No deleterious effect of inundative releases of biological agents on native arthropod assemblages in poultry farms: A mesocosm experiment

Ghais Zriki [a,*], Rumsais Blatrix [a], Liza Dadu [a], Anne-Sophie Soulié [a], Jordan Dijoux [a], David Degueldre [a], Nathalie Sleeckx [b], Lise Roy [a]

[a] *CEFE, Univ Montpellier, CNRS, EPHE, IRD, Univ Paul Valéry Montpellier 3, Montpellier, France*
[b] *Experimental Poultry Centre, Geel, Belgium*

HIGHLIGHTS

- No detected effect of inundative biocontrol in poultry on native predatory mites.
- Biocontrol agents (predatory mites) did not establish under farm-like conditions.
- Poultry mesocosm is an efficient device for population-level impact assessment.

GRAPHICAL ABSTRACT
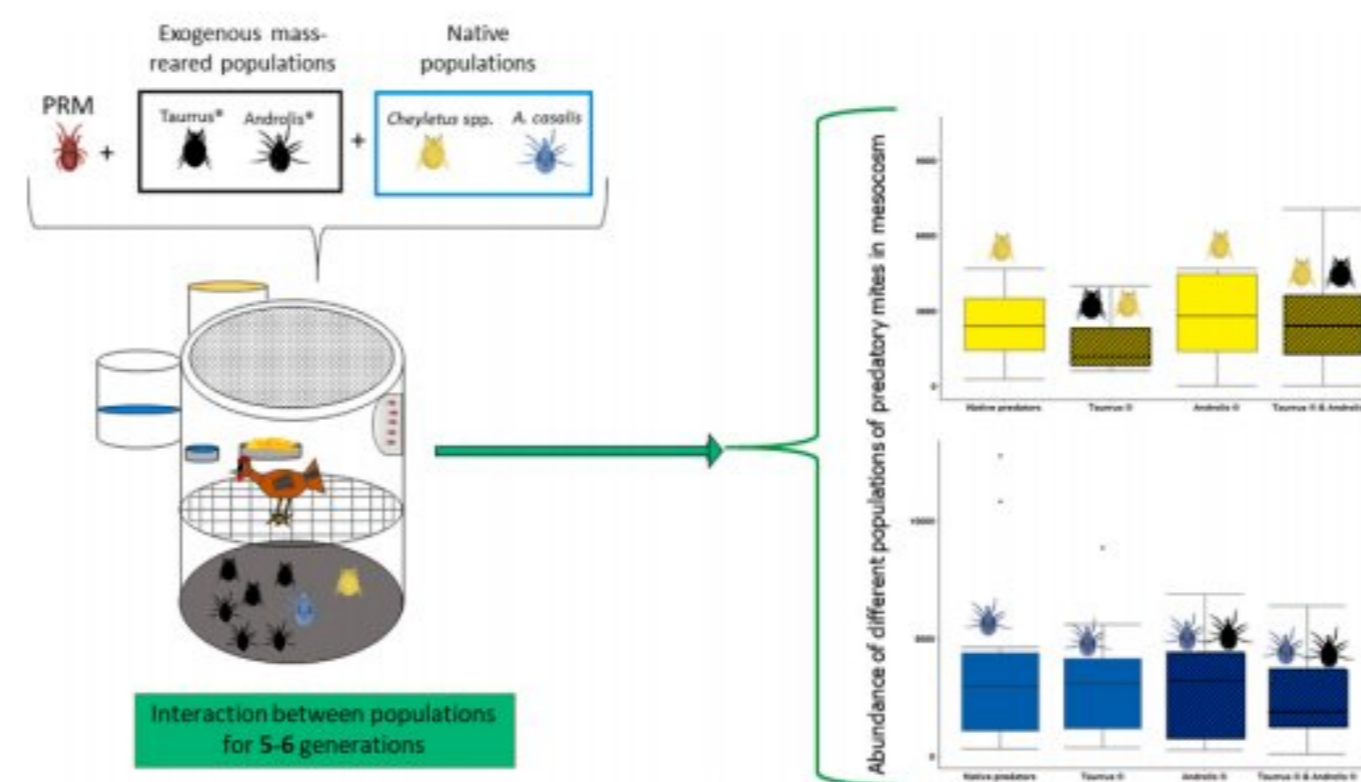
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ABSTRACT

Augmentative biological control relies on the inundative release of natural enemies of pests that are usually mass-reared in the laboratory. This practice substantially reduces the environmental impact of pest control in agriculture by reducing the use of insecticides. However, there are many reasons to expect more or less deleterious effects on biodiversity: if the enemy is not specific to the pest, the release of large populations of predators can directly affect native assemblages through the predation process itself and/or through competition with their native counterparts. In addition, mass-reared populations of enemies generally come from gene pools that are different from native populations and may, through the effects of hybridization, alter their population dynamics. On the other hand, during mass rearing, populations of natural enemies to be released are subject to different selection pressures from those in the field and may be less adapted than native populations to farm ecosystems. These effects are generally very difficult to assess in agro-ecosystems themselves due to the multiplicity of factors. In order to assess the effects of inundative releases of generalist predatory mites on native assemblages that colonize poultry houses from the surrounding environment, we conducted an experiment over several generations of mites using mesocosms mimicking a piece of a henhouse (mite-proof units, each housing one hen). No deleterious effects on native populations of *Androlaelaps casalis* and *Cheyletus* spp. have been detected from the mass introduction of marketed populations of *A. casalis* and *C. eruditus*. The mass introduction of marketed

* Corresponding author.
E-mail addresses: ghais.zriki@cefe.cnrs.fr (G. Zriki), rumsais.blatrix@cefe.cnrs.fr (R. Blatrix), anne-sophie.soulie@cefe.cnrs.fr (A.-S. Soulié), david.degueldre@cefe.cnrs.fr (D. Degueldre), nathalie.sleeckx@provincieantwerpen.be (N. Sleeckx), lise.roy@univ-montp3.fr (L. Roy).

predatory mites against *D. gallinae* appears to be compatible with the conservation of native arthropod assemblages. The mass-reared populations of *A. casalis* and *C. eruditus* did not establish their populations under conditions which otherwise allowed their native counterparts (same taxa) to do so.

1. Introduction

Biological control in general limits the environmental impact of agricultural activity by reducing pesticide inputs while maintaining control of pests on crops and livestock (Tracy, 2015). The conservation or introduction of natural enemies of pests or their augmentation by inundative releases are the main biological control practices (Bale et al., 2008). However, with the exception of conservation biological control, these practices can generate drastic changes in receiving ecosystems through the introduction of non-native species or populations (Louda et al., 2003; Jennings et al., 2017). The native assemblages that develop in the target agro-ecosystems or even in the landscapes that contain them (natural or semi-natural areas surrounding or nested within agroecosystems) may be affected by invasion phenomena by non-native species or other phenomena associated with massive releases of exogenous populations. This may not only affect the regulating (often ignored) ecosystem service provided by natural enemies of pests that naturally occur in agroecosystems, but also the integrity of surrounding natural ecosystems and associated biodiversity. Anticipating the side effects of biological control makes it possible to preserve both the instrumental value of biodiversity and its intrinsic value, two different but complementary points of view (Reyers et al., 2012). Ensuring the integrity of biodiversity per se (intrinsic value) within the framework of biological control should even be the first priority, before that of the instrumental parts according to Simberloff and Stiling (1996).

The risk of deleterious effects on biodiversity is significantly lower when adding populations of natural enemies already present than when introducing non-native natural enemies (Lynch et al., 2001; van Lenteren, 2012). Classical biological control typically relies on the latter, whereas biological control by augmentation relies on periodic inundative releases of mass-produced natural enemies, whether or not they are naturally present in the agro-ecosystems under consideration (Bale et al., 2008). As the Nagoya Protocol imposes a strong constraint on the authorisation of the introduction of non-native natural enemies (Smith et al., 2018), the use of inundative releases of native natural enemies could be substantially increased in the future. While the impact of simple introduction of non-native natural enemies has been the subject of relatively numerous studies (see Silvestri et al., 2020; Myers and Cory, 2017), few studies have focused on the effect of inundative releases of native natural enemies on communities of non-target organisms (Messing et al., 2006). When making inundative releases of natural enemies belonging to a native taxon, side effects can be expected due to the genetic structure of the taxon and the disproportionate size of the introduced population. With regard to genetic structure, mass-produced populations are likely to belong to different sub-populations from those that actually develop in the target agro-ecosystem because the original populations are usually collected from different environments/areas and because mass-produced populations are subject to very different selective pressures under production conditions. Therefore, intraspecific competition and hybridization during secondary contact allowed by the inundative releases may generate more or less important and unpredictable effects. With regard to the size of the introduced population, the massive increase of the local population of the natural enemy may affect indirect interactions, notably density-dependent interactions (Pearson and Callaway, 2005). In the context of inundative augmentation, biocontrol agents are usually not meant to become established in the environment, control is achieved mainly by the individuals that have been released rather than their offspring (Bale et al., 2008). Therefore, these biocontrol agents can be expected to cause only a transient effect in the environment (Lynch et al., 2001; Van Lenteren et al., 2006, 2003).

Nevertheless, it is not excluded that the transient activity drastically affects non-target populations and produces longer-term effects, for instance in case of massive hybridization. In addition, inundative releases of predators or parasitoids in the field may in some cases lead to species establishment (Boivin et al., 2006; Fernando et al., 2010; Newton and Odendaal, 1990). Biocontrol agents have been assessed as posing a high risk to non-target species when they are generalist with a high potential for establishment and interaction with non-target organisms (Van Lenteren et al., 2003). Without going so far as to sound an excessive alarm, as biological control is always more virtuous than 'chemical' control according to van Lenteren (2012), the responsible implementation of new means of pest control implies the upstream assessment of the risks of unwanted side effects, even for biological control by inundative augmentation.

Inundative biological control of the ectoparasite *Dermanyssus gallinae* has been applied for just over a decade in layer hen farming using mass-reared generalist predatory mites (Knapp et al., 2018). *D. gallinae* is the most damaging parasite to egg production in Europe (Chauve, 1998; Sparagano et al., 2014). It is a haematophagous mite that feeds on the blood of resting birds, causing irritation, restlessness and weight loss (Kilpinen et al., 2005), and sometimes anaemia and death of the birds. The annual economic loss associated with *D. gallinae* infestations has been estimated at 130 million Euros in the European Union (Emous et al., 2006). European legislation on public health and the environment increasingly restricts the application of synthetic acaricides, in particular since Regulation (EC) No 396/2005 regulating maximum residue limits in food products of animal origin in 2005. This considerably increases the interest in alternative methods such as biological control (Marangi et al., 2012; Sparagano et al., 2014), which is lagging far behind in livestock production compared to crops (Mul, 2017). As far as we know, the compatibility of an inundative release of predatory mites with the conservation of native arthropod assemblages in poultry farms and their potential effect on the natural regulation of pests has not been studied to date. However, arthropod assemblages in poultry houses are relatively diverse and abundant (Brady, 1970; Lesna et al., 2009; Roy et al., 2017), and include predators of the target pest (Zriki et al., 2020; Roy et al., 2020). In addition, flows of arthropods exist between poultry houses and the surrounding environment via flying insects and phoretic arachnids (Roy et al., 2017). Local and surrounding biodiversity could therefore be substantially affected by inundative biological control of *D. gallinae*.

Androlis® and Taurrus® are commercially available populations of predatory mites inoculated by inundative releases in poultry farms against *D. gallinae* (Knapp et al., 2018). These two populations belong to *Androlaelaps casalis* (Berlese) (Mesostigmata: Laelapidae) and *Cheyletus eruditus* (Schrank) (Trombidiformes: Cheyletidae) respectively. These mite species are generalist predators of other mites, in particular of mites in the families Acaridae and Glycyphagidae (Astigmata; Barker, 1991, 1968; Solomon, 1969). *Androlaelaps casalis* can also feed on other predatory mites, beetle larvae, nematodes and even inert elements such as brewer's yeast (Barker, 1968; Mustafa et al., 2016; Zriki et al., 2020). Unintended effects could therefore be produced by higher-order predation by introduced mites on members of native assemblages. *Androlaelaps casalis* and *Cheyletus* spp. are also among the most frequent and abundant predatory taxa in poultry manure and litter (Brady, 1970; Roy et al., 2017) and engage in reciprocal predation *in vitro* (Zriki et al., 2020). While the ecosystem service of regulation by native predators in laying hen farms has not been unambiguously demonstrated, the existence of interactions between predators and *D. gallinae* in the field suggests that it may exist (Roy et al., 2020). A diversity of interactions

between massively inoculated predatory mites and native predators could occur and affect the regulating service.

Two types of interspecific higher-order predation can affect biological control: intraguild predation (predation by one predator species on another predator with which it shares prey, i.e. a competitor) and hyperpredation (predation between predator species that feed on prey not shared between them) (Chailleux et al., 2014). By simply applying the basic rule "the enemy of my enemy is my friend" and its corollaries, such interactions are expected to impede the biological control exercised by natural enemies. Yet experimental studies show varied effects of interspecific higher-order predation on the pest control service provided by natural enemies, ranging from neutral (if not beneficial) to deleterious effects (Brown, 2003; Obrycki et al., 1998; Colfer and Rosenheim, 2001; Croft and MacRae, 1993; Eubanks et al., 2002; Schausberger and Walzer, 2001). The theory explains these contrasted results by a series of factors related to behaviour, density and spatial heterogeneity (including optimal foraging and prey switching…), the symmetry or asymmetry of predation interactions within assemblages and the variation in the numerical response to prey of different predators (see Chailleux et al., 2014). For example, in intraguild predation, two predators compete for the target extraguild prey (=pest), one of which is also in a prey position for the other (intraguild prey). Depending on whether the higher-order predator or the intraguild prey is the superior natural enemy (which reduces the population of the extraguild prey the most), theory predicts a neutral or deleterious effect of this interaction on biological control (Janssen et al., 2006). If the reciprocal predator-to-predator predation observed *in vitro* by Zriki et al., (2020) with *A. casalis* and *Cheyletus* spp. is expressed in the field as intraguild predation and if any of their native counterparts are the superior natural enemies, the inundative release of populations of these species could paradoxically generate undesired defects of natural regulation. Furthermore, given that the populations of the two species commercialized come from environments other than poultry houses, hybridization between these populations and native populations could alter the demographic dynamics of these natural enemies by producing individuals maladapted to the poultry environment. In particular, the marketed *A. casalis* population is derived from a population collected from wild bird nests and belongs to a different mitochondrial haplotype group (16S rRNA) than populations found in the field (Roy et al., 2017). Finally, certain direct or indirect interactions could be detrimental to the ecosystem in general, beyond the farm buildings. Indeed, the massive introduction of predatory mites in laying hen farms is notably applied from the beginning of the hen flock, just once the empty period is over and when the density of *D. gallinae* is the lowest (Knapp et al., 2018). Since other native arthropods seem to colonize the poultry houses from this moment (Roy et al., 2017), this practice is likely to substantially affect them, and thus the possible ecosystem service provided by predators. This could in turn alter the surrounding biodiversity, through exchanges between the poultry house and the surrounding environment.

The objective of our study is to examine the effect of the inundative release of mass-reared predatory mites *A. casalis* and *C. eruditus*, whether introduced separately or in combination, on their native counterparts. For this we conducted a population-level, multi-generation experiment in small experimental units, hereafter called mesocosms, that mimic the biotic and abiotic conditions of henhouses. We examined the development of commercially available populations of *A. casalis* and *C. eruditus* after inundative releases and determined how the inundative release of these predatory mites affected the development of native arthropod species, with a focus on the most closely related mites (*A. casalis* and *Cheyletus* spp.).

2. Materials and methods

2.1. Study system

2.1.1. Design of poultry mesocosms

Mesocosms were designed to mimic part of a laying hen house in a replicated manner to obtain statistically robust data (10 replicates per modality). They consisted of polyvinyl chloride cylinders, 40 cm in diameter and 39 cm in height, with a plastic bottom and a lid vented by a nylon membrane that was impermeable to mites and insects (80 μm mesh size) (Fig. 1). Each mesocosm was equiped with a circular metal plate (38 cm diameter) with holes, fixed 12 cm from the bottom and fitted with a plastic perch 20 cm long. Each mesocosm housed a chick between one- and seven-week old (specific-pathogen-free chicks; PA12 White Leghorn lineage; Plateforme d'Infectiologie Expérimentale; INRAE, Nouzilly, France). All chicks came from a single clutch. The chick was introduced into the mesocosm through a side door (20 cm in diameter) hermetically closed by a plastic lid sealed with a silicone gasket. Water and feed were supplied *ad libitum* to the chick by tanks that could be filled from the outside. The chicks were fed with organic feed (Evialis poussin B Farine and Evialis pondeuse B Basse cour Farine, Chabeuil, France). Water was provided to the chick through a horizontal Stilla Nipple drinker screwed through the mesocosm wall. Three plastic smartcups (Smart Expresso, SE6006 CLEAR, JMG, Milan, Italy) were fixed against the mesocosm wall, in the upper part, 5 cm below the nylon membrane and filled with successive layers of folded pieces of filter paper and cohesive tape (3.5 cm × 11 cm) (Absopress, Laboratoire Marque Verte, Villers-lès-Nancy, France) to imitate the shelters where *D. gallinae* accumulates in farms. These will later be referred to as 'standardized shelters'.

2.1.2. Native arthropod assemblage

To constitute the native arthropod assemblage in our experiment, we included the most common taxa in manure and litter from poultry houses, based on the inventory previously carried out in France (Roy et al., 2017). The composition of the native assemblage of arthropods is presented in Table 1. The selected arthropod taxa were collected from three laying hen farms located in the Drôme department (Rhône-Alpes-Auvergne region, France) and included in the study by Roy et al. (2017). Arthropods were extracted from manure immediately before being
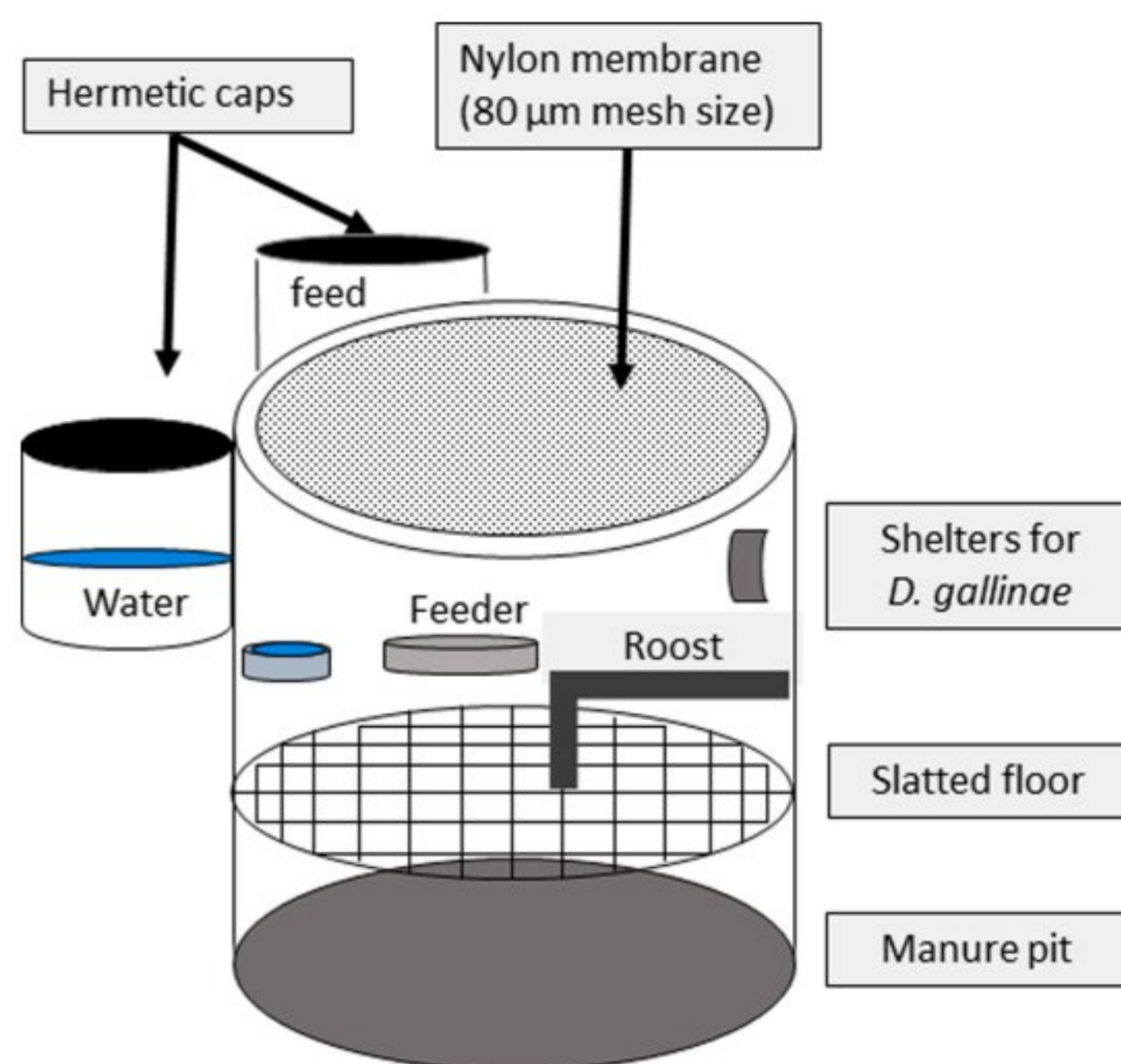

Fig. 1. Schematic representation of an experimental unit, called mesocosm, meant to mimic part of a laying hen house.

Table 1

Identity, trophic position and inoculum size of arthropod species used in mesocosms. BSM, Binocular stereoscopic microscope morpho-species, codes as in Roy et al. (2017).

Species identity	BSM morpho-species	Family	Order	Guild	Inoculum size (individual per mesocosm)
Arthropod native community					
Dermanyssus gallinae	–	Dermanyssidae	Mesostigmata	hematophagous	25 adult females
Dendrolaelaps presepum (dominant species), *Dendrolaelaps* spp. and unidentified Digamasellidae	ME1	Digamasellidae		Predator[#] (nematode and early stages of small arthropods)	20
Androlaelaps casalis	ME2	Laelapidae		Predator[#]	20 adult females
Proctolaelaps parascolyti	ME4	Melicharidae		fungivorous	20
Macrocheles muscaedomesticae	ME7	Macrochelidae		Predator[#] (fly eggs and first-instar larvae	10 adult females
Uropodina spp.	UR2	Uropodidae, Trematuridae, Nenteriidae		predator and detritivorous	50
Cheyletus spp.	–	Cheyletidae	Trombidiformes	Predator[#]	20 adult females
Astigmata	–	Acaridae	Sarcoptiformes	microbivore and detritivorous	2000 Ca
Lamprochernes nodosus	–	Chernetidae	Pseudoscorpionida	Predator[#]	10
Carcinops pumilio	–	Histeridae	coleoptera	Predator[#]	10
Flies	–	sphaeroceridae	Diptera	coprophagous	10
Mass-reared mites					
Androlaelaps casalis (Androlis®)	ME2	Laelapidae	Mesostigmata	predator	300 adult females
Cheyletus eruditus (Taurrus®)	–			predator	300 adult females

#: Predators able to feed on *D. gallinae in vitro* (Zriki et al., 2020).

introduced into mesocosms. The manure samples were dry sieved using a series of stacked sieves with decreasing mesh size (from 1000 μm to 180 μm) and then the live arthropods were collected in Eppendorf tubes containing a piece of moist filter paper. The distinction between mite taxa was based on the binocular stereomicroscope morphospecies defined in Roy et al. (2017) for sorting live mites. Astigmatic mites are ubiquitous microbivorous mites and are one of the most abundant taxa in poultry manure (Brady, 1970; Horn et al., 2018; Roy et al., 2017). In order to have a sufficient number of astigmatic mites for the experiment, a population of mites was extracted from the manure one month before the start of the experiment, kept pure and multiplied in the laboratory on yeast flakes. A single population of *D. gallinae* was sampled in one of the three selected layer farms for mesocosm inoculation.

2.1.3. The commercialized predators

Buckets of Androlis® (*A. casalis*) and Taurrus® (*C. eruditus*) were provided by Koppert Biological Systems (The Netherlands). The predators were extracted from the substrate and separated from the mite prey with which they are packaged following the same protocol as the manure mites (see above).

2.2. Mesocosm experiment

The experiment consisted in introducing predefined arthropod assemblages with known initial numbers of individuals belonging to several taxa into mesocosms, according to five modalities (10 mesocosms per modality): 1. *D. gallinae* + astigmatic mites (control), 2. *D. gallinae* + astigmatic mites + other native arthropods (control), 3. *D. gallinae* + astigmatic mites + other native arthropods + Taurrus®, 4. *D. gallinae* + astigmatic mites + other native arthropods + Androlis®, 5. *D. gallinae* + astigmatic mites + other native arthropods + Taurrus® + Androlis®. A one- to two-week old chick, *D. gallinae* and astigmatic mites were introduced at t0. Other native arthropods and commercialized predators were introduced at t + 7 days. Females of *D. gallinae* were thus given a head start over predators. All arthropods were alive and active at the time of their introduction. The chicks were removed from the mesocoms at t + 50 days. The experiment ended at t + 57 days by placing each mesocosm at −20 °C to inactivate the arthropods prior to extraction. The week without chick allowed *D. gallinae* to digest blood and was meant to reduce the potentially destructive effect of sieving on

freshly blood-fed mites. The experiment was conducted at controlled temperature (26 ± 1 °C during the day and 23 ± 1 °C at night, 10:14 light:dark) and humidity (75 ± 10% RH).

The inoculum size for *D. gallinae* (25 adult females) was fixed so as to obtain a final estimated number of *D. gallinae* per mesocosm smaller than the range of values that had induced chick mortality in preliminary tests. For the other native arthropods, inoculum size (~2000 individuals for astigmatic mites, 10–50 for the other species, Table 1) was fixed to be consistent with the corresponding relative proportions reported from field inventories by Roy et al. (2017). Inoculum size for commercialized predators (300 individuals) was determined by adjusting the producer's recommendations on Androlis® and Taurrus® applications for backyard poultry houses with the surface area available for the chick in the mesocosms (0.126 m²).

2.3. Evaluation of final arthropod population sizes

2.3.1. Extraction and isolation of arthropods from mesocosms

To isolate arthropods from the coarse substrate (manure + dust + feed + feathers) accumulated in the mesocosms, we applied a flotation method used to extract arthropods from soil samples (Edwards, 1991) combined with wet washing and sieving. We adapted the extraction procedure to our specific substrates and to the volume of the mesocosm contents as follows. The nylon membrane, of which the outer surface was thoroughly cleaned, was cut. Standardized shelters were removed from the mesocosm and treated separately (see below) to distinguish aggregated arthropods in this specific small area from those found in other parts of the experimental unit. All internal surfaces of the mesocosm and internal accessories were thoroughly rinsed. The rinsing liquid and the coarse substrate were mixed in water and homogenized by hand. A 20-minute pause allowed complete wetting of the substrate, after which the mixture was subjected to sieving using an electric sieve shaker (AS 200 basic, Retsch GmbH, Haan, Germany) with continuous water rinsing and controlled vibrations (80% amplitude) to maximize the separation of arthropods from organic particles (sieve mesh sizes decreasing from top to bottom: 2000, 1000, 800, 600, 400 and 300 μm). All residues on the six sieves were floated in water saturated with NaCl to isolate arthropods. Flotation was applied separately for filtrates from 2000 to 600 μm sieves and 400–300 μm sieves. The supernatant was filtered through an 80 μM nylon mesh membrane and the arthropods

isolated from each sieve category were collected in vials containing 96% ethanol.

To extract arthropods from the standardized shelters, the smartcups and their contents were rinsed with water. The rinsing liquid was subjected to the same sieving treatment as described above, except that arthropods were recovered directly from the 400–300 μm sieve (no large arthropods in the standardized shelters) into a 96% ethanol flask without passing through a flotation step. As the cups contained only arthropods (no feed or manure), there was no need to separate the mites from other elements.

2.3.2. Counting arthropods and assessing final population size

Arthropods other than mites were directly identified and counted on the large-mesh sieves. For mites, abundance was estimated by extrapolating the number of individuals from counts in four aliquots of $1/33^{rd}$ and four of $1/75^{th}$ volume for artificial shelters and coarse substrate respectively. The mites were counted in each aliquot according to the method of Roy et al (2017): they were filtered through a nylon membrane, spread out on the membrane, identified and counted with a stereomicroscope. As the eggs, larvae and protonymphs cannot be unambiguously attributed to the mite morphospecies, we counted only adult-like individuals (deutonymphs and adult males and females). Astigmatic mites were introduced as a ubiquitous component of the poultry environment (role in manure degradation and potential prey of introduced predators). Thus, their development was assessed by a qualitative control, but abundance was not estimated. They reached a high level of development in all mesocosms.

2.3.3. Discrimination between species

Since we are working on the inundation by species naturally present in the layer farms, we cannot explicitly discriminate marketed mites from the others and therefore cannot directly verify whether they have established themselves or not. Therefore, we compared population growth parameters between test (marketed + native predators) and control modalities (native predators only). However, in the case of the genus *Cheyletus*, we can distinguish the species. Since we know the marketed species (*C. eruditus*), we were able to also compare the specific composition of this predator. Morphological discrimination between species of Cheyletid mites is impossible on living individuals because it requires microscopic preparation. To distinguish *C. eruditus* (the commercialized species) from other Cheyletid mite species, we sampled up to 54 individuals of *Cheyletus* sp. per mesocosm (half from standardized shelters and half from the rest of the mesocosm). Each of these mites was mounted with Hoyer medium on microscopic slides (Jeppson et al., 1975). They were identified using a phase contrast and interference microscope (Leica DMLB, Leica Microsystems SAS, Nanterre, France) and dichotomous keys (Fain and Bochkov, 2001; Gerson et al., 1999; Volgin, 1987). The total number of individuals was estimated for each cheyletid species based on the proportion of each species in the analyzed sample and the total number of cheyletid mites in the mesocosm.

2.4. Data analysis

In order to determine whether or not establishment had occurred, a relative increase index (RII) was calculated for each taxon and within each mesocosm as follows: RII = Nf/Ni with Nf = the final number of individuals and Ni = the initial number of individuals introduced into the mesocosm (inoculum size). A taxon was considered to have successfully established its population if RII > 1. For the following statistical analysis, we included only those mite taxa that developed in almost all mesocosms of all modalities. Taxa that did not develop (RII ≤ 1) or for which development was very sporadic were excluded from the analysis.

All statistical analyses were performed in R software (R Core Team, 2020). To determine the effect of inundative release of mass-reared

predatory mites on the development of native arthropod predators, we tested the effect of modalities (categorical explanatory variable) on the final abundances and RIIs of each taxon (response variables) using generalized linear models specifying a negative binomial error distribution with a log-link function (glm.nb function in the MASS package, Venables and Ripley, 2002). Analyses of variance based on deviance were carried out to assess the effect of modalities on the response variables (anova function in MASS). Tukey's multiple comparisons on the models were done using the glht function in the multcomp package (Hothorn et al., 2008).

3. Results

Of the 50 replicates, only one (in the control modality 1) was excluded from analyses due to a leak in the water reservoir that resulted in the immersion of the substrate in the bottom of the mesocosm. Populations of *D. gallinae*, astigmatic mites, *A. casalis*, *Cheyletus* spp. and *Car. pumilio* successfully established (RII > 1) in 100, 100, 95, 95, 95% of the replicates respectively (Table 2). It should be noted that only larvae and nymphs of *Car. pumilio* were recorded in addition to the adults initially introduced into the mesocosms. The duration of the experiment (50 days) slightly exceeded the duration of egg to adult development in this beetle at 25.5 °C (ca. 40 days; Morgan et al., 1983). This suggests that the population started to develop but was not quite successful in establishing itself under our conditions. Establishment of populations of *M. muscaedomesticae* and Uropodina spp. was irregular and occurred in 12.5% and 57.4% of the replicates respectively. Populations of flies, *Dendrolaelaps* spp., *Proctolaelaps parascolyti* and *Lamprochernes nodosus* did not establish in any of the mesocosms (Table 2).

Morphological identification of cheyletid individuals from developed populations in mesocosms showed the presence of three species in our experiment, namely *C. eruditus*, *C. carnifex* and *C. malaccensis*. *Cheyletus malaccensis* was largely dominant in mesocosms in all modalities with predators (98.3% of *Cheyletus* mites identified). *Cheyletus carnifex* (1.0%) and *C. eruditus* (0.7%) represented a very small portion of the cheyletid mite community (Fig. 2). Since *Cheyletus eruditus* was only detected in mesocosms where Taurrus® had been introduced, the native cheyletid mite community used in this study appears to be composed of *C. malaccensis* and *C. carnifex* only.

The abundance of *Cheyletus* spp. did not differ significantly among modalities with predators (2–5), whether provided with inundative releases of Taurrus® or not (sequential analysis of deviance for GLM: df = 3, deviance residuals = 1.996; df = 36, residual deviance = 48.089, p = 0.573, Fig. 3A). The RIIs of *Cheyletus* spp. were significantly higher in modalities 2 and 4 (native population of *Cheyletus*) than in modalities 3 and 5 (native population of *Cheyletus* spp. + mass-reared population of *C. eruditus*) (sequential analysis of deviance for GLM: df = 3, dev. resid. = 67.080; df = 36, resid. dev. = 45.272, p < 0.001; adjusted p-values of pairwise test (Tukey method) > 0.92 for modality pairs 2–4 and 3–5, and < 0.001 for all other pairs, Fig. 3B). Morphological discrimination between mass-reared and native populations of *A. casalis* is not possible and hybridization between mass-reared and native populations is very likely since the L1 lineage to which Androlis® belongs (Roy et al., 2017) is by far the most frequent in poultry farms (unpublished data). Therefore it did not seem relevant to measure the haplotypic frequency and we were unable to distinguish between mass-reared and native populations of *A. casalis*. However the abundance of *A. casalis* in mesocosms did not differ significantly among the four modalities with predators (sequential analysis of deviance for GLM: df = 3, dev. resid. = 1.720; df = 36, resid. dev. = 44.937, p = 0.632, Fig. 4A). In contrast, RIIs were significantly higher in modalities 2 and 3 (native population of A. *casalis*) than in modalities 4 and 5 (native + mass-reared population of A. *casalis*) (sequential analysis of deviance for GLM: df = 3, dev. resid. = 95.628; df = 36, resid. dev. = 44.238, p < 0.001; adjusted p-values of pairwise test (Tukey method) > 0.40 for modality pairs 2–3 and 4–5, and < 0.001 for all other pairs, Fig. 4B). Finally, the abundance of *Car. pumilio* did not

Table 2
Distribution of successful population establishment in mesocosm according to modality and arthropod species. Establishment is considered successful when the relative increasing rate is >1. Values are numbers of mesocoms with successful establishment.

Treatment	Arthropod species										
	D. gallinae	Dendrolaelaps spp	A. casalis	P. parascolyti	M. Muscadomesticae	Uropodina spp.	Cheyletus spp.	Acaridae	L. nodosus	Car. pumilio	Flies
1) PRM control	9/9	–	–	–	–	–	–	9/9	–	–	–
2) Native-community control	10/10	0/10	10/10	0/10	3/10	4/10	10/10	10/10	0/10	10/10	0/10
3) One mass-reared predator (Taurrus®)	10/10	0/10	10/10	0/10	2/10	4/10	10/10	10/10	0/10	10/10	0/10
4) One mass-reared predator (Androlis®)	10/10	0/10	9/10	0/10	1/10	7/10	9/10	10/10	0/10	8/10	0/10
5) Two mass-reared predators	10/10	0/10	9/10	0/10	0/10	8/10	9/10	10/10	0/10	10/10	0/10

N/n = number of replicate with successful development/number of valid replicates in the treatment.
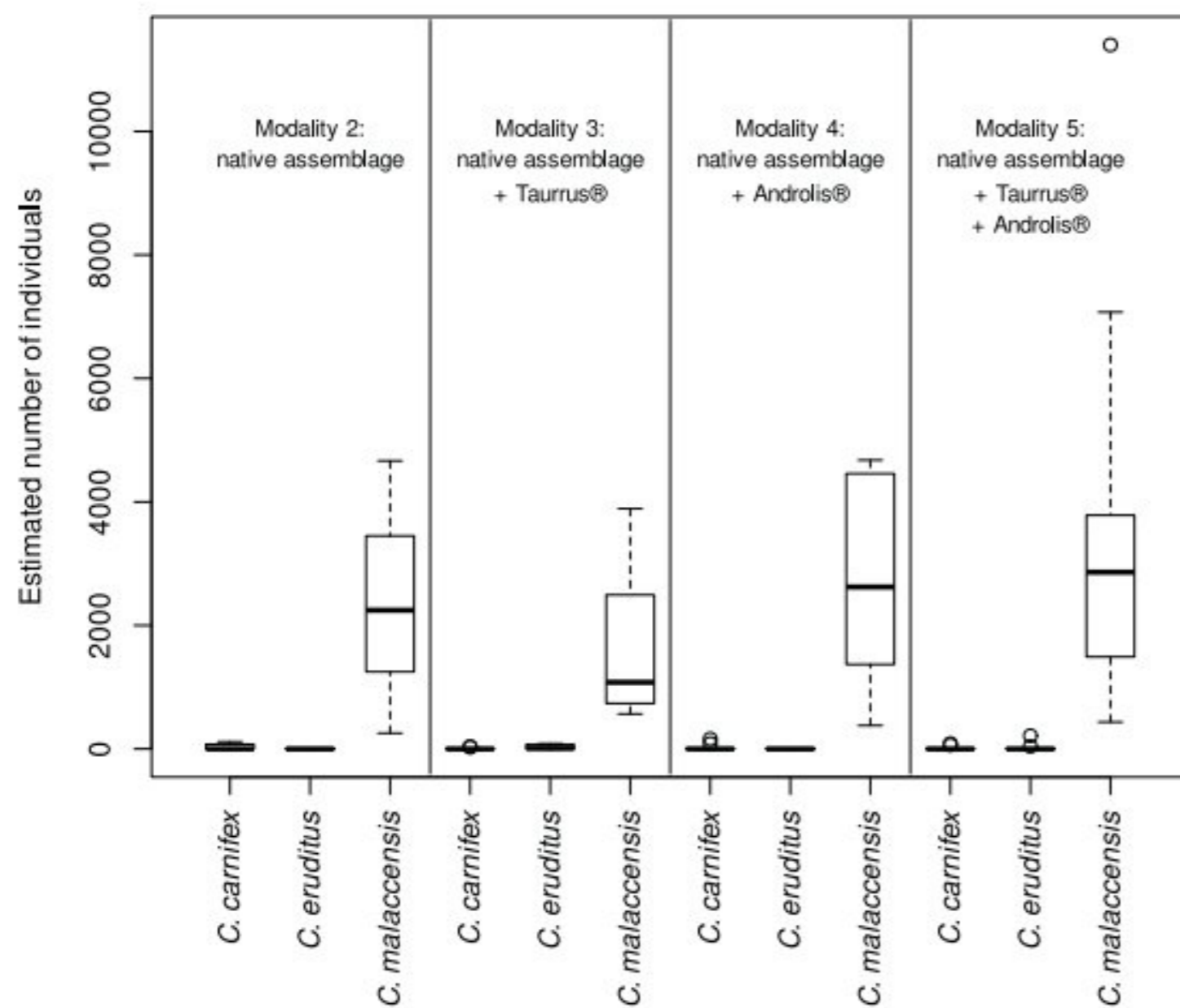
Fig. 2. Estimated abundance of each *Cheyletus* species after 50 days of development time in mesocosms according to four modalities. Boxes represent interquartile range with the median as a thick line, whiskers represent data range and empty circles represent outliers.

differ significantly between modalities with predators (sequential analysis of deviance for GLM: df = 3, dev. resid. = 4.501; df = 36, resid. dev. = 47.501, p = 0.212).

4. Discussion

This study tested for the first time the effect of inundative releases on native arthropod fauna in a poultry context. The three focal taxa, the prey *D. gallinae* and the predators *A. casalis* and *Cheyletus* spp., have developed substantially in the mesocosms, with an increase in adult-like individuals of more than a hundredfold within a 50-day interval for both predators. Considering the mean egg-to-adult development times according to Barker (1968) and Mustafa et al. (2016) between 25.0° and 26.5 °C in *A. casalis* and according to Toldi et al. (2017) in *C. malaccensis*, the first native predator may have produced about 7 consecutive generations while the second probably produced <3 during our experiment. However, the fecundity of the former is much lower than that of the latter (ten times less eggs laid per female in *A. casalis* than in
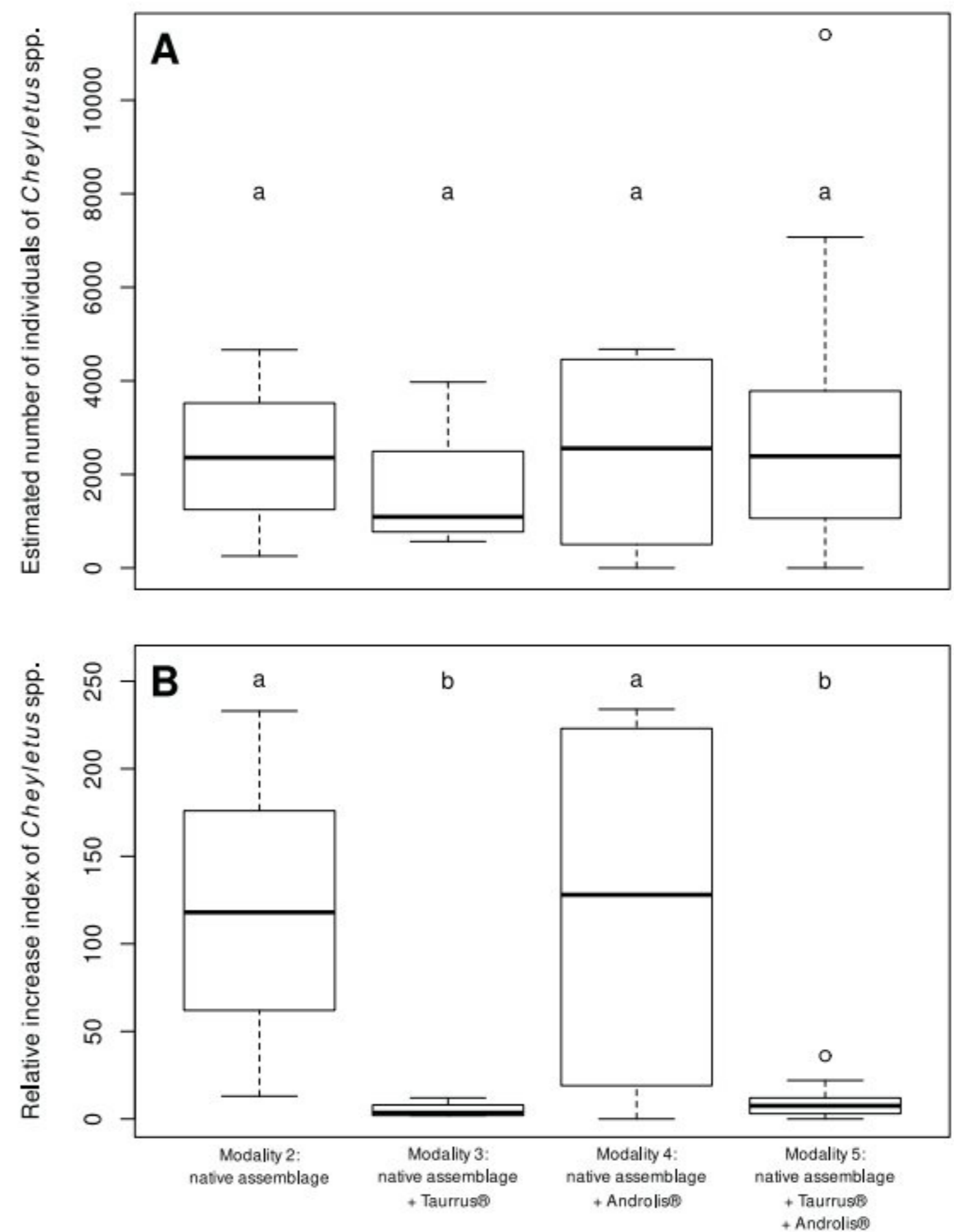

Fig. 3. Population development of *Cheyletus* spp. in the four modalities with predators after a 50 days of development time in mesocosms. (A) Total abundance estimated based on final counts. (B) Relative increase index (final abundance/size of initial inoculum). Different lowercase letters indicate significant differences.

C. malaccensis according to Mustafa et al., 2016; Toldi et al., 2017), which probably explains the RIIs within comparable ranges.

4.1. Mass-reared populations of predators did not establish in the mesocosms

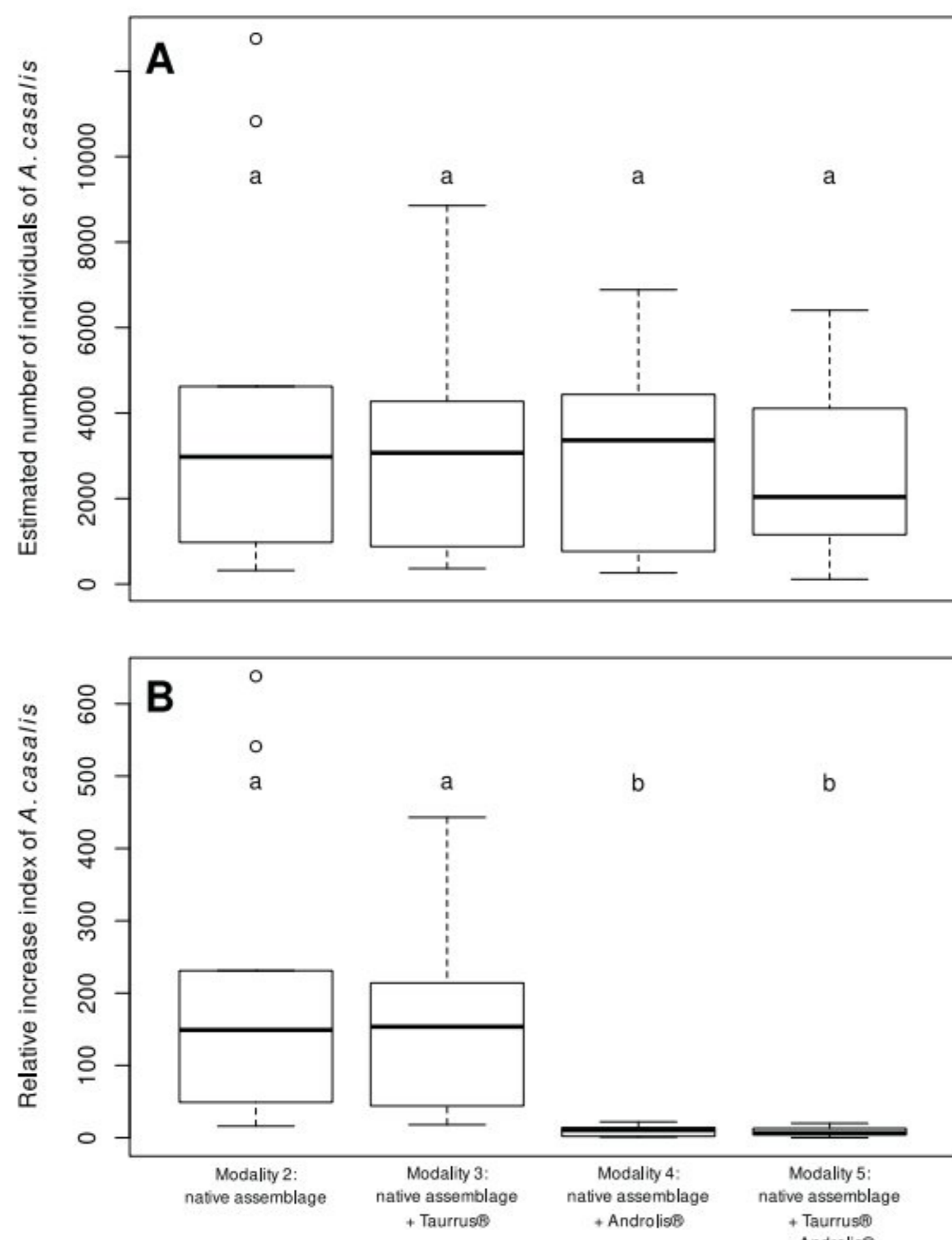The successful development of native *A. casalis* and cheyletid mites

Fig. 4. Population development of *A. casalis* in the four modalities with predators after a 50 days of development time in mesocosms. (A) Total abundance estimated based on final counts. (B) Relative increase index (final abundance/size of initial inoculum). Different lowercase letters indicate significant differences.

(*C. malaccensis* and *C. carnifex*) in the mesocosms confirms the suitability of biotic and abiotic conditions for the development of these taxa. Moreover, the results support a system capacity limit that was far from being reached at the beginning of the experiment even in the "inundation" modalities. This is an important point because starting from a number of individuals greater than or close to the carrying capacity of the system is in itself likely to lead to the collapse of populations and would not leave room for the natural dynamics of the assemblages. The final numbers of adults in each species of predator greatly exceeded the number of mites initially introduced, even in the "inundation" modalities: the median numbers of *Cheyletus* and *A. casalis* adults counted at the end in the "native assemblage" modality were >2000 while we introduced only 320 Cheyletus in the inundation modalities by Taurrus (20 native *Cheyletus* + 300 marketed *C. eruditus*) and 320 *A. casalis* in the inundation modalities by Androlis (same ratio). Even if the carrying capacity of a system can be transiently slightly exceeded, the exceedance ratio (>6 times) does not seem to be consistent with such transient exceedance. We therefore argue that our mesocosms, under our conditions, can carry much more than the initial number of individuals, even in inundation modalities. Consequently, we consider that we can exclude the hypothesis of a collapse of predator populations because of the limit capacity reached.

At the end of the experiment, *C. eruditus* (the mass-reared species) was rare and represented only 0.7% of the cheyletid mite community in the mesocosms. In contrast, *C. malaccensis* was the most abundant, even in modalities inoculated with Taurrus®. As Taurrus® is composed exclusively of *C. eruditus*, we are confident that the population of mass-reared *Cheyletus* did not establish in the mesocoms. As discrimination was not possible between mass-reared and native populations of

A. casalis, we cannot state the origin of the individuals that occurred in the mesocosms at the end of the experiments for this species. If the mass-reared populations had established, RII should have been similar between the two modalities as the preys were unlikely to be limiting (astigmatic mites were highly abundant), yet they were much higher in modalities without inundation (inoculum = 20 individuals) than with inundation (inoculum = 320 individuals). Thus, it seems unlikely that the mass-reared population of *A. casalis* established in the mesocosms, although we cannot exclude that it has replaced or hybridized with the native population to some extent.

Given that biotic and abiotic conditions in mesocosms were suitable for the development of native populations of *A. casalis* and cheyletid mites (*C. malaccensis* and *C. carnifex*), the developmental failure of the mass-reared populations of *C. eruditus* and *A. casalis* may be due to their maladaptation to the biotic and/or abiotic conditions of a poultry environment in which their native counterparts develop very well. This can be explained not only by the non-poultry origin of the initial populations (Androlis® stems from a population taken from nests of wild starlings; Koppert, pers. comm.), but also by the involuntary selection exerted by mass rearing: artificial rearing conditions may lead to the alteration of traits important for their ability to survive, mate, feed and reproduce efficiently under field conditions (Bertin et al., 2017; Cerutti and Bigler, 1995; Hopper et al., 1993) as a result from inbreeding and random genetic drift with loss of genetic diversity (Paspati et al., 2019; Poe and Enns, 1970; Rasmussen et al., 2018). Marketed populations of *A. casalis* and *C. eruditus* may have suffered some loss of genetic diversity as demonstrated for the predatory mite *Amblyseius swirski* (Paspati et al., 2019). This may be accompanied by the loss of traits important for their development under field conditions.

4.2. Inundative releases of mass-reared predators are unlikely to affect native populations of the same taxa and biodiversity.

The failure of commercial populations to establish within the 50 days of the mesocosm experiment is consistent with the transient presence of the massively released biocontrol agents previously noted in culture systems (Lynch et al., 2001). In addition, the mass introduction of populations of *A. casalis* and *C. eruditus*, alone or in combination, had no detectable effect on the development and establishment of native populations of the corresponding taxa. This too is consistent with the few published studies on the unintended effects of inundative biological control identified by Lynch et al. (2001) on crops. Since *A. casalis*, *C. eruditus* and *C. malaccensis* are generalist predators (Barker, 1991; Cebolla et al., 2009; McKinley, 1963; Sinha, 1988), we may have expected competition and/or intraguild predation to occur between mass-reared and native populations, although it was unlikely given the non-limiting density of shared astigmatic mite prey. At least for cheyletid mites we can tell that such processes did not impact native populations as mostly individuals of the native species remained at the end of the experiment. Conversely, the elimination of mass-reared populations by native predators is not excluded for either taxa.

In a previous experiment, native *Cheyletus* spp. and *A. casalis* showed high predation rates on *D. gallinae* in in-vitro tests, and both preferred *D. gallinae* over astigmatic mites (Zriki et al., 2020). In addition, abundances of *Cheyletus* spp. and *A. casalis* covaried with that of *D. gallinae* in farm buildings, suggesting trophic relationships between populations of these two predators with *D. gallinae* in the field (Roy et al., 2020). These two studies used *Cheyletus* samples from the same source buildings as in the present experiment, and thus dealt most likely with the species *C. malaccensis*. As *C. malaccensis* and *A. casalis* are among the most frequent and abundant predatory mites of *D. gallinae* in poultry farms and in bird nests (Faleiro et al., 2015; Silva et al., 2013; Lesna et al., 2009; Brady, 1970; Roy et al., 2017), we may expect a regulating service of their native populations on *D. gallinae* on farms, although such an effect has still not been demonstrated. As no effect of inoculation with Androlis® and Taurrus® was detected on these native predatory species,

we assume that their potential regulating service on *D. gallinae* should not be affected by the massive release of their mass-reared counterparts.

Given the wild origin of Androlis® and its development over dozens of generations in the laboratory, an important genetic differentiation could be expected between *A. casalis* from Androlis® and *A. casalis* native to poultry buildings. Indeed, the mass-reared population exhibits a mitochondrial haplogroup (16S rRNA) different from the two present in poultry farms (*A. casalis* L1 and L2; Roy et al., 2017). As for *Cheyletus* spp., the differentiation between mass-reared and native populations seems considerable (different Linnaean species). In the present experiments, reciprocal introgression by cross-breeding between them (secondary contact) may have occurred in modalities with inundation with the mass-reared population. Hybridization may be expected to either boost (heterosis) or reduce (outbreeding depression) population growth. Although the initial propagule was 16 times larger in inundation mesocosms than in no-inundation mesocosms, the final population sizes were similar in the different modalities. Therefore, it is rather unlikely that heterosis occurred. On the other hand, the effect of depression is not entirely excluded for *A. casalis*. It can be excluded for *Cheyletus*, because if hybrids of the Linnaean species had been present in large numbers, it is likely that we would have been confronted with morphological ambiguities. This suggests a very reduced risk of pollution of the native populations of *A. casalis* and *Cheyletus* and of the surrounding biodiversity by exogenous gene pools.

4.3. System incompleteness and limitations of our study

Despite the remarkable potential of our experimental system to mimic a portion of a poultry farm, taxa other than our focus predators failed to develop. Although mesocosms did not exactly mimic abiotic conditions of poultry houses, we believe biotic incompleteness of the ecosystem is likely to have strongly impeded development of the failing taxa. As we did not inoculate nematodes or fungi and birds were specific-pathogen-free, typical nematode preys of *Dendrolaelaps* spp. and *Macrocheles muscaedomesticae* and some fungi potentially fed upon by *Proctolaelaps parascolyti* and Uropodina were probably absent. Furthermore, the manure layer is produced by the chick as it grows, so it was very thin and relatively dry at the beginning. This can substantially hinder the development of fly eggs and larvae at the beginning of the experiment, and consequently hinder the development of their predators, namely *M. muscadomesticae*, *Car. Pumilio* and maybe pseudoscorpions. Even though we have shown that *L. nodosus* was able to feed on *D. gallinae in vitro* (Zriki et al., 2020), typical conditions in poultry houses, available prey types in particular, may not suit this species since it seems quite infrequent in poultry farms (Roy et al., 2017) and has been classified as a nidixenous species only occurring accidentally in bird nests (Christophoryová et al., 2011).

In short, we are aware that the biocenosis of the mimed ecosystem was incomplete in our experimental system and we remind that our objective was not to mimic the entire system. We cannot be entirely certain that inundative releases of mites into poultry houses do not have a deleterious effect on all non-target wildlife since we have tested only part of the possible interactions in a poultry ecosystem.

5. Conclusion and perspectives

Our study undeniably provides crucial answers to the questions raised. Although *A. casalis* and *Cheyletus* spp. are generalist predators capable of mutual predation, inundative releases of their mass-reared populations did not show any detectable effect on their native populations. This result is even more important that a recent study suggests the native populations could be involved in the ecosystem service of parasite regulation (Roy et al., 2020). Short-term persistence of mass-reared populations in the environment is likely to explain their lack of effect on native populations, as with other mass-released biocontrol agents in agriculture. The mass introduction of mass-reared predators of *D. gallinae* appears to be compatible with the conservation of native mite communities, whether they are likely to provide ecosystem services (instrumental value of natural enemies) or not (intrinsic value of biodiversity). However, we tested only a small subset of possible interactions in a poultry ecosystem. Inundative releases of the two tested control agents may still have deleterious effects on other non-target wildlife.

These results are one more example of the low risk associated with biological control by inundative augmentation already observed in cropping systems (Lynch et al., 2001). In addition, the study provides valuable information as it is the first of its kind on a livestock production system. Commercial poultry houses are a major interface between agricultural production and the environment since they are largely colonized by arthropods from the surrounding environment, but also since poultry manure is widely spread in agricultural landscapes as an organic fertilizer. Further investigation should focus on the consequences of inundative releases on more complete reconstructions of avicultural ecosystems and of putative introgression of native populations by inoculated populations. Taking advantage of the results of our mesocosm experiment, hen house scale investigation could now be conducted to address these issues.

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