young (< 1 year) (Nutter et al., 2004).	
		One additional paper than those considered in this report exists (Dutch paper provided by the experts: De Moor et al., 2019a,b), showing a higher number (but not significantly) of C-sections in young cats (0–1 year age) than in older females.	
		<u>Judgement</u> EFSA considers that queens should not be bred before skeletal maturity. This is because puberty may come before skeletal maturity and animals should not be bred before they are fully grown (the energetic demand for a pregnancy would impact the growth of the mother).	
		There is a large diversity in the timing of puberty in cats, varying from 4 to 18 months. This variation is affected by breed, but there is also variation within breeds.	
2	There is scientific evidence to support that, from an animal welfare perspective: - the minimum breeding age	Overall statement if supported or not from literature From the scoping literature search, no studies were found about minimum breeding age for cats in relation to welfare.	Further research is needed to suggest a minimum breeding age for queens.
	for queens is 12 months	Reasoning However, there is scientific evidence about optimal delivery ages, for instance 18 months and 24 months for larger breeds (Goericke- Pesch and Packeiser, 2022).	
		Provisions from national legislations exist, for example:	
		 Sweden: minimum age is 10 months (The Swedish Board of Agriculture's regulations and general advice for keeping cats and dogs^(a)). 	
		<u>Judgement</u> From the retrieved literature, EFSA considers that there is no evidence to suggest a minimum breeding age of 12 months.	



		EFSA Judgement	
#	Statement	Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
3	 There is scientific evidence to support that, from an animal welfare perspective: controlling the frequency of pregnancies have beneficial impact on health and welfare of queens by preventing physical exhaustion 	Overall statement if supported or not from literature From the scoping literature search, no studies were found to support that queens will get	Frequency of pregnancy should be controlled. To prevent exhaustion, physical state of the queens should be checked before breeding through assessment of general health status and body
		and poor maternal behaviour (Schmidt et al., 1983; Furthner, 2023) <u>Judgement</u> Although no evidence was found that queens might get physically exhausted, controlling frequency of pregnancy is important for queen welfare.	Mating of lactating cats should be prevented.
4	 There is scientific evidence to support that, from an animal welfare perspective: a minimum of 12 months is needed between two kittenings 	Overall statement if supported or not from <u>literature</u> From the scoping literature search, no studies were found supporting the minimum period of 12 months. <u>Reasoning</u> Some provisions from national legislations exist, for example: The Netherlands: 'A cat shall have a maximum of 2 litters in a consecutive period of 12 months. Or a maximum of 3 litters in 24 months (Dutch Animal Welfare Act ^(b))'.	Further research is needed to suggest a minimum period of time between two kittenings.
	These is esignific evidence to	Judgement From the retrieved literature there is no evidence to suggest a minimum period of 12 months between two kittenings. However, legislative provisions recommend that queens should have a maximum of 3 litters in 2 years.	The mature age should
5	There is scientific evidence to support that, from an animal welfare perspective: - the mature age is a relevant element of welfare in older queens	Overall statement if supported or not from <u>literature</u> From the scoping literature search, evidence was retrieved to suggest that mature age is a relevant element of welfare in queens <u>Reasoning</u> For breeding cats of more than 6 years, a check is needed because of risk of pyometra and stillbirths (Ström and Frössling, 2009; Hollinshead and Krekeler, 2016; Gatel et al., 2020; Holst, 2022). With age the rate of C-sections increases and the occurrence of C-sections is a welfare issue (Dutch paper provided by the experts: De	The mature age should be considered as a relevant element to ensure good welfare in queens. After the age of 6, queens should be checked by a veterinarian for general health status and body condition score.
		Moor et al., 2019a,b). Mature age is depending on the breed so it is very difficult to fix a threshold.	

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
		<u>Judgement</u> EFSA considers that the mature age is a relevant element to ensure good welfare in queens.	
		After the age of 6 years, queens should be checked by a veterinarian before breeding, as commonly done in the commercial practice.	
		Although cats have longer lifespan than dogs, they are also more prolific. Therefore, after the age of 6, queens will have already had several litters (more than bitches).	

(a): https://jordbruksverket.se/languages/english/swedish-board-of-agriculture/animals(b): https://www.rvo.nl/onderwerpen/dieren-houden-verkopen-verzorgen/wet-dieren

4.3. Reproduction in dogs

4.3.1. Introduction part 1: reproductive physiology in dogs

Canids are monoestrous breeders (Concannon, 2011), typically non-seasonal as their reproductive cycle is not limited to a certain time in the year (England and Russo, 2006; Concannon, 2011). However, seasonality in canine breeds is still a matter of debate (Bouchard et al., 1991; Kustritz, 2012; Wigham et al., 2017) and to date, the only dog breed for which a seasonal pattern in oestral cycles has been scientifically proven is the Basenji breed, which shows oestral cycles exclusively in autumn (Fuller, 1956).

Bitches are polytocous, spontaneous ovulators and the canine cycle is classically divided into four phases: proestrus, oestrus, metoestrus (or dioestrus) and anoestrus (Concannon, 2011). Besides hormonal changes, bitches experience also physiological and behavioural changes during the different phases of oestrus cycle (Concannon, 2011). During proestrus, vulval swelling is visible and is usually accompanied by serosanguinous vulval discharge. Proestrus typically lasts 9 days (ranging from 5 to 20 days, due to individual, size and breed effects), during which vulval turgor and vaginal secretions of pheromones attracting males increase. Despite the bitch attracting males, during proestrus she refuses to be mounted. Normally, as proestrus progresses, the bitch passes from aggressive refusals of the male attempts to mount, to ambivalent and playful responses to mounting males. Passing from proestrus to oestrus, the bitch's behaviour is characterised by proactive receptivity to mounting males and increased male-seeking behaviour. Oestrus lasts a variable length of time, between 5 and 15 days and the anatomical signs may then wane slowly or rapidly at 5–10 days from its occurrence. From an anatomical point of view, oestrus is reflected in the initial wrinkling and crenulation of the vaginal mucosa 1 day from the luteinising hormone (LH) peak. Metoestrus is the first portion of the luteal phase and starts when oestrus behaviour ceases and ovulation occurs. Corpora lutea produce progesterone and the luteal phase (i.e. metoestrus or dioestrus) lasts typically 64 ± 1 days similar in length to the duration of pregnancy (i.e. 65 ± 1 days). During metoestrus, progesterone induces changes in the uterus to prepare a suitable environment for early embryo development. Those changes include endometrial proliferation, increased uterine glandular secretions and decreased myometrial contractions, as well as a temporary drop in uterine cellular immune defences. All these progesterone-driven changes are factors predisposing to the occurrence of pyometra which is a potentially fatal uterine infection caused by either hormonal or bacterial factors (Dow, 1959). Pyometra is a common infection in bitches, with around 24% of non-neutered females experiencing it by 10 years of age (Egenvall et al., 2001). Another potential risk factor for pyometra is pseudopregnancy (also referred to as phantom pregnancy, false pregnancy or pseudocyesis), which is another pathology linked to the luteal phase of the oestrus cycle (Fidler et al., 1966). During pseudopregnancy, bitches show behavioural and clinical signs typical of the peri and post-partum period of pregnancy, despite not being pregnant. The exact aetiology of this condition is still unknown but it is reported as being linked to the exposure and decline of plasma progesterone during metoestrus, high-plasma

concentrations of prolactin, increased tissue sensitivity to prolactin or the existence of prolactin molecular variants with varying bioactivities (Root et al., 2018). The regulation of progesterone in dogs is highly dependent on prolactin. Indeed, while in other species the regulation of the cyclic corpus luteum is influenced by luteolytic factors, such as Prostaglandin F2a produced from the endometrium, this is not present in dogs. In bitches, the first stage of the luteal phase is completely autonomous, while the second depends on pituitary factors, mainly prolactin. This is why the regulation of the oestrus in bitches is considered unique among animal species (Butinar et al., 2004).

If neither pseudopregnancy nor pyometra has occurred, the metoestrous is followed by the anoestrus. However, the end of metoestrus and the onset of anoestrus (late metoestrus/early anoestrus) are difficult to define and variable. Some consider this passage as when uterine endometrium has undergone histological 'repair', when mammary enlargement in response to luteal phase progesterone recedes or when serum progesterone declines to levels persistently below 1 or 2 ng/mL (Concannon, 2011). Usually, the anoestrus is considered the phase of the rest of the ovarian activity between two oestrous cycles associated with uterine regeneration and endocrine preparation of the following oestrous cycle (Wanke et al., 2006). Aoestrus lasts 2-10 months, and when it is reduced to a few months (e.g. 2 months), it can lead to breeding failures (Wanke et al., 2006) due to the insufficient time for the uterus to recover from the previous heat. The interval between two consequent oestrus cycles is referred to as interoestrus interval, and as canine anoestrus is highly variable, also this period may have large variations among bitches. As a rule, the typical intercestrus interval varies between 5 and 12 months, with an average of 6-7 months (Bouchard et al., 1991; Concannon, 2011). Interoestrus intervals shorter than 4 months or longer than 11 months have been associated with infertility and subfertility (Bouchard et al., 1991). Considerable differences were found between the interoestrus interval of different breeds, for instance, German Shepherd, Basset Hound and Cocker Spaniel bitches have an interoestrous interval significantly shorter than Toy Poodle, Pekingese, Boston Terrier and Beagle bitches (Sokolowski et al., 1977; Linde-Forsberg and Wallén, 1992). However, interoestrus intervals of a single bitch are highly variable suggesting that several factors affect the interval between two oestrus cycles (Bouchard et al., 1991). Overall, the duration and the occurrence of the oestrus cycles in the canine species is highly variable due to different effects of the size, the breed but also the individual. It remains therefore poorly understood and studies are needed on the endogenous cycle in affecting the duration of anoestrus and the 6-12 month interoestrus intervals (Concannon, 2011).

4.3.2. Introduction part 2: skeletal and sexual maturity in bitches

Puberty is defined by Gobello (2014) as the process of physical changes by which an animal matures into an adult capable of sexual reproduction. However, the exact definition of puberty may be puzzling in some species. Only a few fundamental studies in literature have evaluated the factors that influence puberty attainment in domestic canids. In bitches, because of their important physical manifestations, the onset of the first proestrus is generally considered puberty. However, the actual situation is not so simple, since pubertal bitches are likely to manifest split or silent heat the first time they cycle. Split heat is defined as an abnormally short duration of proestrus or oestrus, accompanied by a low level of progesterone, due to lack of ovulation, followed by a normal ovulatory oestrous cycle after only 1–2 months (Meyers-Wallen, 2007). Consequently, the onset of puberty is still a matter of debate and Gobello (2014) listed factors that should be considered for defining puberty or sexual maturity and that may happen in different periods of life: the first follicular phase, as shown by physical signs or vaginal cytology; the first time the bitch permits mating; the first time she has a biphasic oestrous cycle, as evidenced by ovulation; the first time she has a normal luteal phase; or, finally, the first time she becomes pregnant.

The developmental process that leads to puberty and the next step – that is sexual maturity – is based on an extraordinarily complex series of inter-related intrinsic and extrinsic factors (Ojeda and Skinner, 2006). Pubertal oestrus usually occurs at 6–14 months of age in most breeds, depending on the size of the breed and the breed itself. Indeed, body weight is a major parameter affecting puberty onset, and consequently sexual maturity. Body weight itself is dependent on several variables such as the genotype, body condition score, season of the year, social cues and diet. Each of these factors can contribute differently to the final attainment of sexual maturity. Small and miniature breeds of dogs, which have steep growth curves, normally reach puberty at the age of 5–6 months (Gobello, 2014). Conversely, large and giant dog breeds are still 'large-sized puppies' at that age, attaining puberty only after 18 months. Differences among breeds occur and may be due to different development of their

endocrinological system. For example, German Sheperd female dogs show the first heat much earlier than Greyhounds probably because they are exposed to endogenous oestrogens earlier than Greyhounds (Gustafsson et al., 1975). The first oestrus period in medium-sized dog breeds occurs at about 1 year of age; for example, Beagle females have the first oestrus at 10–14 months (Andersen, 1965). There is consequently a great variability related to the onset of sexual maturity in dogs.

Sexual and skeletal maturity are not concurrent in all mammals. A comprehensive review by Kilborn et al. (2002) compared the timing of growth plate closure and sexual maturity in different laboratory animals, including dogs. The synchronisation between skeletal maturity and sexual maturity was expressed by Kilborn et al. (2002) with a ratio between growth plate closure and sexual maturity (Growth Plate closure age to Sexual Maturity age ratio = GP: $SM \times 100$) (Kilborn et al., 2002). Rodents have for example a ratio of 5 (considering male rats and their age at growth plate closure in femurs), suggesting that bone growth is present during the largest proportion of life expectancy. In contrast, dogs were given a ratio of around 1, as for the authors these animals are like incredibly young adults at the time of physis closure, with synchrony between sexual maturity and age at cessation of bone growth (Kilborn et al., 2002). During growth, the metaphyses of the long bones remodel continuously to create a mature skeleton. Although most mammals are assumed to experience growth-plate closure and cessation of bone growth soon after puberty (Kilborn et al., 2002), the cessation of growth is secondary to epiphyseal closure – through the action of the sex steroids, and primarily oestradiol in both sexes (Lee and Witchel, 1997; Frank, 2003). Therefore, the height at the withers and other skeletal morphometrical measurements can be considered markers of sexual maturity as their growth is also influenced by sexual hormones. Sex-steroid stimuli hasten skeletal maturity, thus determining adult height. This is why often adult height is considered as a proxy of puberty (Mukasa-Mugerwa & Ezaz, 1992), and it is considered that an animal is sexually mature when it has reached \sim 80% of the adult height (Johnson, 2022).

Abundant work has also shown that puberty is more closely related to body weight (assuming a normal dietary intake) than age or height in domestic animals (Kilborn et al., 2002). As a provisional figure, a mean of 70–72% and 81% of the mature body weight was calculated for the onset of puberty in Labrador and Beagle dogs (England and Russo, 2006; Taha et al., 1981). Differences in growth patterns, and therefore sexual maturity, however, exist among small, medium and large breeds (Hawthorne et al., 2004; Menchetti et al., 2020). It was shown that medium and large dog breeds reached 80% of the adult weight at 10–12 months, while small-sized breeds reached 80% of their final weight at about 7 months of age (Hawthorne et al., 2004). Consistently, in another study, puberty occurs at about 6 months of age in smaller-sized breeds, at about 10 months in medium-large breeds (Helmink et al., 2000), and at 24 months of age in giant breeds (England and Russo, 2006).

4.3.3. Introduction part 3: methods to assess sexual and skeletal maturity in bitches

As skeletal and sexual maturity are synchronised in dogs X-rays to evaluate the growth plate closure in dogs may be a viable tool also to evaluate the onset of sexual and skeletal maturity. The latest bones completing growth plate closure are radius and femur (Sumner-Smith, 1966). In previous studies, the completion of the epiphyseal fusion at the level of proximal femur and distal radius occurred between 6 and 9 months of age depending on the canine breed (Oviawe et al., 2018; Sumner-Smith, 1966). Canine breeds have, however, highly variable progression of skeletal development due to their morphological variability. A recent study performed by Roccaro et al. (2021) demonstrated that even breeds of the same size and morphotype displayed different progressions of the limb ossification centres over time. These authors stated that there is a need for further research on the exact time windows of ossification centres and growth plate closure in the different canine breeds as the majority of the information was mainly obtained from old studies performed on small samples of German Shepherds, Greyhounds and Beagles. Despite these shortcomings, radiographic images proved to be a useful tool to check the closure of the growth plates in limb long bones (such as the radius and femur) and can be used as a tool to determine whether a dog has reached skeletal maturity.

Assessing hormonal shifts in serum plasma may also be useful to evaluate skeletal maturity in bitches. A marker of skeletal maturity is the level of alkaline phosphatase (ALP) isoenzymes. Three ALP isoenzymes, liver ALP, bone ALP (BALP) and corticosteroid-induced ALP are present in dogs (Hoffman et al., 2019; Mahaffey & Lago, 1991). Allen et al. (2000) reported that BALP provided the main

contribution to the total ALP activity in dogs less than 1 year of age. Trangerund et al. (2007) documented the growth patterns of four large dog breeds (Newfoundland, Labrador Retriever, Leonberger and Irish wolfhound) found that valid growth-related clinical variables were the following: (1) total serum alkaline phosphatase (ALP), provided the main contribution to the total ALP activity in dogs less than 1 year of age; (2) the circumference of the distal radius and ulna (CDRU) reached maximum size at 6 months of age while in skeletally immature animals, the activity of BALP were still different, and the age of the dogs was the major explanatory variable of the change in total ALP concentration during growth. The study concluded on the importance of estimating the concentration of BALP at different ages during growth and that total ALP resulted inversely related with age. In addition, the differences observed among breeds diminished with increasing age, while no significant effect of breed and sex were found., Skeletal maturity in dogs seems to be successfully assessed using radiography or the determination of BALP level.

Skeletal maturity and body weight are associated with the development and weight increase of ovaries and, consequently, with sexual maturity. Ovarian weight was indeed demonstrated to change with the progression of age and the increase in body weight in dogs (Stott et al., 1971).

Doppler evaluation of the uterine and ovarian blood flow may also be of interest to test whether the bitch has reached sexual maturity. During oestrous cycle, uterine arteries show changes in the blood flow, passing from only systolic peaks during anoestrus to biphasic with a systolic peak followed by a small continuous flow diastolic peak in proestrus (Aires et al., 2021). Doppler velocimetric parameters from uterine and ovarian arteries were found to strongly vary according to the number of parities and pregnancies, the breed and size and the clinical history of the animal (Aires et al., 2021; Freitas et al., 2017).

Hormonal concentrations (oestradiol-17/3, LH or progesterone) can also be used to assess sexual maturity. Wildt et al. linked behavioural, ovarian and endocrine profiles in seven bitches at first oestrus and compared the results with previous data obtained on multiparous females (Wildt et al., 1981). They showed a difference between the level of oestradiol-17/3, LH and progesterone between the first and the second oestrous cycle. In particular, the levels were higher during the second cycle over concentrations observed at the pubertal oestrus. Aberrant results observed in young females may have been due to incomplete developmental processes that normally occur during the ontogeny of the adult cycle. Reproductive performance is often reduced in animal breeds at the pubertal oestrus. Consequently, many commercial breeders recommend that the female should be allowed to experience at least one oestrous period before mating (Wildt et al., 1981).

Ovarian activity and consequently sexual maturity can be assessed also determining Anti-Müllerian hormone (AMH) plasma concentrations (Hill et al., 2018). This hormone is a glycoprotein belonging to the transforming growth factor- β superfamily, and in females is secreted by granulosa cells of small growing follicles. In female mammals, AMH is produced and released in the circulating blood after puberty and has the key role of preventing the recruitment of primordial follicles into the pool of growing follicles. As this hormone is directly related to the ovarian follicular reserve, AMH concentrations have been associated with fertility, and its concentrations are higher during puberty and in highly fertile females. A study by Hill et al. (2018) significantly different levels of AMH in prepubertal and spayed females vs. pubertal bitches.

4.3.4. Minimum breeding age and effects of breeding of bitches before they reach skeletal and sexual maturity

A strong nexus exists between overall health status in animals and reproductive efficiency. Several studies exist assessing measures linked to reproductive performance (e.g. litter size, mortality rates) in relation to the overall health and welfare of bitches. Studies were found, for example, about the incidence of health problems experienced by bitches mated at different ages.

Depending on the breeds, bitches as young as 6 months of age may already become pregnant but with negative outcomes (Dejneka et al., 2020). A recent retrospective study by Dejneka et al. (2020) evaluated 914 cases of canine dystocia reported at Wrocaw University between 1981 and 2019. Among them, 79 cases that originated from unwanted matings were further investigated to find possible risk factors for dystocia. The age of the bitch and the parity order were strongly associated with dystocic births, as 14 out of the 76 cases (18.4%) came from bitches younger than 12 months. The authors suggested that these bitches were too young to be mated, as they could have developed dystocia due to fetomaternal disproportion. The increased risk of fetomaternal disproportion in juvenile bitches was also pointed out in the Handbook of Veterinary Obstetrics by Jackson (2004).

In addition to health problems and issues encountered during parturition, several studies have addressed and compared reproductive performance in bitches of different ages (Andersen, 1965; Mandigers et al., 1994; Mugnier et al., 2020; Strasser & Schumacher, 1968; Tønnessen et al., 2012). Decreased litter sizes and impaired puppies' survival rates were noticed in Beagle bitches younger than 18 months at delivery (Andersen, 1965). A study performed on two colonies of Beagles evaluated the reproductive performances of Beagles kept in two colonies that were mated at different ages for a total of six parities with variable interpartum periods (500 days for Colony I and 200 days for Colony II) (Andersen, 1965), Beagle bitches belonging to Colony I had their first parity at 750 days of age (2 years) and had six parities up to the age of 3,250 days (almost 9 years). Beagle bitches in Colony II also had six parities, but their breeding career started at 400 days (about 14 months) of age and lasted up to 1,400 days (almost 4 years). Bitches from Colony II that gave birth at 14 months of age, had the lowest number of puppies weaned per dam in 100 days, with a survival rate per dam/ 100 days of 0.4 pups. Bitches in Colony I first mated at 2 years of age, showed a number of puppies weaned per dam in 100 days of 1.1 (Andersen, 1965). The huge difference in the number of puppies weaned between the two colonies indicates that breeding at 14 months in female Beagles should be avoided as it leads to small litters and high mortality rates.

Similarly, Tønnessen et al. (2012) showed that highest early neonatal mortality rates occurred in bitches delivering at an age less than 1 year. In another study, the negative effect related to the low age in primiparous bitches aged less than 1 was only observed in miniature and small breeds, while no effects of young ages were found in large and giant breeds (Borge et al., 2011). This may be explained by the fact that bitches belonging to large and giant breeds reach sexual maturity at higher ages and have larger uteruses and vaginal canals even at a young age, thus allowing for less problematic pregnancies and deliveries. In addition, it is to be noted that, in perspective, puppies from smaller breeds are larger for the mothers.

Overall, differences in the occurrence of sexual maturity exist among breeds. For small- and medium-sized breeds, the studies seem to suggest that females should have reached at least an age of 1 year of life before giving birth, as younger bitches may have increased risks of dystocic births and perinatal mortality rates in puppies. Larger or giant breeds should instead be mated at older ages, but more studies are needed to indicate thresholds for large and giant-sized breeds. The literature is in line with the minimum breeding age already established by some Kennel Clubs. For example, UK Kennel Club establishes that puppies born from bitches younger than 1 year at the time of parturition cannot be registered in the studbook ('The Kennel Club Assured Breeders Standard', 2019).

Overall, evidence was retrieved to suggest that breeding bitches should not be mated before they have reached skeletal maturity to safeguard their and their puppies' welfare.

4.3.5. Effects of frequent pregnancies on the health and welfare of bitches

Very few studies have been found for the direct effect of frequency of pregnancies on welfare.

Different interpartum periods were evaluated by Andersen (1965) (see Section 4.3.4). The reproductive efficiency and the survival rate of the puppies were not related to the different interpartum periods, while the main factor affecting the reproductive performances of the bitches was their age. Based on these results, therefore, a 7-month interpartum period does not seem to affect the health and welfare of the bitches and puppies in Beagles. Tonnessen et al. (2012) suggested in a cohort study interpartum periods of longer than 1 year. Overall, breeding practices in Northern Europe countries do not seem to be intensive. Instead, in the USA, back-to-back breeding seems to have become particularly popular in recent years among breeding practices. This breeding practice consists of having females mating at each successive heat without skipping heats after whelping, in order to obtain three or four successive births (Van Soom's Personal Communication), with an interpartum interval which varies among breeds based on the physiological inter-oestrus interval. Although at the moment the benefits and disadvantages of back-to-back breeding practice are not supported by scientific studies, dog breeders suggest that this practice could avoid the occurrence of pyometra resulting from the effect of progesterone during metoestrus in non-pregnant bitches (because progesterone is the pregnancy hormone preparing the uterus for the pregnancy and if pregnancy does not occur, the uterus will only be exposed to mucus and bacteria which will predispose to infection and pyometra). Indeed, pregnancies seem to have a protective action on the canine endometrium (Romagnoli, 2002). The exact reason for this protective effect is however unknown (Romagnoli, 2002).

During post-partum period, bitches may also have a greater risk of health problems, including postpartum haemorrhage, uterine prolapse, postpartum metritis/endometritis, pyometra, mastitis, eclampsia (hypocalcaemia/puerperal tetany) and subinvolution of the placental sites (Al-Bassam et al., 1981; Kempisty et al., 2013; Smith, 1986). Eclampsia is a serious condition that can be fatal if not treated properly (Pathan et al., 2011), caused by poor nutrition, insufficient albumin levels in the blood, excessive milk production and parathyroid gland dysfunction. The occurrence of eclampsia is higher during the first 3 weeks of lactation in small-sized dogs nursing larger litters (Pathan et al., 2011; Smith, 1986). Romaniuk et al. (2022) highlighted an increased incidence of intestinal parasitosis in bitches at 4 and 8 weeks post-partum (Romaniuk et al., 2022). The percentage of dams with intestinal parasites increased from 11% and 4% at 6- and 1-week prepartum, respectively, to 23% and 15% at 4 and 8 weeks postpartum. Therefore, the authors stated that bitches may experience impaired immune defences during postpartum and may have a greater risk of health problems. All health problems can impair the functionality of the reproductive tract and the breeding career of the bitches, affecting their welfare, and should therefore be prevented or early diagnosed and treated.

Some studies were found on how to determine the uterine conditions after a pregnancy. During pregnancy (63 days ± 1 days) and lactation (7 or 8 weeks) bitches need extra energy and an accurate feeding regime (Fontaine, 2012) to ensure their health and welfare and their normal cyclicity. In bitches, the complex endocrinological, physiological and environmental factors determining the onset of a new oestrous cycle and the termination of the long obligatory anoestrus (either due to pregnancy or not) are still not fully understood (Concannon, 2011). It seems that the return to heat after delivery is not affected by the pregnancy and the lactation, but it is more linked to the physiological interoestrous interval of the individual bitch (Conze et al., 2022) and the activation of the hypotalamicpituitary-adrenal axis (Concannon, 2011). There are indeed different pharmacological protocols to induce oestrus, such as dopamine agonists (anti-prolactinergic drugs like cabergoline and bromocriptine) and GnRH agonists (such as deslorelin). However, induction of heat is not easy to obtain and the longer the interval from the previous oestrus is, the better the expected results of the induction are (Maenhoudt et al., 2018). However, a healthy and regular bitch will return to heat based on her normal cyclicity and there is no need for induction. The best way to identify when the bitch is ready to be mated again could be done by vaginal cytology, vaginal endoscopy, ovarian and uterus ultrasound and hormonal assessment. Among those practices, vaginal cytology is considered the simplest and the most practical tool to identify the phase of the oestrous cycle and identify the presence of inflammatory cells in vaginal smears. In addition, the assessment of progesterone can be used to predict the timing of insemination and ovulation. (Romagnoli, 2014).

Accurate monitoring of the bitches during post-partum aiming at deciding whether the bitch can be mated again can be performed using ultrasonography or radiology (Pharr & Post, 1992). Al-Bassam et al. (1981) observed placental subinvolution at various intervals following parturition and others conducted ultrasonography to follow post-partum involution of the uterus (da Cunha Barbosa et al., 2013; Pharr & Post, 1992; Yeager & Concannon, 1990), and uterine abnormality in bitches (Melandri et al., 2019). Ultrasonography evaluation was performed starting week one until week five after birth, and after that, the examination of uterus was conducted between 1-to-3-week intervals until the uterus maintained fixed ultrasonography appearance. In this study, uterine involution was completed after the 15th week postpartum. By this period, the uterus horns appear hypoechoic, and have a similar appearance. da Cunha Barbosa et al. (2013) conducted an evaluation measuring the postpartum uterine involution in bitches using B-mode and Doppler ultrasonography. And found that the stratification of the uterine wall was most clearly observable within the 2nd week postpartum while after 4th week it was not possible to distinguish the walls. Overall, it may take up to 12 weeks to have a normal uterine involution in bitches (Al-Bassam et al., 1981), this is why it is better to monitor the reproductive tract of the bitches post-partum using ultrasonography than radiology.

Ultrasonography is helpful not only to evaluate uterine involution but also to detect any abnormalities (Fulton, 2021) such as cystic endometrial hyperplasia, uterine collections and uterine masses (1.3%). (Melandri et al., 2019). The authors concluded that it is crucial to perform ultrasonography examinations in breeding bitches after one-third of their life. Radiology is not specific like ultrasonography but can also help in diagnosis of reproductive pathology particularly conditions like pyometra. Finally, in order to decide whether the bitches can be mated again after pupping, evaluation of vaginal smear or vaginal cytology can also be helpful in identifying disorders in oestrous cycle and vaginal or uterine infections. When associated with other clinical signs and behavioural changes, the presence of neutrophils, lymphocytes, eosinophils, erythrocytes and bacteria in vaginal smears may be

indeed an indicator of vaginal and uterine infections or neoplastic tissues (Solano-Gallego & Masserdotti, 2016; Wright & Parry, 1989).

4.3.6. Mature age in bitches

In female animals, reproductive performance generally tends to decline with increasing age due to various physiological changes and factors. As female mammals age, their fertility typically decreases. This decline is primarily attributed to the ageing of the reproductive organs, particularly the ovaries. The ovaries contain a finite number of eggs, and as the animal gets older, the quality and quantity of these eggs diminish. This can result in reduced fertility and an increased likelihood of infertility or difficulties in conceiving. Ageing can also impact the production and regulation of reproductive hormones in female animals. Hormones such as oestrogen and progesterone play crucial roles in ovulation, pregnancy maintenance and overall reproductive health. As an animal ages, the production and balance of these hormones may become less efficient, leading to disruptions in the reproductive cycle and reduced fertility. Older female animals are more prone to developing reproductive disorders or conditions that can affect their reproductive performance (Dow, 1959; Egenvall et al., 2001; Gibson et al., 2013; Knauf et al., 2018; Niskanen & Thrusfield, 1998; Sasidharan et al., 2021). Examples include pyometra, cysts, tumours or structural abnormalities in the reproductive organs. These conditions can interfere with normal reproductive processes, impair fertility and increase the risk of complications during pregnancy.

Pyometra is a severe condition, potentially fatal and with a high incidence in non-neutered bitches. Consecutive oestrus cycles increase over time the risk of cystic endometrial hyperplasia, which predispose to the development of pyometra (Gibson et al., 2013; Kempisty et al., 2013). Age is, therefore, a key factor increasing the risk of pyometra, and this condition has been shown to predominantly affect middle-aged to older dogs, with a mean age at presentation ranging from 6.4 to 9.5 years (Dow, 1959; Egenvall et al., 2001; Gibson et al., 2013; Niskanen & Thrusfield, 1998). (Gibson et al., 2013) identified in a large crossectional study. A median age of 7.9 years found in pyometra cases, with most of the cases occurring between 5 and 10 years. Some breeds showed to be more prone to develop pyometra, with a significantly lower age at presentation, such as Dogue de Bordeaux (mean age 3.3 years) and Bullmastiffs (mean age 5.4 years). Accordingly, a study by Blendinger and Bostedt (Blendinger & Bostedt, 1991) reported that 70% of bitches affected by pyometra were aged 5–10 years. Similarly, there is an increased incidence of ovarian cysts in bitches aged over 6–8 years (Knauf et al., 2018; Sasidharan et al., 2021). It is therefore highly advisable for bitches over 5 years old to be checked more frequently by a veterinarian, especially if they belong to breeds at higher risk of developing pyometra and ovarian cysts.

The need for veterinary treatment and caesarean sections also increases with the bitches' age (Linde Forsberg and Persson, 2007), with older bitches showing more uterine inertia (Thomassen et al., 2006) and problems during parturition, such as dystocia and prolonged parturition (Münnich & Küchenmeister, 2009). In particular, dystocic births seem to be significantly more frequent in bitches aged more than 6 years, if they had not experienced parturition before (Thomassen et al., 2006). This increased risk of problematic births in primiparous older (> 6 years) bitches may be caused by increased uterine inertia. In agreement with the results reported by Thomassen et al. (2006), a study performed by Gavrilovic et al. (2008) is in agreement with this fact as this study showed that Drever bitches mated for the first time at 4 years of age or more had impaired reproductive performances when compared with younger bitches of the same breed.

Age is a risk factor for dystocic births even in cases of multiparous bitches. In the retrospective study on dystocia cases in bitches (Dejneka et al., 2020), it was shown that that apart from bitches younger than 12 months, older age of the bitch was a risk factor for dystocia. Among the 76 cases examined, 29 (29/76; 38.2%) were bitches aged 8 years or more. Bitches of that age often give birth to litters consisting of a single pup. Singleton puppy pregnancies are associated with increased risks of dystocic births and uterine inertia as singleton pupps tend to overgrow in the uterus due to the absence of siblings in the uterine horns. Differences however exist among breeds, with some genetic lines or breeds being more prone to have dystocic births even at at younger ages and for other reasons: the risk of dystocic births in Boxer increased with increasing age of the bitch from 4 years of age (Linde Forsberg and Persson, 2007). In another study comparing the prevalence of dystocic birth in guide dogs (Cornelius et al., 2019) of different breeds (Labrador Retrievers, German Shepherd Dogs, Golden retrievers and mixed breeds), increased risks of dystocic births were noticed for small-sized litters (< 5 pups) and for older bitches Curly-coated Retrievers and had a fivefold increased likelihood

of having dystocia births when compared with Golden Retrievers. The major risk factors for dystocia identified by the study of Cornelius et al. were maternal age and litter size (Cornelius et al., 2019). Overall, all studies document a higher risk of dystocia associated with the ageing of the bitches.

Ageing increases the likelihood of pregnancy losses in female animals. Older females may experience higher rates of miscarriages, stillbirths or early embryonic deaths due to a decline in the quality of their eggs or decreased uterine health (Thomassen et al., 2006). This can result in reduced litter sizes or decreased success rates in producing viable offspring, increasing the risk of puppy's perinatal mortality. Litter size is indeed dependent on maternal age, with bitches aged more than 6 years being more prone to deliver smaller sized litters (Borge et al., 2011). However, small litters are not a welfare problem for the bitch as such.

With age, the bitches have smaller litters and this may increase the risk of peripartum problems such as uterine inertia and dystocia (because in smaller litters puppies are bigger).

These results are supported by other studies (Mandigers et al., 1994; Schrack et al., 2017). Mandigers et al. (1994) showed in purebred Kooiker dogs that litter size decreases with the increase in maternal age: the mean age of the bitches was 3.96 ± 2.0 years, suggesting that most of the breeders mated their female dogs until they reached the age of ± 6 years. About 11% was mated more than four times, suggesting that mating bitches aged more than 6 years and with four or more parities is the minority of the target population in that study.

Schrack et al. (2017) studied the mortality rates and litter size (915 litters from 202 sires and 348 dams) in Entlebucher Mountain dogs and reported a mean age at parturition was 52.9 \pm 20.2 months (about 4 years), ranging from 21 to 146 months (from less than 2 years to 11 years); A negative correlation between the age of the bitch and the reproductive performances was observed as an increase in the age of the dam by 1 month resulted in a reduction of 0.01 puppies at birth (p < 0.001) and of 0.02 puppies at registration (p < 0.001).

Maternal age also affects the number of weaned puppies. Andersen (1965) showed a negative trend after 3 years of age in Beagle bitches. After 3 years of age the number of puppies weaned per dam started decreasing. Besides impaired reproductive performance there was increased mortality as three bitches died at 6 years of age (Andersen, 1965). A study by Strasser & Schumacher (1968) showed an impaired reproductive performance in Beagle bitches as of 5 years old.

Bitch's age and litter birth weight as well as survival rate of puppies are associated. Mugnier et al. (2020) showed that the age of the bitch at whelping a significant effect on the survival rate of lowbirth-weight litters, which was reduced when the maternal age was > 6 years and the litter had at least one stillborn puppy.

The cohort study by Tønnessen et al. (2012) reported the highest risk of stillbirth, early neonatal mortality and total perinatal mortality in litters with increasing age of the bitches (6 years or more). Gavrilovic et al. (2008) observed small sized litters if bitches were more than 5 years old.

As a consequence, the impaired reproductive performance in ageing bitches is linked to their lower fertility. Assessing AMH plasma concentration in ageing bitches may help predict the reproductive performances over the breeding career. A recent study on plasma AMH concentrations reported that the concentration of AMH decreases as bitches get older (Hollinshead et al., 2017). The authors therefore suggest that AMH could be used to select the bitches that are fitted for further breeding. However, it is not a practical method and not always reliable.

The literature cited above is in line with several kennel clubs that establish a maximum breeding age for bitches. Some kennel clubs do not accept to register puppies born from old bitches. For example, Swedish Kennel Club prohibit the use of Irish Wolfhound bitches aged more than 7 years for breeding (Urfer, 2009), or UK Kennel Club does not accept puppies born from bitches aged more than 8 years ('The Kennel Club Assured Breeders Standard', 2019).

4.3.7. EFSA judgement of mandate statements related to reproduction in dogs

The outcomes of the judgement of the mandate statements are reported in Table 14.

Table 14: EFSA Judgement of mandate statements related to access to reproduction in dogs with examples of the retrieved evidence

		EFSA Judgement	0
#	Statement	Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	 There is scientific evidence to support that, from an animal welfare perspective: breeding of bitches before they reach skeletal and sexual maturity should be avoided 	Overall statement if supported or not from literature From the scoping literature search, evidence was retrieved supporting that breeding of bitches before they reach maturity should be avoided. <u>Reasoning</u> Several papers (e.g. Andersen et al., 2021) reported more dystocia and loss of litters due to too young age (e.g. because of low quality maternal behaviour. Sexual maturity starts with hormonal release, inducing the closing of growth plates over some time. Some papers suggest checking for skeletal maturity through the assessment of closure of growth plates (e.g. in femur) which can be checked by X-rays.	Bitches should not be bred before skeletal maturity. Skeletal maturity is breed-specific and should be checked before breeding is initiated.
		Judgement EFSA considers that bitches should not be bred before skeletal maturity. This is because sexual maturity may come before skeletal maturity and animals should not be bred before they are fully grown (the energetic demand for a pregnancy would impact the growth of the mother).	
2	There is scientific evidence to support that, from an animal welfare perspective: - the minimum breeding age for bitches is 18 months	 Overall statement if supported or not from literature From the scoping literature search, no studies were found about 18 months as minimum breeding age for dogs. <u>Reasoning</u> There is evidence that bitch welfare is impaired when they are bred at the first oestrus (Andersen, 1965; Dejneka, 2020). However, there is no univocal age for all breeds (e.g. 18 months may be fine for chihuahuas and too early for large breeds). Normally, first oestrus occurs before 24 months of age (about 6 months of age in smaller-sized breeds, about 10 months in medium-large breeds, Helmink et al., 2000). However, larger breeds tend to have their first oestrus at an older age which can be even 24 months (England and Russo, 2006). In the latter case, it is not a problem to mate at the first oestrus. Provisions from national legislations exist, for example: Sweden: minimum age is 18 months (The Swedish Board of Agriculture's regulations and general advice for keeping cats and dogs^(a)) 	No univocal minimum age can be suggested, however for dogs of small breeds 18 months can be suggested (because they will be fully grown by that age) while for larger breeds the skeletal maturity is the pre-requisite for breeding and should be checked before. The first oestrus should be avoided, the bitch can be bred from second oestrus onwards.



#	Statement	EFSA Judgement Is this statement supported by the	Overall recommendations
		studies from the scoping literature search and/or other sources?	on this statement
		 Netherlands: minimum age is 18 months (Dutch Animal Welfare Act^(b)). Judgement 	
		EFSA considers that for dogs of small breeds 18 months can be suggested as minimum breeding age. For larger breeds it depends on the growth and is therefore not possible to indicate a minimum age. In general, breeding in the first oestrus should be avoided and the bitch can be bred from the second oestrus.	
3	There is scientific evidence to support that, from an animal welfare perspective: – controlling the frequency of	Overall statement if supported or not from literature From the scoping literature search, no studies were found to support that bitches will get physically exhausted from the frequency of	Frequency of pregnancy should be controlled. To prevent exhaustion, physical state of the bitch should be checked before breeding through assessment of general health and body condition score.
	pregnancies have beneficial impact on health and welfare of bitches by preventing physical exhaustion	pregnancies. <u>Reasoning</u> Additional information was provided by the experts: studies report that after occurrence of pyometra following an oestrus and following medical treatment, breeding immediately at the next oestrus is a preventive measure for pyometra (Jurka et al., 2010; Melandri et al., 2019).	
		<u>Judgement</u> EFSA considers that, although no evidence was found, controlling frequency of pregnancy is important for bitch welfare. Physical exhaustion is dependent on the physical state of the bitch, which should be checked before breeding through assessment of general health and body condition score.	
4	There is scientific evidence to support that, from an animal welfare perspective:	Overall statement if supported or not from literature From the scoping literature search, no studies	Further research is needed to suggest a minimum period of
	 a minimum of 12 months is needed between two whelpings 	 were found about minimum breeding age for dogs in relation to welfare. <u>Reasoning</u> Provisions from national legislations exist, for example: Sweden: maximum 2 L in 12 months followed by a rest period (The Swedish Board of Agriculture's regulations and general advice for keeping cats and dogs^(c)) The Netherlands: limit to one litter per 12 months (Dutch Animal Welfare Act^(d)) Belgium: maximum 3 litters in 2 years (Flemish Animal Welfare Council^(b)) Judgement From the retrieved literature, EFSA considers that there is no scientific evidence to suggest a minimum period of 12 months between two whelpings. However, legislative provisions 	


#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
		suggest bitches should have a maximum of 3 litters in 2 years, followed by a 1 year resting period.	
5	There is scientific evidence to support that, from an animal welfare perspective: - the mature age is a relevant element of welfare in older bicker	Overall statement if supported or not from literature From the scoping literature search, evidence was retrieved supporting that mature age is a relevant element of welfare in queens.	The mature age should be considered as a relevant element to ensure good welfare in bitches.
	in older bitches	<u>Reasoning</u> Age is a key factor increasing the risk of pyometra, and this condition has been shown to predominantly affect middle-aged to older dogs, with a mean age at presentation ranging from 6.4 to 9.5 years (Dow, 1959; Egenvall et al., 2001; Gibson et al., 2013; Niskanen and Thrusfield, 1998).	After the age of 8 the bitch should be checked by a veterinarian for general health status and body condition score before breeding.
		It is also demonstrated that older bitches show more uterine inertia (Thomassen et al., 2006) and problems during parturition, such as dystocia and prolonged parturition (Münnich & Küchenmeister, 2009).	
		<u>Judgement</u> EFSA considers that the mature age is a relevant element to ensure good welfare in bitches.	
	https://jordbr.kg.orket.co//apguages/opg	After the age of 8 years the bitch should be checked by a veterinarian before breeding, as commonly done in the commercial practice.	

(a): https://jordbruksverket.se/languages/english/swedish-board-of-agriculture/animals

(b): https://www.rvo.nl/onderwerpen/dieren-houden-verkopen-verzorgen/wet-dieren

(c): https://www.gov.ie/en/publication/fe3427-code-of-practice-for-sellers-of-pet-animals/#

(d): https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388895/ COPAnimalsFullPrint.pdf

5. Judgement 3: painful procedures

5.1. Question to address and guidelines from the EU AW platform

The question to be addressed is:

Do surgical interventions, such as ear cropping, tail docking, partial or complete digit amputation, and resection of vocal cords or folds, have detrimental effect on welfare of cats and dogs?

The recommendations from the EU AW Platform in relation to painful procedures were:

- 'Surgical mutilations, including declawing, of cats and kittens are not permitted unless it is deemed necessary and certified in writing by a veterinarian for medical purposes (FECAVA, 2004). Neutering of cats (and ear tipping as a neutering mark, where necessary) is the only exception'.
- 'Surgical mutilations (as defined in the national legislation), including debarking, tail docking and ear cropping, of dogs and puppies are not permitted. It is only allowed if it is deemed necessary and certified in writing by a veterinarian for medical purposes (FECAVA, 2004)'.

5.2. Convenience surgeries

5.2.1. Introduction

The term 'convenience surgery' is used to describe surgical interventions in pets that are not justified from a veterinary medical perspective (Quartarone et al., 2012). Convenience surgeries are also called 'cosmetic surgeries' and include e.g. declawing in cats and ear cropping, tail docking, debarking (removal or resection of vocal cords) which are performed in dogs. These are the ones that have been assessed in the following sections (see Table 15 and Section 1.2).

Currently, there is no specific EU legislation as such on convenience surgeries of dogs and cats, some provisions are reported in the European Convention for the Protection of pet animals (ETS 125), but the matter remains under the competence of MSs. Examples of national legislative provisions (also beyond the EU MSs) on the abovementioned convenience surgeries are reported in Appendix D.

There might be cases where certain surgeries (e.g. docking part of the tail) in pets are necessary to treat specific medical conditions (WSAVA, 2018); in these cases the procedures are not considered 'convenience surgeries'.

 Table 15:
 Convenience surgeries that have been assessed in detail with indication of the animal species

Convenience surgery	Dogs	Cats
Declawing (partial or complete digit amputation)		Х
Tail docking	Х	
Ear cropping	Х	
Debarking (resection of vocal cords or folds)	Х	

5.2.2. Declawing in cats

In cats, declawing also known as onychectomy involves the removal of the distal phalanx using a guillotine-type nail clipper, surgical blade or laser (Kogan et al., 2016).

5.2.2.1. Why is declawing performed?

Declawing is an elective surgical procedure requested by cat owners or advocated by veterinarians primarily to prevent property damage or injury to humans from scratching (Patronek, 2001). Scratching behaviour can fulfil different functions (a means of defence or to attack prey, or to avoid falling or slipping). In all cases the behaviour can cause injuries to humans involved and/or damage furniture (Landsberg, 1991). Unwanted scratching is a quite common behavioural problem in domestic cats to an extent where owners may decide to get rid of the animal (Houpt, 1991). Thus, declawing is considered a way to prevent unwanted scratching and its consequences.

5.2.2.2. Information on specific national legislation on declawing

Declawing is illegal in more than 25 countries (including the UK, EU MSs, Australia, New Zealand, Brazil and Israel), whereas it is instead still performed in North America (Sandøe et al., 2015). In Canada and in the USA, the procedure is practised, as it is considered as an alternative to abandonment or killing of the cats (Kogan et al., 2016; Ruch-Gallie et al., 2016).

Declawing may be requested by owners and be performed simultaneously with neutering (AVMA, 2016).

5.2.2.3. Scientific evidence on the welfare implications of declawing

Scratching is a natural behaviour of cats. Kittens begin to retract their claws at about 4 weeks of age, and scratching behaviour begins at about 35 days. Environmental or territorial scratching probably serves four different functions: claw conditioning, visual territorial marking, olfactory territorial marking and stretching for the front limbs. The motivation to perform this behaviour is so strong that kittens declawed shortly after birth and also declawed adult cats have been observed to perform sham-scratching. The procedure of declawing stops the unwanted consequences, but will also prevent cats from performing this highly motivated natural behaviour (Landsberg, 1991).

Short and long term welfare implications of declawing have been reviewed by Patronek (2001). One of the first short-term consequences was pain, which led to lameness, less willingness to use the paws

and a reduction in eating and play behaviour. However, this pain can be managed by provision of analgesics after the procedure. How the surgery is performed (blade vs laser) also seems to be associated with different complications, such as risk of bleeding, infection, swelling, limping, formation of abscesses and development of cystitis. Long-term consequences were also reported, as some lameness lasted even up to 4–6 months, in particular when declawing was performed with disarticulation amputation. Apart from severe lameness, signs of persistent stress and an increase in other behavioural problems were reported. Some cats were reported to start biting instead of scratching and defecation and urination in unwanted places were also common in declawed cats (Patronek, 2001).

Ruch-Gallie et al. (2016) conducted an online survey in the USA to estimate the proportion of veterinarians working with feline patients in private practices that eventually perform onychectomy and assess attitudes regarding this procedure. The questionnaires were distributed to 33,000 veterinarians and a total of 3,441 responses were collected. The majority (79%) of the veterinarians performed onychectomy. The surgery procedures varied among the respondents and most of them prescribed analgesia after surgery, also because 70% of the veterinarians reported that pain was present after the procedure. Numerous short-term and long-term complications were reported, with haemorrhage and pain being the most common early postoperative complications. Other reported complications that can occur later included claw regrowth, chronic draining tracts, radial nerve paralysis secondary to tourniquet use, infection, wound dehiscence or incomplete healing, protrusion or loss of the second phalanx, tissue necrosis from improper bandage placement, development of palmigrade stance and persistent lameness. Changes in behaviour, such as foot shaking, unwillingness to jump and a lack of hunting behaviour after the procedure, were reported and interpreted by the respondent veterinarians as signs of neuropathic pain.

Similarly, Kogan et al. (2016) investigated the perception of Ontario veterinarians towards the procedure. At the time of the study, declawing was allowed in Canada and 90% of pet veterinarians practised it regularly. Many veterinarians experienced that declawing involved a significant amount of pain, leading to concerns about appropriate anaesthesia and analgesia. Other concerns included the belief that declawed cats were more likely to exhibit other behavioural problems including aggression, biting and house soiling. Apart from pain after surgery, many veterinarians were worried due to the other complications which often were reported in the cats after the surgery.

The veterinarians' concerns were confirmed by Martell-Moran et al. (2018) who conducted a casecontrol study. They compared declawed cats vs. non declawed cats to examine possible associations between surgery and health and behavioural problems. Significant increases in the odds of signs of back pain (odds ratio [OR] 2.9; 95% confidence interval [CI]: 1.5–5.5), periuria/perichezia (OR 7.2; 95%CI: 3.6–14.3), biting (OR 4.5; 95%CI: 2.1–9.8) and barbering (OR 3.1; 95%CI: 1.1–8.8) occurred in declawed compared with control cats. Of the 137 declawed cats, 86 (63%) showed radiographic evidence of residual P3 fragments. The odds of back pain (OR 2.66; 95%CI: 1.1–6.4), periuria/ perichezia (OR 2.52; 95%CI: 1.2–5.3) and aggression (OR 8.9; 95%CI:1.2–69.1) were significantly increased in declawed cats with retained P3 fragments compared with those declawed cats without. Declawed cats remained at increased odds of biting (OR 3.0; 95%CI: 1.1–8.2) and unwanted habits of elimination (OR 4.0; 95%CI: 1.7–9.3) compared with controls. The authors concluded that declawing was associated with health and behavioural issues.

5.2.2.4. EFSA judgement of mandate statements related to declawing

The outcomes of the judgement of the mandate statements are reported in Table 16.

Table 16:	EFSA Judgement of mandate statements related to declawing in cats with examples of
	the retrieved evidence

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	There is scientific evidence to support that, from an animal welfare perspective:	Overall statement if supported or not from literature From the scoping literature search, scientific publications were found reporting that	Declawing in cats should not be performed, unless necessary for the
	 the surgical intervention of declawing has a detrimental effect on the welfare of cats. 	1 5	health of the animals.
		Reasoning Examples of these studies:	
		 Landsberg (1991), reported that the motivation to perform the behaviour is so strong that kittens declawed shortly after birth and many adult cats that are declawed still go through the scratching motions, performing sham-scratching. A case-control study by Martell-Moran et al. (2018) found increased odds of signs of back pain (odds ratio (OR) 2.9), periuria/perichezia (OR 7.2), biting (OR 4.5) and barbering (OR 3.06) occurred in declawed compared with control cats. 	
		Declawing is illegal in more than 25 countries (including UK, EU MSs, Australia, New Zealand, Brazil and Israel).	
		<u>Judgement</u> EFSA considers that declawing has a detrimental effect on the welfare of cats.	

5.2.3. Tail docking in dogs

Tail docking refers to the amputation of part or the whole tail of an animal (Bennett and Perini, 2003a,b).

The procedure is usually performed on young dogs, between three and 5 days of life, and generally using surgical amputation or a 'banding' method. For surgical amputation, with or without local anaesthetics, the puppy can be only restrained manually. The hair around the site of amputation may be clipped. Part or all of the tail is then removed using sharp scissors or a blade. One or more sutures may be applied. The banding method performed by the application of a tight rubber ring around the tail which serves to occlude blood vessels supplying tissue distal to the ring, resulting in ischaemia, necrosis and, later, loss of the tail (Bennett and Perini, 2003a,b).

5.2.3.1. Why is tail docking performed?

The issue of tail docking, including the reasons why it is performed and the consequences it has on the health and welfare of the dogs, have been extensively reviewed by different authors (Morton, 1992; Wansbrough, 1996; Bennett and Perini, 2003a,b; Mills et al., 2016a; Mellor, 2018).

Traditionally, tail docking has been a widespread practice among pure breed dogs. Evidence in support of this practice are very limited and 'prophylaxis' and 'tradition' are the two main reasons reported for tail-docking. Lederer et al. (2014) reported that tail docking may have a preventive role for tail injury in hunting dogs; however, according to Noonan et al. (1996) there is no scientific evidence supporting this theory. In addition, not all puppies of hunting breeds will become dogs used for hunting; as many of the puppies do not show the required attitudes for hunting and are kept as companion dogs. Docking is considered necessary to prevent the pain and discomfort associated with the possible adult tail damage (Bennett and Perini, 2003a,b), but this seems inconsistent across breeds

and was never supported by data; whereas, other characteristics, such as the angle of the tail, were reported to be associated with tail injuries (Diesel et al., 2010).

Some dog owners want to dock tails of their working dogs for hygienic reasons; but although the prevention of accumulation of faecal material is reported for sheep and cattle as an effect of tail docking, there is no evidence supporting a similar effect in dogs (Quartarone et al., 2012). Regarding this concern, Wansbrough (1996) stated that regular clipping of long-haired areas and 'feathers' keeps dogs clean.

Overall, the available evidence suggests that dogs are tail-docked mainly for human preference and cosmetic reasons (Morton, 1992) and tail docking does not bring direct benefit for the puppy. Mills et al. (2016a) reported that breeders are pro-docking in order to preserve tradition and out of fear that some puppies remain unsold if they are not tail-docked.

5.2.3.2. Information on specific national legislation on tail docking

Tail docking for cosmetic or convenience purposes is banned in several the EU MSs (for details, see Appendix D, Table D.1). However, exemptions exist and there seems to be large variation among countries (see Sinmez et al., 2017).

In the UK, cosmetic surgery is addressed in the Animal Welfare Act (2006), which bans ear cropping in dogs, but still allows tail docking in specific breeds (Packová et al., 2021) with differences among the states. In 2006, Scotland was the only state to introduce a ban of tail docking in all breeds, while it was still possible to tail-dock specific purebred dogs in England, Wales and Northern Ireland. In the same year, also the Royal College of Veterinary Surgeons opted to support a total ban on non-therapeutic docking of dogs' tails (RCVS, 2006).

Currently, tail docking is forbidden in all breeds in New South Wales, Australia and it is still allowed in all States in the USA (with some restrictions in some States; see also Mills et al., 2016a).

5.2.3.3. Scientific evidence on the welfare implications of tail docking

Cameron et al. (2014) studied dogs that had visited one of 16 veterinary practices located in Scotland between 2002 and early 2012 and the overall prevalence of any tail injury among dogs of all breeds was 0.59%. Working dog breeds were 1.7 times (95% CI 1.49-2.06) more likely to sustain at least one tail injury than non-working dog breeds (p < 0.001) over the examined period. Pointers/ setters/retrievers were the most affected. Amputation was reported in about 20% of cases due to tail injury (i.e. unspecified injuries [n = 51] or lacerations [n = 38]). The remaining tail injury cases were treated with a combination of antibiotics and dressings or recorded as untreated. The overall prevalence of tail injuries severe enough to require amputation was 0.12% (95% CI 0.10–0.14%). The prevalence of amputation in working breeds ranged from 0.4% (95% CI 0.28-0.57%) in Spaniels to 0.11% (95% CI 0.07–0.18%) in Retrievers. Compared with nonworking breeds, Spaniels and hunt point Retrievers were 4.57 (95% CI 2.95–7.09; p < 0.001) and 3.76 (95% CI 1.37–10.32; p = 0.03) times more likely to have sustained a tail injury that required amputation. Overall, working breeds were 2.19 (95% CI 1.53–3.14; p < 0.001) times more likely to have sustained a tail injury that required amputation than non-working breeds. Even though from this study it was clear that the number of tail amputations due to tail injuries had increased after the ban, it was calculated that the number of dogs to be docked to prevent a later amputation was high and it did not justify performing tail docking to all puppies even in the most affected working breeds (i.e. between 81 and 135 pointer/ setter, hunt point retriever or spaniel puppies would need to be docked to prevent one tail injury that required a veterinary examination in those breed groups). It was therefore estimated that a minimum of 320 (95% CI 207-527) pure breed working puppies would have needed to be docked to prevent one tail amputation in that breed (Cameron et al., 2014). Therefore, in the opinion of EFSA experts, tail docking can be considered as an effective measure to prevent tail lesions in hunting and working dogs, as dogs will not get any lesions if the tails are docked. However, the low incidence of tail injuries, combined with the consequences of docking in terms of pain and lack of tail function, does not justify tail docking as standard procedure.

Diesel et al. (2010) performed a similar study to quantify the risk of tail injury, evaluate the extent to which tail docking reduces this risk, and identify other major risk factors for tail injury in dogs in Great Britain. They conducted a nested case–control study during 2008 and 2009. Data were obtained from a stratified random sample of veterinary practices throughout Great Britain, and from questionnaires sent to owners of dogs with tail injuries and owners of a randomly selected sample of dogs without tail injuries. Two hundred and eighty-one tail injuries were recorded from a population of 138,212 dogs attending 52 participating practices and the weighted risk of tail injuries was 0.23% (95% CI: 0.20–0.25%) during this period. The risk of tail injury was therefore considered low by the authors, although some specific breeds (i.e. Spaniels, Greyhounds and Lurchers) were deemed at higher odds of injury. Moreover, the majority of the injuries were treatable and did not require amputation. The study highlighted that there are animal-related factors (such as breed and angle of the tail) which are positively associated with tail injury, while the working use was not a significant factor in the model. Overall, based on the study by Diesel et al. (2010), docking appeared to have a protective effect against injury, as expected; however, it was calculated that 500 dogs would need to be docked in order to prevent one tail injury.

Different results were obtained by Lederer et al. (2014). These authors used an online survey to estimate the prevalence of tail injuries; assess the risk of tail injuries in docked and undocked working dogs; and identify risk factors for owner-reported tail injuries in Scotland. A total of 1,035 respondents participated in the survey, of whom 848 (81.9%) completed the whole questionnaire. Tail-docked dogs were less likely to have a tail injury, but the length of the docking was not significant. To prevent one tail injury in all working breeds in one shooting season, the number of dogs which needed to be docked (NNT) would be between 5 and 54, depending on the breed. Instead, to prevent one tail injury in Spaniels or hunt point Retrievers in one shooting season, the NNT would be between two and 18. Consequently, Lederer et al. (2014) proposed that tail docking could have been considered as a preventive measure in working Spaniels and hunt point Retrievers, but they also acknowledged that the study contained respondent bias because working dog breeders' associations are advocates for tail docking.

Noonan et al. (1996) documented the behaviour of 50 puppies during and after tail docking. The puppies were restrained by an assistant while a surgeon docked the tail (using a pair of large surgical scissors) at a length considered appropriate for that breed. A single suture was placed in the stump for the close docked breeds (e.g. Rottweilers, Dobermans and Bouviers) to prevent uneven healing ('proud dock') of the tail. Bleeding was minimised by the application of Condy's Crystals (potassium permanganate) to the tail stump. A researcher observed the puppy and recorded the number and type of vocalisations (either shrieks or whimpers) in the time taken for the docking. The puppy was returned to the littermates and the behaviour was recorded in 5 s intervals for the first minute and then in 10 s intervals until an end-point of sleeping was reached. The puppies vocalised intensely ('shrieking') at the time of amputation of the tail, averaging 24 shrieks (range of 5–33). The average number of minor vocalisations ('whimpers') made during docking was 18 (range of 2–46). There were no shrieks recorded during the recovery period. The puppies settled quickly (average 3 min), but the time to settle was positively correlated with the frequency of vocalisations. This study was important to document the possible pain involved. It is worth highlighting that tail docking involves the removal of all or part of the tail using cutting or crushing instruments; muscles, tendons, four to seven pairs of nerves and sometimes bone or cartilage are severed. Wansbrought (1996) reported that the initial pain, from the direct injury to the nervous system, would be intense. While the nervous system of some young animals is not fully mature at birth, as some nerves are not completely myelinated, this does not mean that the nerves that conduct painful impulses are not present, rather their speed of conduction is slower than in mature animals (Morton, 1992). This difference in the speed of conduction means a puppy will perceive pain roughly a guarter of a second later than a mature animal (Morton, 1992). Since the nervous system is immature, this means that there is also a lack of inhibitory nerves, so the pain may actually be even more intense in the puppies than in adult animals (Bennett and Perini, 2003a,b).

Moreover, the transaction of peripheral nerves often leads to the formation of neuromas, potentially exposing the animal to persistent painful conditions (Mellor, 2018). Neuromas have been frequently reported in livestock which have been tail docked (Herskin et al., 2016; Sandercock et al., 2019), whereas they have been less reported in dogs, but these figures could be just due to under-reporting (Mellor, 2018). Gross and Carr (1990) reported six cases of neuromas due to tail docking and the authors suggested that neuromas should be always considered as a possible differential diagnosis of causal pain in all tail-docked dogs. Overall, even if there is a lack of well-designed cross-control studies, no evidence was retrieved to argue or exclude the possibility that there is chronic pain and the development of neuromas in docked dogs, as in other species (Bennett and Perini, 2003a,b). Since then, comparative evidence strongly suggests that neuromas are formed after tail docking also in puppies.

Tail docking do not only affect the health of the animals, but also other aspects of their behaviour. The tail is not a mere inconsequential appendage (Wansbrough, 1996) and docked dogs may have problems in balancing and in communicating since the tail is a means of balance and communication (Siniscalchi et al., 2018). The position of the tail and the way it is moved signal different emotional states, from positive states to fear, and from aggression to submissiveness; thus tail docking can affect the interaction of dogs with other animals and man (Wansbrough, 1996; Mellor, 2018).

Leaver and Reimchen (2008) recorded and analysed 492 interactions between off-leashed dogs approaching a model dog composed of a black synthetic fur-like material covering a wire frame and cotton-stuffed body. The model had a shoulder height of 50 cm, head height of 62 cm, a body length of 80 cm, an appearance similar to that of a Labrador Retriever. Two tail lengths (9 and 30 cm) were used and two tail status (still or wagging) were tested. Dogs responded with an elevated head and tail to a long/wagging tail model relative to the long/still tail model but did not show any response to tail motion when the model's tail was short. The authors concluded that a long tail is an effective communication method to send intraspecific cues.

Overall, the retrieved evidence supports that tail docking for convenience/cosmetic reasons has detrimental effects on dog welfare.

5.2.3.4. EFSA judgement of mandate statements related to tail docking

The outcomes of the judgement of the mandate statements are reported in Table 17.

Table 17:	EFSA Judgement of mandate statements related to tail docking in dogs with examples of
	the retrieved evidence

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	There is scientific evidence to support that, from an animal welfare perspective: - the surgical intervention of tail docking has a detrimental effect on the welfare of dogs	Overall statement if supported or not from literature From the scoping literature search, scientific publications were found reporting that tail docking has a detrimental effect on the welfare of dogs.	Tail docking in dogs should not be performed, unless necessary for the health of the animals.
		 Examples of these studies: Noonan et al. (1996) found increased vocalisation after amputation. Overall effect of tail docking on the welfare of dogs are reported by several authors, e.g. Morton (1992), Wansbrough (1996), Bennett and Perini (2003), Mills et al. (2016a), Mellor (2018). Mellor (2018) reported that tail docking has welfare impact in dogs also later in their lives (e.g. with development of neuromas). Tail docking for cosmetic purposes is banned in most EU MSs and other countries, although some exemptions exist (see Appendix D, 	
		Table D1).JudgementEFSA considers that tail docking has a direct detrimental impact on the welfare of puppies.Based on experience with other animal species, and evidence in dogs, it is likely that tail docking has welfare impact in dogs also later in their lives (e.g. with development of neuromas).	
		Although some breeds have a slightly higher risk to injure their tails, the number of adult dogs that would have injured tails does not justify applying tail docking to all puppies of these breeds.	

5.2.4. Ear cropping in dogs

In dogs, ear cropping involves reshaping the appearance of the external ear, usually by removing up to half of the caudal portion of the pinna (auricula). This surgery allows a naturally drooping ear to stand upright (AVMA, 2013). Following the removal of the pinna, the ears are taped and splinted to facilitate healing in the desired shape (Mills et al., 2016a). This procedure is typically performed when puppies are between 9 and 12 weeks old after they have received their initial vaccinations. Most often, dogs are anaesthetised during the procedure and may or may not be given analgesics afterwards.

5.2.4.1. Why is ear cropping performed?

Ear cropping originated as a way to reduce pinna injury during dog fighting and hunting.

It has been suggested that it may reduce susceptibility to otitis limiting the accumulation of debris but based on the observation by Rosser (2004), there are more patients with long pendulous ears that have never developed otitis externa than those with such conformation that have developed otitis externa. Many working breeds, such as Spaniels and Retrievers, have naturally pendulous ears, and they do not seem to be at a higher risk of otitis or ear injuries (Mills et al., 2016a).

Other advocates suggest that the procedure improves hearing, though supporting evidence is lacking (Mills et al., 2016a).

In another survey study, it was clear that respondents perceived dogs with shorter ears and tails as more aggressive, more dominant, and less playful than dogs with long ears and tails (Mills et al., 2016b). This is one of the reasons why breeders of many security or guard dogs, such as Doberman, are pro ear cropping since they believe that these puppies are sold quicker if their appearance suggest better protection (AVMA, 2013).

5.2.4.2. Information on specific national legislation on ear cropping

The provisions in place in some countries are described in Appendix D, Table D.1.

Even though ear cropping has been illegal in the UK since 2007, photographs of dogs with cropped ears are still present on social media (Norris et al., 2023) and data from ~ 500 veterinary clinics from October 2015 to March 2022 revealed that 132 dogs were checked for consultation on ear cropping in UK. There was a peak of consultation in 2020 and 2021, and the majority of these dogs were imported from eastern Europe, some of them illegally without microchips and necessary documents. The most common breeds were: the American Bulldog (32 cases, 0 controls), the Dobermann (26 cases, 0 controls), the Italian Mastiff (Cane Corso) (17 cases, 0 controls), the Bulldog (10 cases, 3 controls) and the Mastiff (5 cases, 2 controls). These breeds were all significantly (p < 0.01) overrepresented in cases compared to the control population.

Ear cropping is legal in the USA, and the American Kennel Club deems cropping (and tail docking) 'acceptable practices integral to defining and preserving breed character, enhancing good health and preventing injuries'. Scientific evidence on the welfare implications of ear cropping.

In their ethical review of Sandøe et al. (2015) suggested that post-surgery associated pain after ear cropping is present and it may last many weeks. Anaesthetic complications can occur, as well as secondary problems including infection or 'unsuccessful pinnae shaping' that may require subsequent surgery (AVMA, 2013; Norris et al., 2023). During the post operative care, puppies experience some discomfort during healing, stretching, retaping and bandaging, and other manipulations after surgery. Indeed, puppies need their ears bandaged or taped upright for days to months, and they may be isolated from other dogs during this period (AVMA, 2013). Besides the direct effect of the surgery on the dog, some have suggested that ear cropping may impact inter-canine communication well beyond any period of surgical recovery. The erect, forward ear position that results from cropping generally indicates interest, attention and approach, while a more caudal ear positioning, restricted by cropping, depicts appeasement or fear (Siniscalchi et al., 2018). So ear-cropped dogs may in extreme cases be hard to interpret.

Overall, even if well-controlled studies addressing the animal welfare implications of cropping dogs' ears do not exist, evidence was retrieved to support that this procedure is associated with negative welfare consequences such as pain, with the risk for puppies to be experience isolation stress injury.

5.2.4.3. EFSA judgement of mandate statements related to ear cropping

The outcomes of the judgement of the mandate statements are reported in Table 18.

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement
1	There is scientific evidence to support that, from an animal welfare perspective:	Overall statement if supported or not from literature From the scoping literature search, scientific	Ear cropping in dogs should not be performed, unless necessary for the health of the animals.
	 the surgical intervention of ear cropping has a detrimental effect on the welfare of dogs. 	publications were found reporting that ear cropping is associated with potential negative welfare consequences (e.g. pain and handling stress).	
		 <u>Reasoning</u> Examples of these publications are: Sandøe et al. (2015) reported that post-surgery associated pain after ear cropping is present and it may last many weeks. During the post operative care, puppies experience welfare consequences such as discomfort (e.g. during healing, stretching, retaping, etc.); as they also need their ears bandaged or taped upright for days to months, they may be isolated from other dogs during this period (AVMA, 2013), thus, experience isolation stress. 	
		Ear cropping is forbidden in many countries, e.g. EU MSs and UK (see Table D.1 in Appendix D).	
		Judgement EFSA considers that that ear cropping has a detrimental effect on the welfare of dogs.	

Table 18: EFSA Judgement of mandate statement related to ear cropping in dogs with examples of the retrieved evidence

5.2.5. Debarking in dogs

Debarking, also known as devocalisation, ventriculocordectomy or vocal cordectomy is the complete or partial removal of the vocal folds to avoid or minimise vocalisations. It can be performed via the oral cavity or by a ventral laryngotomy.

Whilst less or non-invasive methods (e.g. the use of citronella collars, see Wells, 2001) are generally advocated as a solution to this problem, surgical devocalisation has been employed for many years. The effectiveness of debarking has shown great variation depending on the breed (Mills et al., 2016a). The results of 25 devocalisations via ventral laryngotomy was considered an effective low-morbidity procedure (Franklin et al., 2011).

5.2.5.1. Why is debarking performed?

Barking is a natural behaviour of dogs which can be of disturb to some people to a level that becomes a problem for them (so called 'nuisance barking'). Debarking is considered a measure to avoid nuisance barking and it is generally not performed in puppies.

However, it is worth highlighting that barking is often a consequence of other behavioural problems such as separation anxiety and compulsive disorders, which are all treatable using behavioural therapies and training. An example of an efficient and applicable dog training system used to reduce excessive barking is reported by Yin et al. (2008).

5.2.5.2. Information on specific national legislation on debarking

Removal of vocal cords is forbidden in several countries and EU MSs (for details, see Appendix D, Table D.1). However, exemptions exist (e.g. in New Zealand) and according to Andersen et al. (2021) it is allowed in most States of USA.

5.2.5.3. Scientific evidence on the welfare implications of debarking

Glottic stenosis has been reported as a complication of vocal fold resection (Holt and Harvey, 1994). Another potential long-term complication of devocalisation in dogs is formation of a laryngeal web that obstructs airflow and may require corrective surgery (Mills et al., 2016a). Being an invasive surgical procedure, as with the aforementioned, there are risks associated with it, including post-surgical pain. As per tail docking and ear cropping, debarking can affect dog communication with humans, other dogs and other species. It is well known that dogs rely heavily on vocalisations to express motivation and for intra- and inter-species communication (Siniscalchi al., 2018).

Overall the evidence retrieved supports that surgical debarking for convenience reasons is an invasive procedure which impairs dogs' welfare.

5.2.5.4. EFSA judgement of mandate statements related debarking

The outcomes of the judgement of the mandate statements are reported in Table 19.

#	Statement	EFSA Judgement Is this statement supported by the studies from the scoping literature search and/or other sources?	Overall recommendations on this statement	
1	There is scientific evidence to support that, from an animal welfare perspective:	Overall statement if supported or not from literature From the scoping literature search, scientific	Surgical debarking in dogs should not be performed, unless	
	 the surgical intervention of debarking has a detrimental effect on the welfare of dogs 		necessary for the health of the animals.	
		Reasoning Examples of these studies are: Holt and Harvey (1994); Mills et al. (2016a); Siniscalchi et al. (2018).		
		Removal of vocal cords is forbidden in several countries (see Appendix D, Table D.1).		
		Judgement EFSA experts considers that surgical debarking has a detrimental effect on the welfare of dogs.		
		Not-invasive measures against nuisance barking exist and could be used to avoid excessive barking.		

Table 19: EFSA Judgement of mandate statement in relation to debarking in dogs with examples of the retrieved evidence

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Abbreviations

ABMs ALPHA AMH ATP BOAS BCS CBE CI ETS GP IATA IOP LCT LED Im Ix MSs NEFA OR PRL TCZ TG TNZ	animal-based measures Animal & Plant Health anti-Mullerian hormone adenosine triphosphate brachycephalic obstructive airway syndrome body condition score commercial breeding establishment confidence interval European Treaty Series grant procurement international air transport association intraocular pressure lower critical temperature light-emitting diode lumens lux Member States non-esterified fatty acids odds ratio prolactin thermal comfort zone tasking grant thermal neutral zone terms of reference
ToR UCT	terms of reference upper critical temperature

Appendix A – Protocol developed for assessing welfare of cats and dogs in commercial breeding establishments

#	Assessment questions	Sub-questions		
1)	Should accommodating of cats and dogs permanently in tiered boxes and crates be avoided for welfare of these animals?	1. Identify and select the relevant evidence describing how the welfare of cats is affected by permanent accommodation in tiered boxes and crates.	2. Describe how the welfare of cats is affected by permanent accommodation in tiered boxes and crates.	3. Is there scientific evidence to agree/ partially agree/disagree with the ban (interpreted as avoidance) of tiered boxes and crates for accommodating cats permanently?
		4. Identify and select the relevant evidence describing how the welfare of dogs is affected by permanent accommodation in tiered boxes and crates.	5. Describe how the welfare of dogs is affected by permanent accommodation in tiered boxes and crates.	6. Is there scientific evidence to agree/ partially agree/disagree with the ban (interpreted as avoidance) of tiered boxes and crates for accommodating dogs permanently?
2)	Do dogs have the need to exercise and socialise outdoors on a daily basis?	7. Identify and select the relevant evidence describing the effects that a lack of physical exercise outdoors on a daily basis has on the welfare of dogs.	8. Describe the main effects that a lack of physical exercise outdoors on a daily basis has on the welfare of dogs.	9. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation of providing physical exercise outdoors on a daily basis in dogs?
		10. Identify and select the relevant evidence describing the effects that a lack of expressing social and locomotor behaviour, outdoors daily, has on the welfare of dogs	locomotor behaviour, outdoors daily, has on the	12. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation of providing opportunities for dogs to express social and locomotor behaviour outdoors on a daily basis
3)	Is there scientific evidence in the literature to suggest a thermal comfort zone between 15 and 26°C in adult cats?	13. Identify the thermal comfort zone (TCZ) and the thermal neutral zone (TNZ) in cats and describe the welfare implications of living within TCZ and TNZ based on the scientific evidence.	14. Describe the main physio and physio- pathological effects occurring in adult cats kept at environmental temperatures below 15°C or above 26°C.	15. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation of keeping adult cats within the thermal comfort zone between 15°C and 26°C?
4)	Is there scientific evidence in the literature to suggest a thermal comfort zone between 10 and 26°C in adult dogs?	16. Identify the thermal comfort zone (TCZ) and the thermal neutral zone (TNZ) in adult dogs and describe the welfare implications of living within TCZ and TNZ based on the scientific evidence.	17. Describe the main physio and physio- pathological effects occurring in adult dogs kept at environmental temperatures below 10°C or above 26°C.	18. Is there scientific evidence to agree/ partially agree/disagree with the recommendation of keeping adult dogs within the thermal comfort zone between 10°C and 26°C?
5)	Is there scientific evidence in the literature to suggest a thermal comfort zone between 22 and 28°C	19. Identify the thermal comfort zone (TCZ) and the thermal neutral zone (TNZ) in kittens of less than 21 days and	20. Describe the main physio and physio-pathological effects occurring on the welfare of kittens kept at	21. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation of keeping kittens for the

Table A.1:	Sub-questions developed for topic 1: housing of cats and dogs



#	Assessment questions	Sub-questions		
	during the first 21 days of life in kittens?	describe the welfare implications of living within TCZ and TNZ based on the scientific evidence.	environmental temperatures below 22°C or above 28°C during the first 21 days of life.	first 21 days of life in the kittening areas at environmental temperatures between 22°C and 28°C?
6)	Is there scientific evidence in the literature to suggest a thermal comfort zone between 22 and 28°C during the first 10 days of life in puppies?	22. Identify the thermal comfort zone (TCZ) and the thermal neutral zone (TNZ) in puppies of less than 10 days and describe the welfare implications of living within TCZ and TNZ based on the scientific evidence.	23. Describe the main physio and physio- pathological effects occurring in puppies kept at environmental temperatures below 22°C or above 28°C during the first 10 days of life.	24. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation of keeping puppies during the first 10 days of life within the thermal comfort zone between 22°C and 28°C?
7)	Are there other considerations regarding thermal regulation in relation to certain breeds or types of cats and dogs?	25. Document how animal-related factors (i.e. long or short-haired; small/medium/large sized; brachycephalic) may affect the thermoregulation of cats.	26. Describe how cat characteristics (i.e. long or short-haired; small/ medium/large sized; brachycephalic) may impair thermoregulation in cats and consequently they require different ranges of TCZ and TNZ.	27. Is there scientific evidence to agree/ partially agree/disagree with the recommendation of providing different temperatures in relation to the cat breed or type (i.e. long or short-haired; small/medium/large sized; brachycephalic)?
		28. Document how animal-related factors (i.e. long or short-haired; small/medium/large sized; brachycephalic or dolicomorphic) may affect the thermoregulation of dogs.	brachycephalic or dolicomorphic) may impair thermoregulation	30. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation of providing different temperatures in relation to the dog breed or type (i.e. long or short-haired; small/medium/large sized; brachycephalic or dolicomorphic)?
8)	Is setting thermal comfort zone for indoor accommodation of cats and dogs according to specifics of various category of animals relevant for welfare of these animals?	31. Identify and select the relevant evidence describing the effects of different temperatures on the welfare of different categories or life stage of cats. (i.e. pregnant and lactating queens, newborn kittens, kittens, female and male adult cats).	32. Describe the main effects of different temperatures on the welfare of different categories or life stage of cats (i.e. pregnant and lactating queens, newborn kittens, kittens, female and adult cats).	33. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation of providing different temperatures in relation to the category or life stage of cat (i.e. pregnant and lactating queens, newborn kittens, kittens, female and adult cats)?
		34. Identify and select the relevant evidence describing the effects of different temperatures on the welfare of different categories or life stage of dogs (i.e. pregnant and lactating bitches, female and male adult dogs, puppies).	35. Describe the main effects of different temperatures on the welfare of different categories or life stage of dogs (i.e. pregnant and lactating bitches, female and male adult dogs, puppies).	36. Is there scientific evidence to agree/ partially agree/disagree with the recommendation of providing different temperatures in relation to the category or life stage of dog (i.e. pregnant and lactating bitches, female and male adult dogs, puppies)?

#	Assessment questions	Sub-questions		
9)	Is access to natural daylight important to ensure welfare of cats and dogs?	37. Identify and select the relevant evidence describing the effects of different light intensities and photoperiods on the welfare of cats.	38. Describe the main effects of different light intensities and photoperiods on the welfare of cats.	39. Is there scientific evidence to agree/ partially agree/disagree with the recommendation of ensuring a minimum intensity of 50 lx during at least 16 h per day to cats?
		40. Identify and select the relevant evidence describing the effects of different light intensities and photoperiods on the welfare of dogs.	41. Describe the main effects of different light intensities and photoperiods on the welfare of dogs.	42. Is there scientific evidence to agree/ partially agree/disagree with the recommendation of ensuring a minimum intensity of 50 lx during at least 16 h per day to dogs?

Table A.2:	Sub-questions developed for topic 2: Health of cats and dogs	
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#	Assessment questions	Sub-questions	Sub-questions		
1)	Should breeding of bitches and queens before they reach skeletal and sexual maturity be avoided?	1. Identify and select the relevant evidence on how to objectively identify when a queen reaches skeletal and sexual maturity.	2. Identify a possible method to identify skeletal and sexual maturity in queens.	3. Describe the main welfare effects of breeding queens which have not reached skeletal and sexual maturity.	4. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation suggesting a test to identify skeletal and sexual maturity in queens?
		5. Identify and select the relevant evidence on how to objectively identify when a bitch reaches skeletal and sexual maturity.	6. Identify a possible method to identify skeletal and sexual maturity in bitches.	7. Describe the main welfare effects of breeding bitches which have not reached skeletal and sexual maturity.	8. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation suggesting a test to identify skeletal and sexual maturity in bitches?
2)	Can controlling the frequency of pregnancies have beneficial impact on health and welfare of bitches and queens by preventing physical		the effects that s (more frequent	10. Describe the main effects that frequent pregnancies (more frequent than one per year) have on the welfare of queens.	11. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation of providing a minimum period of 12 months between two kittenings?
	exhaustion?	12. Identify and sele evidence describing frequent pregnancie than one per year) h of bitches.	the effects that s (more frequent	13. Describe the main effects that frequent pregnancies (more frequent than one per year) have on the welfare of bitches.	14. Is there scientific evidence to agree/ partially agree/ disagree with the recommendation of providing a minimum period of 12 months between two whelpings?

#	Assessment questions	Sub-questions		
3)	Is the mature age a relevant element of welfare in older bitches and queens?	15. Identify and select the relevant evidence describing the effects that pregnancies have on the welfare of queens of different ages.	16. Describe the main effects that pregnancies have on the welfare of queens of different ages.	15. Is there scientific evidence to agree/partially agree/disagree with the recommendation to suggest a threshold based on the age to stop the reproductive career of queens?
		16. Identify and select the relevant evidence describing the effects that pregnancies have on the welfare of bitches of different ages.	17. Describe the main effects that pregnancies have on the welfare of bitches of different ages.	18. Is there scientific evidence to agree/partially agree/disagree with the recommendation to suggest a threshold based on the age to stop the reproductive career of bitches?

Table A.3:	Sub-questions developed	for topic 3: Painful	surgical procedures
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#	Assessment questions	Sub-questions		
1)	Do cosmetic and convenience surgical interventions, such as ear cropping, tail docking, partial or complete digit amputation and	1. Identify and select the relevant evidence describing the effects of cosmetic surgical interventions on the welfare of cats.	2. Describe the main effects of cosmetic surgical interventions on the welfare of cats.	3. Is there scientific evidence to agree/ partially agree/ disagree with the ban (interpreted as avoidance) of cosmetic surgical interventions in cats?
	resection of vocal cords or folds, have a detrimental effect on welfare of cats and dogs?	4. Identify and select the relevant evidence describing the effects of cosmetic surgical interventions on the welfare of dogs.	5. Describe the main effects of cosmetic surgical interventions on the welfare of dogs.	6. Is there scientific evidence to agree/ partially agree/disagree with the ban (interpreted as avoidance) of cosmetic surgical interventions in dogs? Is there any welfare benefit in performing cosmetic surgery or recommending them for specific breeds (e.g. tail docking in hunting dogs to minimise tail injury)

Appendix B – Literature search strings, inclusion and exclusion criteria for the assessment of welfare of cats and dogs in commercial breeding establishments

Key word combination of the "topic search"	TS = (cat OR dog AND welfare)
Defined time period	1980–2023
Exclusion criteria	 Exclusion of articles other than research and review articles Exclusion of articles in a language other than English Exclusion of articles discussing Ethical issues or addressing cats and dogs breeding and health from an ethical point of view Exclusion of articles on animal species other than cats and dogs Exclusion of attitude studies addressed at investigating veterinarians' and owners' perception Exclusion of articles with abstract missing. Exclusion of articles that did not address or mention issues concerning housing, reproduction or cosmetic and convenience surgeries
Topics	 Housing, Health, Surgical
Subtopics	 For Housing: Space (e.g. cages, outdoors access, social behaviour), TNZ (thermoneutral zone, factors affecting thermoregulation) and Light For Health: Reproduction (e.g. physio and physiopathology of reproduction, dystocia, neonatal mortality), Skeletal maturity and Sexual maturity (e.g. grow rate, puberty) For Surgical: Cosmetic surgery (e.g. tail docking)
Criteria for ranking the relevance of the retained articles	 1: Articles and reviews that directly addressed the topics of interest (housing, reproduction or cosmetic and convenience surgeries) were ranked as essential to study 2: Articles that reported in their experimental design factors related to the topics of interest (housing, reproduction or cosmetic and convenience surgeries) were ranked as important and must-read. 3: Articles that addressed in general to the welfare of dogs and cats were ranked as to be considered 4: Articles that addressed topics that are indirectly related to the topics of interest were ranked as limited interest (e.g. surveys administered to dog and cat owners concerning factors that could influence the welfare of these animals)
Outcome	Out of 2,532 search results, 127 articles were retained for further evaluation. Among the 127 articles, 54 were ranked as '1-essential to study'; 22 were ranked as '2-important'; 34 were ranked as '3-to be considered'; 17 were ranked as '4-of limited interest'.

Table B.1: Description of the general literature search strings and screening methods

Table B.2: List of search terms for the literature review about topic housing in dogs inserting terms related to the thermal comfort zone

Key word combination of the "topic search"	TS = (dog AND ((heat AND stroke) OR (cold AND stress) OR (thermal AND stress)))
Defined time period	1980–2023
Exclusion criteria	 Exclusion of articles other than research and review articles Exclusion of articles in a language other than English Exclusion of articles discussing Ethical issues or addressing dog breeding and health from an ethical point of view Exclusion of articles on animal species other than dogs Exclusion of attitude studies addressed at investigating veterinarians' and owners' perception Exclusion of project description only, no results given. Exclusion of articles with abstract missing.

Criteria for ranking the relevance of the retained articles	 1: Articles and reviews that directly addressed the topics of interest (heat-related diseases, heat stress, cold stress, etc) were ranked as essential to study 2: Articles that reported in their experimental design factors related to the topics of interest (temperature and humidity) were ranked as important and must-read. 3: Articles that addressed in general to the welfare of dogs and cats in relation to environmental parameters were ranked as to be considered 4: Articles that addressed topics that were somewhat indirectly related to the topics of interest were ranked as limited interest (e.g. surveys administered to dog owners)
Outcome	Out of 67 search results, 19 articles were retained for further evaluation. Among the 19 articles, 13 were ranked as '1-essential to study'; 3 were ranked as '2-important'; 1 was ranked as '3-to be considered'; 2 were ranked as '4-of limited interest'.

Table B.3: List of search terms for the literature review about topic housing in cats inserting particular terms related to the thermal comfort zone

Key word combination of the "topic search"	TS = (cats AND ((heat AND stroke) OR (cold AND stress) OR (thermal AND stress)))
Defined time period	1980–2023
Exclusion criteria	 Exclusion of articles other than research and review articles Exclusion of articles in a language other than English Exclusion of articles discussing Ethical issues or addressing cat breeding and health from an ethical point of view Exclusion of articles on animal species other than cats Exclusion of attitude studies addressed at investigating veterinarians' and owners' perception Exclusion of project description only, no results given. Exclusion of articles with abstract missing.
Criteria for ranking the relevance of the retained articles	 1: Articles and reviews that directly addressed the topics of interest (heat-related diseases, heat stress, cold stress, etc) were ranked as essential to study 2: Articles that reported in their experimental design factors related to the topics of interest (temperature and humidity) were ranked as important and must-read. 3: Articles that addressed in general to the welfare of dogs and cats in relation to environmental parameters were ranked as to be considered 4: Articles that addressed topics that were somewhat indirectly related to the topics of interest were ranked as limited interest (e.g. surveys administered to cat owners)
Outcome	Out of 275 search results, only 2 articles were retained and ranked as '1-essential to study'.

Table B.4:	List of search terms for the literature review about topic health inserting particular terms
	related to light and circadian rhythm in dogs and cats

Key word combination of the "topic search"	TS = (cats OR dogs) AND ((circadian AND rhythm) AND light)
Defined time period	No limitation due to the limited number of records
Exclusion criteria	 Exclusion of articles other than research and review articles Exclusion of articles in a language other than English Exclusion of articles discussing Ethical issues or addressing cat and dog breeding and health from an ethical point of view Exclusion of articles on animal species other than cats and dogs Exclusion of attitude studies addressed at investigating veterinarians' and owners' perception Exclusion of project description only, no results given. Exclusion of articles with abstract missing.

Criteria for ranking the relevance of the retained articles	 1: Articles and reviews that directly addressed the topics of interest (i.e. light, photoperiod, circadian rhythm) were ranked as essential to study 2: Articles that reported in their experimental design factors related to the topics of interest were ranked as important and must-read. 3: Articles that addressed in general environmental factors were ranked as to be considered 4: Articles that addressed topics that were somewhat indirectly related to the topics of interest were ranked as limited interest (e.g. surveys administered to dog and cat owners) 			
Outcome	Out of 46 search results, 21 articles related to light were retained for further evaluation. Among the 21 articles, 14 were ranked as '1-essential to study'; 2 were ranked as '2-important'; 3 were ranked as '3-to be considered'; 2 were ranked as '4-of limited interest'.			

Table B.5: List of search terms for the literature review about topic health in dogs and cats inserting particular terms related to skeletal and sexual maturity

Key word combination of the "topic search"	TS = ((dog OR cats) AND (sexual AND maturity) AND ((growth AND curve) OR (growth AND rate) OR (skeletal AND development)))		
Defined time period	No limitation due to the limited number of records		
Exclusion criteria	 Exclusion of articles other than research and review articles Exclusion of articles in a language other than English Exclusion of articles discussing Ethical issues or addressing cat and dog breeding and health from an ethical point of view Exclusion of articles on animal species other than cats and dogs Exclusion of attitude studies addressed at investigating veterinarians' and owners' perception Exclusion of project description only, no results given. Exclusion of articles with abstract missing. 		
Criteria for ranking the relevance of the retained articles	 1: Articles and reviews that directly addressed the topics of interest (i.e. growth curve and sexual maturity) were ranked as essential to study 2: Articles that reported in their experimental design factors related to the topics of interest were ranked as important and must-read. 3: Articles that addressed in general sexual maturity were ranked as to be considered 4: Articles that addressed topics that were somewhat indirectly related to the topics of interest were ranked as limited interest (e.g. surveys administered to dog and cat owners) 		
Outcome	Out of 13 search results, 3 articles related to skeletal maturity were retained for further evaluation, all ranked as `1-essential to study'.		

Table B.6: List of search terms for the literature review about topic health in dogs and cats inserting particular terms related to puberty in queens and bitches

Key word combination of the "topic search"			
Defined time period	No limitation due to the limited number of records		
Exclusion criteria	 Exclusion of articles other than research and review articles Exclusion of articles in a language other than English Exclusion of articles discussing Ethical issues or addressing cat and dog breeding and health from an ethical point of view Exclusion of articles on animal species other than cats and dogs Exclusion of attitude studies addressed at investigating veterinarians' and owners' perception Exclusion of articles with abstract missing. 		
Criteria for ranking the relevance of the retained articles	 - 1: Articles and reviews that directly addressed the topics of interest (i.e. puberty and oestrus cycle) were ranked as essential to study 		

	 2: Articles that reported in their experimental design factors related to the topics of interest were ranked as important and must-read. 3: Articles that addressed in general puberty were ranked as to be considered 4: Articles that addressed topics that were somewhat indirectly related to the topics of interest were ranked as limited interest (e.g. surveys administered to cat and dog owners)
Outcome	Out of 99 search results, 24 articles were retained for further evaluation. Among the 24 articles, 18 were ranked as '1-essential to study'; 3 were ranked as '2-important'; and 3 were ranked as '3-to be considered'. The 24 articles were categorised in the following sub-topics: 1 in 'Skeletal maturity'; 14 in 'Sexual maturity'; 8 were more in general related to 'Reproduction'; and 1 was categorised into the Housing sub-topic of 'Light' as the abstract addressed the effect of photoperiod on cats' reproduction.

Table B.7: List of search terms for the literature review about topic painful procedures in dogs inserting particular terms related to cosmetic and convenience surgeries

Key word combination of the "topic search"	TS = (dog AND ("cosmetic surgery" OR "ear cropping" OR "tail docking" OR debarking OR "wrinkle removal"))		
Defined time period	1980–2023		
Exclusion criteria	 Exclusion of articles other than research and review articles Exclusion of articles in a language other than English Exclusion of articles discussing Ethical issues or addressing dog breeding and health from an ethical point of view Exclusion of articles on animal species other than dogs Exclusion of attitude studies addressed at investigating veterinarians' and owners' perception Exclusion of project description only, no results given. Exclusion of articles with abstract missing. 		
Criteria for ranking the relevance of the retained articles	 1: Articles and reviews that directly addressed the topics of interest (tail docking, ear cropping, debarking and cosmetic surgery) were ranked as essential to study 2: Articles that reported in their experimental design factors related to the topics of interest (e.g. diseases in tail-docked dogs) were ranked as important and must-read. 3: Articles that addressed in general the welfare of dogs in relation to cosmetic surgery were ranked as to be considered 4: Articles that addressed topics that were somewhat indirectly related to the topics of interest were ranked as limited interest (e.g. surveys administered to dog owners) 		
Outcome	Out of 50 search results, 9 articles were retained for further evaluation. Among the 9 articles, 7 were ranked as '1-essential to study'; 1 was ranked as '2-important'; 1 was ranked as '3-to be considered'.		

Table B.8: List of search terms for the literature review about the topic of painful procedures in cats inserting particular terms related to cosmetic and convenience surgeries

Key word combination of the "topic search"	TS = (cats AND ("cosmetic surgery" OR "ear cropping" OR "tail docking" OR declawing OR "wrinkle removal"))		
Defined time period	1980–2023		
Exclusion criteria	 Exclusion of articles other than research and review articles Exclusion of articles in a language other than English Exclusion of articles discussing Ethical issues or addressing cat breeding and health from an ethical point of view Exclusion of articles on animal species other than cats Exclusion of attitude studies aiming at investigating veterinarians' and owners' perception Exclusion of project description only, no results given. Exclusion of articles with abstract missing. 		



Criteria for ranking the relevance of the retained articles	 1: Articles and reviews that directly addressed the topics of interest (tail docking, ear cropping, declawing and cosmetic surgery) were ranked as essential to study 2: Articles that reported in their experimental design factors related to the topics of interest (e.g. problems in declawed cats) were ranked as important and must-read. 3: Articles that addressed in general to the welfare of cats in relation to cosmetic surgery were ranked as to be considered 4: Articles that addressed topics that were somewhat indirectly related to the topics of interest were ranked as limited interest (e.g. surveys administered to cat owners)
Outcome	Out of 19 search results, 4 articles were retained for further evaluation, and were all ranked as `1-essential to study'.

Appendix C – Uncertainty assessment

Table C.1: Sources of uncertainty (in a non-prioritised order) associated with the assessment of welfare of cats and dogs kept for commercial breeding

Source of uncertainty	Nature or cause of the uncertainty
Literature search – Language	The search was performed exclusively in English. More studies could have been identified by including references with abstracts in languages other than English.
Literature search – Publication type	The studies considered included research articles and reviews (identified through the literature search and known to the EFSA Experts, but an extensive search of the grey literature was not conducted. Therefore, there may be reports and other guidance documents on animal welfare of which the EFSA Experts were not aware off.
Literature search – Search strings	Although the search criteria were thoroughly discussed, some synonyms may have not been used in the search strings, and thus less hits might have been retrieved.
Literature search –data source	The search was limited to Scopus. Although the search was complemented by those reported in the Report and in the guidelines and from knowledge of the experts, some articles published in journals not included in Scopus could have been considered
Literature search – inclusion and exclusion criteria	The screening phase might have led to the exclusion of certain studies that could have included relevant information. Papers without abstracts were excluded, but they may have been relevant.
Expert group – number of experts, type of experts	For this scientific report, preparatory work on literature for dogs and cats (housing, health and surgical procedures) was executed by a tasking grant framework contract on Animal Welfare (GP/EFSA/ALPHA/2021/10 Animal Welfare TG). The appraisal of the literature was carried out by an hoc EFSA Working group with 3 species-specific experts. Due to time constrains and the type of appraisal that could be carried out for this scientific report, the lack of a multidisciplinary group of experts and consequently the lack of multidisciplinary review process – as normally done for a full assessment process – may have result in uncertainties and different interpretations/discussions in relation to the risk questions.
Time constraint	The time and resources allocated to execute the method (rapid review) were limited and additional time for reflection would have facilitated a more in-depth discussion of some of the aspects.
Inability to retrieve some full-text documents	Some full articles were not accessible using University access and other platforms (e.g. Research gate) and could not be read in full.

Appendix D – Examples of legislative provisions on convenience surgeries in dogs and cats

Currently, there is no specific EU legislation as such on convenience surgeries of dogs and cats.

The European Convention for the Protection of pet animals (ETS 125),⁸ signed on 13 November 1987, stipulates, *inter alia*, as basic principles for animal welfare that '*Nobody shall cause a pet animal unnecessary pain, suffering or distress'* (Art 3, 1). In addition, Art 10 specifies that '*Surgical operations for the purpose of modifying the appearance of a pet animal or for other non-curative purposes shall be prohibited and, in particular: the docking of tails; the cropping of ears; devocalisation; declawing and defanging'. Exceptions to these prohibitions are also listed in the article referring to animal health and welfare aspects but not to 'cosmetic' reasons.*

In the absence of a EU legislation, the matter remains under the competence of MSs. To date, 19 EU MSs and seven other Countries have ratified the Convention.⁹ However, some MSs have made specific reservations on Art 10 and have for example granted for a period of time an exception for tail docking.

In 1995, with the Resolution on surgical operations in pet animals adopted by the Multilateral Consultation of the Parties to the Convention ETS No. 125¹⁰, the Parties to the European Convention for the Protection of Pet Animals determined to made strong efforts to abolish such practices, in particular docking of tails and cropping of ears, for the purpose of modifying the appearance of a pet animal or for other non-curative purposes, and agreed to: (i) promote awareness particularly among judges, breeders, veterinarians and keepers that mutilation should not be carried out; (ii) strongly encourage breeding associations to revise breeding standards in the light of Art 10 of the Convention and thereby to contribute to the discontinuation of surgical alterations of pedigree dogs' appearances' and (iii) consider the possibility of phasing out the exhibition and the selling of animals having been subjected to these operations.

To identify variations in the legal framework of welfare regulation in pets in different countries, Andersen et al. (2021), compared the legislation on convenience surgeries for dogs existing in seven EU MSs, England, USA, New South Wales (Australia) and New Zealand.

Table D.1 reports examples of national legislations on this matter from EU MSs and other Countries; however, it needs to be noted that this list is not exhaustive.

Country	Tail docking	Ear cropping	Removal of vocal cords
Austria	Forbidden	Forbidden	Forbidden
Denmark	 Forbidden Exceptions in puppies if it is performed: by a veterinarian with anaesthesia and subsequent pain treatment, before the puppy is < 4 days old and of the following breeds: Wirehaired pointer Shorthaired pointer Weimaraner Vizsla Brittany 	Forbidden	Forbidden

Table D.1: Examples of provisions on convenience surgeries related to the welfare of companion dogs in 11 countries (modified by Andersen et al., 2021)

⁸ European Convention for the Protection of Pet Animals. Strasbourg, 13 November 1987. European Treaty Series – No 125 available online: https://rm.coe.int/168007a67d

⁹ The chart of signatures and ratifications of Treaty 125 is available at: https://www.coe.int/en/web/conventions/full-list? module=signatures-by-treaty&treatynum=125 - visited on 14 June 2023.

¹⁰ Multilateral consultation of Parties to the European Convention for the Protection of pet animals, Strasbourg, 7–10 March 1995. Available online: https://rm.coe.int/168008c37b



Country	Tail docking	Ear cropping	Removal of vocal cords
England	Forbidden	Forbidden	Forbidden
	Except for: • certified working dogs, • the following dog types: – Breeds of hunt/point/retrieve dogs – Spaniels – Terriers		
Germany	Forbidden Hunting dogs can be exempted from this	Forbidden	Forbidden
Italy	Forbidden	Forbidden	Forbidden
New South	Forbidden	Forbidden	Forbidden
Wales (Australia)			 However, it can be allowed if: the owner has been required to stop the dog's bothersome barking and behavioural treatments have been unsuccessful and the only remaining solution is killing, performed by a veterinarian.
New Zealand	Forbidden	Forbidden	 Not forbidden, but: it must be performed by a veterinarian, and only if other attempts at treating the barking behaviour have been unsuccessful.
Norway	Forbidden	Forbidden	Forbidden
Sweden	Forbidden	Forbidden	Forbidden
The Netherlands	Forbidden	Forbidden	Forbidden
USA	Allowed	Allowed	Allowed in most States