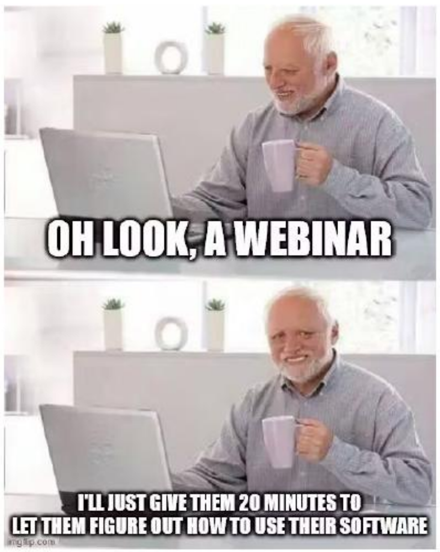
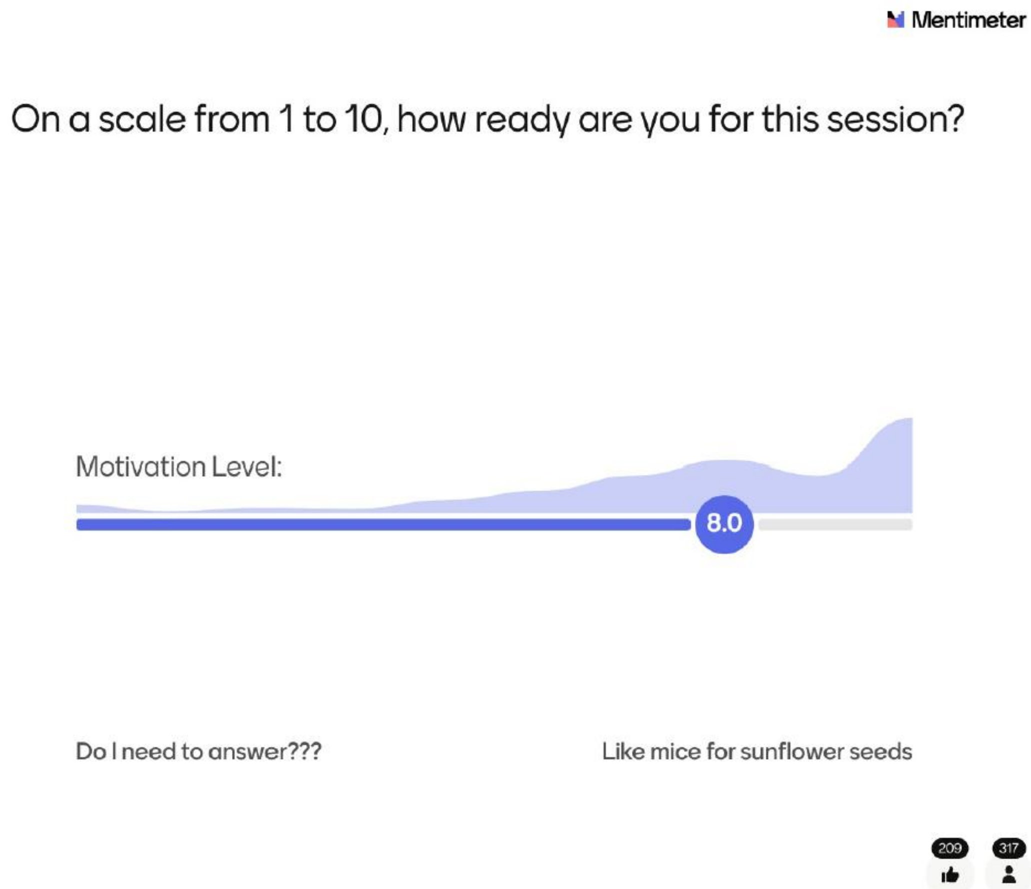
Data Driven Breeding

Reducing Surplus Laboratory Animals Through Smart Planning

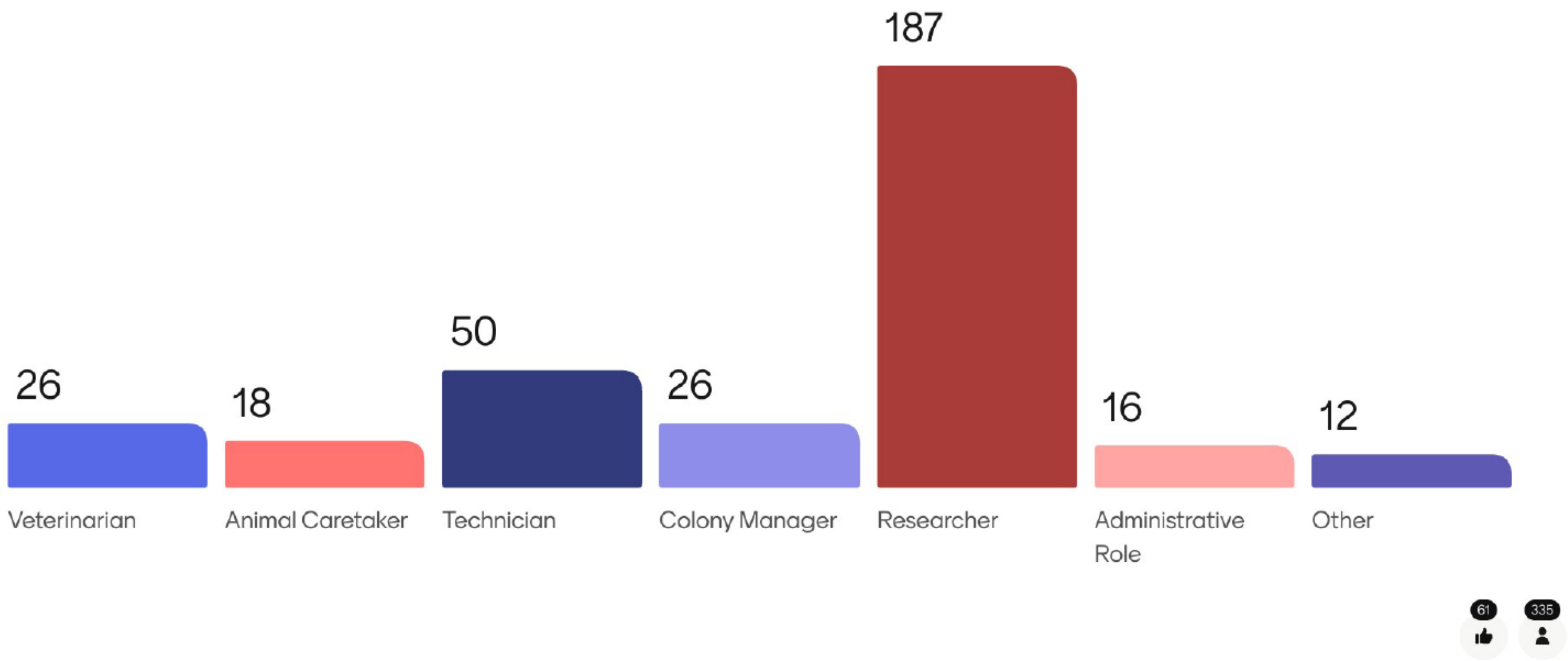
Keeping things interactive ☺





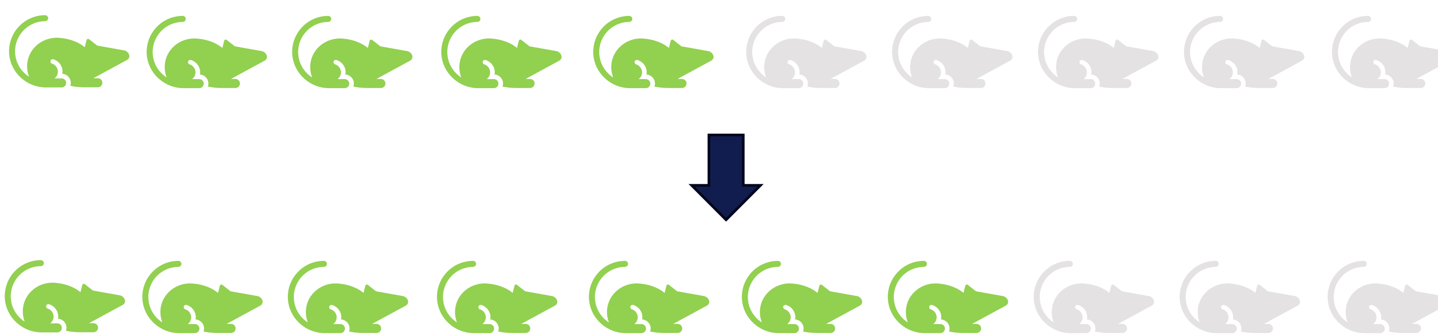
Mentimeter

What's your primary role when it comes to animal research or breeding?



Outline

1. Why surplus matters
2. Root causes
3. Data-driven breeding basics
4. Case-study Himberg
5. Practical take-aways and short Q&A



What are surplus animals?

Surplus animals are animals that were bred but not used and may need to be culled or rehomed (if eligible) due to:

🧬 **Unneeded genotype**
(e.g., wild-type or heterozygous littermates not used in the study)

🐭 **Only one sex required**
(e.g., male-only cohorts for behavior studies)

🕓 **Excessive age**
(e.g., missed cohort window or delayed experiments)

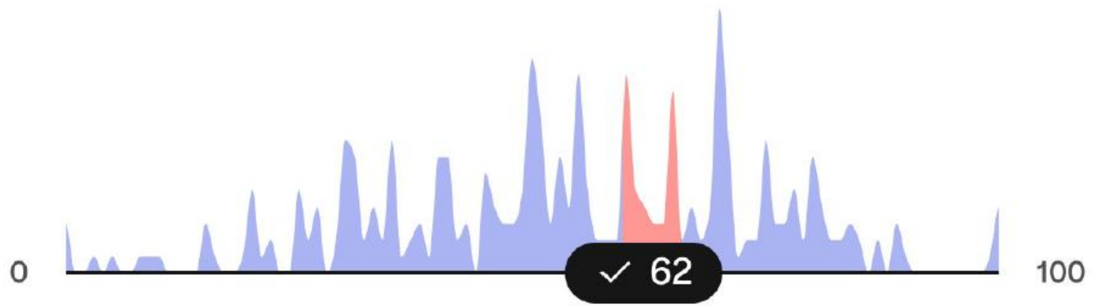
⚠️ **Poor breeding management or study planning**
(e.g., overestimation of need, unclear timelines)

🔄 **Maintenance of genetically altered lines**
(e.g., due to a lack of cryopreservation availability)

💉 **Compromised health status**
(e.g., infections, welfare concerns)

Mentimeter

"For 2022, what percentage of all research mice bred in the EU-27 were never used in an experiment (i.e., were 'surplus' and culled)?



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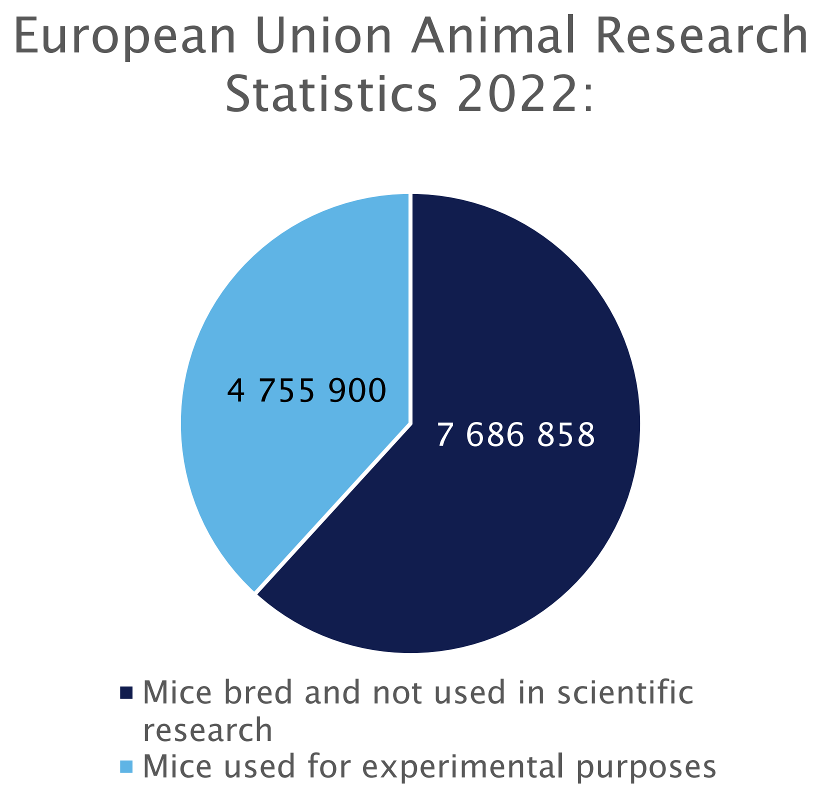
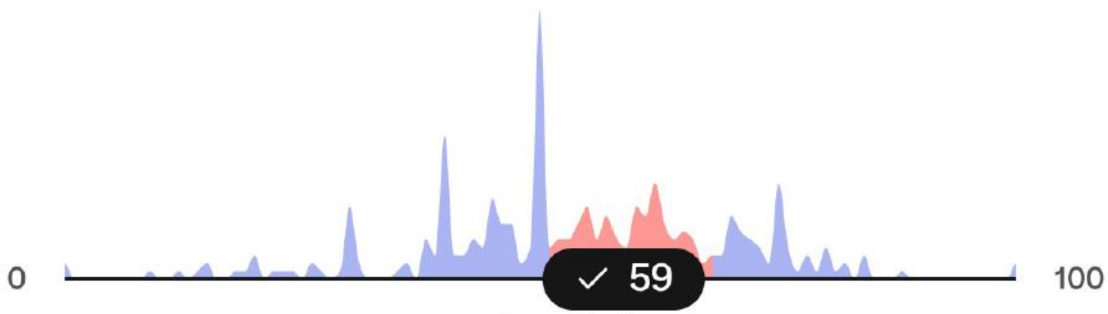
Surplus mice – Europe



→ For every mouse used for experimental purposes 1,61 mice are bred, killed and not used in procedures

Mentimeter

"For 2022, what percentage of all research mice bred in Austria were never used in an experiment (i.e., were 'surplus' and culled)?

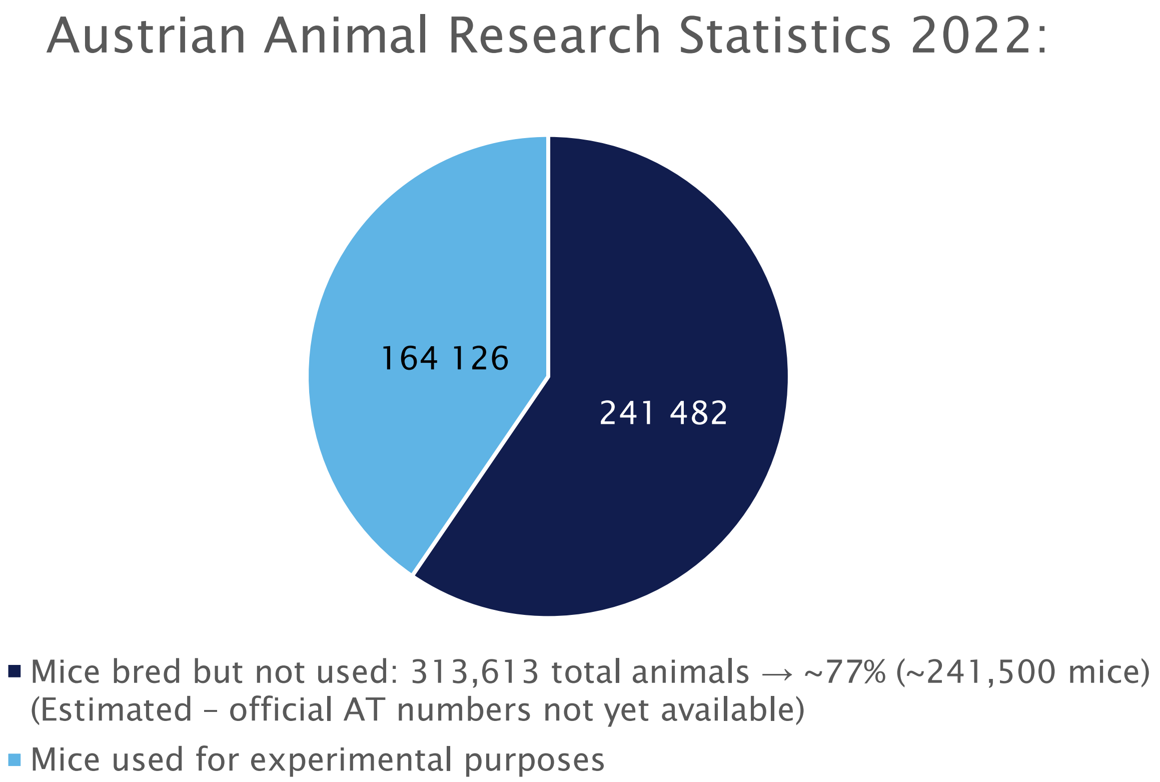


Accepted answers:

51 - 67

49 309

Surplus mice - Austria



→ Slightly below EU-27 average

Where is potential for reducing surplus mice?

- Some mice are still being used for research although not in procedures, reducing should focus on unused surplus, as sometimes breedings are set up for organ/tissue collection

Category	1. Number of genetically normal animals (wild type offspring) produced, bred and killed as a result of a creation of a new genetically altered (GA) line			2. Number of animals bred and killed for the maintenance of an established genetically altered (GA) line			3. Number of conventional animals, bred, killed and not used in procedures (excluding those involved in the creation of maintenance of a genetically altered animal (GA) line)			
Species	Collection of organs and tissue	No collection of organs and tissue	Total	Collection of organs and tissue	No collection of organs and tissue	Total	Collection of organs and tissue	No collection of organs and tissue	Total	**Overall total 2022**
Mice	20 632	150 699	171 331	297 785	4 693 602	4 991 387	1 042 956	1 481 184	2 524 140	**7 686 858**
Zebrafish		12 139	12 139	743	491 968	492 711	41 083	136 820	177 903	**682 753**
Rats	27	1 525	1 552	5 925	163 812	169 737	113 086	212 424	325 510	**496 799**
Domestic fowl				646	14 545	15 191	21 713	115 106	136 819	**152 010**
Other fish				312	39 007	39 319	53 945	35 855	89 800	**129 119**
Sea bass					2 335	2 335	1 668	87 728	89 396	**91 731**
Guinea-Pigs					52 376	52 376	3 102	24 636	27 738	**80 114**
Rabbits				130	56 212	56 342	1 930	8 432	10 362	**66 704**
Other amphibians		716	716	23 895	20 975	44 870	7 342	3 312	10 654	**56 240**
Salmon, trout, chars and graylings					7 657	7 657	7 308	37 405	44 713	**52 370**
Xenopus	15	67	82	45	2 651	2 696	3 345	28 484	31 829	**34 607**

Unavoidable surplus: Mendelian math sets the ceiling

- Some surplus is unavoidable.
- Example: experiment requires only female homozygous transgenic mice and litter-mate wild-type controls (het × het mating):

Genotype	% of litter	Male	Female
TgTg (homozygous TG)	25 %	12.5 %	12.5 %
TgWT (heterozygous)	50 %	25 %	25 %
WTWT (homozygous wild-type)	25 %	12.5 %	12.5 %

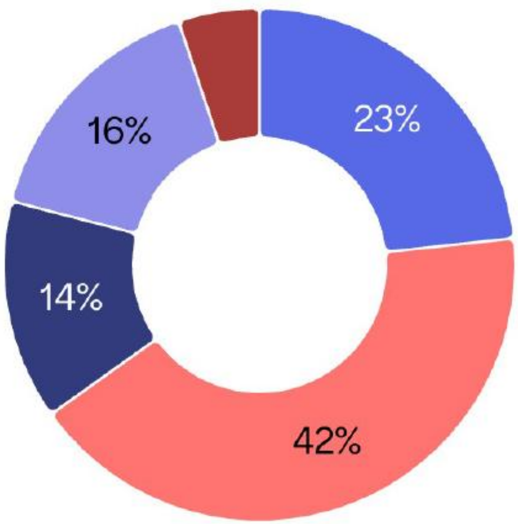
--> 75% of all bred mice are not required.

Avoidable Surplus: Typical Causes

- Lack of training with regard to colony management
- Decentralized breeding management
- Overbreeding due to poor or a lack of planning
- Continuous breeding without real demand
- Lack of communication between caretakers and responsible person
- Keeping unused or rarely used lines on maintenance
- Using only one sex unnecessarily

Mentimeter

How is breeding typically organized in your animal facility?*(Who is primarily responsible for planning and managing the colonies?)*



- 23% Centrally managed by dedicated colony managers or facility staff
- 42% Managed by individual PhD students or technicians within research groups
- 14% Managed by principal investigators (PIs)
- 16% Shared responsibility within research groups (no single person in charge)
- 5% Other

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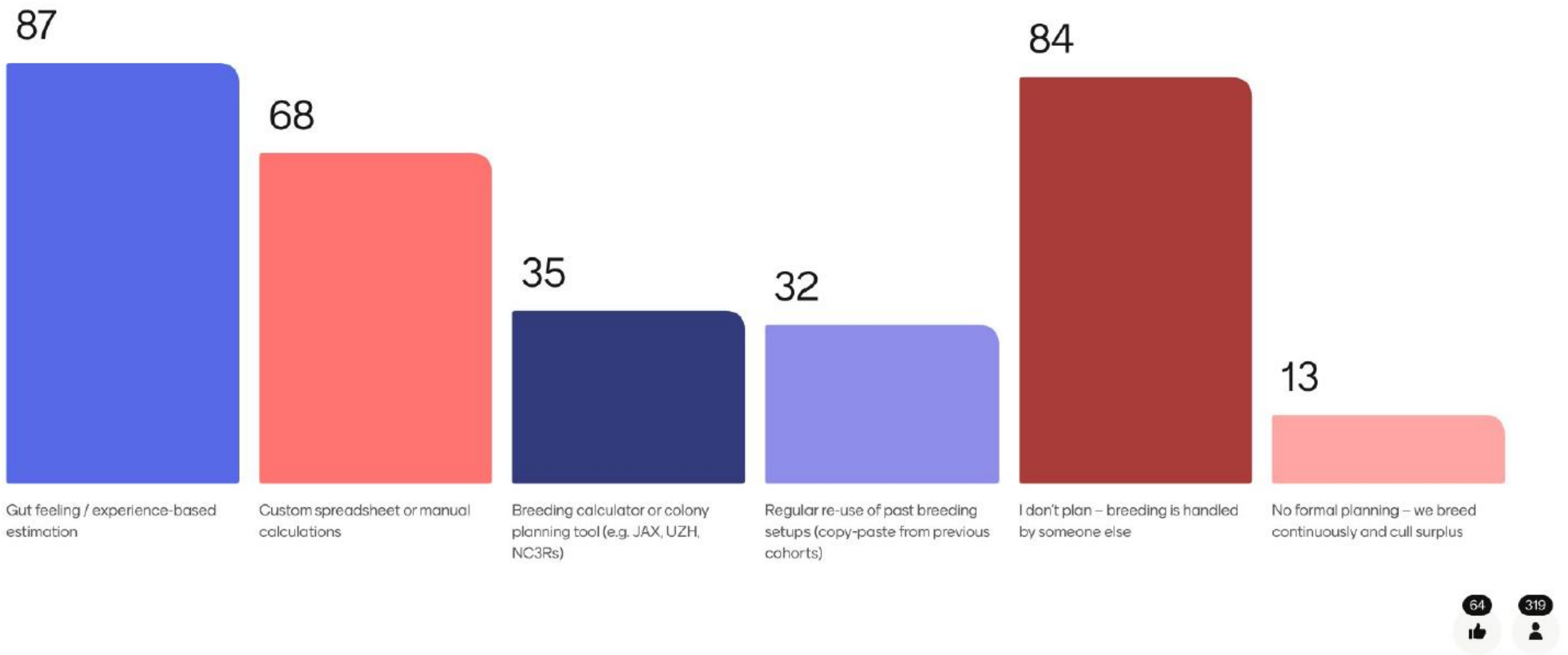
Mentimeter

How do you currently plan your mouse breedings for colony management?*(Select the method that best describes your typical approach)*



Data Driven Breeding –what does that mean?

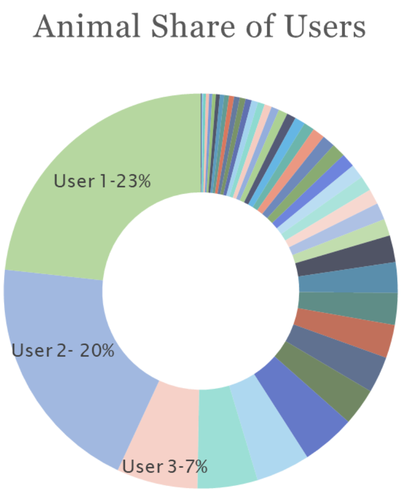
A structured approach to colony management based on real performance data, not guesswork.
It consists of **three key steps**:

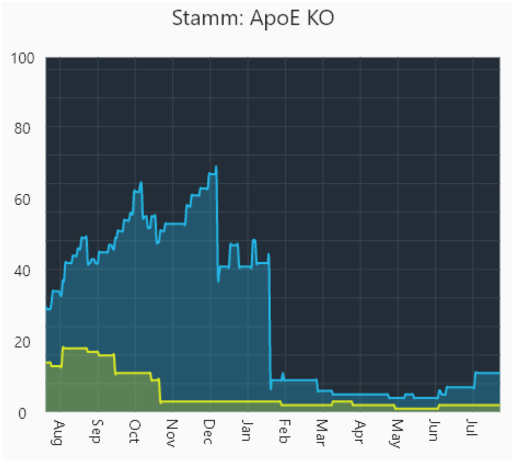
1. **Analyze colony data:** Identify major sources of surplus
2. **Develop targeted strategies:** Use the data to plan actions
3. **Measure outcomes and adjust:** Reassess after implementation

Prerequisite: Animal facility management software or proper documentation!

Data Driven Breeding – which data is useful?

- Breeding vs. Demand:
  - How many animals are used, how many are bred?
  - Are lines not used at all and therefore candidates for cryopreservation?
- Animal share of research groups:
  - Are lines kept in parallel by multiple research groups?
  - Small amount of research groups responsible for majority of animal usage – prioritizing and streamlining communication

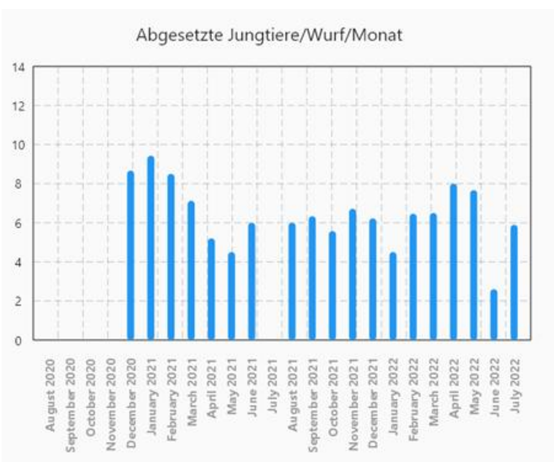


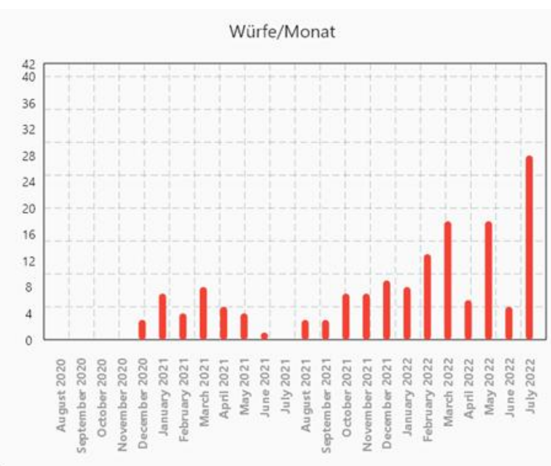


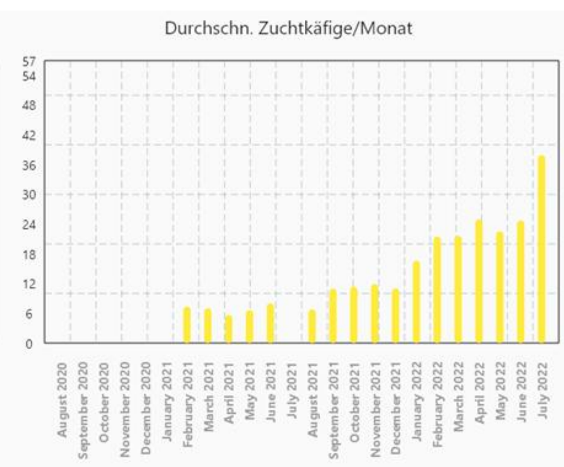
Data Driven Breeding – which data is useful?

- Animal usage – historical data:
  - Seeing trends in demand and react in a timely fashion
- Breeding performance:
  - Weaned pups/litter
  - Litters per month
  - Set up breedings per month

<u>Average litter size:</u> pups per litter
<u>Fertility:</u> How many breedings produce offspring







Data Driven Breeding: Optimizing with Breeding calculators

Various tools to calculate the amount of necessary breedings are available:

- Various Punnett Square Calculators: useful for calculating with multiple mutations
- The Jackson Laboratory Breeding Colony Size Planning Worksheet (additionally takes replacement of breedings into account)
- University of Zürich: Institute of Laboratory Animal Science Breeding Calculator: https://www.ltk.uzh.ch/en/Breeding.html (confidence intervals)
- and the corresponding R-package from Milchevskaya and collaborators: https://github.com/VladaMilch/breedingCalculator (confidence intervals and highly adaptable)

How can I further optimize my breedings using a data driven approach?

- Multiple factors may affect breeding performance:
  - External factors (room climate, lighting, noise, vibration, food, etc.)
  - Internal factors (health status, genotype, stress)
- Breeding performance data that can be found in literature therefore rarely reflects the real performance – calculations can be optimized by using data from animal facility software

Optimisation example – Conventional calculation

<u>A research group needs 100 mixed-sex 129/SvJ:</u>

- Literature breeding performance: 5.9 pups/litter.
- Strain fertility: 80 %.
- Needed breeding cages: 100/(5.9*0.8) → <u>**22 breeding cages**</u>

Optimisation example – New calculation

A research group orders 100 mixed-sex 129/SvJ:

- Actual avg. performance: 7 pups/litter.
- Avg. Strain fertility: 95 %.
- Needed cages: 100/(7*0.95) → **16 breeding cages**

Conventional vs. new – comparison

- Conventional: 22 cages → 154 animals → 54 surplus.
- New: 16 cages → 112 animals → 12 surplus.

→ **37,5% less animals!**

CAUTION: Why under-planning can be even worse

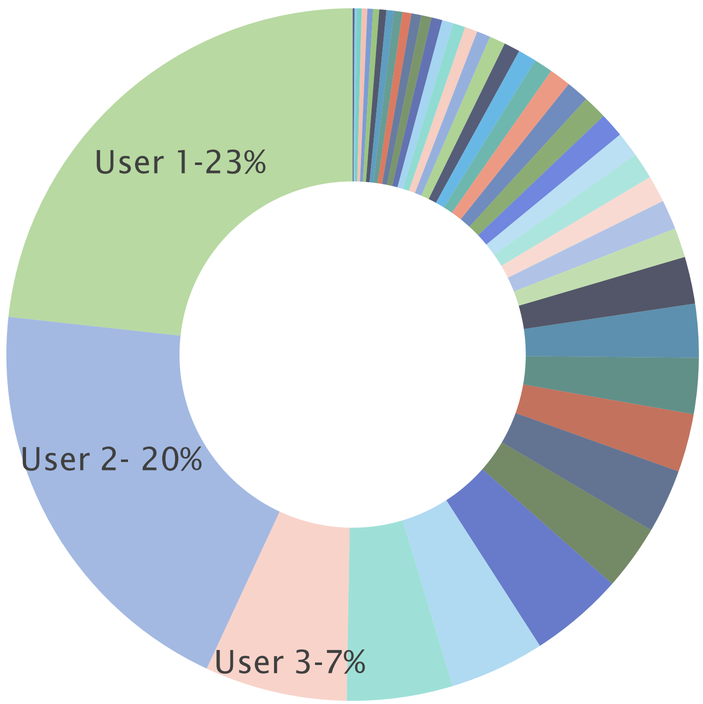
- Too few breeding cages, risk running short → study delay → second breeding round.
- “catch-up” round often doubles the surplus (old cohort can’t be used, new cohort overshoots).
- Goal: avoid both over- and underplanning extremes

Another Example: Lab Animal Facility in Himberg– 2022

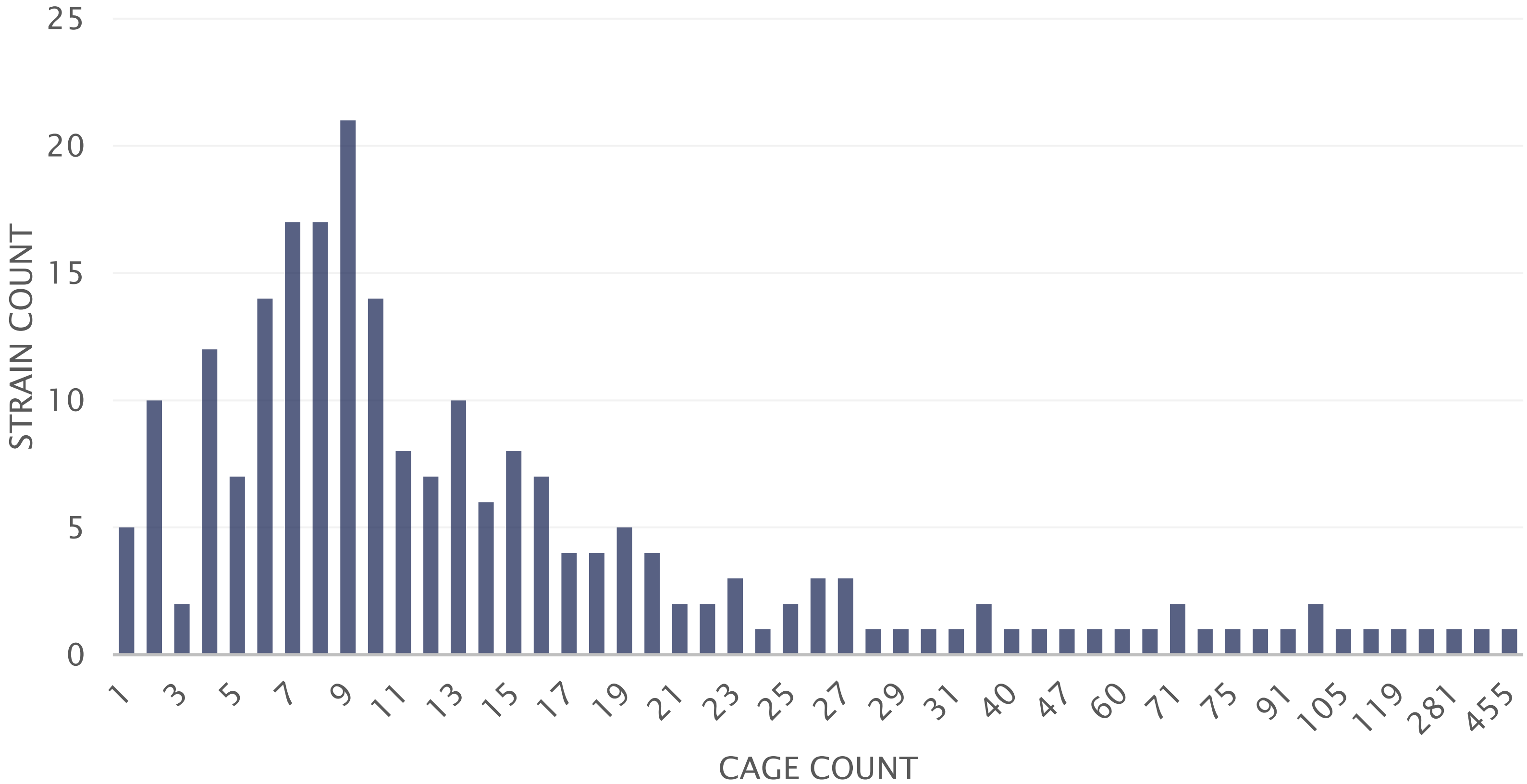
- Centralized breeding facility of the Medical University of Vienna
- Research groups mostly responsible for managing their colonies themselves
- Some colonies – especially shared lines – managed by dedicated colony management team
- 13 000 animals: 12 500 mice, 500 rats
- 220 strains, ≈ 190 genetically modified
- 43 researchers/users

Data Driven Breeding Step 1: Analyzing

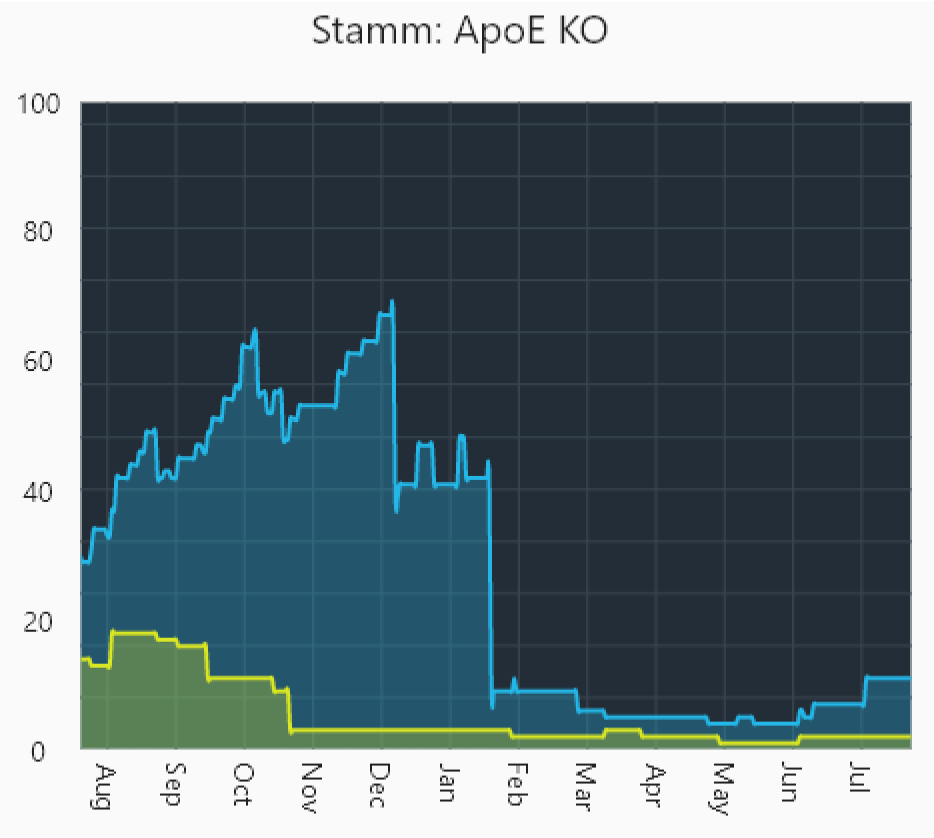
Animal Share of Users



Distribution of strains by number of cages per strain



Individual Strain History



Data Driven Breeding Step 1: Analyzing our Findings

- Animal Share:
  - Few research groups are responsible for the majority of animals
- A lot of research group managed strains are bred on maintenance level:
  - Further analysis shows that some were not ordered for over a year → **Second largest contributor**
- Highly fluctuating demand for wildtype breedings – **<u>largest contributor!</u>**
- Strain level animal history shows that some strains were poorly managed: high breeding cages and frequent culling of surplus

Data Driven Breeding Step 2: Possible solutions at Himberg

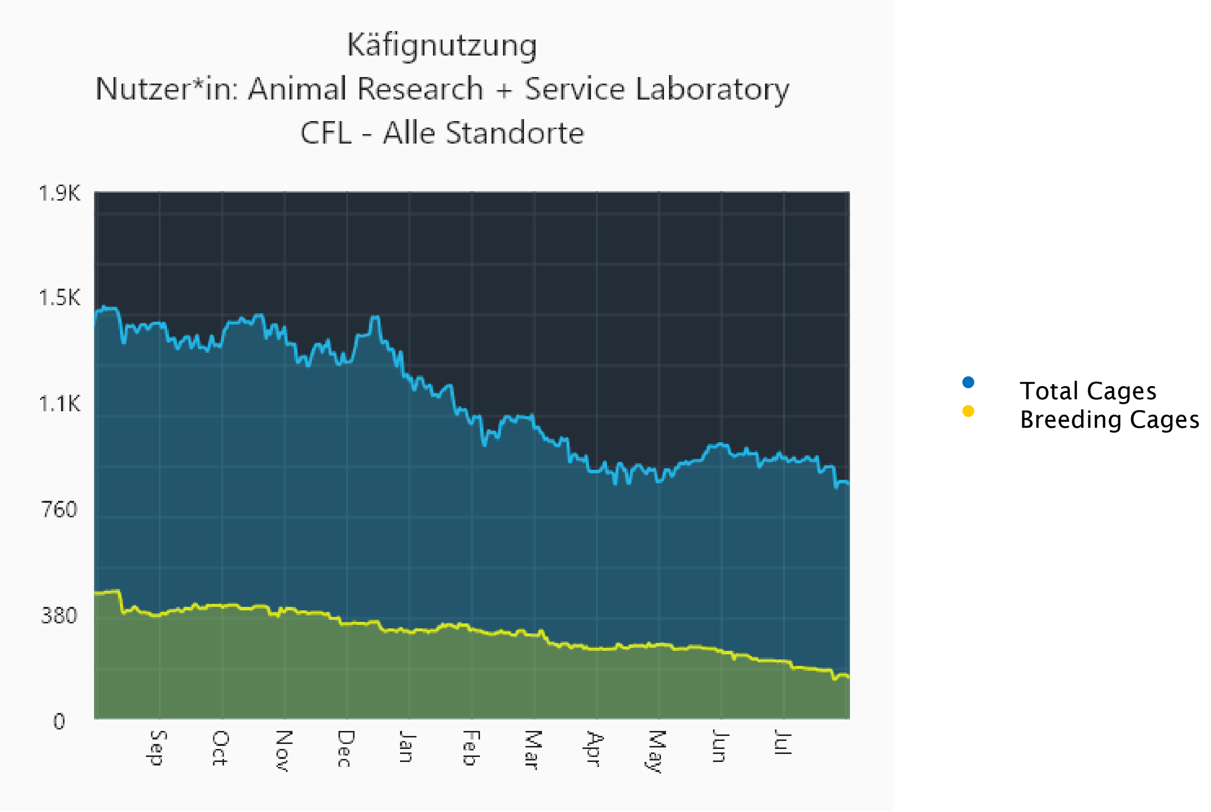
- Outsourcing of centralized breeding of wildtype lines to a commercial breeder
- Increasing the dedicated colony management team to better assist research groups so they can focus on the actual research
- Stronger focus on cryopreservation of unused lines
- Earlier, better communication of real animal demand & cohort planning in dedicated meetings with colony managers

Data Driven Breeding Step 3: Reassess and improve

- Overall reduction in 1 year: 30%
- Communication with research groups was continually improving
- Six lines archived or discontinued → status quo: An additional 20 lines were crayopreserved, more are planned
- An increase of the colony management team was planned: Status quo: we increased the team from 2 to 5 people
- Data retrieval for the facility was a lot of work: “Early-warning” system that pulls data from colony software and visualises trends → development of automated processes

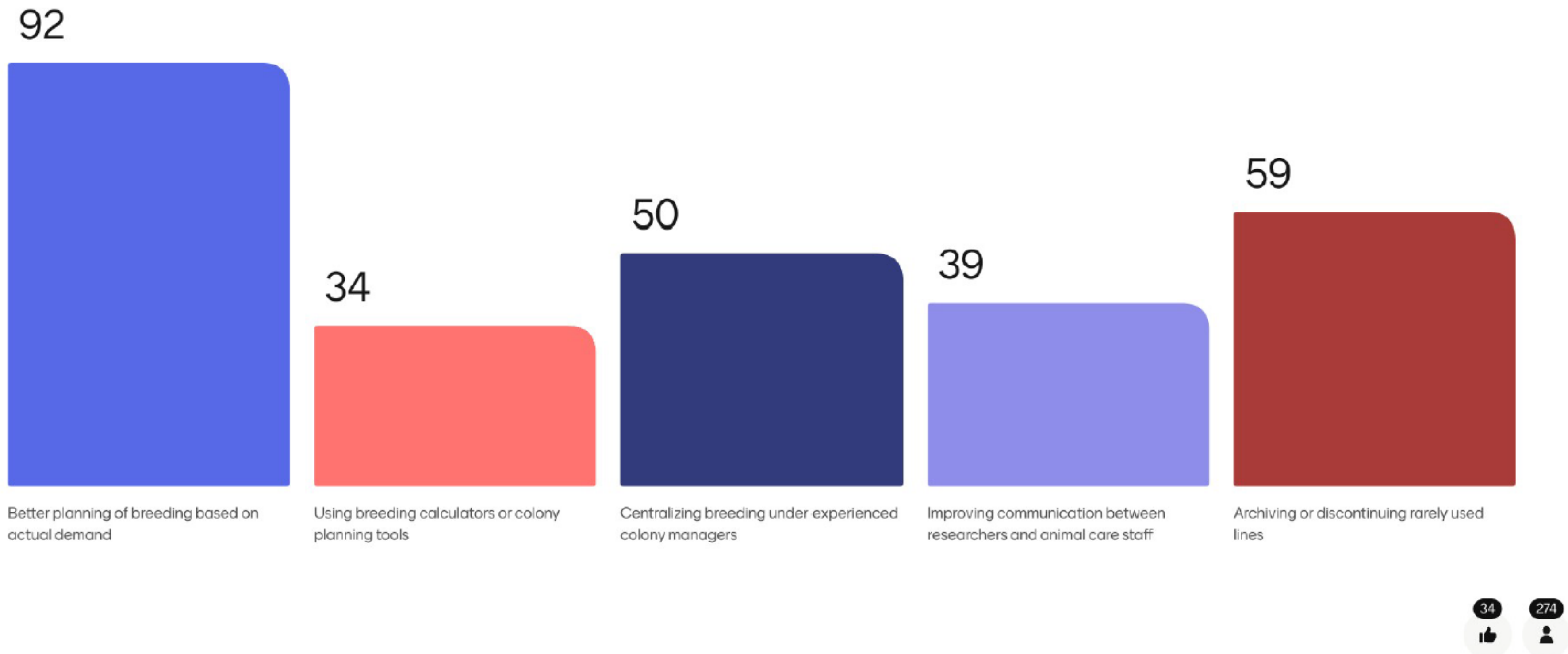
Data Driven Breeding Step 3: Reassess and improve

- Almost halved centrally managed animal numbers within one year.



Mentimeter

Where do you see the greatest potential to reduce surplus animals in your facility?



Take-home message

- Avoid gut-feeling breeding management → collect data for optimisation
- Try to centralize breedings whenever possible
- Good documentation is key for data driven approaches!
- Take your time: Analyze – Implement - Reassess
- Better communication cannot be replaced by automation!
- Outsourcing wildtype breedings to commercial providers might help
- Cryopreservation of unused lines is more often than not the most effective way to vastly reduce animal numbers

Questions?

Resources

- Summary Report on the statistics on the use of animals for scientific purposes in the Member States of the European Union and Norway in 2022: https://d8aaf127-0203-427a-b8b6-1f1b942cd1af.usrfiles.com/ugd/d8aaf1_cf831339d46f4fe4bfcfd0b0d1eb1f37.pdf
- Milchevskaya, V. breedingCalculator. breedingCalculator https://github.com/VladaMilch/breedingCalculator (2022)
- Breeding colony size planning work sheet. The Jackson Laboratory https://www.jax.org/-/media/jaxweb/files/jax-mice-and-services/colonysizewksht.pdf?la=en&hash=8C1CB11B342A9E8177B31DD2D7A7BFC6414628AC (2023).

Thank you for your attention!