



The widespread vulnerability of *Hydra oligactis* to tumourigenesis confirms its value as a model for studying the effects of tumoural processes on the ecology and evolution of species

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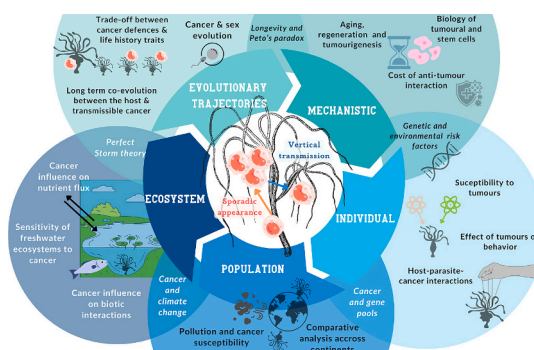
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HIGHLIGHTS

- Tumoral processes are important to understanding evolutionary biology and ecosystem dynamics
- There is a need for good laboratory models in a context of increased oncogenic pressures
- We show that *Hydra oligactis* has widespread geographical vulnerabilities to tumorigenesis
- This species is thus a great model to study the effect of tumours on ecosystem functioning
- We discuss the implications of using this model in future comparative studies

GRAPHICAL ABSTRACT



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ABSTRACT

Tumoural processes, ubiquitous phenomena in multicellular organisms, influence evolutionary trajectories of all species. To gain a holistic understanding of their impact on species' biology, suitable laboratory models are required. Such models are characterised by a widespread availability, ease of cultivation, and reproducible tumour induction. It is especially important to explore, through experimental approaches, how tumoural processes alter ecosystem functioning. The cnidarian *Hydra oligactis* is currently emerging as a promising model due to its development of both transmissible and non-transmissible tumours and the wide breadth of experiments that can be conducted with this species (at the individual, population, mechanistic, and evolutionary levels). However, tumoural hydras are, so far, only documented in Europe, and it is not clear if the phenomenon is local or widespread. In this study we demonstrate that Australian hydras from two independent river networks develop tumours in the laboratory consisting of interstitial stem cells and display phenotypic alterations (supernumerary

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tentacles) akin to European counterparts. This finding confirms the value of this model for ecological and evolutionary research on host-tumour interactions.

1. Introduction

Tumoural processes appeared early in the evolution of metazoan species and are omnipresent in the tree of life (Aktipis et al., 2015; Albuquerque, 2018; Boutry et al., 2022c). The necessity for metazoans to prevent the appearance and proliferation of cheater cells that overuse resources and replicate out of control has led to the evolution of various anti-tumoural defences (e.g., DeGregori, 2011). Species must invest significant amount of resources to maintain these anti-cancer defences (e.g., it is one role of the immune system in vertebrates, Nilsson et al., 2007). As such, anti-cancer defences form a complex protective system in which natural selection optimises trade-offs between tumour suppression, reproduction, and other maintenance functions (Boutry et al., 2020; Dujon et al., 2024; Dujon et al., 2023; Jacqueline et al., 2017). When anti-cancer defences fail, the consequences of tumour progression are often dramatic for individuals (e.g., cancer), significantly increasing their mortality rate, reducing their fitness, and reshaping their interactions with other species (Arnal et al., 2017; Boutry et al., 2022a; Hollings et al., 2016). Thus, it is crucial to determine the role of tumoural processes in the ecology and evolution of species in order to gain a comprehensive understanding of their impact on ecosystem functioning (Dujon et al., 2021a; Vittecoq et al., 2013). Understanding this relationship is also particularly relevant and timely because human activities are increasing oncogenic pressures which promote the emergence of tumoural cells in wildlife (Baines et al., 2021; Dujon et al., 2021d, 2021e; Giraudeau et al., 2018; Pesavento et al., 2018).

Despite the well-established impact of tumoural processes on the physical condition of individuals and their widespread occurrence in the animal kingdom (Aktipis et al., 2015; Albuquerque, 2018; Vincze et al., 2022), it remains paradoxical that our understanding of the extent of their effects and consequences, both empirically and theoretically, is still limited (Thomas et al., 2017). A notable exception is the case of transmissible cancers, which, due to recent research breakthroughs, have garnered significant attention from ecologists (Beer et al., 2024; Boutry et al., 2022a, 2022b; Hollings et al., 2014; Lachish et al., 2009). For example, field studies on the Tasmanian devil were used to demonstrate that the elimination of a keystone species by a transmissible cancer can cause an important trophic cascade and proliferation of invasive species and associated diseases (Cunningham et al., 2020; Hollings et al., 2016). The bivalve transmissible neoplasia (likely spread by human activities and able to jump between species causing mass mortality events in their host) is studied through extensive screening of wild or farmed bivalves (Bramwell et al., 2021; Hammel et al., 2024; Hammel et al., 2022). While precious, these *in natura* models have, so far, mostly led to observational studies. This is because the complexity of ecosystems rarely allows for the testing of the effect of one factor at a time on biological processes (Sugihara et al., 2012). For this purpose, laboratory models are often indispensable to establish causal links before translating them to the ecosystem level (Imai et al., 2013). Ideally, a good laboratory model for this purpose should be easy to sample, have a widespread geographical distribution, be easy to maintain and manipulate, be capable of multiplication in the laboratory, and result in the production of replicable results when subjected to experimental treatments (Swearengen, 2018). For example, mice (*Mus musculus*) and zebrafish (*Danio rerio*) are commonly used for the study of human cancer (Roth et al., 2021; White et al., 2013) and are also used for evolutionary ecology experiments (e.g. Boutry et al., 2024a; Gouzerh et al., 2022). Other species, such as planaria (*Girardia tigrina*) or drosophila (*Drosophila melanogaster*) have also been used to investigate the ecological effects of tumoural processes (Arnal et al., 2017; Dawson et al., 2018; Duneau and Buchon, 2022; Klaasen et al., 2024). In those species, tumours are primarily induced

through genetic modifications which can introduce confounding factors when studying the effects of tumour progression on the ecology of species (i.e., it is not always clear if the measured effects are due to the tumour or the genetic modification by itself). Another particularly promising model, *Hydra oligactis*, a freshwater cnidarian capable of both sexual and asexual reproduction, has recently caught the attention of researchers. Indeed, in this species it is possible to induce both non-transmissible and vertically transmissible tumours without the use of genetically modified organisms. These tumours are made of abnormally proliferating interstitial cells, the stem cell lineage from which most cell types (including gametes) are derived from in adult hydra (Boutry et al., 2023; Diehl and Burnett, 1964, 1965; Domazet-Lošo et al., 2014; Nishimiya-Fujisawa and Kobayashi, 2012). These characteristics allow the effect of tumours on hydra biology to be studied and to establish proof of concepts with the aim to translate them to ecosystem levels (Boutry et al., 2022a, 2022b; Domazet-Lošo et al., 2014; Tissot et al., 2023).

Hydra oligactis has recently been used as a model to investigate how tumoural processes affect predator-prey interactions, the hydra microbiome and commensals, alter their life history traits, and to study the de novo-emergence of transmissible cancers (Boutry et al., 2024b; Boutry et al., 2024a; Boutry et al., 2022b; Rathje et al., 2020; Tissot et al., 2024a). This species is also widely used to study the effect of aging, body regeneration, and the biology of stem cells (Bosch, 2009; Galliot et al., 2018; Tomczyk et al., 2015; Yoshida et al., 2006). When cultured in the laboratory, tumours are induced in this species using one of following methods: overfeeding the animals, manipulation of their microbiome, or through the grafting of tumour fragments (Boutry et al., 2024b; Dujon et al., 2022; Rathje et al., 2020; Tissot et al., 2023). However, tumours in *Hydra oligactis* have only been reported in European laboratories (in France, Hungary, and Western Russia, see Boutry et al., 2023; Domazet-Lošo et al., 2014) despite the species being present on nearly all continents (Martínez et al., 2010). This is very likely due to a lack of attempts to replicate the tumour induction protocols on a different continent. The discovery of additional *Hydra oligactis* populations undergoing tumorigenesis outside of Europe in laboratory settings would significantly enhance the relevance of this species as model for understanding the impact of tumoural processes on ecosystem functioning.

In this publication we describe the occurrence of spontaneous tumours in the laboratory for wild Australian *Hydra oligactis* from two different river systems. We describe the phenotype of tumoural hydras, identify the proliferating cell types, and document the appearance of supernumerary tentacles. We then discuss the implications of this discovery in the light of recent insights obtained with this model in Europe.

2. Material and methods

2.1. Sampling and mass culture

Australian wild *Hydra oligactis* were sampled in January 2023 in Monbulk Creek (37°55'23"S 145°19'45"E) and Werribee River (37°41'26"S 144°27'38"E), each part of two independent catchments in the state of Victoria. We confirmed that the sampled individuals were of the *Hydra oligactis* species based on the following phenotypic criteria: 1) absence of symbiotic zoochlorellae; 2) tentacles less than five times the length of the body column; 3) polyps with a well-defined foot; 4) holotrichous isorhizas that are narrowly oval or cylindrical (with width less than half the length) wound with thread longitudinally throughout but without transverse coils; and 5) tentacles on buds arising successively in a fixed pattern with lateral tentacles appearing first (Forrest, 1963; Lenhoff, 1983; Schuchert, 2010).

Sampled individuals were transported to the laboratory at Deakin

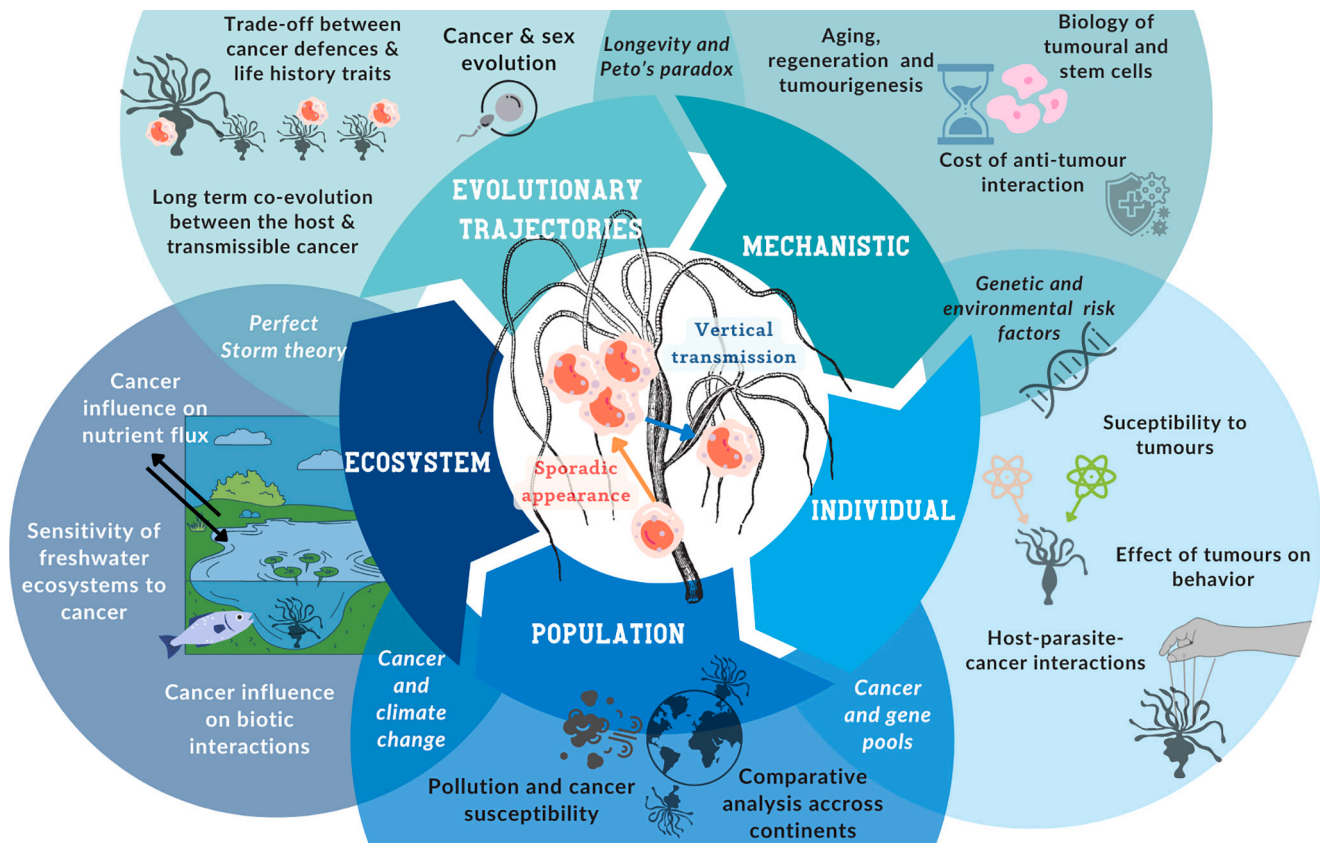


Fig. 1. Summary of the key areas in which the *Hydra oligactis* model can be used to improve our understanding of the effect of tumourigenesis on the ecology and evolution of species. The following references were used to create the figure: individual (Boutry et al., 2023; Boutry et al., 2022a; Boutry et al., 2022b; Domazet-Lošo et al., 2014; Rathje et al., 2020; Tissot et al., 2024a; Tissot et al., 2023), population (Boutry et al., 2022a; Dujon et al., 2021e, 2024), ecosystems (Dujon et al., 2024; Dujon et al., 2021c; Quinn et al., 2012; Tissot et al., 2022), evolutionary trajectories (Thomas et al., 2017), and mechanistic (Tomczyk et al., 2015; Yoshida et al., 2006).

University, Waurn Ponds campus, and placed in mass culture conditions in 400 mL glass crystallisers filled with hydra culture media (1 mM CaCl_2 , 0.1 mM MgCl_2 , 0.08 mM MgSO_4 , 0.5 mM NaHNO_3 , 0.03 mM KNO_3 , in distilled water) under a 12/12-h day night/cycle at 18 °C in a Thermoline Scientific refrigerated incubator. Five times a week in the morning (Monday-Friday), the mass cultures were fed ad libitum with the nauplii of freshly hatched *Artemia salina*. In the afternoon, the mass cultures were cleaned of food debris and dead *artemia nauplii*, and if needed, their water supplemented with fresh media. This five time per week feeding regime is known to increase the rate of spontaneous tumour emergence in the European strains (Tissot et al., 2023). The *artemia nauplii* were hatched by mixing 0.5 g of eggs (AquaLabs, Australia) in a bottle containing 400 mL of reconstituted salt water (36 g.L⁻¹ in distilled water, Ocean Salts) and bubbled with an aquarium pump overnight under a lamp in a 30 °C warm bath. Over the weekends the bottle was left bubbling at ambient temperature on a laboratory bench (20 °C) to obtain freshly hatched *nauplii* on Mondays. If needed, the mass cultures were fully cleaned (every 2–3 weeks depending on bacterial film development) by transferring the hydras into a clean crystalliser (Lenhoff, 1983). The commensal protozoa *Kerona pediculus* commonly observed living on the European strains (Boutry et al., 2022a; Coleman, 1966; Tissot et al., 2024b) was not observed in any of the mass cultures. All hydras used in this study are a mix of clonal offsprings from the founding individuals used to establish the mass cultures. It is not known if those founding individuals were themselves clones or genetically distinct individuals born from sexual reproduction.

2.2. Classification of hydra phenotype

In Europe, *Hydra oligactis* develops tumours made of proliferating interstitial cells located in the ectoderm of the animal leading to the appearance of abnormal growths that can be visually identified (Boutry et al., 2023; Domazet-Lošo et al., 2014; Tissot et al., 2023). We thus screened weekly the mass cultures of Australian hydras under a Zeiss Stemi 2000-C dissecting microscope to detect hydras presenting a similar phenotype. *Hydra oligactis* is gonochoric, meaning individuals are genetically distinct male or female, but phenotypically indistinguishable in the asexual phase. However, the sex of each hydra can be visually identified during the sexual phase: males develop testes, which appear as nipple-shaped organs on the body column, while females develop eggs, which form as a round structure on the side of the body column. In mature sexual males, functioning sperm, with a flagellum can be observed (Lenhoff, 1983; Littlefield et al., 1991). During this experiment the hydras were separated into three classes: 1) tumoural when the presence of abnormal growths was observed; 2) males undergoing testes development; or 3) asymptomatic if the hydra was neither tumoural nor undergoing testes development (see Fig. 2 for a more detailed description of the phenotypes). Since it is not possible to determine the sex of hydras sampled in the field unless they have developed reproductive organs, there is a likelihood of sampling only one sex because hydras are known to form clusters with high sex ratio biases (Kaliszewicz, 2018). This is the case in this study and no female hydra (identified by the presence of an egg developing on the individual) was observed.

For each observed hydra we also determined the number of tentacles because the presence of supernumerary tentacles in hydra is a significant

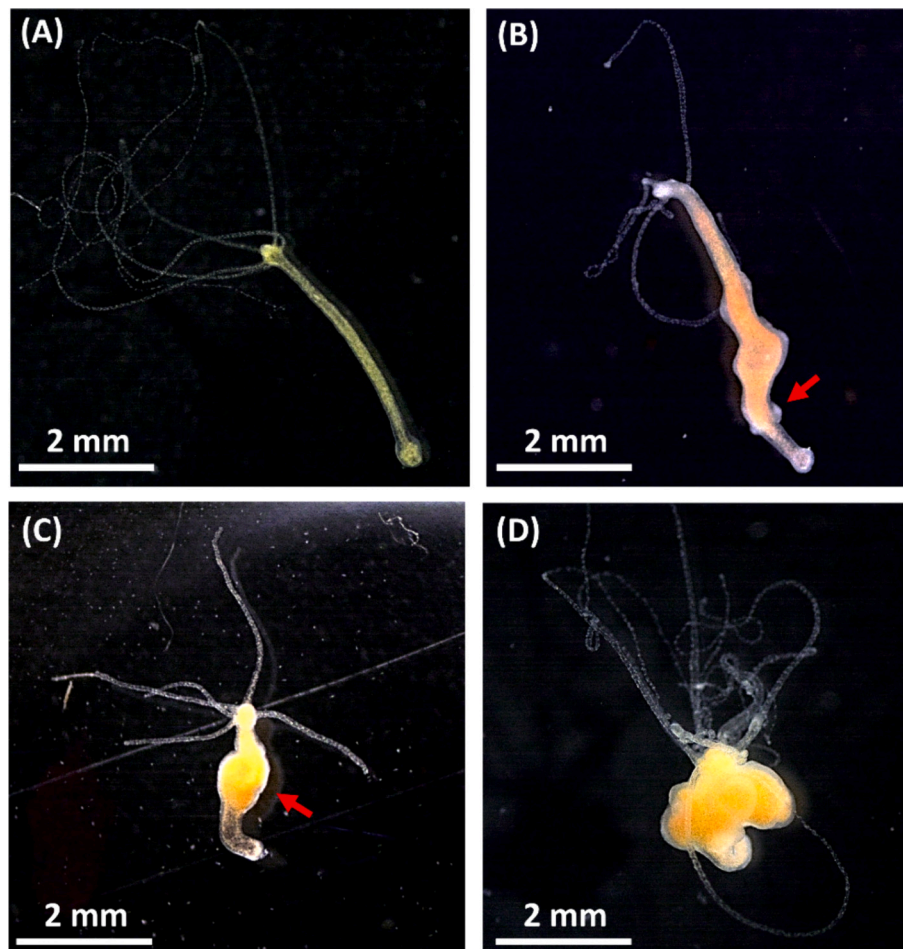


Fig. 2. Photographs of Australian *Hydra oligactis*: (A) an asymptomatic hydra, with a very thin body column. This is the typical phenotype of an hydra not undergoing sexual reproduction; (B) a male undergoing gonadogenesis with a formed testis (red arrow), the ectoderm exhibiting a translucent thickening not present in tumoural hydras, and with the presence of nipple-shaped testes; (C) an individual with a tumour of medium size (red arrow) showing a non-translucent thickening of the ectoderm and an absence of testes/eggs; and (D) an individual with a large tumour and supernumerary tentacles displaying a body column that is completely distorted by the tumour, multiple times the width of an asymptomatic hydra. Prior to taking the photographs, hydras were relaxed in a solution of 1 mM of linalool (Goel et al., 2019). As tumour development progressed hydras tended to lose their ability to relax. The phenotypes described above were common to the two sampling sites.

deviation from their homeostasis. Supernumerary tentacles are present in the European Saint Petersburg line which harbours a vertically transmissible tumour and has been cultivated in the laboratory for >15 years (Boutry et al., 2024a, 2024b; Domazet-Lošo et al., 2014). In Europe, supernumerary tentacles were, so far, not documented for Hungarian or French individuals which developed spontaneous tumours in the laboratory (Boutry et al., 2023).

2.3. Macerations and cell type counts

To determine which cell type(s) proliferated in Australian tumoural hydras, asymptomatic, tumoural, and males undergoing testes development were dissociated using a commonly used maceration protocol (David, 1973). Individual hydras were placed in 1.5 mL Eppendorf tubes and macerated into 50 μ L of a mix of 1:1:12 glycerol:acetic acid:water for 30 min at 30 °C. After incubation the hydras were dissociated by gently tapping the bottom of the Eppendorf tubes with a finger until the formation of a cloudy solution, and 50 μ L of 4 % paraformaldehyde was added to the mix to fix the cells. Then 100 μ L of macerate was spread on ~ 2 cm² at the surface of a glass slide pre-coated with a mix 0.5 % pork gelatine and 0.05 % Chromium (III) potassium sulphate dissolved in milli-Q water. The macerations were left to dry for at least 12 h and observed on a Zeiss Axio microscope, using differential interference

microscopy to take pictures at a magnification of X400. The pictures were then used to count the individual number of small interstitial cells, large interstitial cells, and epithelial cells (Fig. 3) using DotDotGoose, version 1.7.0 (Erst, 2024). At least 200 cells were counted per individual hydra. Interstitial cells were identified as the cellular type forming tumours in the European strain (Boutry et al., 2023; Domazet-Lošo et al., 2014). These counts were subsequently used to calculate the ratio between the numbers of the three cell types (Boutry et al., 2023). These ratios are then presented as proportions for statistical analyses. Here, the number of epithelial cells was used to standardise cell counts as this number remains relatively stable in well-developed, well-fed hydras (Holstein and David, 1990) with a ratio of 0.34 to 0.56 between large interstitial cells and epithelial cells and of 0.46 to 0.87 between small interstitial cells and epithelial cells (measured by Flechtner and Lesh-Laurie, 1984). In *Hydra oligactis* large interstitial cells divide into small interstitial cells which then differentiate, among other cell types, into germinal cells (Diehl and Burnett, 1964; Nishimiya-Fujisawa and Kobayashi, 2012). The area of the small and large interstitial cells (in μ m²) was also determined using the photographs for 30 individual cells of each type per individual with the help of ImageJ, version 2.1.0 (Eliceiri et al., 2012).

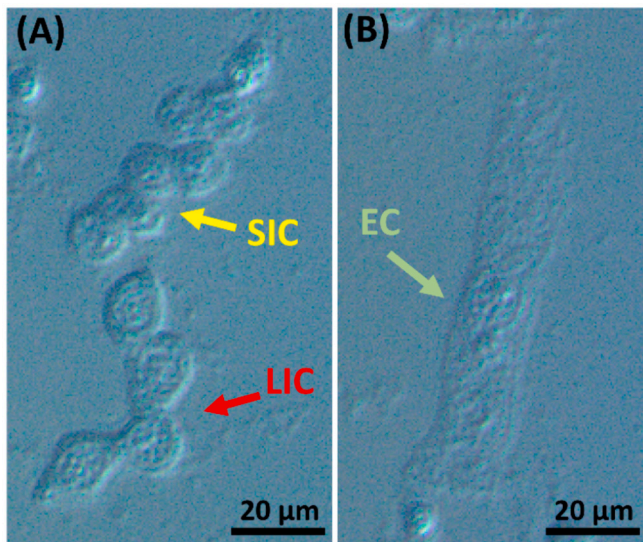


Fig. 3. Australian *Hydra oligactis* cells: (A) Seven small interstitial cells (SIC), four large interstitial cells (LIC); and (B) an epithelial cell (EC) obtained by macerating an individual and visualised using differential interference microscopy.

2.4. Histology

To visualise cellular proliferation in hydra tissues, we performed histology on asymptomatic and tumoural hydras from both sampling sites. The hydras were first relaxed in a solution of 1 mM of linalool (an anaesthetic of freshwater hydra, Goel et al., 2019) and then fixed in a 4 % formaldehyde solution. The fixed hydras were then embedded in paraffin, sectioned transversally, and stained using toluidine blue by the Melbourne University histology platform (Lenhoff, 1983). The resulting slides were then observed using a Zeiss Axio microscope under bright field light and photographed.

2.5. Statistical analyses

We used generalised linear models (GLM) with a Gaussian distribution to compare the average cell ratios between asymptomatic, male undergoing testes development, and tumoural hydras (the distribution of ratios being close to a Gaussian distribution and the cell count used to calculate the ratio for each individual being relatively large). In this model we included the study site and the hydra type as explanatory variables along with an interaction term between these two categorical variables.

To compare the average cell sizes, we used a generalised linear mixed effect model (GLMM) with a Gaussian distribution. As explanatory variables, we included the study site and the hydra type along with an interaction term between these two categorical variables. Each macerated hydra was given a unique categorical identifier which was included in the models as a random intercept to account for repeated cell size measurements within individuals (Zuur et al., 2009).

The average number of tentacles per hydra was compared between the three hydra types and the two sites using a generalised linear model with a Poisson distribution (Zuur et al., 2009). As for the previous models, the hydra type and the study site along with an interaction between the two were included as response variables.

We applied a model selection procedure to determine which explanatory variables were important in the models described above. For this purpose, we used the Akaike Information Criterion (AIC), Δ AIC and AIC weights, retaining the models with the lowest AIC, Δ AIC and the highest AIC weight (Symonds and Moussalli, 2011).

All statistical analyses conducted in this study were completed using

the R statistical (R Development Core Team, 2020) software using the RStudio graphical user interface (Team RStudio, 2020). Linear models were fitted with the glmmTMB package (Brooks et al., 2017) and the assumption of the models (residual distribution and variance, presence of outliers) were checked using the DHARMA package (Hartig, 2020). Across this manuscript effect sizes are given with their standard deviation ($\pm$ SD).

3. Results

In July 2023, less than six months after sampling, tumoural hydras appeared in all the mass cultures from both study sites, concomitant with the appearance of a small number of male hydras undergoing gonadogenesis (evidenced by the appearance of testes along their body columns, Fig. 3b). Visually, the phenotype of tumoural Australian hydras is similar to the phenotype of European strains with a deformed upper body column, and with a tumour that is often multilobed in later stages (pumpkin-shaped alike) (Fig. 3c-d). All the observed individuals were males, but no sperm (evidenced by the presence of a well-formed flagella) was observed in either the macerations or mass cultures water, suggesting that male gonadogenesis was incomplete.

The tumoural hydras from both sites were characterised by a proliferation of small interstitial cells, and to a lesser extent of large interstitial cells. Indeed, the ratio between small interstitial cells and epithelial cells increased from an average of 0.8 ± 0.5 in asymptomatic hydras to 2.0 ± 0.4 in tumoural hydras. For large interstitial cells, the ratio with epithelial cells increased from 0.5 ± 0.2 for asymptomatic hydras to 1.0 ± 0.2 in tumoural hydras. Cellular proliferation is potentially slightly more pronounced for small interstitial cells as evidenced by an increase in the ratio of small to large interstitial cells from 1.5 ± 0.3 in asymptomatic hydras to 2.2 ± 0.2 in tumoural hydras (Fig. 4a-b, Gaussian GLM, $p = 0.053$). Both interstitial cell types also proliferated significantly more in males undergoing testes development compared to asymptomatic and tumoural hydras, with a ratio of 6.1 ± 0.4 between small interstitial and epithelial cells, 1.9 ± 0.2 between large interstitial cells and epithelial cells and 3.7 ± 0.3 between small and large interstitial cells (Gaussian GLM, $p < 0.001$). This suggests the cell proliferations observed in tumoural hydras is an abnormal stage between an asymptomatic hydra and a male hydra undergoing tested development. The average area of the small ($41 \pm 2 \mu\text{m}^2$) or large interstitial cells ($94 \pm 2 \mu\text{m}^2$) remained the same between the three hydra types and the two study sites (Gaussian GLMM, $p > 0.05$, Fig. 4c-d), thus the size of tumours is not driven by an increase in cell size but by the increase in the number of interstitial cells. Full details of the fitted statistical models are presented in Supplementary tables 1–10. Histological analyses indicate that proliferating interstitial cells are located within the ectoderm of hydras (Fig. 5).

The average number of tentacles of asymptomatic hydras was 4.9 ± 0.8 for Monbulk creek hydra and 5.1 ± 0.8 for Werribee River. These numbers were close to those of male hydras undergoing testes development with an average 4.6 ± 1.1 tentacles for Monbulk creek and 4.6 ± 1.2 tentacle for Werribee River (Poisson GLM, $p > 0.05$). In contrast the average number of tentacles was higher in tumoural hydras compared to the two other types with an average of 5.5 ± 1.8 tentacles for Monbulk creek and 5.7 ± 2.1 for Werribee River (Poisson GLM, $p < 0.001$). These differences are driven by the presence of tumoural individuals with supernumerary (> 7) tentacles, with a maximum number of 12 observed tentacles (Fig. 6). No such supernumerary tentacles were observed in asymptomatic hydras or males undergoing testes development. The average number of tentacles was similar between the two study sites for each of hydra type (Poisson GLM, $p > 0.05$, Fig. 6). Full details of the fitted statistical models are presented in Supplementary tables 11 and 12.

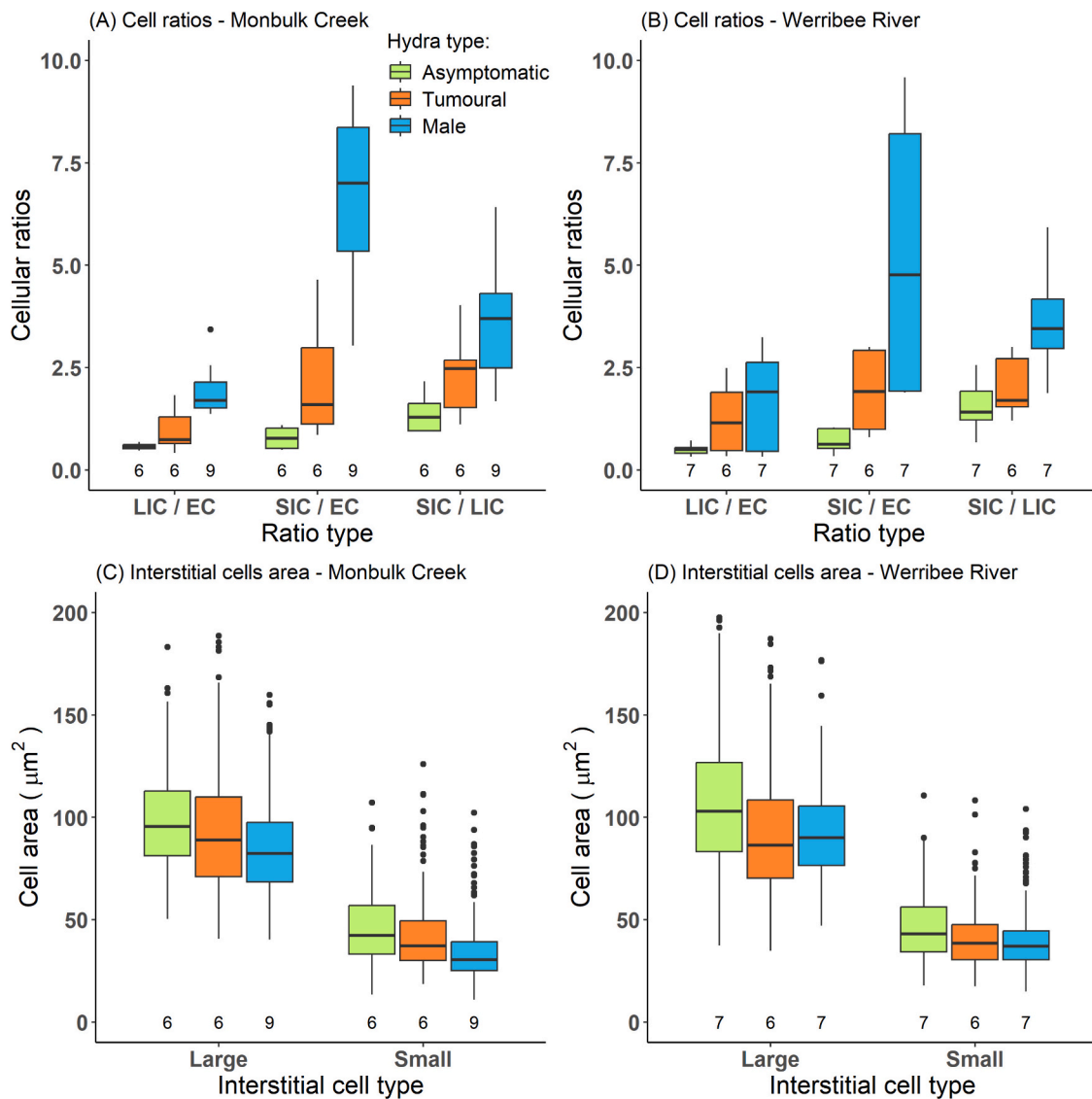


Fig. 4. (A–B) Ratios between large interstitial cells (LIC), small interstitial cells (SIC), and epithelial cells (EC) for asymptomatic, tumoural, and male hydras undergoing testes development from Monbulk Creek and Werribee River. (C–D) Large interstitial and small interstitial cells' area for asymptomatic, tumoural, and male hydra from the same two study sites. The numbers under the boxplots indicate their respective sample sizes.

4. Discussion

4.1. Tumoural processes are widespread in *Hydra oligactis*

With this study we observed for the first time the emergence of spontaneous tumours in Australian *Hydra oligactis*, providing evidence that vulnerabilities to tumoural processes are widespread in this species. The tumours we observed, based on their morphology and cellular proliferation patterns, are phenotypically similar to those observed in the European strains (Boutry et al., 2023; Domazet-Lošo et al., 2014). We found evidence that the proliferation of interstitial cells represented an abnormal phenotype intermediate between an asymptomatic individual and a male undergoing gonadogenesis, suggesting that the tumours form as part of a disruption of the reproduction (as previously suggested, Domazet-Lošo et al., 2014). We also observed the appearance of supernumerary tentacles in tumoural hydras. So far supernumerary tentacles were only documented in the Saint Petersburg laboratory strain which was maintained for more than ten years in the laboratory and for which the timing of the appearance of supernumerary tentacles is uncertain (Domazet-Lošo et al., 2014) and which was not documented in any wild strain despite investigations. With this second documented

example, we show that supernumerary tentacles can appear relatively quickly after the appearance of the first tumours in the populations.

Two main hypotheses could explain the appearance of supernumerary tentacles in tumoural hydras. In the first scenario, the phenomenon is a compensatory response to the appearance of tumours; the additional tentacles allow the hydra to consume more food to compensate for the increased energetic cost of bearing tumours. In the second scenario, and under the assumption that the tumours are vertically transmitted (which is the case in the Saint Petersburg line, see Domazet-Lošo et al., 2014), the supernumerary tentacles are the result of an adaptation, with a parasite-like tumour that manipulates the host to maximize its transmission in the population. Indeed, in hydras, the budding rate is positively correlated with the amount of food ingested (Stiven, 1965) and an increased budding rate may favour tumour transmission. Recent experiments suggest it is the case in the Saint Petersburg line (Boutry et al., 2024b). In our mass cultures the proportion of individuals with supernumerary tentacles was low. We believe that the time between the emergence and the spread of this trait in the mass cultures through asexual budding was too low for it to be fixed in the population. In addition, our mass cultures were established from a mix of different individuals, and thus contained multiple clonal lines

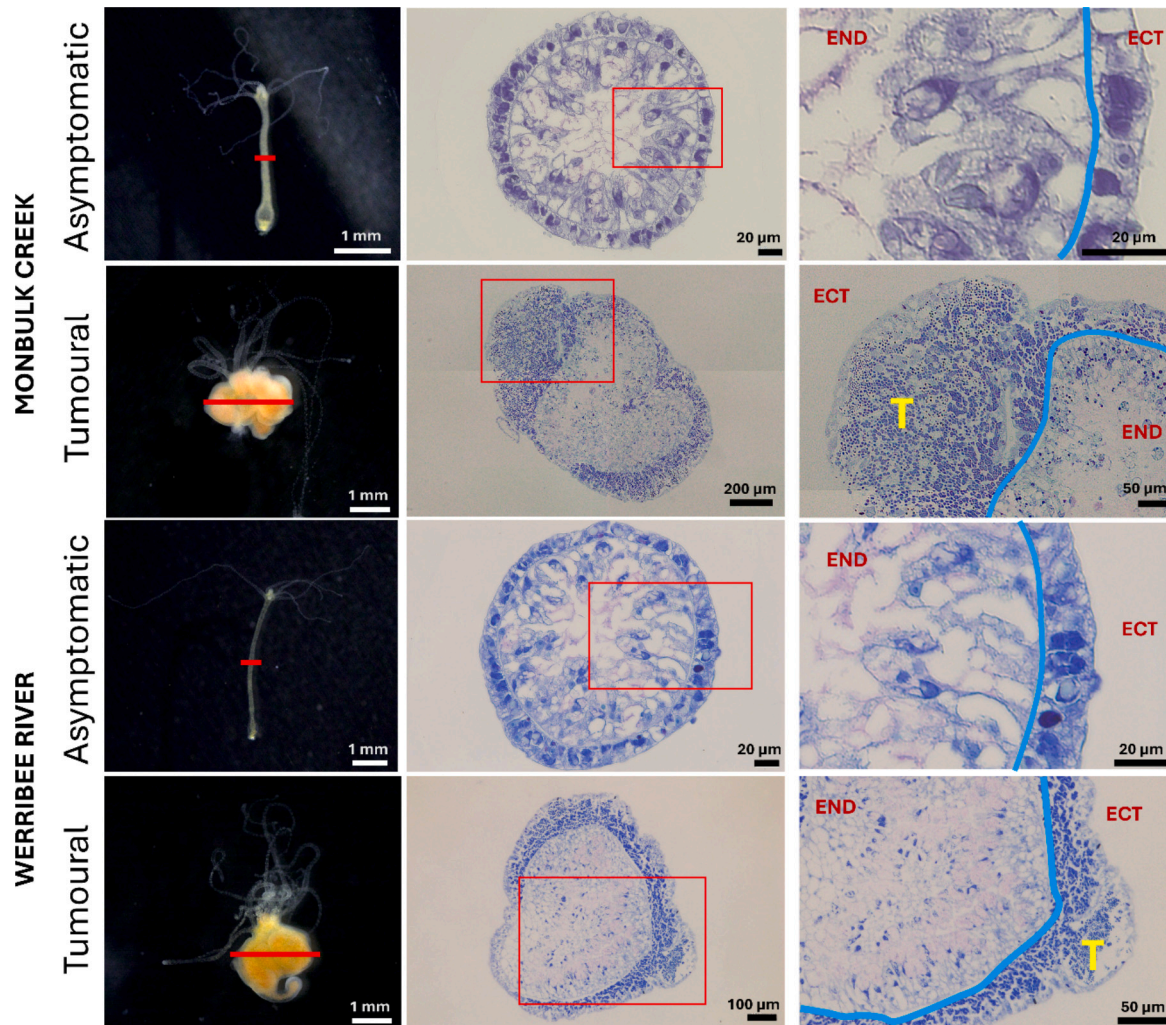


Fig. 5. Photography of an asymptomatic and tumoural hydra from Monbulk creek and Werribee River along with their associated transverse histology section (approximate position represented by the horizontal red lines). A closer detail of the structure of each hydra (indicated by the red rectangles) is also provided with the mesoglea highlighted with a solid blue line, the endoderm (END) and ectoderm (ECT) sides marked in red, and the locations of the tumours formed by proliferating interstitial cells with a yellow T. Slides were stained with toluidine blue which highlight interstitial cells. In tumoural hydra, the proliferation of interstitial cells is visible in the ectoderm. Note the difference in width between healthy and tumoural hydras as shown by the first column of images (black background) of the entire hydra.

potentially slowing down the spread of the trait to the whole population. Future studies could investigate the rate at which supernumerary tentacles appear in tumoural hydra and how fast this trait spreads in the population.

We did not observe any females undergoing gametogenesis in the mass cultures over the course of the experiment. It is likely that only males were sampled in the field. Wild hydra populations can under certain environmental circumstances be dominated by male individuals, for example during autumn in Europe (Kaliszewicz, 2018). While the sex ratio patterns of Australian *Hydra oligactis* are not known, strong biases could also occur (here hydras were sampled in late summer). An alternative explanation could be that differential mortality between sexes occurred in laboratory conditions. Nevertheless, both male and female tumoural hydras are observed in Europe (Boutry et al., 2023), thus it is very likely they can also be observed in Australia.

4.2. Implications for the study of the effect of tumoural processes on the ecology and evolution of species

The discovery of hydras undergoing spontaneous tumourigenesis in Australia opens the possibility to conduct comparative studies between

populations located 15,000 kms apart. Both European and Australian ecosystems can differ significantly in term of climate, hydrology, flora and fauna, evolutionary history of species, and anthropogenic pressures (Braithwaite, 1990; Westoby, 1988), but also share similarities (Flores-Moreno et al., 2023). Such large-scale studies, with a wide range of measurable endpoints (Quinn et al., 2012), would allow us, among many other applications (Fig. 1), to quantify how environmental variation impacts the emergence of tumours in a wide range of environmental conditions (see an example on cancer in sea turtles, Dujon et al., 2021c). This is a key step in improving our understanding of how tumoural progression, from the activation of anti-tumoural defences to late-stage lethal cancer, will affect the health and functioning of ecosystems. The model is also the only species for which the emergence of a vertically transmissible cancer was documented twice, allowing the study of life-history trait evolution in hosts newly harbouring transmissible tumours (Tissot et al., 2024a). This also means that the hydra model can be used to perform manipulative experiments to further our understanding of the perfect storm that leads to outbreaks of transmissible cancer in wildlife populations (Dujon et al., 2021b; Tissot et al., 2022; Ujvari et al., 2016).

By providing evidence that the emergence of tumours in *Hydra*

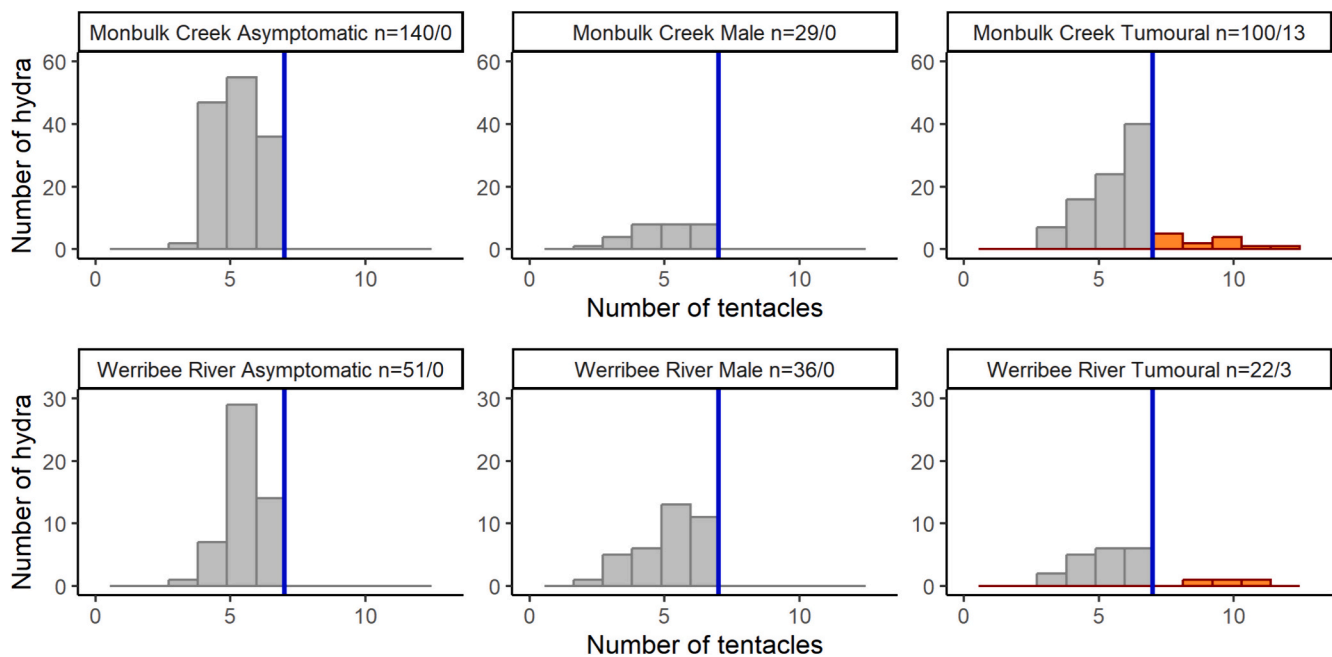


Fig. 6. Distribution of the number of observed tentacles for asymptomatic, tumoural and male undergoing testes development hydras from Monbulk Creek and Werribee River. Individuals with supernumerary tentacles were observed only in tumoural hydras (orange bars). The vertical blue line represents a tentacle number of 7. Above 7 a hydra is considered as having one or more supernumerary tentacles. Sample sizes are given as $n = \text{total number of hydras} / \text{number of hydras with supernumerary tentacles}$.

oligactis is not confined to Europe we have shown that the phenomenon is replicable in the laboratory. Because of the ease of *Hydra oligactis* maintenance in the laboratory, we believe that this species is a promising model to study the effects of tumoural processes on the ecology of species.

Author contribution

AMD designed and conducted the study which included maintaining the mass cultures, performing the macerations and microscopy analyses, analysing the data and writing the manuscript with input from all authors. AMD, JB, and AM conducted the field work sampling. AM assisted in maintaining the mass cultures. ST, JM, JB, JT, and FT provided their expertise on the care and diagnosis of tumours in hydras, the interpretation of microscopy and histology, and the interpretation of the results. BU provided her expertise on the ecology and evolution of cancers.

CRediT authorship contribution statement

Antoine M. Dujon: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Justine Boutry:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Sophie Tissot:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Jordan Meliani:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Anna Miltiadous:** Writing – review & editing, Validation, Methodology, Investigation. **Jácint Tokolyi:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Beata Ujvari:** Writing – review & editing. **Frédéric Thomas:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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