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# Tumors (re)shape biotic interactions within ecosystems: Experimental evidence from the freshwater cnidarian *Hydra*

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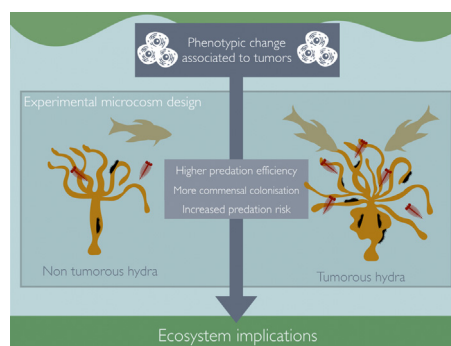
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## HIGHLIGHTS

- Tumors alter hydra's phenotype.
- The tumorous phenotype has increased predation abilities.
- Commensal ciliates colonize preferentially and more effectively tumorous hydras.
- Tumorous hydras are preferentially eaten by a fish predator.
- Tumoral phenotype can affect biotic interactions within ecosystems.

## GRAPHICAL ABSTRACT



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## ABSTRACT

While it is often assumed that oncogenic processes in metazoans can influence species interactions, empirical evidence is lacking. Here, we use the cnidarian *Hydra oligactis* to experimentally explore the consequences of tumor associated phenotypic alterations for its predation ability, relationship with commensal ciliates and vulnerability to predators. Unexpectedly, hydra's predation ability was higher in tumorous polyps compared to non-tumorous ones. Commensal ciliates colonized preferentially tumorous hydras than non-tumorous ones, and had a higher replication rate on the former. Finally, in a choice experiment, tumorous hydras were preferentially eaten by a fish predator. This study, for the first time, provides evidence that neoplastic growth has the potential, through effect(s) on host phenotype, to alter biotic interactions within ecosystems and should thus be taken into account by ecologists.

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## 1. Introduction

Over the last few decades, great attention has been devoted by ecologists to understanding the relationships between biological community

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dynamics and the functioning of ecosystems (Loreau, 2010; Massol et al., 2011; Petchey and Gaston, 2002; Thompson et al., 2012). It is now clearly established that the outcome of species interactions and their ecosystem consequences are often strongly influenced by variation in the functional traits of the organisms (Chalmandrier et al., 2017; Kishida et al., 2010; Raffard et al., 2019; Wright et al., 2016). Factors influencing individual phenotypic variability are numerous, including genetic and epigenetic ones in interaction with external biotic and abiotic variables (e.g., predation, food availability, see Chalmandrier et al., 2017; Raffard et al., 2019; Reed et al., 2010), as well as internal biotic factors associated with the holobiont. For instance, host-microbe interactions are known to influence animal behavior and life-history traits (Alcock et al., 2014; Walters et al., 2020; Wong et al., 2015). Besides, many manipulative parasites have the capacity to alter a broad range of phenotypic traits of their hosts, from color to morphological traits and behavior (Hughes et al., 2012; Hughes and Libersat, 2019; Moore, 2013; Thomas et al., 2011). Because alterations in the phenotype of parasitized hosts can be substantial, it has been argued that manipulated hosts can be ecologically equivalent to new organisms in the ecosystem, involved in novel direct and/or indirect interactions with other species (Thomas et al., 1998; see also Mouritsen and Poulin, 2010; Pascal et al., 2020).

In addition to microbiota and parasites, multicellular organisms also evolve with another category of living entity inside their body: the community of neoplastic cells (Aktipis and Nesse, 2013; Thomas et al., 2017). Neoplastic cell transformation affects most, if not all, multicellular organisms, from hydras to whales (Aktipis et al., 2015; Albuquerque et al., 2018; Madsen et al., 2017). Prior to being, in some cases, fatal for their hosts, tumors often result in the alteration of phenotypic traits in their hosts, e.g. in morphology (Barr et al., 2016; Jones et al., 2016), physiology (Argilés et al., 2014), body odors (Buszewski et al., 2012), reproductive activities (Arnal et al., 2017), as well as social interactions (Dawson et al., 2018; Hamilton et al., 2020). Despite this large range of phenotypic alterations and the omnipresence of neoplastic processes in the wild, the ecological consequences of neoplastic processes on ecosystems and animals' life histories remain largely unexplored (Giraudeau et al., 2018).

To fill this gap, we developed a novel model system, an artificial (tripartite) microcosm, which allows empirical testing of whether bearing tumors may have implications for biotic interactions within ecosystems. The freshwater cnidarian *Hydra oligactis* (further referred to as hydra) is naturally found in aquatic habitats attached to aquatic vegetation or submerged rocks. Hydras reproduce asexually by budding but can also perform sexual reproduction in autumn, which is usually followed by a post-reproductive senescence. Within its ecosystem, hydras are involved in a variety of interactions with invertebrates and vertebrates. For instance, they are predators of various zooplankton species or even fish spawn (Elliott et al., 1997; Rivera-De la Parra et al., 2016). They can also serve as host for commensal (Coleman, 1966) or parasite (Stiven, 1965) species. Finally, they can be a prey for fish or mollusks grazing on plants (Baumga and Constance, 2005; Cuker and Mozley, 1981).

Domazet-Lošo and co-authors (Domazet-Lošo et al., 2014) have demonstrated that *H. oligactis* polyps can spontaneously develop tumors. These neoplasia not only severely alter the polyp's body shape, but tumor-bearing individuals also show a shift in their microbiota and have a higher number of tentacles (see after and also Domazet-Lošo et al., 2014; Rathje et al., 2020). These tumors reduce the hosts' fitness but rarely kill them, and are vertically transmitted to the buds when polyps reproduce asexually, resulting in the spread of the neoplasia to the new lineage. Conversely offspring resulting from sexual reproduction are tumor free (Domazet-Lošo et al., 2014). The prevalence of tumor-bearing individuals in the natural environment is unknown, but the observation of tumorous hydras in strains derived from wild sampled individuals suggests that hydras in natural populations can be susceptible to tumor development (Fig. 1). These tumors appear after 3 to 4 months of maintenance in stable laboratory conditions and

seem to be composed of aberrant germinal cells, like the tumors already described. However, more work is required to understand the causes of tumors' emergence in hydras.

Given the substantial phenotypic differences between healthy and tumorous hydras (Fig. 2), we hypothesized that tumorigenesis might influence the biotic relationships involving hydras. To test this hypothesis, we experimentally tested for the first time the consequences of tumor associated phenotypic alterations on three types of biotic interactions: hydras' ability to catch prey, their capacity to serve as a host for a commensal ciliate and their vulnerability to predators. Through this pioneer study, we aim to improve our understanding of the impact of tumorigenesis on biotic interactions.

## 2. Materials and methods

Clonal non-tumorous and tumorous individuals of *Hydra oligactis* strains (St. Petersburg strains as control and tumorous lineages from the laboratory of Thomas Bosch<sup>2</sup>) were used for all experiments (Domazet-Lošo et al., 2014). Polyps were maintained at 18 °C in Volvic® water and fed three times per week with freshly hatched *Artemia salina* nauplii according to standard protocols in tanks of 500 mL (Lenhoff and Brown, 1970). *Artemia* nauplii were obtained by adding 0.5 g of eggs microcyst (*Artemia salina*, Planktovie S.A.S., Marseille, France) in 400 mL of seawater prepared with 36 g/L of sea salt (Reef Crystals, Aquarium systems, Sarrebourg, France) and oxygenated with an aquarium pump. After 24 to 48 h of incubation at 30 °C, nauplii hatched and were collected with a pipette, rinsed with a filter and suspended in a 200 mL beaker of Volvic® water. All the experiments took place in Montpellier between the 06/15/20–09/15/20.

### 2.1. Hydra predation ability

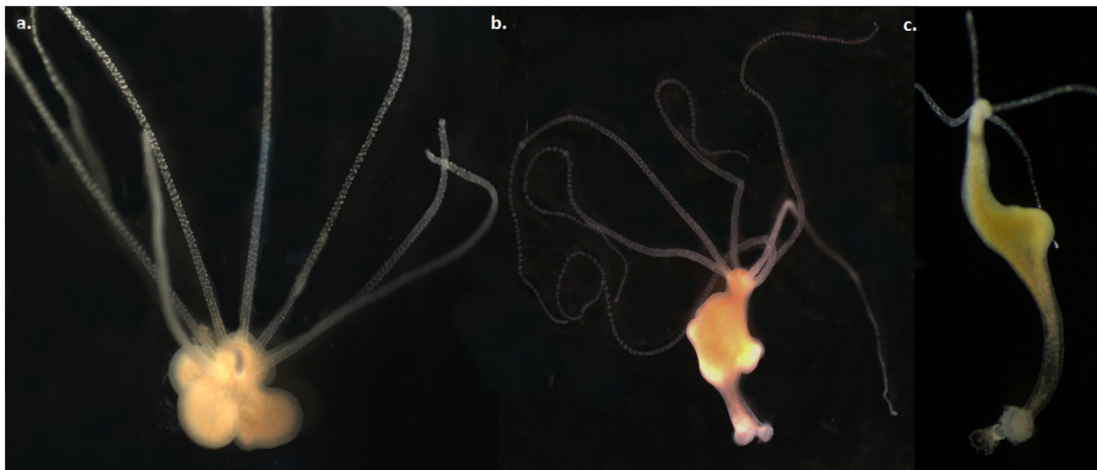
For each experiment, one hydra was placed in the experimental tank (20 mL well of a 6 well-plate; Thermo Scientific®) and left for 2 min of habituation to allow the polyp to reattach to the substrate. Prey were added at two different density levels: In ad libitum feeding (by adding 250 µL taken from the bottom of the *Artemia* beaker, representing more than hundred nauplii) or restricted feeding (by adding 250 µL of Volvic® water from the upper part of the beaker containing around 10 nauplii in total). The total number of nauplii added in restricted feeding was re-counted at each experiment (since some individuals can sometimes remain attached to the pipette tips, or conversely additional individuals can be introduced) and variations were taken into account in the analysis. In each experimental group (non-tumoral in restricted feeding, non-tumoral ad libitum, tumoral in restricted feeding, tumoral ad libitum) 42 hydras were measured. One tumoral hydra of the restricted feeding condition was excluded because of an error in the nauplii number introduced. The predation capacity of hydras was calculated by counting the number of preys captured in their tentacles in 10 min under the dissection microscope.

### 2.2. Hydra colonization by commensals

The ciliates *Kerona pediculus* (named ciliate thereafter) are naturally present on hydras both in the lab and in the wild, and considered as commensals (Coleman, 1966) and hence were used here as a model to test commensal interactions. We collected *K. pediculus* individuals on polyps from a *H. oligactis* C2/7 strain previously sampled in Hungary and maintained in J. Tökölyi's laboratory.<sup>3</sup> Ciliates were taken from C2/7 strain hydras using a one-milliliter pipette tip, under a binocular magnifier, by mildly aspirating the water around the hydra. In a first

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**Fig. 1.** Pictures of different individuals harboring spontaneous tumors in strains established from field sampling.

a. An individual sampled at Blyes in France ( $45^{\circ}50'55.5''\text{N}$ ;  $5^{\circ}14'10.1''\text{E}$ ) on the 09/17/2020 and developed tumoral masses four months later. b. This individual was sampled near Debrecen in Hungary on the 09/23/2019 and developed tumoral masses two months later. c. An individual of a lineage established from a field sampled individual at Tiszadorogma in Hungary, ( $47^{\circ}67'12''\text{N}$ ,  $20^{\circ}86'41''\text{E}$ ), this lineage is prone to develop tumors spontaneously in routine cultures.

experiment (free-choice), we introduced one ciliate into the 1.5 mL well of a microplate (12 well-plate, Thermo Scientific©) containing one tumorous and one non-tumorous hydras (both free from ciliates). After 4 h, we recorded on which hydra the ciliate was present, this experiment was replicated 42 times. A second experiment consisted in inoculating a single ciliate into a 1.5 mL well containing one hydra, tumorous or non-tumorous. We first verified the presence of the ciliate 15 h after the inoculation, before the ciliate started to asexually reproduce. The number of ciliates on each individual was counted daily over six days on 36 non-tumoral and 36 tumoral individuals. The trials for which ciliates failed to colonize the host were removed to focus the analysis on colonization dynamics.

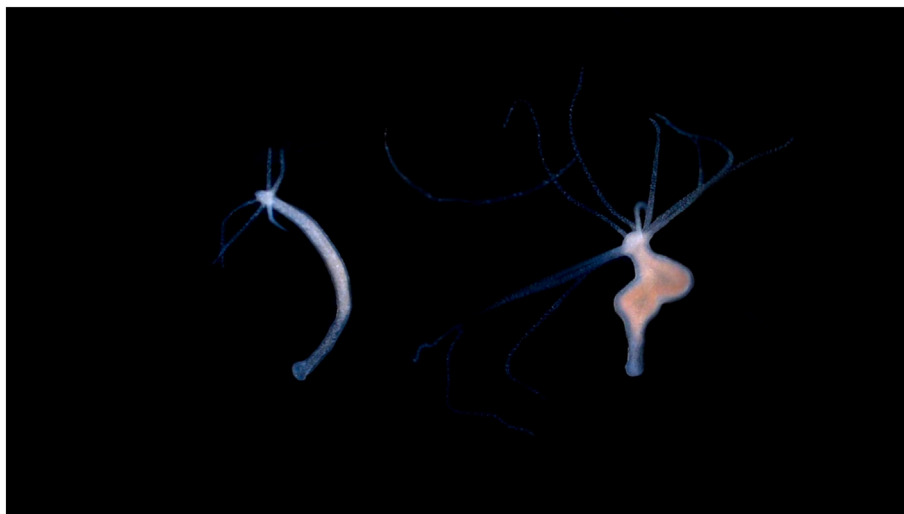
### 2.3. Predation risk on hydra

To model the predation interactions, we used Siamese Fighting fish (*Betta splendens*) as predator. In the aquarist circle, some fishes are well-known to consume hydras efficiently (Sharpe, 2020) but it is poorly documented in the literature (Baumga and Constance, 2005; Cuker and Mozley, 1981). Adult females were obtained from pet shops (Oxyfish©

Verlinghem and Botanic©, Clapiers, France). Each fish was introduced into 500 mL plastic tanks ( $16 \times 8.4 \times 6.5$  cm) in Volvic© water at  $25^{\circ}\text{C}$  at least 1 h before the test. A tumorous and a non-tumorous polyp were introduced together in the central well (20 mL) of a standard six well plastic plate for at least 10 min of acclimation. The plate containing the two polyps was then introduced progressively in the container with the fish and pressed the bottom against the wall in front of the camera to allow recording and visualization of each hydra during the test. The open part of the plate faced the tank to let the fish consume the polyps attached in the central well of the plate. Two observers identified precisely the position of each hydra during the test to recognize which one was consumed at each predation event. The test stopped after the predation of the two hydras or after 45 min in any case (i.e., no predation or only one specimen predated). This test was replicate 51 times and each replicate was recorded on a camera (GoPro©).

### 2.4. Statistical analysis

The effect of the tumorous phenotype on hydra predation was assessed by comparing the total number of *Artemia nauplii* captured



**Fig. 2.** Phenotypic differences between non-tumorous and tumorous hydras used in the present experiment.

The non-tumorous hydra is on the left and the tumorous one is on the right. An increase in body size and number of tentacles can be observed.

by the two types of hydras (tumorous and non-tumorous). For each model we started by representing the distribution of the variable to identify the relevant law to use, the normality was tested with Shapiro tests. When the variable was a proportion, we used binomial laws. When it was a count data we used Poisson law or a negative binomial in case of overdispersion with a Poisson model. The selection of random and fixed effects to include in the analyses was made by model comparison (see Zuur et al., 2013). The quality of models was evaluated by the package DARHMa, testing the homogeneity of the distribution, the distribution of the residuals, the overdispersion, the presence of deviant outlier and the quantile deviation.

In the ad libitum condition we used a Poisson generalized linear mixed model (glmm). In the restricted feeding condition, we used a binomial glmm taking into account the proportion of artemias consumed over the number introduced initially since small variation can exist between trials. In both analyses, the hydra type was a fixed effect. Random effects of the date was also included in ad libitum condition (see model choice in Supplementary material). The number of tentacles per polyp of each group was compared using a Wilcoxon test.

The effect of the supernumerary tentacles of the tumorous phenotype on hydra predation was assessed by comparing the total number of nauplii captured reported to the total number of tentacles. In the ad libitum condition, we used a log-normal linear mixed model with the tumorous status as a fixed factor and the date as a random effect (see model choice in Supplementary material). In the restricted feeding condition, we used a negative binomial generalized model taking into account the proportion of nauplii consumed reported to the total number of tentacles, the tumorous status was included as a fixed factor (see model choice in Supplementary material).

The proportion of tumorous to non-tumorous hydras colonized by ciliates was compared to the number expected under a no preference hypothesis (50%, binomial test). The verification has been done that there is no effect of the day of measurement using a Fisher exact test. We used a Poisson glmm to compare the number of ciliates in each group across time (in days) with the group as a fixed effect and the individual as a random effect (see Supplementary material).

The proportion of tumorous hydras captured by the predator was compared to the expected number under a random choice, using a binomial test. The verification has been done that there was no effect of the measurement day, using a Fisher exact test. We excluded from the analysis fish that attacked none of the hydras. The delay between the first and the second prey consumption was compared using generalized model with a Poisson law (see supplementary material). All analyses were performed using Rstudio (version 1.3; RStudio Team, 2021) and R (version 4.1.0; R Core Team, 2017). The list of packages used and the explained code are available in Supplementary material.

### 3. Results

#### 3.1. Hydra predation ability

The number of tentacles was significantly higher in the tumorous group ( $9.35 \pm 1.14$  for tumorous,  $5.71 \pm 0.75$  for non-tumorous; mean  $\pm$  standard deviation, W-value = 13,  $p < 2.2e-16$ ). Since the chief function of the tentacles is capturing prey, we hypothesized that the tumorous polyps might have a different predation capacity compared to the healthy polyps. We tested this hypothesis in two feeding experiments - using ad libitum and restricted amount of prey. In both feeding conditions, tumorous hydras captured significantly higher number of nauplii than non-tumorous ones (ad libitum: Fig. 3a., Incidence ratio rates (IRR) =  $1.31 \pm 0.07$ ;  $Z = 5.245$ ;  $df = 81$ ,  $p < 0.001$ ); restricted feeding condition: Fig. 3b., Odds ratio (OR) =  $1.54 \pm 0.22$ ;  $Z = 3.091$ ;  $df = 80$ ;  $p = 0.002$ ).

We also evaluated the importance of tentacle numbers on the feeding performance by reporting the predation abilities of each hydra to their number of tentacles. In ad libitum condition, tumorous hydras

captured significantly less prey than non-tumorous ones reported to their respective number of tentacles (Fig. 3c., Estimate =  $-0.17 \pm 0.08$ ;  $df = 81$ ,  $p = 0.025$ ). In the restricted feeding condition, there was no significant difference (Fig. 3d., IRR =  $0.89 \pm 0.15$ ;  $df = 80$ ,  $p = 0.488$ ).

#### 3.2. Hydra colonization by commensals

When given the choice between a tumorous and a non-tumorous hydra, the ciliates colonized preferentially the tumorous one (Fig. 4a.,  $81\% \pm 13\%$ ,  $p = 6.877e-05$ , alpha = 0.01, binomial exact test,  $n = 42$ ), without any effect of the day of measurement ( $p = 0.1081$ , alpha = 0.01, Fisher exact test,  $n = 42$ ). Of the 36 individuals inoculated with ciliates in the second experiment, 24 non-tumorous and 33 tumorous were colonized successfully by ciliates. Long-term observation of the colonization dynamics uncovered that the number of ciliates on hydras increased faster over time and then reached higher densities on tumorous hydras than on the non-tumorous ones (Fig. 4b., IRR =  $2.03 \pm 0.46$ ;  $df = 336$ ,  $p = 0.011$ ).

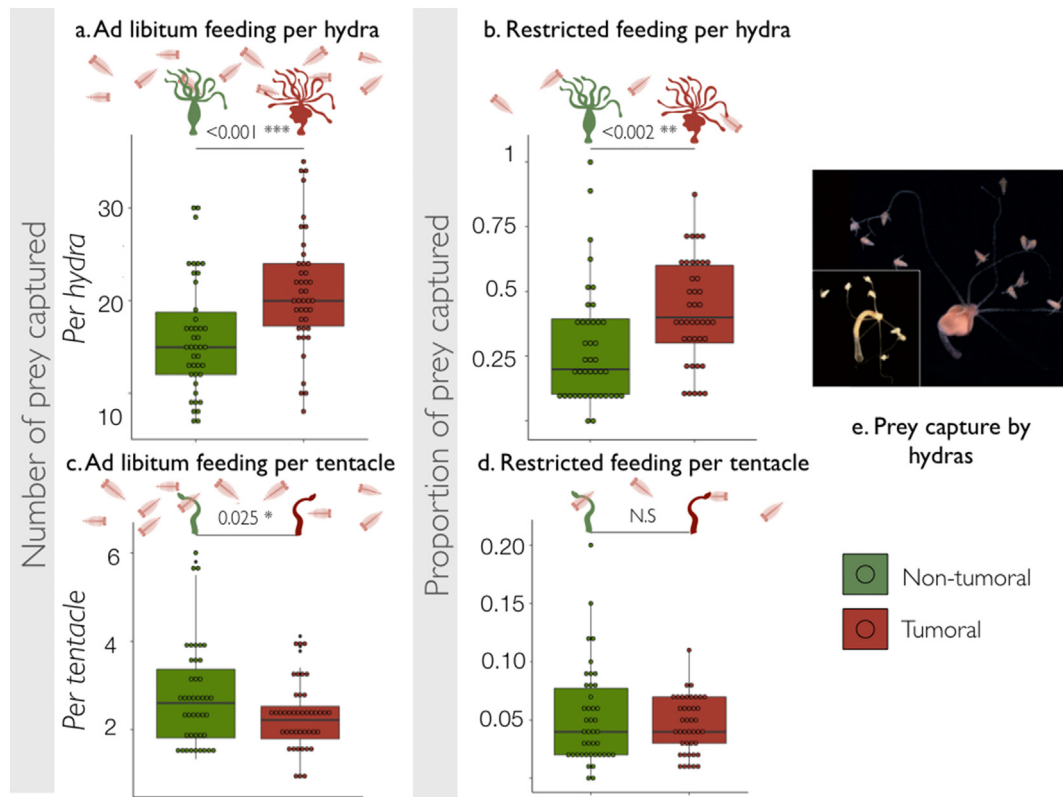
#### 3.3. Predation risk on hydra

Siamese fish ate hydras in 37 out of 51 trials and the first hydra consumed was significantly more often the tumorous one (Fig. 5a., i.e. 73% of cases, 27 times over 37 trials,  $p = 0.008$ , binomial exact test). Nine fish preyed only on the tumorous hydra and none of them preyed only on the non-tumorous one (Fig. 5b.). There was no influence of the measurement day ( $p = 0.650$ , Fisher exact test,  $n = 37$ ). When the first hydra consumed was a non-tumorous one, the time needed to observe the second predation event was significantly shorter than when the first hydra captured was the tumorous one (Fig. 5c.,  $715 \pm 738$  s vs.  $55 \pm 98.3$  s; IRR =  $0.08 \pm 0.04$ ,  $p < 0.001$ ,  $df = 26$ ).

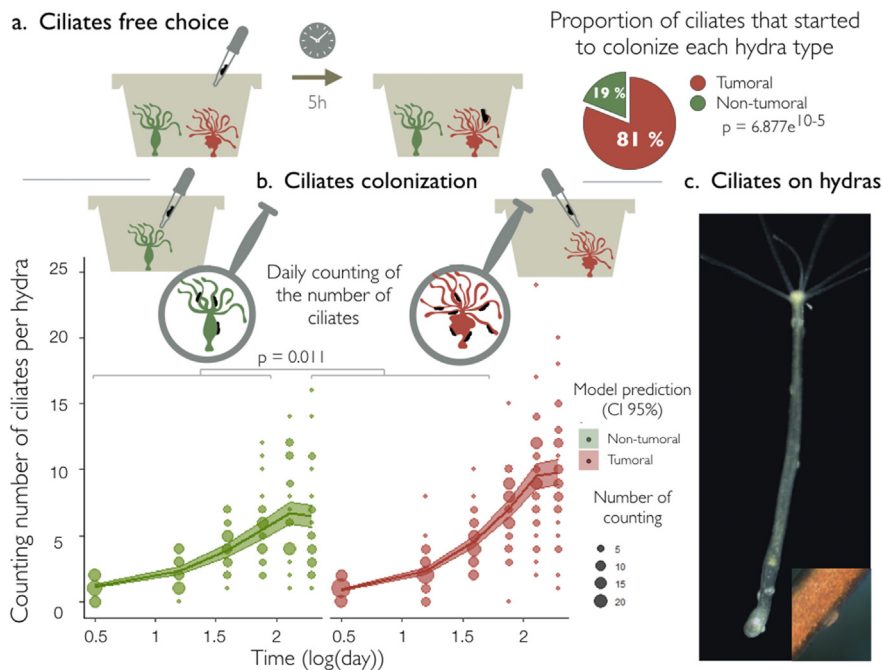
### 4. Discussion

Ecological implications of oncogenic processes, while theoretically important in ecosystem functioning (Vittecoq et al., 2013), have never been studied experimentally until now. This study provides empirical evidence for the first time for the hypothesis that the phenotypic consequences associated with the presence of tumor(s) in an organism reshape, qualitatively and/or quantitatively, various types of biotic interactions within the ecosystem. We found that (i) the predation ability of tumorous hydras was increased compared to non-tumorous ones, which is explained by their increased number of tentacles, (ii) the tumorous phenotype is more often colonized by a ciliate and with a faster colonization and (iii) tumorous hydras were preferentially eaten by predatory fish. Although tumorous and non-tumorous hydras used in our experiments differ in the presence of tumors and their phenotypic consequences, as well as their microbiota (Rathje et al., 2020), they originated from the same ancestral polyp and are genetically identical (clonal). Therefore, the alterations of biotic interactions reported here are most likely caused by the phenotypic alterations induced by the tumors (i.e. non-tumorous hydra being here a perfect control).

The significantly higher ability of tumorous hydras to capture prey may first appear counterintuitive given that neoplasms are usually associated with reduced host performances (Arnal et al., 2015; Perret et al., 2020). The most parsimonious explanation here is that this higher performance is due to the increased number of tentacles in tumor-bearing hydras compared to non-tumorous ones (see also Domazet-Lošo et al., 2014). Precise causes behind this novel phenotype are unclear and could have different origins since it could benefit the host (e.g. compensatory mechanisms, see for instance Thomas et al., 2020), the tumor (e.g. host manipulation, see for instance Tissot et al., 2016) or be beneficial for both the host and the tumor (see for instance Ewald, 1980). Deciphering the molecular cross-talk between the host and the tumor during the window when additional tentacles are growing could help



**Fig. 3.** Tumorous hydras have higher predation abilities. (a.) The number of prey captured per hydra in ad libitum feeding. (b.) the proportion of prey captured per hydra in restricted feeding. (c.) the number of prey captured per tentacle in ad libitum feeding and, (d.) the proportion of prey captured per tentacle in restricted feeding (b.) were counted during 10 min. Boxplots represent the first, median and third quartiles on the 95% extent. A point represents each measurement with the non-tumorous group in green (on the left) and the tumorous in red (on the right). (e.) Pictures of tumorous and non-tumorous hydras capturing prey.



**Fig. 4.** Ciliates established easier on tumorous hydras than on non-tumorous ones. (a.) Five hours after the introduction of a ciliate into an experimental tank containing both hydra types, we found 81% of the ciliates attached to the tumoral host instead of the non-tumoral one. (b.) After the introductions of one ciliate into the well of a tumoral or a non-tumoral individual, we counted daily the number of ciliates per hydra. Each point represents the number of ciliates measured on a given day; the size of the point depends on the number of hydras recorded at this measurement. The black line represents the average value predicted by the model and the colored areas the confidence interval at 95% predicted by the model. In green, on the left, the non-tumorous group and in red, on the right, the tumorous group. (c.) Pictures of eleven ciliates attached to a hydra at  $\times 20$  and zoomed picture of one ciliate on a hydra.



to explore concerns the ecological implications of these phenomena at the ecosystem level. Previous studies on parasites, notably manipulative ones, have for instance shown that many ecological consequences may result from the novel phenotype displayed by parasitized hosts (Lafferty et al., 2008; Lefèvre et al., 2009). This topic has been rarely addressed in the context of cancerous pathologies (Hamede et al., 2020; Roche et al., 2017; Vittecoq et al., 2013). Because tumorous hydras, compared to healthy ones, are both more efficient predators and more vulnerable prey, stronger trophic cascades could be for instance expected in the presence of tumorous hydras in the ecosystem, a process that is also likely to speed up energy flow and nutrient cycles as observed for other parasitic species (Mischler et al., 2016; Shurin and Seabloom, 2005; Wood et al., 2007). Similarly, by boosting the population dynamics of their commensal ciliates, tumorous hydras probably enhance the probability of other hydra species to be colonized by this generalist ciliate. Further studies manipulating the prevalence of tumorous hydras in the field and/or in mesocosms are necessary to explore and properly quantify these ecological consequences.

We developed and present here a unique experimental system, with tripartite microcosm composed of two interacting organism with, one containing tumoral cells considered as a third living entity impacting the interaction. The set-up allows assessing the impact of tumor burden on multiple biotic interactions within an ecosystem. Moreover, it can provide new insights for the mechanistic understanding of these dynamics. The simplicity of the system and its accessibility offers unlimited perspective for ecological, behavioral or even biochemical studies.

Beyond the present demonstration that the tumorigenesis has the potential to alter biotic interactions, further experiments manipulating in microcosms the frequency of tumor-bearing individuals in *H. oligactis* populations would be necessary to simulate the possible cascade effects on the whole aquatic community. As suggested above, these experiments should also test if the influence of tumors in biotic interactions depends on the phenotypic variation already existing in tumor-free individuals, especially natural size variability of non-tumorous hydras or variation of their tentacle numbers and/or predation performances. Our experiments also uncovered a truly surprising phenomenon: bearing a tumor may appear advantageous on particular aspects, all other things being equal. The evolutionary significance of this principle and its impact on the species' life history may be also studied in long-term experiments.

The results presented here suggest that neoplastic processes in biological communities should be fully considered by ecologists to understand and predict the evolutionary ecology of species interactions, as well as their consequences on ecosystem functioning. This research direction is likely to become crucial in the future given that ecosystems are increasingly exposed to oncogenic pollution resulting from anthropic activities (Giraudeau et al., 2018).

### CRediT authorship contribution statement

JB, MG, BU and FT designed the study. JB, JM, LB and LF collected the data, while JB, JT and AD performed the analyses. JT and AK provided biological material and technical training. JB wrote the manuscript, and all authors significantly contributed to revisions.

### Data availability statement

Data supporting the results will be archived in Hal should the manuscript be accepted. Readers should get in contact with the journal if they discover issues with data.

### Ethics statement

All experimental animals were treated in accordance with French and European Union regulations (directive 2010/63/UE). All the behavioral observations were included in the project authorization APAFIS: 29961. No fish were injured or killed during the experiments.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.149923>.

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