COREMI COST Action FA1404

A structured design approach with focus on the PRM problem in laying hen facilities.

Summary and results of ‘a short introduction into’ or a ‘tasting of’ the structured design approach focussing on the PRM problem in laying hen facilities.

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FOREWORD AND INTRODUCTION

Dear all,

I thank you for attending the COREMI design session last Wednesday April 12th in London. I summarize the afternoon session as informative on the limited knowledge available on *Dermanyssus gallinae*'s biology, but also informative on the wide variety of possibilities for manipulating the mite population in laying hen facilities.

Our aim was to give a short introduction into/ provide you a 'tasting' of the **structured design** approach **with focus on the PRM problem in laying hen facilities**. The design approach method aim to assist the methodical process of design by ensuring that developers "avoid jumping to conclusions, obtain a good overview of the stages in the designing process, reduce the chances for overlooking essential items, facilitate the taking of justifiable decisions, and to increase the chances on feasible design" (Siers, 2004). As a form of structured design, the Reflexive Interactive Design (in Dutch: Reflexief Interactief Ontwerpen or RIO) approach has been successfully used in the design of new concepts for husbandry systems for pigs, laying hens, broiler hens, goats, and dairy cows (Bos, 2010) and of semi structures in animal facilities (Van Weeghel, Bos, Spoelstra, Groot Koerkamp, 2016), of which some have been further developed and realized by industry actors (e.g. Spoelstra et al., 2013). RIO as described by Bos, Groot Koerkamp, Gosselink, and Bokma (2009) is an interdisciplinary interactive method for structured design and consists of three stages: 1) "system and actor analysis", 2) "structured design" and 3) "anticipating niche and structural change". These three stages of RIO each consist of several steps. The stages are shown in Fig. 1 with the three circles intended to show how, upon realising new insights, stages may be repeated.*)

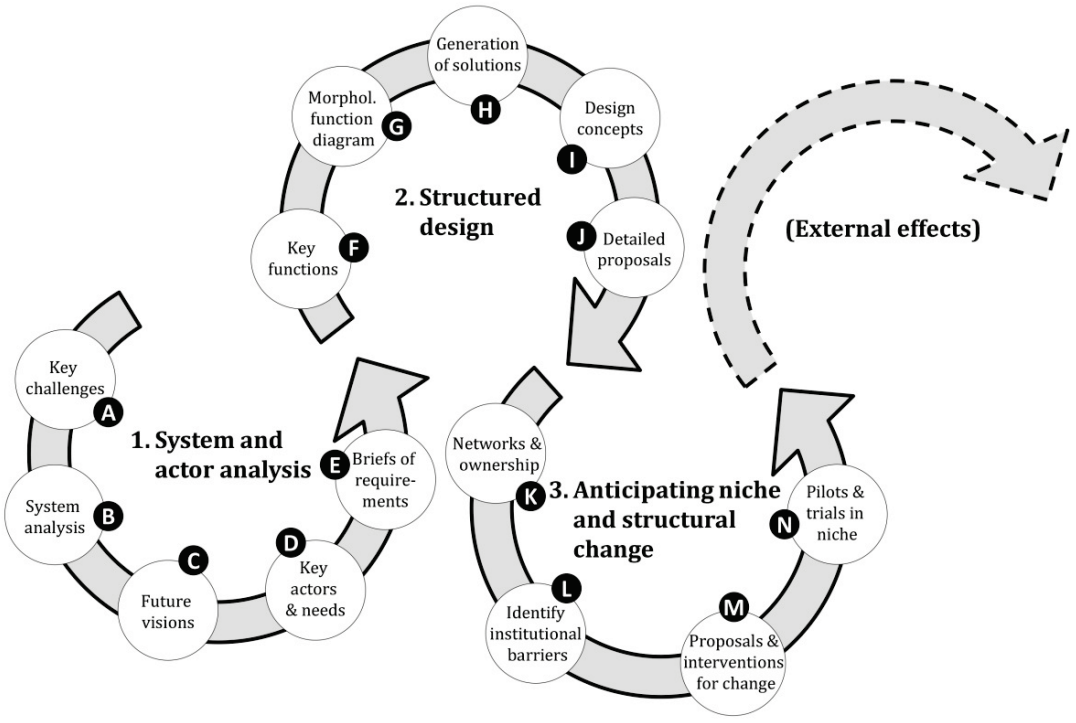


Figure 1 Scheme of the design approach used in Reflexive Interactive Design (in Dutch: RIO) (Bos et al. 2009)

*)Partly from Mul MF, Ploegaert JPM, George DR, Meerburg BG, Dicke M, Groot Koerkamp PWG (2016) Structured design of an automated monitoring tool for pest species. Biosyst Eng 151: 126-140

Together we worked on parts of B (system boundary: egg producing laying hen husbandry system), C (the aim we described together) , D (needs of the key actor poultry red mite *Dermanyssys gallinae*), E (Brief of requirements of the poultry red mite) and H (generation of solutions). Below the discussion and the results of each step of the design session is summarized.

We hope you enjoy reading this document. Do not hesitate to disseminate the findings and to encourage people and organisations to explore the solutions and putting it forward with the final aim to put the ideas mentioned in this document into practice.

Wageningen, Monique Mul

SUMMARY AND RESULTS

C. AIM

Our ideal was to have no poultry red mites (PRM) in the egg laying hen facilities. However we concluded that it is more realistic to aim for the presence of poultry red mites in egg laying hen facilities without negative effects on a) production , yield, costs, b) staff, working, health and c) on laying hen welfare and health.

D. PRM NEEDS

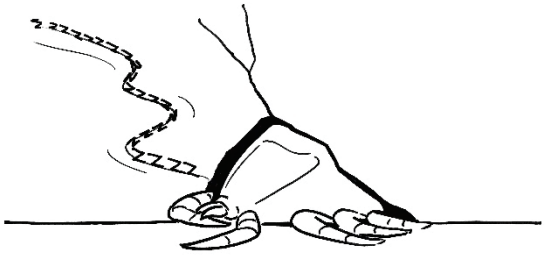
We discussed the needs of the poultry red mite *Dermanyssus gallinae*. With taking into account the discussion, the needs of the poultry red mite are now described in the table 1. It was suggested to make a difference in the needs and the more specific requirements for female mites and male mites.

Table 1 Concept version 5 of the needs of the poultry red mite; their individual needs, a description of the need and an example of a more specified requirement of the described need

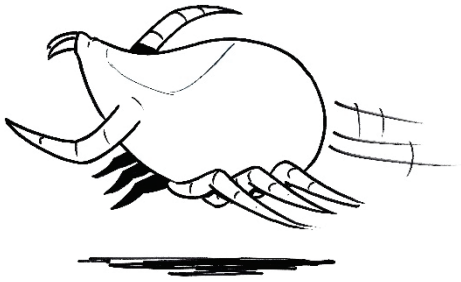
Individual need	**Description**	*Example of a requirement*
Saturation	*Dermanyssus gallinae* need blood to fulfil their lifecycle and to reproduce	*Access to food*
Rest/ Thigmotactics	*D. gallinae need to rest (post prandial)*	*Place to rest, place close to the host, presence of conspecifics*
Movement	*D. gallinae moves to find food, water, a resting/ hiding place and to explore the environment.*	*Space and place to move*
To map and explore the environment	*D. gallinae need to map and explore the environment to identify e.g. conspecifics, food, laying hen, and to avoid dangerous situations.*	*Methods and means to orientate e.g. sensors, attractants, repellents*
Respiration	*D. gallinae needs to diffund air to live*	*Good air (quality)*
Health	*D. gallinae need to be healthy (function normally) and in good condition without diseases, wounds, damage.*	*Conditions to stay healthy, absence of conditions leading to disease, wounds or damage*
Excretion	*D. gallinae excretes the remains of a digested blood meal*	*Place to excrete*
Suitable living environment for the poultry red mite	*D. gallinae need physical comfort for a maximum longevity*	*Temperature, humidity, structure*
Population need	**Description**	**Example of a requirement**
Reproduction	*D. gallinae populations need to reproduce / lay eggs*	*Presence of males and females and place to reproduce*
Development	*D. gallinae need to fulfil their lifecycle*	*Food, temperature, humidity*
Aggregation/ Social structure	*D. gallinae* form aggregations and live in social structures	*Presence of other D. gallinae*

The needs of *Dermanyssus gallinae* individuals are depicted on page 3-6.

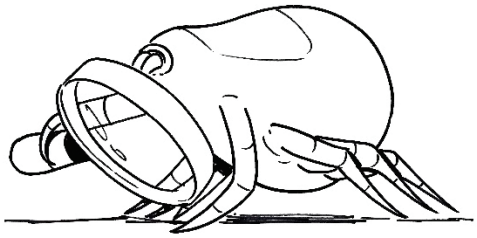
Saturation: *Dermanyssus gallinae* need blood to fulfil their lifecycle and to reproduce.



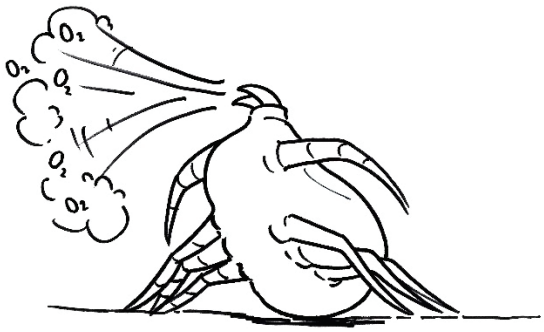
Rest/Thigmotactics: *Dermanyssus gallinae* need to rest.



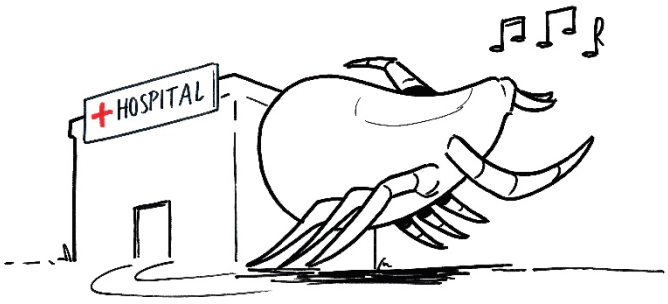
Movement: *Dermanyssus gallinae* moves to find food, water, a resting/ hiding place and to explore the environment.



To map and explore the environment: *Dermanyssus gallinae* need to map and explore the environment to identify e.g. conspecifics, food, laying hen, and to avoid dangerous situations.

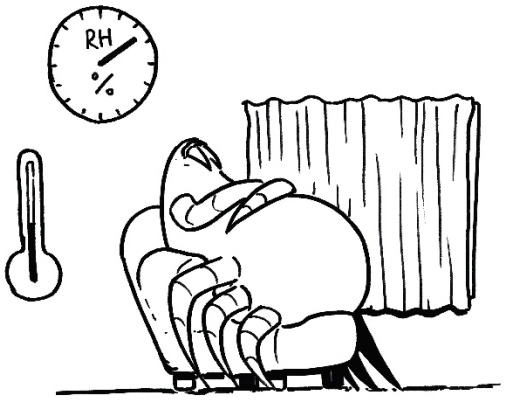


Respiration: *Dermanyssus gallinae* need to diffund air to live.



Health: *Dermanyssus gallinae* need to be healthy (function normally) and in good condition without diseases, wounds, damage.

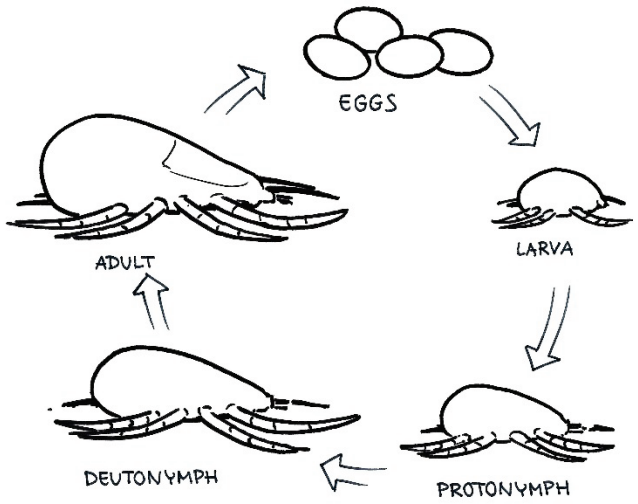
Excretion: *Dermanyssus gallinae* excretes the remains of a digested blood meal.



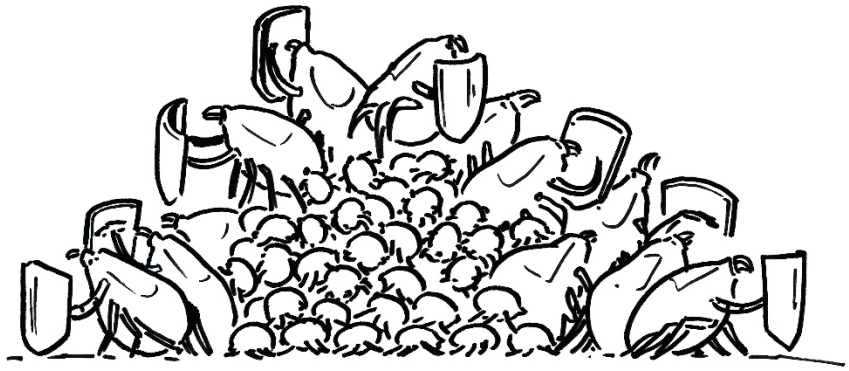
Suitable living environment: *Dermanyssus gallinae* need physical comfort for a maximum longevity.

The next three drawing depict the needs of *Dermanyssus gallinae* populations.

Reproduction: For survival of a *Dermanyssus gallinae* population reproduction is a need.



Development: For survival of a *Dermanyssus gallinae* population individuals fulfilling their lifecycle is a need.



Aggregation/social structure: For survival of a *Dermanyssus gallinae* population aggregations and living in social structures is a need.

E. PRM BRIEF OF REQUIREMENTS

We shortly discussed the brief of requirements (Version 4, see appendix). In this brief of requirements table, some data was collected from the literature and some data was expert knowledge. This list with specifications was not easy to read by the participants. The participants suggested to re-arrange this Brief of requirement. One suggestion was to split this table into a table with literature knowledge and a table with expert knowledge. It must be kept in mind that not all knowledge acquired in laboratory is applicable in the hen house situation.

KNOWLEDGE QUESTIONS

Along the trajectory we came across knowledge questions. A selection of the question are listed below.

Knowledge questions:

- Clustering is not uncommon in Mesostigmatid mites such as the poultry red mite *Dermanyssus gallinae*, but is it a need for them?
- How do females and males communicate?
- What does a female need to lay her eggs
- When and how do females and males come together
- What are the advantages of aggregations. Is there a social need?
- Do mites show maternal care?
- What does PRM excrete?
- Is there a need for water (any kind; humidity or fluid water)
- What do we know about life expectancy and what knowledge is missing
- Do PRM need darkness in rest or is darkness only related to the activity of the hen, or related to "safety"?
- What is the effect of the different spectrums of light on the mites activity?
- Are they hindered in rest with vibration?
- Are there less attractive hens? And is it possible to breed less attractive hens?

We concluded that not much is known about the poultry red mite and that, to find new solution for the PRM problem or new ways of PRM control, research should focus on e.g. the mite preferences, behaviour and biology, including potential diapause and reproduction, physiology, host searching, host location and host acceptance mechanisms.

INTERVENTION POSSIBILITIES

For three "Needs" of PRM we explored the possibilities to intervene in the PRM problem in egg producing laying hen husbandry systems; for the need "rest", "movement" and "saturation". Interventions approached in two ways; what to do and what not to do.

Rest

We started with the need "rest" and looked at the requirements in the table: resting place, hiding in cracks & crevices. Intervention were:

- Make sure no hiding places are available
- Create preferred hiding places
- Provide a PRM resting place nearby the laying hens nightly resting place
- Make a PRM resting place at a long distance from the laying hens nightly resting place
- Provide a preferred resting place
- No resting places are available close to the laying hens nightly resting place
- Place a physical barrier between the laying hen and the mites resting place (like water)
- Hinder the resting process

Movement

Requirements related to movement are: space, air velocity, darkness (?), surface. Suggested intervention were:

- Physical hindering of movement: air, water, electricity
- Affect the mites orientation mechanism
- Move towards something attractive
- Move PRM
- Move hens
- Manipulate circadian rhythm, dark-light perception, force to move outside the comfort (dark-light)

Saturation

Requirements related to the need saturation are: blood, presence of the laying hen. A requirement was added to the list: PRM needs a membrane to puncture.

Suggested intervention were:

- Absence of laying hens
- Blood is toxic for PRM
- No mouth parts to puncture
- Make hen blood less attractive
- Provide a more attractive blood source
- Something in the blood that triggers
- PRM without need for blood or less blood

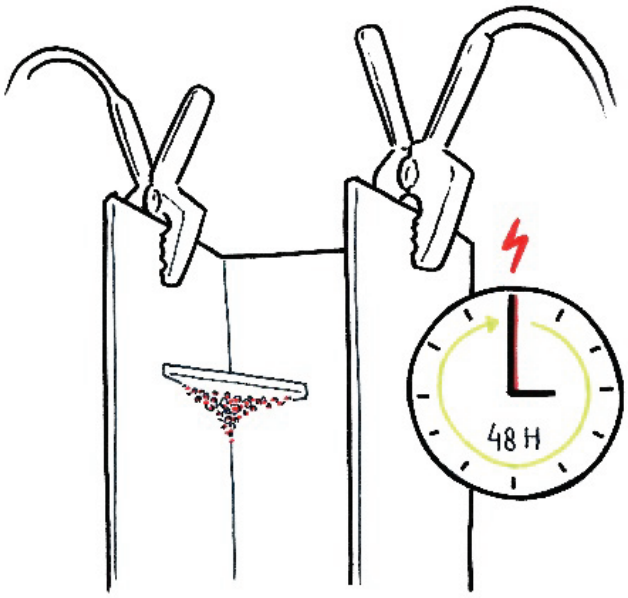
H. GENERATION OF SOLUTIONS

How the found interventions can be realised is the next step of the design. For each need we explored how we can intervene, and thus find solutions, by using technologies, humans, nature and animals.

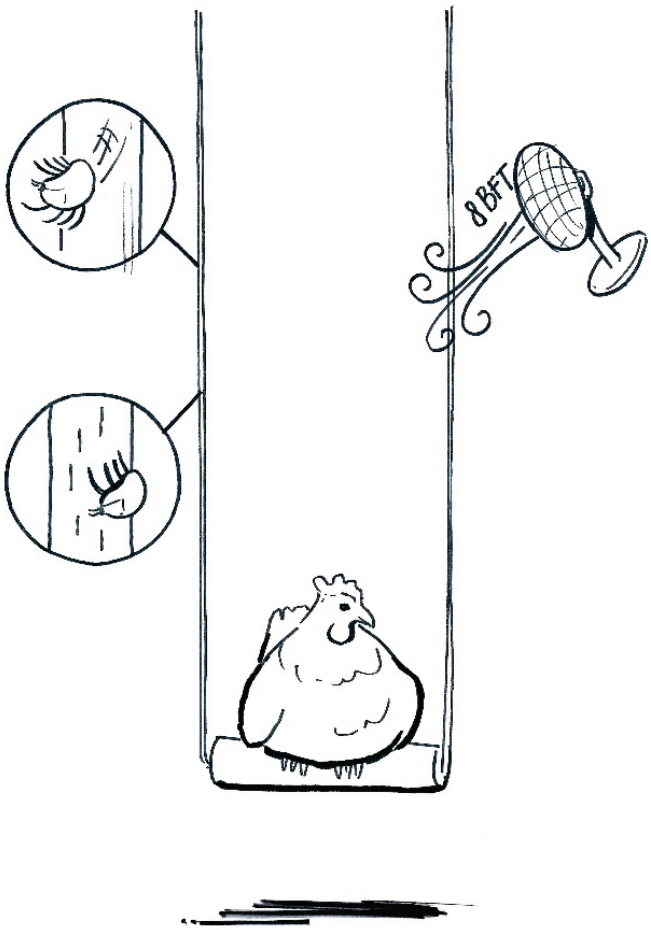
Rest

Found solutions related to the intervention as 1) providing hiding places for *Dermanyssus gallinae*, 2) making sure no hiding places are available in the housing systems, and 3) making a long distance from the mites hiding place to the laying hens nightly resting place are depicted in the Solution 1-3.

Solution 1: Producers of housing systems discussing the *Dermanyssus gallinae* problem together with *Dermanyssus gallinae* experts to find the best material and construction methods enabling to avoid creation of hiding places in the housing system.



Solution 2: Creating a suitable living environment in the vertical post/beam and apply electrocution to kill the collected *Dermanyssus gallinae* population

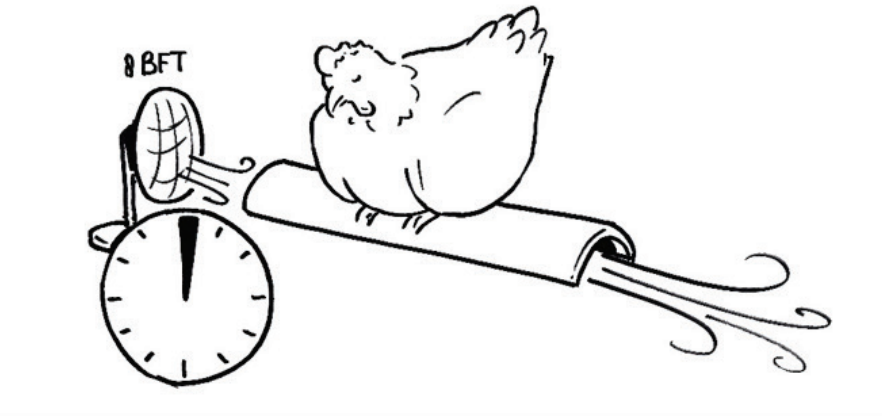


Solution 3: Creating a long distance between the hiding place of *Dermanyssus gallinae* and the birds sleeping place during darkness and obstructing the route by making it slippery, sticky or creating circumstances obstructing *Dermanyssus gallinae* to walk such as blowing with 8 Bft.

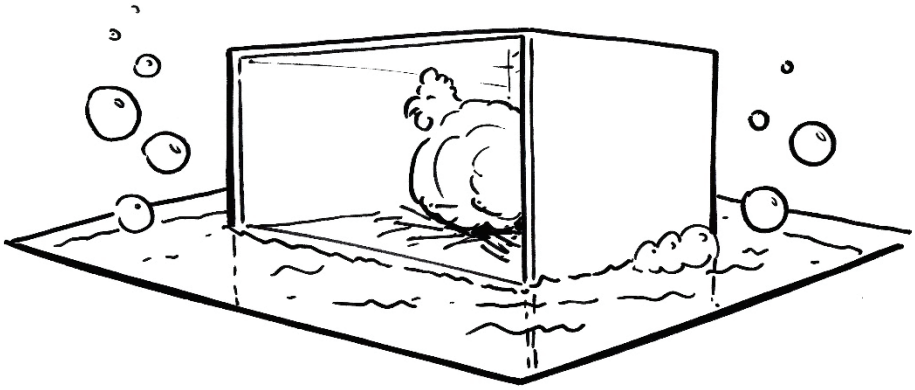
Movement

Found solutions to physically hinder movement of *Dermanyssus gallinae* (with for example water, air, electricity) are depicted in Solution 4-6.

Solution 4: Hindering *Dermanyssus gallinae* to use the vertical beam or post as a highway from their hiding place to the laying hen during the dark hours.

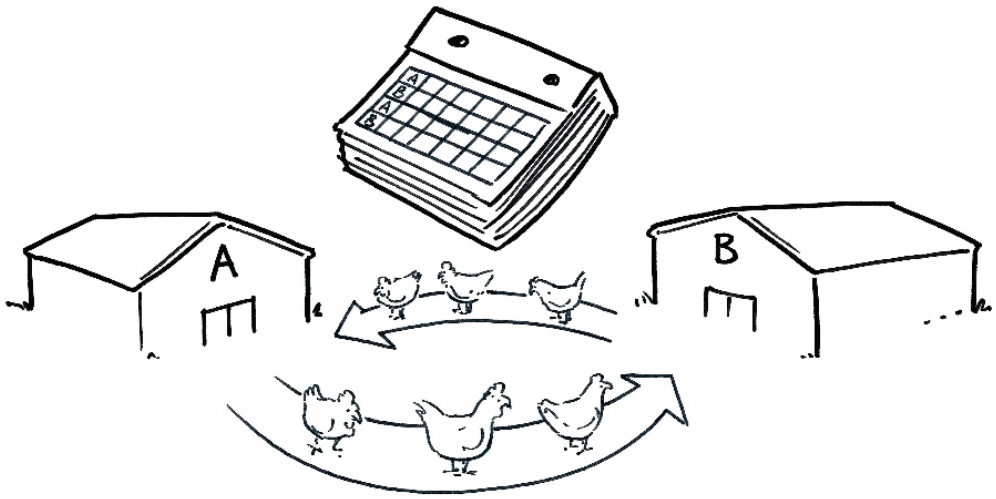


Solution 5: Obstructing walking of *Dermanyssus gallinae* in a hollow perch by blowing wind with an air velocity of 8 Bft. Or providing a hiding place under the perch and cleaning the hiding place by irregularly blowing wind with an air velocity of 8 Bft. Another idea was illumination of UV light under the perch.



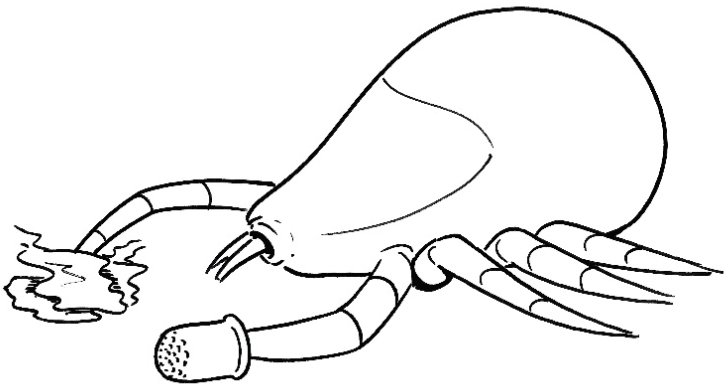
Solution 6: Obstruct movement from *Dermanyssus gallinae* towards the birds resting places (laying nests, perch) using canals with water and detergent, or bio oils.

Found solution to move laying hens is depicted in Solution 7.

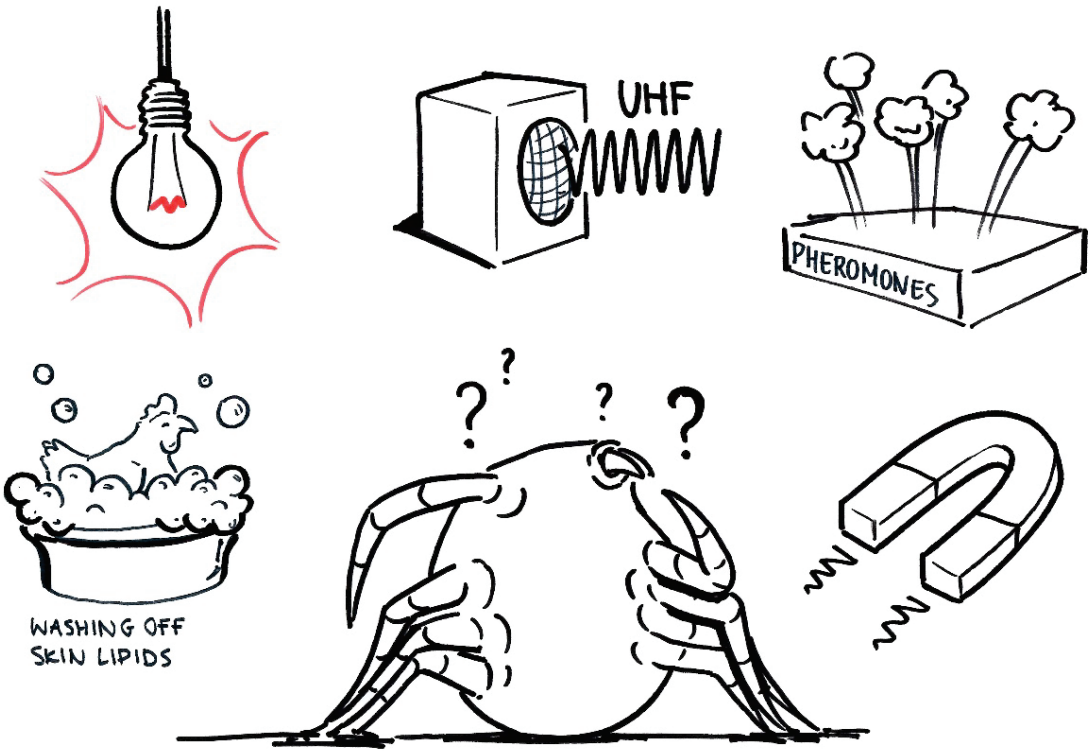


Solution 7: Moving hens on a weekly bases to another hen house enabling cleaning and creating long distances and disturbing the behaviour of *Dermanyssus gallinae*.

Found solutions to affect the orientation mechanisms of *Dermanyssus gallinae* are depicted in Solution 8 and 9.



Solution 8: Affecting the orientation mechanisms of *Dermanyssus gallinae* by blocking the sensors of *Dermanyssus gallinae* using "gloves'', "thimbles", or (volatile) substances attached to the sensors.

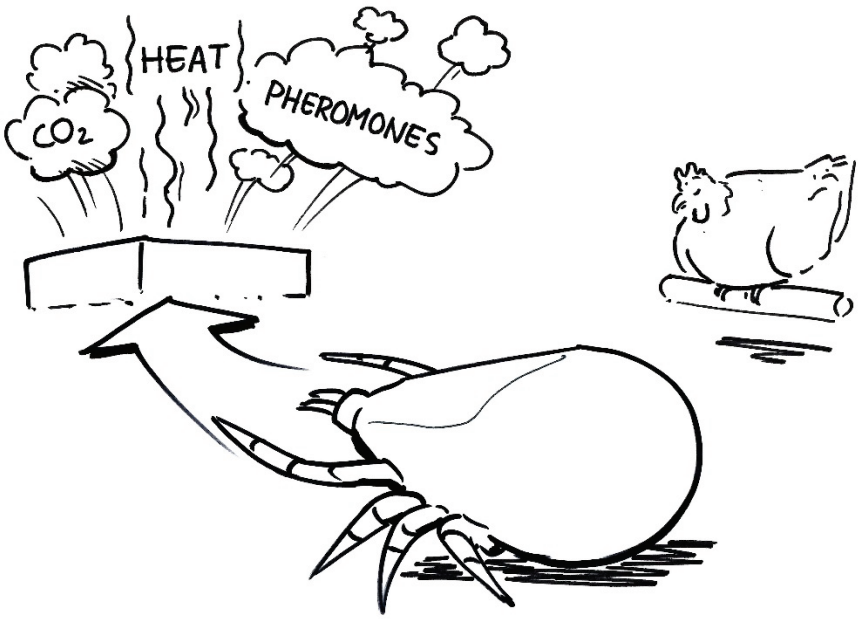


Solution 9: Affecting the orientation of *Dermanyssus gallinae* by, for example, red light or infra red light, ultra high frequency sound, pheromones, magnetics fields or washing of skin lipids (non proven ideas).

Found solution to move *Dermanyssus gallinae* is depicted in Solution 10.

Solution 10: A hollow perch providing a hiding place during the dark hours rotating during the afternoon enabling the hens to feed on *Dermanyssus gallinae* thus reducing the mite population.

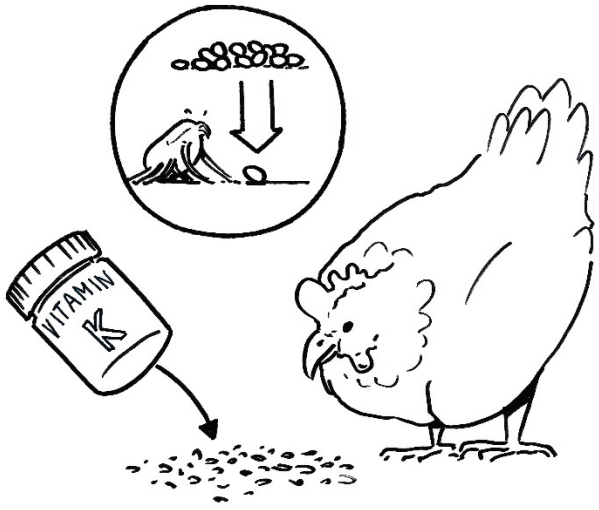
Found solution to move *Dermanyssus gallinae* to something more attractive is depicted in Solution 11.



Solution 11: Luring *Dermanyssus gallinae* away from the laying hen using attractive boxes with CO2, heat, and pheromones.

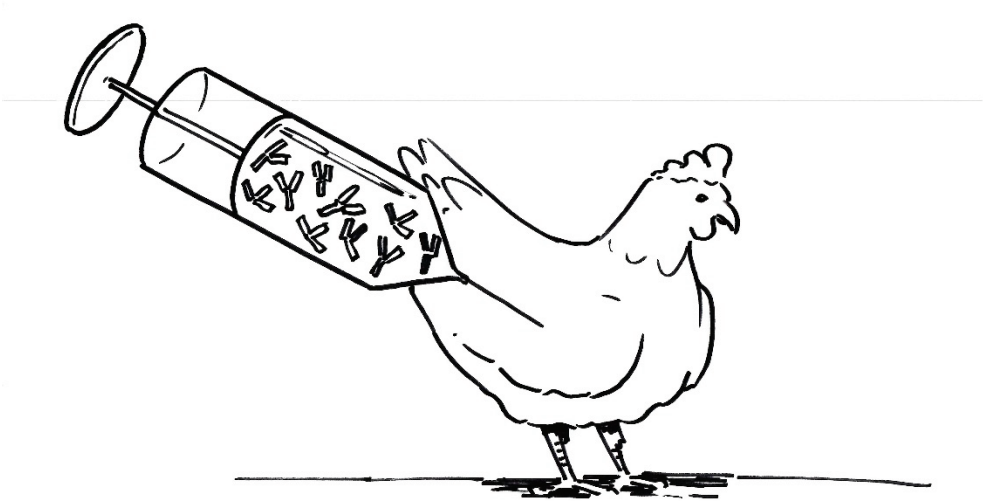
Saturation

Found solutions to alter the hens blood are displayed in Solution 12-14.



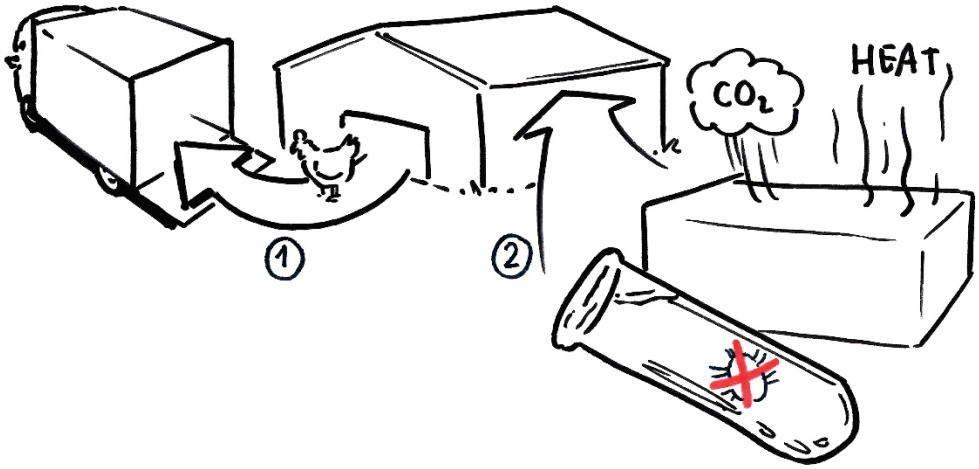
Solution 12: Feeding vitamin K will make the hens blood viscous. Most likely it affects the egg production of *Dermanyssus gallinae*.

Solution 13: Adding *Dermanyssus gallinae* unfriendly products to the hens feed or water.



Solution 14: Vaccinating the laying hen with antibodies against *Dermanyssus gallinae*.

Found solutions to create alternatives for hens blood to feed *Dermanyssus gallinae* are displayed in Solution 15.



Solution 15: In between two production flocks, when the laying hen house is empty, *Dermanyssus gallinae* present in the hen houses and hiding in the cracks and crevices are attracted towards a membrane and product affecting the survival of Dermanyssus galllinae (e.g. mite toxins, lysotomes).

CONCLUSION

There is limited knowledge on the biology of the poultry red mite *Dermanyssus gallinae.*
Within an afternoon we were able to generate multiple solutions for reducing the number of PRM or limiting the negative effects of PRM bites on laying hens in egg producing laying hen husbandry system.

FOLLOW UP

The found solutions need to be further detailed. Organisations looking for new solutions, however, can use the findings.
More and other solutions can be generated using the same structure for the needs which were not dealt with (e.g. health and reproduction).

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Bos AP, Groot Koerkamp PWG, Gosselink JMJ, Bokma S (2009) Reflexive interactive design and its application in a project on sustainable dairy husbandry systems. Outlook Agric 38 (2): 137-145

APPENDIX 1
Brief of Requirements of the Poultry Red Mite Version 4: April 10 2017

Code	Need/ requirement	specification of the need	Further specification	Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
Saturation								
ST1	*Dermanyssus gallinae* need blood to fulfil their lifecycle and to reproduce	Protonymphs need blood	Quantity of blood	±0.023 mg blood		Sikes and Chamberlain, 1954	Lab	Without blood the mite can survive for as long as 9 months. Nordenfors et al., 1999 Bird blood is preferred but blood from mice and other warm blooded vertebrates are also considered as food Sikes and Chamberlain, 1954
ST2		Deutonymph need blood	Quantity of blood	±0.052 mg blood		Sikes and Chamberlain, 1954	Lab	
ST3		adults need blood	Quantity of blood	±0.204 mg blood		Sikes and Chamberlain, 1954	Lab	
		Presence of sufficient blood/ hosts						
ST5	D. gallinae need water	Presence of water (?)				Roy pers. observation		
Rest/ thigmotactics								
	D. gallinae need to rest							
R1		Place to rest	crack and crevices, in dust, trash, under manure			Wood, 1917	Lab	D. gallinae remain in crevices for three days including oviposition
R2		Place to rest	short distance from food/ host			Kilpinen Pers.		
R3		Place to rest	where conspecifics are present					
R4		Place to rest		Under filter paper/ objects	open	Entrekin + Oliver (1982) lab		

Code	Need/ requirement	specification of the need	Further specification	Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
Movement								
	D. gallinae moves to find food, water, a resting/ hiding place and to explore the environment.							
M1		Space to move, turn, …	Required space?					
M2		Air velocity	Mites move with an air velocity	< 7 or 8 Bft	>7-8 Bft	Mul et al., 2016	lab	
M3		Darkness	Mites move during darkness (preferred see remarks)	Darkness	Light	Maurer et al. 1988	Semi practice	Hungry mites leave their hiding place an hour after the onset of the darkness and return to their hiding places within half an hour after the onset of the light period. A few days after starvation, mites will also come out to feed in day light
M4		Surface to move	Mites move vertically, horizontally and upside down			Maurer et al., 1988 Mul et al., 2016	Semi practice lab	
M5		Surface to move	Mites are able to climb on slippery surfaces					
M6		Time to last bloodmeal	Movement is affected by time of last blood meal	< 10 days post feeding	>10 days post feeding			At 10 days post feeding the mites become more static to safe energy

Code	Need/ requirement	specification of the need	Further specification	Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
M7		CO2	Mite movement stops with CO_2 and light		5% CO_2 and 100% CO_2 pulses during light	Kilpinen, 2005		
M8		Muscles	Muscles are used for movement					
To map and explore the environment								
	D. gallinae need map and explore the environment to identify e.g. conspecifics, food, laying hen, and to avoid dangerous situations.							
ME1		Methods to orientate	See sensors					
ME2		Attractants for mites	Conspecifics			Koenraadt and Dicke, 2010	lab, dark, 20 degrees	
ME3		Attractant unfed mites	Aged feathers			Koenraadt and Dicke, 2010	lab, dark, 20 degrees	
ME4		Attractant fed mites	CO_2 stream (2.5%)			Koenraadt and Dicke, 2010	lab, dark, 20 degrees	
ME5		Attractant fed mites	CO_2 stream + aged feathers			Koenraadt and Dicke, 2010	lab, dark, 20 degrees	
ME6		Attractant fed mites	Fresh feathers + CO_2			Koenraadt and Dicke, 2010	lab, dark, 20 degrees	
ME7		Host finding fed mites	The speed of temperature change () (0.005 degrees / second)			Kilpinen (2001)	Lab, light	Temperature change will not activate D. gallinae

Code	Need/ requirement	specification of the need	Further specification	Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
ME8		Host finding mites	Skin lipids			Zeman 1988	in vitro feeding, dark room	
ME9		Mite feeding stimulant	Diol esters of fatty acids prepared from secretion of the uropygial (preen) glands of hens			Zeeman 1988	in vitro feeding, dark room	
ME10		Conspecifics pheromones fed mites		mites	No mites	Koenraadt + Dicke, 2010	lab, dark, 20 degrees	
ME11		Sex pheromones				Entrekin + Oliver (1982)	lab	
ME12		Synthetic component attractant: Guanine				Entrekin + Oliver (1982)	lab	
ME13		Spots of previous aggregation of D. gallinae				Soler Cruz et al., 2005	SEM microscopy	
ME14		Temperature		33 °C compared to 20 °C		Mul et al 2016		
ME15		Repellent	Thyme oil			Pritchard et al., 2016		
Respiration								
	D. gallinae need to diffund air to live							
RP1		Air quality	% CO_2					
RP2			%O_2					
RP3			%??					
RP4		Tracheal opening		Open	closed			

Code	Need/ requirement	specification of the need	Further specification	Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
Health								
	D. gallinae need to be healthy and in good conditions without diseases, wounds, damage.	*Conditions to stay healthy, absence of conditions leading to disease, wounds or damage*						
H1		Bacteria leading to disease	Bacteria: Bacillus Thuringiensis;	absence	presence	Nordenfors, 2000; Chauve, 1998;	lab	
H2		Fungi leading to disease, wounds and damage	Fungi: Beauveria bassiana; Metarhizium anisopliae; Trichoderma album; Paecilomyces fumosoroseus	absence	presence	Kaoud, 2010; Steenberg and Kilpinen, 2003; Steenberg et al., 2006; Tavassoli et al., 2011; Immediato et al 2015	Lab + semi practical	Limited results in practice
H3		Material leading to skin damage, legg damage	Silica dust diatomaceous earth	absence	presence	Maurer et al., 2009; Kilpinen and Steenberg, 2009;	Semi practical, lab	
H4		Sunlight		absence	presence	Wood, 1917	lab	mites were placed on a board in direct sun light
H5		Predators leading to wound or damage	Alphitobius diaperinus, Cheyletus eriditus, Androlaelaps casalis, Stratiolaelaps scimitus	absence	presence	Maurer and Hertzberg, 2001; Chauve, 1998; Kozlov, 1970; Maurer and Hertzberg,		

Code	Need/ requirement	specification of the need	Further specification	Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
						2001; Lesna et al., 2012		
H6		Chemicals leading to damage	Acaricides causing brain damage			???		
Safety/ social behaviour								
	D. gallinae need to be safe avoiding to be eaten, damaged or become wounded.							
S1		Safe place	crack and crevices, in dust, trash, under manure			Wood, 1917		
S2		Safe place	Transparancy	Non-transparant	transparant	Mul et al, 2016	Lab; practical = no difference	
S3		Safety through Social structure	Females to protect non adult stages in aggregations			Entrekin and Oliver (1982)	lab	
Excretion								

Code	Need/ requirement	specification of the need	Further specification	Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
Suitable living environment for the poultry red mite								
	D. gallinae need physical comfort for a maximum longevity							
PH1		Temperature		10 °C	20-25 °C	Nordenfors et al, 1999; Bucher, 1998	Lab, semipractice	
PH2		Material surface		Smooth surface	Rough surface	Bucher, 1998	Semi practice	With hen in box
Code	**Population need**	**specification of the need**		Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
Reproduction								
PRP1	*D. gallinae* populations need to reproduce / lay eggs	Presence of males and females		Male + females				
PRP2		Place to reproduce						
PRP3		Place to lay eggs	Cracks, crevices, dust					
PRP4		Temperature for egg laying		28.6 °C 20-25°C	<5°C > 40 °C	Maurer and Baumgärtner, 1992. Nordenfors et al, 1999	lab	65-75% RH
PRP5		RH for egg laying		70-90%		Nordenfors et al., 1999		
PRP6		RH for egg laying		20-100% (preferred 70%)		Maurer and Baumgärtner, 1992, Nordenfors et al, 1999	lab	
PRP7		Temperature for oviposition		20-30 °C	15 °C	Tucci et al., 2008	Lab	70-85% RH

Code	Need/ requirement	specification of the need	Further specification	Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
PRP8		Temperature pre oviposition		20-35 °C	15 °C	Tucci et al., 2008	lab	70-85% RH
PRP9		Preferred blood (type of species) for reproduction		fowl blood, most successful	human blood unsuccessful	Sikes and Chamberlain, 1954	lab	
		Space to reproduce	Enough (safe) places					
			(Safe) places in the vicinity of the host					
Development								
PDV1	D. gallinae need to fulfil their lifecycle	eggs need to hatch into larvae	Temperature	30 °C	›37°C < 10 °C	Tucci et al., 2008	lab	70-85%
PDV2			humidity	95%				
PDV3			RH (humidity) for hatching	20-100% (preferred 70%)		Maurer and Baumgärtner, 1992, Nordenfors et al, 1999	lab	
PDV4		Larvae need to moult into protonymph	temperature	30 °C 35 °C	›35°C <5, > 40 °C	Tucci et al., 2008 Maurer and Baumgartner, 1992	Lab Lab	70-85% 65-75%
PDV5		Protonymph need to moult into deutonymph	temperature	30 °C	15°C <4.8 °C	Tucci et al., 2008	Lab	70-85%
PDV6			Food	Fowl blood	Human blood	Sikes and Chamberlain, 1954	lab	

Code	Need/ requirement	specification of the need	Further specification	Requirement (+)	Requirement (-)	Source/ reference	Type of research (lab/ practice/ semi practice)	Clarification/ remarks
PDV7		Deutonymph need to moult into adults	temperature	30 °C	< 15 °C ›35°C <5.7 °C	Tucci et al., 2008 Maurer and Baumgartner, 1992	Lab lab	70-85% 65-75%
PDV8			Food	Fowl blood	Human blood	Sikes and Chamberlain, 1954	lab	
Social structure								
PSS1	*D. gallinae* form aggregations and live in social structures.	*Presence of other D. gallinae*	Presence of mixture of juvenile stage and adult stage			Entrekin + Oliver (1982)	lab	Fed nymphs: aggregate quicker; below protected by females ; touch each other in clumps
PSS2			Females to protect non adult stages in aggregations			Entrekin and Oliver (1982)	lab	