



The 8th edition of *the Guide For The Care And Use Of Laboratory Animals* states that single housing of a social species should be the exception to housing standards. Based upon these guidelines we have developed a process of pair housing our colony of New Zealand White rabbits. Adult non-related females in addition to male and female related weanlings have been effectively paired using our pair housing process.

Previous studies have found adequate environmental enrichment may reduce anxiety and stress reactivity. Therefore, we hypothesized that environmental enrichment would facilitate greater success in pair housing by decreasing aggressive behaviors that arise around the age of sexual maturity (12-17 weeks).

We carefully track the rabbits' ages such that we can increase enrichment around 12-17 weeks and closely observe the rabbits for visual cues of behaviors that may precede aggression. These behaviors include excessive urine spraying, barbering and increased chasing. All cages are provided with at least 1 toy plus food treats as approved by our environmental enrichment committee. If any of these aggressive behaviors are observed, extra enrichment is added to the cage from that point forward. This additional enrichment includes loose hay daily in addition to novel interaction items three times per week (Figure 4).

- 63 pairs of New Zealand White rabbits bred in house (26 male pairs and 37 female pairs)
- Rabbit Pairing Enrichment Sheet (Figure 1)
- Double cages with dividers - Allentown and Techniplast cages were used (Figure 2)
 - Allentown Inc., Allentown, NJ., and Techniplast, West Chester, PA.
- Dedicated room and staff to ensure consistent care, enrichment and monitoring
- Spray water bottle to discourage negative behaviors
- Thick leather gloves to intervene in aggressive interactions
- Nontoxic Ketchum markers for identification
 - Ketchum Manufacturing Inc., Brockville, ON.

[illegible]

A photograph showing two white rabbits inside a wire cage. They are both reaching towards a brown paper bag that is placed on a dark surface. The rabbit on the left is looking at the bag, while the rabbit on the right is reaching out with its front paws. The cage floor is covered with green hay. The cage walls are made of metal wire.



Poster

Figure 4. Novel Enrichment Items

a. Hay stuffed ball

b. Bag stuffed with hay, treats or toys

c. Brush for grooming

d. Box stuffed with hay, treats or toys

e. Cardboard tube stuffed with hay

f. Cardboard tube stuffed with Crink-I-Nest™

-The Andersons Inc, Maumee, OH

g. Various Toys

h. Wood Gnawing block

-Bio-Serv, Flemington, NJ

i. Manzanita Wood Gnawing Stick

-Bio-Serv, Flemington, NJ

j. Lavender essential oil applied to toys or linens

k. Frozen fruit

l. Classical music

m. Carrot and parsley stuffed ball

- 34 successful pairs (22 female and 12 male pairs)
- Oldest current male and female pairs both 42 weeks old

Reasons Pairs Were Separated

Reason	Percentage
Fighting	6%
Lab Use	40%
Currently Paired	54%

Sex	Fighting	Lab Use	Currently Paired
Male	5	13	15
Female	2	17	25

Age When Negative Behaviors First Noted
**Gray Bar Denotes Age of Sexual Maturity 12-17 Weeks*

Pair Designation	Age (In Weeks)	Behavior Type
a	27	Separated for Fighting - 50% during sexual maturity
b	29	Separated for Fighting - 50% during sexual maturity
c	27	Separated for Fighting - 50% during sexual maturity
d	13	Separated for Fighting - 50% during sexual maturity
e	9	Separated for Fighting - 50% during sexual maturity
f	17	Separated for Fighting - 50% during sexual maturity
g	19	Separated for Fighting - 50% during sexual maturity
h	34	Separated for Fighting - 50% during sexual maturity
i	18	Separated for Fighting - 50% during sexual maturity
j	27	Separated for Fighting - 50% during sexual maturity
k	22	Separated for Fighting - 50% during sexual maturity
l	13	Separated for Fighting - 50% during sexual maturity
m	13	Separated for Fighting - 50% during sexual maturity
n	22	Separated for Fighting - 50% during sexual maturity
o	14	Separated for Fighting - 50% during sexual maturity
p	18	Separated for Fighting - 50% during sexual maturity
q	40	Separated for Lab Use - 33% during sexual maturity
r	13	Separated for Lab Use - 33% during sexual maturity
s	32	Separated for Lab Use - 33% during sexual maturity

Legend:
■ Separated for Fighting - 50% during sexual maturity
■ Separated for Lab Use - 33% during sexual maturity
■ Currently Paired - 25% during sexual maturity

- Pair housed rabbits exhibit less abnormal behaviors and more locomotor behaviors than singly housed rabbits (Chu, Garner & Mench, 2004), and environmental enrichment can enhance the environment and well-being of laboratory animals (Baumans, 2005). Utilizing both of these ideas, we were able to improve the rabbits' quality of life by maintaining the pair housed experience for as long as experimentally possible.
- Figure 5 shows that since increased enrichment has been utilized on a regular basis with pairs exhibiting aggressive behaviors, only four pairs have had to be separated due to fighting (6%). Even after male pairs were found with minor fight wounds, we were able to de-escalate aggressive interactions and maintain the pairs with increased enrichment (n=4). Additionally, adding extra enrichment prevented the reoccurrence of new fighting-related lesions. One of these pairs was marked for increased enrichment but due to accidental oversight did not receive it, which led to them ultimately being separated for fight wounds. This data shows that increased environmental enrichment does have a positive effect on troubleshooting aggressive behaviors in pair housed rabbits.
- Figure 6 shows that we have had success maintaining both female and male pairs. Though all four pairs that had to be separated for fight wounds were males, there are still 12 successful male pairs in the program (35% of maintained pairs).
- Only 30% of the pairs demonstrated aggressive behaviors and Figure 7 specifically addresses when aggression was first noted among that 30%. Of those, at minimum a quarter of negative interactions began during sexual maturity (12-17 weeks). When also considering the weeks surrounding sexual maturity (+/- 3), 47% of negative interactions began during this time. This shows the importance of monitoring ages of pairs to watch closely for signs of aggressive behaviors in the weeks surrounding sexual maturity.
- One of the main challenges that we faced during this project was determining which aggressive behaviors were serious enough to warrant separation and which pairs could be monitored and maintained. We ultimately decided to go on a case-by-case basis to determine which pairs could stay together and which needed to be separated. The determining criteria was the location and the severity of the fight wounds (e.g. genital vs. superficial ear lesion). If veterinary staff determined that the pair could be maintained, we increased the enrichment even further and monitored closely several times per day.
- Working closely with the laboratory personnel was vital during this project to educate them on what constitutes a separation worthy behavior. They were taught how to submit an animal treatment report (ATR) to the veterinary staff to report aggressive behaviors instead of automatically separating the rabbit pairs. This eliminated the unnecessary separation of pairs that could have been kept together with increased enrichment and monitoring.

To decrease aggressive behaviors and increase the number of pairs that are maintained successfully past sexual maturity

- Increase monitoring around the age of sexual maturity
- Increase environmental enrichment
- Utilize a variety of enrichment items to maintain novelty

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