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REVIEW

A review of the methods used to induce cancer in invertebrates to study its effects on the evolution of species and ecosystem functioning

Antoine M. Dujon^{1,2,3}  | Justine Boutry^{2,3} | Sophie Tissot^{2,3} | Jordan Meliani^{2,3} |
Lena Guimard^{2,3} | Océane Rieu^{2,3} | Beata Ujvari^{1,2}  | Frédéric Thomas^{2,3} 

¹Deakin University, Geelong, School of Life and Environmental Sciences, Centre for Integrative Ecology, Waurn Ponds, Victoria, Australia

²CANECEV-Centre de Recherches Ecologiques et Evolutives sur le Cancer (CREEC), Montpellier, France

³CREEC, MIVEGEC, UMR IRD 224-CNRS 5290-Université de Montpellier, Montpellier, France

Correspondence

Antoine M. Dujon

Email: antoine.dujon@yahoo.fr

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Abstract

1. Cancer is an understudied but important process in wildlife that is predicted to have a significant effect on the evolution of metazoan species due to negative effects on host fitness. However, gaining understanding of the impact of cancer on species and ecosystems is currently relatively slow as the development of both animal models in which cancer can be induced and experiments that can be performed in an ecological setting are required. Invertebrates, because they are widely available and relatively easy to manipulate, are promising animal models.
2. In this review we examine how tumours can be induced in invertebrates to use them as experimental models to study the effects of cancer on the ecology and evolution of species. We identified four main groups of invertebrates (planarian, bivalves, hydra and drosophila) in which such inductions are performed. We then reviewed the types and effectiveness of the methods employed to induce tumours in those groups.
3. Cancer alters the phenotype of the host. We review how experiments using invertebrate models can be used to investigate the impact of cancer on tumour-bearing individuals for their movement, reproduction, feeding behaviours, social interactions, holobiont and predation risk.
4. We provide recommendations to facilitate the development of new invertebrate models. We also highlight a series of key questions on the ecology and evolution of cancer that could be answered with the use of invertebrate models.

KEYWORDS

biotic interactions, neoplasm, parasite, pollution

1 | INTRODUCTION

Cancer is a group of diseases in which cells break cellular collaboration and start to proliferate without control, invade surrounding tissues

and organs, and eventually spread throughout the body (i.e. metastasis) (Hanahan, 2022; Hanahan & Weinberg, 2011; Ujvari et al., 2017). Cancer is increasingly seen as a speciation event in which a new parasitic species emerges, initiating a clade and consuming the host's resources

Justine Boutry and Sophie Tissot contributed equally.

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(Capp & Thomas, 2020; Duesberg et al., 2011; Pienta et al., 2020; Vincent, 2010). During their progression, cancers often modify their host's phenotype, altering, for example, their morphology, physiology, and/or behaviour (Nicholson et al., 2018; Stark et al., 2012; Ujvari, Beckmann, et al., 2016; Vittecoq et al., 2015; Walker & Borniger, 2019). While phenotypic changes are obvious with well-advanced cancers (thinness, extreme fatigue, cachexia etc.), they can also, albeit often more subtly, occur early during the tumorigenesis (Thomas et al., 2017, 2018). As a result, tumour-bearing individuals often have modified interactions with other individuals or species present in the ecosystem (e.g. predator–prey interactions, host–parasite interactions, and/or intra- and interspecific competition, Boutry, Mistral, et al., 2022; Boutry, Tissot, et al., 2022; Comte et al., 2020; Cunningham et al., 2018; Hollings et al., 2014). By impacting the host's fitness, these changes are predicted to have significant effects on the evolutionary ecology of species (Boutry et al., 2020; Burioli et al., 2021; Dujon, Gatenby, et al., 2020; Jones et al., 2008; Perret et al., 2020; Russell et al., 2018). In the vast majority of cases, cancers die with their host, being also sometimes directly, or indirectly, responsible for host's death. For instance, prey with cancer are, for several reasons (e.g. poor condition, different morphology etc.), predicted to be quickly eliminated by predators, or to become preferential hosts for parasitic species. Similarly, predators unable to sustain tumour burdens have enhanced risk of death (Boutry, Tissot, et al., 2022; Duneau & Buchon, 2021; Perret et al., 2020). However, not all cancers die with their host. Noticeable exceptions include transmissible cancers (with one in dogs, two in the Tasmanian devil and seven in marine bivalves), that have fully evolved to behave like a parasitic species able to infect multiple hosts (Burioli et al., 2021; Ganguly et al., 2016; Garcia-Souto et al., 2022; Loh et al., 2006; Metzger et al., 2015; Metzger & Goff, 2016; Pye et al., 2016; Skazina et al., 2021; Yonemitsu et al., 2019). Transmissible cancers in bivalves are even able to cross the barrier between species (Metzger et al., 2015; Yonemitsu et al., 2019). Such transmissible cancers can cause mass mortalities in their host populations. For example, they caused a >85% population decline in ≥ 20 years in Tasmanian devils (*Sarcophilus harrisii*, Hawkins et al., 2006; McCallum et al., 2009) and are causing epizootic outbreaks in marine mollusc populations (Carballal et al., 2015). Therefore, there is increasing evidence that oncogenic processes are playing a role in ecosystem functioning (Hamede et al., 2020; Pesavento et al., 2018; Vittecoq et al., 2013).

Human activities significantly increase oncogenic pressures on ecosystems (Acevedo-Whitehouse & Duffus, 2009; Giraudeau et al., 2018; Pesavento et al., 2018), and understanding the effects of cancer on species and ecosystems remains a key question to answer in order to mitigate their consequences on biodiversity and ecosystem functioning (Dujon, Aktipis, et al., 2021; Hamede et al., 2020; Michael & Noble, 2017; Vittecoq et al., 2013). While cancers are frequently reported and/or studied in vertebrate species (Madsen et al., 2009; Vincze et al., 2021), they also affect a wide range of invertebrate species (Newton & Lewbart, 2017; Scharrer & Lochhead, 1950). Invertebrates are key components of Earth's ecosystems, representing the majority of the animal biomass on the planet, and they strongly influence ecological processes being, for instance, involved in predator–prey interactions, nutrient cycling and the flow of matter

and energy (Bar-On et al., 2018). Because of their abundance and distribution, invertebrates are widely exposed to a range of environmental disturbances and contaminations increasing their risk of developing malignancies (Dujon, Ujvari, & Thomas, 2021; Michael & Noble, 2017; Møller et al., 2013; Møller & Mousseau, 2009). As such, they are interesting models to study oncogenic processes and their ecological consequences, notably in human-altered ecosystems.

Here, we reviewed the different methods used to induce tumours in invertebrates, compared their relative efficacy, and detailed recent advances in how they are being used to study the effects of cancer on the ecology of host species and ecosystem functioning. In addition, and since we expect the use of invertebrate models at the interface of ecology and oncology to increase (Fernández Robledo et al., 2019; Knakievicz, 2014), we discussed challenges to address when performing experiments using invertebrates. Finally, we included in this article a short list of some of the key questions invertebrate models would help to answer to gain insight on the effect of cancer on species and ecosystems.

2 | REVIEW OF THE INDUCTION OF CANCER IN INVERTEBRATES

Using a previously established protocol (see Bramwell et al., 2021; Dujon, Bramwell, et al., 2020), we performed a comprehensive literature review. We searched the Thomson Reuters ISI Web of Science™ database, the Scopus database and Google Scholar for relevant publications with specific terms in the topic field, which included the title, abstract, keywords and keywords plus (i.e. words that frequently appear in the titles of articles cited within a publication). We used the following terms: 'neoplasm', 'tumor', 'tumour', 'cancer' and 'invertebrate', 'bivalve', 'hydra', 'planarian', 'gastropod', 'mollusk', 'mollusc', or 'arthropods'. To locate potential articles missed by the initial search, we checked the references and cited lists of relevant papers based on the predefined keywords. We included studies describing benign and/or malignant tumours, as well as hyperplasia (Boutry, Tissot, et al., 2022).

For each study, we extracted the species' name, the type of carcinogenic substances used and their concentration or mass, the sample size (expressed as number of individuals in each trial), the prevalence of individuals which developed cancer, the duration of the exposure to carcinogenic substance(s) or risk factor(s) and the duration of the trials. These results were then aggregated for each taxon (Figure 1).

3 | RESULTS AND DISCUSSION

3.1 | Examples of invertebrates in which cancer can be induced

3.1.1 | Planarians

Planarians are bilateral flatworms that have colonized both terrestrial and aquatic ecosystems. They are present in most freshwater

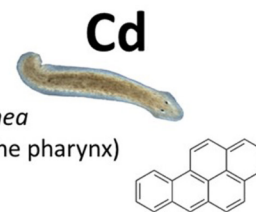


FIGURE 1 Summary of the main methods used to induce cancer in planarians, bivalves, hydras and drosophila. The duration of exposure (when relevant), the sample size of the experiments and the prevalence of cancer observed are provided for each group. Source: Planarians: (Foster, 1963; Hall et al., 1986; Plusquin et al., 2012; Schaeffer, 1993; Van Roten et al., 2018; Voura et al., 2017); Hydra: (Domazet-Lošo et al., 2014; Rathje et al., 2020), Justine Boutry personal communication; Bivalves (Gardner et al., 1991, 1992; House, 1997; Khudoley & Syrenko, 1978; Mateo et al., 2015; Walker et al., 2009); Drosophila: (Enciso et al., 2018; Enomoto et al., 2021; Enzmann, 1951; Hartung, 1942; Martorell et al., 2014; Pagliarini & Xu, 2003; Read et al., 2005; Shu et al., 2017; Willecke et al., 2011). Photo credits: Planarian, Eduard Solà, modified; Bivalve, Ecomare/Oscar Bos, modified; drosophila, Sanjay Acharya, modified (under CC BY-SA 4.0 License, <https://creativecommons.org/licenses/by-sa/4.0/legalcode>); Hydra, Jordan Meliani, modified

PLANARIANS

Heavy metals:

In *Dugesia dorocephala*, *Dugesia tigrina*, *Schmidtea mediterranea* (0.15-10 ppm $\text{CdSO}_4 \cdot \text{H}_2\text{O}$ or CdCl_2 , sometimes with TPA)
Exposure duration: 14-21 days (n = 9-126)
Cancer prevalence: 23%-90%



Polycyclic aromatic hydrocarbons:

In *Dugesia dorocephala* and *Bdellocephala brunnea* (saturated alcoholic solution or crystal inserted in the pharynx)
Exposure duration: 2-12 weeks
Cancer prevalence: 7%-36% (n = 14-90)

HYDRAS

Tumour graft:

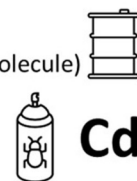
In *Hydra oligactis* and *Pelmatohydra robusta*
Cancer prevalence: high (\approx 50%-75%)



BIVALVES

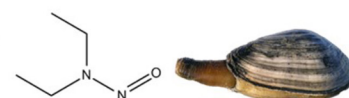
Cocktail of heavy metals, hydrocarbon, and pesticides:

In *Crassostrea gigas* (0.0229-5130 ppm, depending of the molecule)
Exposure duration: 1-4 months (n = 40-295)
Cancer prevalence: 9%-25%



Amines:

In *Unio pictorum* (200-400 ppm)
Exposure duration: 51-152 days (n=26-103)
Cancer prevalence: 8%-68%



Bivalve transmissible neoplasia:

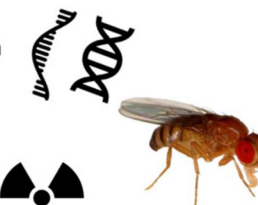
In *Mya arenaria* ($2.10^4 - 1.10^{6.3}$ BTN cells / 0.1 mL injected)
Exposure duration: 4-5 months (n = 65-104)
Cancer prevalence: 12%-16%



DROSOPHILA

Genetic modifications in *Drosophila melanogaster* :

Apc-Ras pathway activation
Scrib¹-Ras^{v12} or Ras^{v12}-Scr pathways activation
Ret overexpression
RNA interference (Dlg-RNAi, Igl-RNAi)
All with high prevalence



Radiation (UVs, X-Rays):

Dose: 9000 $\mu\text{J} \cdot \text{cm}^2$ (n = 60), 1500 R (n = 923)
Cancer prevalence: <10%, 48.3%



environments all over the world, with the exception of Antarctica and some islands (Vila-Farré & Rink, 2018). They are primarily predators that actively hunt (sometimes as a group, Cash et al., 2017) small invertebrates, aquatic arthropods, snails, oligochaetes, but also amphibian eggs and other items of animal origin (Hay & Ball, 1979; Vila-Farré & Rink, 2018). Cannibalism is also frequent among planarian species (Best, 1960; Hay & Ball, 1979; Hull, 1947). At the ecosystem level, planarian activity contributes to bioturbation and nutrient cycling processes (Majdi et al., 2016), and under certain conditions can induce direct (i.e. predation, Mckee et al., 1997) but also indirect effects on their prey (e.g. reduced growth rates and reduction in detritivorous leaf-consumption rate, Bordalo et al., 2018).

Planarians are widely studied invertebrates, primarily because they possess a pool of stem cells called neoblasts that enable them to regenerate their body after sustaining considerable damage (Gentile

et al., 2011). As a consequence, the protocols to maintain planarian colonies are well-established and described (Merryman et al., 2018; Oviedo et al., 2008) and research on their cellular biology (Gentile et al., 2011), genetics (Grohme et al., 2018), ecotoxicology (Best & Morita, 1991) and behaviour (Deochand et al., 2018) have been conducted for decades. Their biology is, therefore, relatively well documented compared to other invertebrate species (Vila-Farré & Rink, 2018).

Naturally occurring tumours have been observed in planarian species (Stephan, 1962). In the 1960s, Lange observed that two planarian species (*Dugesia etrusca* and *Dugesia ulvani*) that grew in polystyrene tubes and tap water developed tumours when they were fed twice a week (Lange, 1966). These early observations were followed by a series of studies investigating potential carcinogenic substances for planarians. It has since been found that tumours in

planarians can be induced by exposing them to heavy metals such as cadmium (Schaeffer, 1993; Voura et al., 2017) or to a relatively large range of aromatic hydrocarbon molecules (Foster, 1963; Hoshina & Teshirogi, 1991; Schaeffer, 1993). However, there is also evidence that not all species of planarians develop cancer when exposed to cadmium and that some planarians must also be exposed to a tumour promotor (Hall et al., 1986; Plusquin et al., 2012; Van Roten et al., 2018). In addition, exposure to ultraviolet is likely to be a relatively inefficient way to induce tumours in these organisms (Kalafatic et al., 2006; Schaeffer, 1993).

3.1.2 | Hydras

Hydras are freshwater cnidarians common in various types of aquatic habitats (rivers, lakes, ponds), living attached to aquatic vegetation or submerged rocks. They reproduce asexually by budding but can also reproduce sexually under specific environmental conditions (i.e. seasons, temperature, low food availability). In certain species, like *Hydra oligactis*, individuals then suffer from post-reproductive senescence and die (Yoshida et al., 2006). On the contrary, most other hydra species do not suffer from any kind of senescence, which makes them virtually immortal (Bosch, 2009). From an ecological perspective, hydras are involved in a variety of interactions with invertebrates and vertebrates. They can, for instance, serve as hosts for commensal (Coleman, 1966) or parasitic species (Stiven, 1965). They are also predators of various zooplankton species, aquatic insect larvae and even fish spawn (Elliott et al., 1997; Rivera-De la Parra et al., 2016), and are themselves prey for fish or molluscs grazing on plants (Baumgärtner & Rothhaupt, 2005; Cuker & Mozley, 1981). In at least two groups of hydras, *H. oligactis* and *Pelmatohydra robusta*, polyps can spontaneously develop tumours in a laboratory environment (Domazet-Lošo et al., 2014) which significantly alter the polyp's body shape (Figure 2). In addition, tumour-bearing individuals present changes

in their microbiota composition and also possess a higher number of tentacles (Figure 2, see also Domazet-Lošo et al., 2014; Rathje et al., 2020). Interestingly, tumours in hydras (e.g. in those presenting in laboratory conditions, see Domazet-Lošo et al., 2014) are vertically transmitted to the buds when polyps reproduce asexually, resulting into the spread of the tumours in the lineage. Recent studies (Boutry et al., in preparation) have shown that these transmissible tumours are associated with changes in the host's life-history traits: hydras emerging from a tumoural polyp maximize their budding rate before developing tumours themselves, after which they experience a reduced budding rate and a shorter life span. The prevalence of tumour-bearing individuals in the natural environment is unknown, but presumably very low, as only about 3% of field individuals brought to the laboratory develop tumours after 3–4 months of maintenance in stable conditions (Boutry and Tokolyi unpublished field observations). Tumours observed in the field appear to be composed of aberrant germinal cells, similar to the tumours described in the laboratory strain studied by Domazet-Lošo et al. (2014). More work is currently needed to understand the causes of tumour appearance in hydras, as well as why they remain rare in the wild compared to the laboratory. A possible reason for their scarcity could be the higher predation rate by fish on tumorous hydras compared to healthy ones, as recently demonstrated by Boutry, Mistral, et al. (2022) in experimental conditions. This study also showed that the phenotypic differences induced by tumours influence other biotic interactions, as, for example, tumorous hydras catch more prey than healthy ones and serve as preferential hosts for commensal ciliates. Although several methods (e.g. exposure to various pollutants, UV and bacteria) are currently used to induce tumours in hydras, there is currently no evidence of success to our knowledge. Currently, the most efficient way to generate tumours in *H. oligactis* and *P. robusta* is to experimentally transplant pieces of neoplasms from tumorous individuals to healthy ones (Rathje et al., 2020, see also Figure 3 and Video S1).

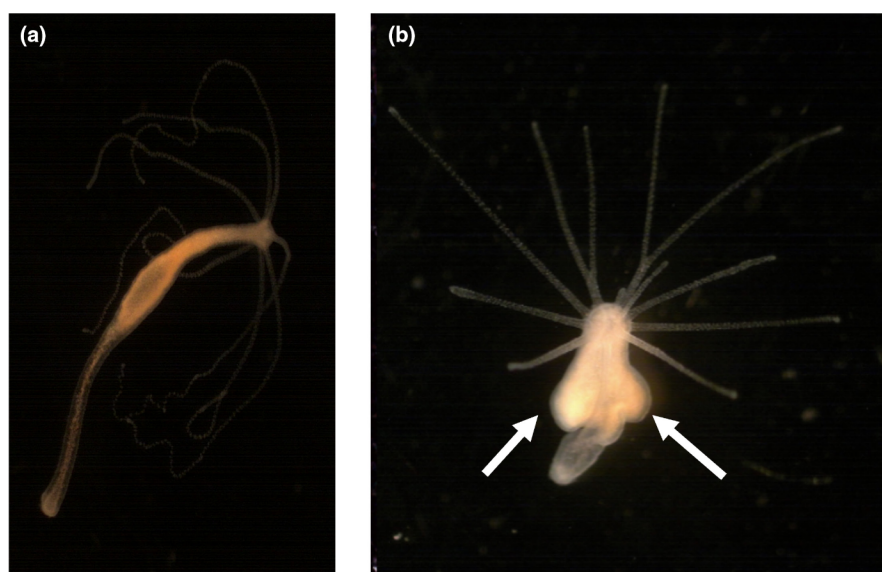


FIGURE 2 Example of healthy (a) and tumorous (b) hydras. Note in (b) the tumour masses, indicated by white arrows, and a greater number of tentacles (photo credit: Jordan Meliani)

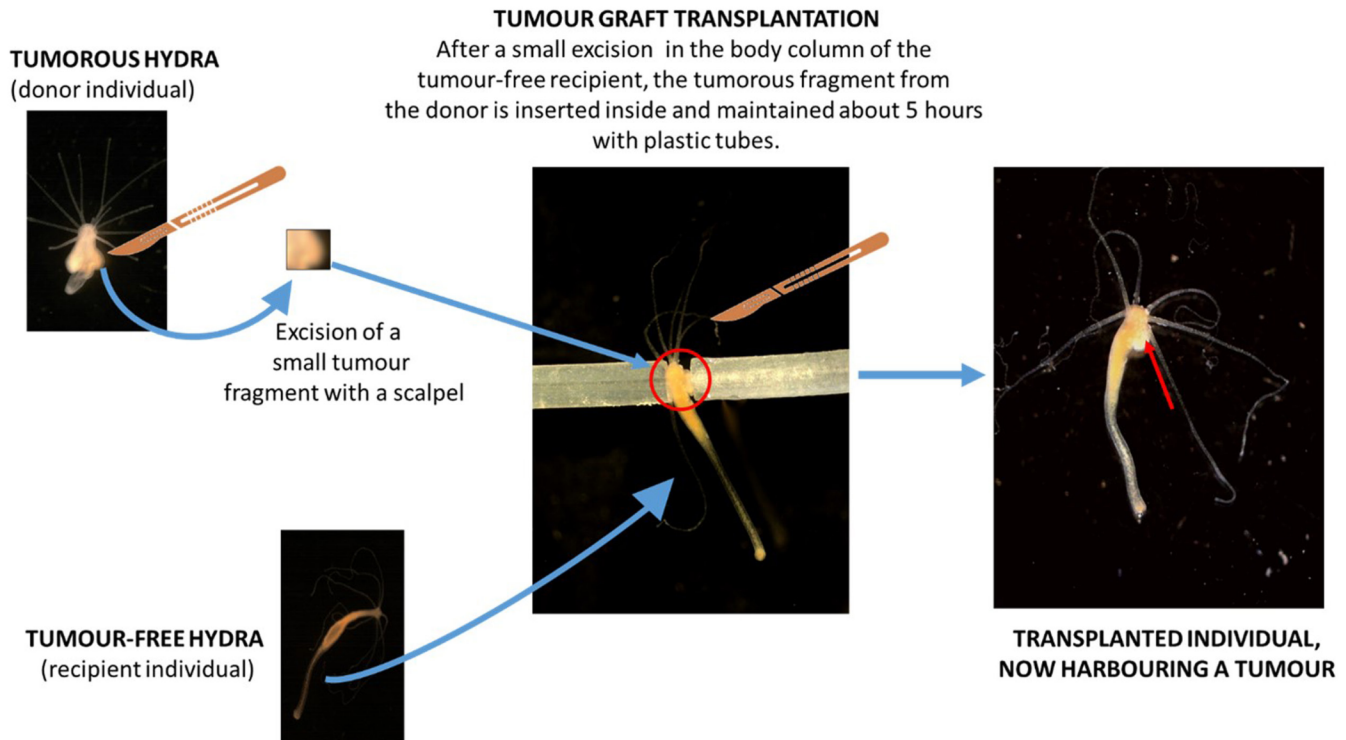


FIGURE 3 Visual description of the protocol used to graft tumours between hydras (photo credit: Lena Guimard and Jordan Meliani). The red arrow indicates a tumour. See Video S1 for a video version of the grafting protocol (video credit: Océane Rieu).

3.1.3 | Bivalves

The effect of bivalves on aquatic ecosystems is important because they are often engineer species (Vaughn & Hoellein, 2018) offering habitat to a large diversity of species (Wijsman et al., 2018). They impact nutrient cycling, bioturbation processes (Vaughn & Hakenkamp, 2001) and bioaccumulate pollution and carcinogenic molecules (Porte & Albaigés, 1994; Zuykov et al., 2013). Bivalves are, therefore, exposed to a wide range of contaminants in the wild. As such they make interesting candidates to study the effect of cancer on engineer species.

Non-transmissible neoplasia is observed in both freshwater and marine bivalves species (Carballal et al., 2015; Carella et al., 2016). In addition it is in marine bivalves that the largest number of transmissible cancers (an abnormal proliferation of circulating cells called bivalve transmissible neoplasia) have been documented with seven lineages so far (Garcia-Souto et al., 2022; Hammel et al., 2022; Metzger et al., 2015, 2016; Yonemitsu et al., 2019). This number is likely underestimated (Dujon, Bramwell, et al., 2021). Transmissible cancers in bivalves behave very similarly to other marine parasites. For example, it castrates the host in the blue mussel *Mytilus edulis* (Burioli et al., 2021), which could allow the cancerous cells to use energy that would otherwise have been invested in reproduction. In addition, circulating cancerous cells are able to survive for periods ranging from days to weeks in seawater under the right conditions, allowing their dispersion (Burioli et al., 2021; Giersch

et al., 2022; Sunila & Farley, 1989). For those reasons, bivalve transmissible neoplasia can lead to mass mortality events that have been reported in multiple oceans (Green et al., 2008; Mateo et al., 2015; Villalba et al., 2001) with the frequency and severity of outbreaks predicted to increase in the decades to come (Bramwell et al., 2021).

Non-transmissible neoplasia have been induced in bivalves by exposing them to contaminated marine sediments (Gardner et al., 1991), to N-nitroso compounds (Khudoley & Syrenko, 1978), or to a relatively complex cocktail of aromatic and chlorinated hydrocarbons, amines, and metals (Gardner et al., 1992). It is also worth mentioning that 5-bromodeoxyuridine, concomitant with a potential retrovirus infection, has been proposed to induce neoplasia in *Mya arenaria* haemocytes (Oprandy & Chang, 1983). However, those results require further investigation because bivalve transmissible neoplasia has since been demonstrated to be a transmissible cancer and likely the most common neoplastic disease in this species (Metzger et al., 2016).

Currently, bivalves are the only invertebrate model in which it is possible to study the effect of horizontally transmissible cancers. Transmission experiments demonstrate that cancerous circulating cells can be injected in healthy individuals to artificially infect them with a transmissible cancer (House, 1997; Mateo et al., 2015; Twomey & Mulcahy, 1988; Walker et al., 2009). In addition, individuals intentionally placed in the field near infected individuals can contract the disease allowing for the design of field experiments (Mateo et al., 2015; Weinberg et al., 1997).

3.1.4 | *Drosophila melanogaster*

The fruit fly, *Drosophila melanogaster*, is a widely used animal model in science, including for the study of cancer because it replicates most steps of the hallmarks of cancer observed in humans and mammals (Brumby & Richardson, 2005; Hanahan & Weinberg, 2011; Mirzoyan et al., 2019). The effects of cancer on the ecology of this invertebrate model have been relatively well documented in the past few years. For example, it has been observed that cancer alters drosophila social behaviour (Dawson et al., 2018). Indeed, cancerous drosophila interacted considerably less with healthy individuals and exhibited a strong social attraction towards other cancerous individuals, particularly at the beginning of tumour development which mediated cancer progression. Tumour progression was slower when cancerous drosophila interacted together compared to when they interacted with healthy individuals (Dawson et al., 2018). In addition, female drosophila with developing tumours also reproduce earlier during their life compared to healthy flies, a life-history change that alleviates the negative effects of cancer on fitness (Arnal et al., 2017). Interaction between cancer progression and concomitant pathogen infection also suggests that bacterial infection can slow down cancer progression in this model (Jacqueline et al., 2020).

It is possible to induce tumours in drosophila by exposing them to radiation. For example, Enzmann (1951) and Enciso et al. (2018) induced melanotic tumours by exposing newly laid eggs and larvae to ultraviolet light, but they only achieved a very low prevalence (<10%). Depending on the strain used, exposing drosophila to X-rays can induce tumours in almost half of the exposed individuals (Hartung, 1942). Currently, the main way to induce tumours in drosophila is through genetic modifications (e.g. using the mosaic analysis with a repressible cell marker [MARCM] method, Wu & Luo, 2006). Indeed tumours can be induced in the intestine by modifying the APC and Ras pathways to create APC–Ras clones in adults (Martorell et al., 2014) or in larvae's eyes by altering the scrib¹/Ras^{v12} pathway (Pagliarini & Xu, 2003). Similarly, the concomitant activation of both the Ras^{v12} and Scr pathways, or the overexpression of the Ret receptor tyrosine kinase can be used to induce malignant tumours in the eye (Enomoto et al., 2021; Read et al., 2005). In parallel with activating oncogenes, RNA interference can be employed to downregulate genes, thus facilitating the apparition of tumours (e.g. downregulating genes involved in maintaining cell polarity such as the determinant Discs-large [Dlg] or lethal giant larvae [lgl] genes, Willecke et al., 2011; Shu et al., 2017).

3.2 | Considerations when running an experiment with invertebrates

Using invertebrates as models to study the effect of cancer on the evolutionary ecology of species and on ecosystem functioning is promising but is not without challenges. Indeed, a range of factors must be considered before running an experiment.

It is first important to clearly define and understand what are control groups when attempting to induce cancer (Harris et al., 2014; Lipsitch et al., 2010). Since the induction of non-transmissible cancers in planarians and bivalves is achieved through the exposure to one or more carcinogenic substances, it is important to be able to distinguish the toxic effects of the molecules on the animal (e.g. damage to tissues, or disruption of physiological pathways not directly leading to cancer) from the effects caused by the initiation and growth of tumours (e.g. damage to the DNA, physical growth of the tumour, consumption of resources by the tumour cells). It can, however, be difficult during an experiment (i.e. without killing the animals) to determine if the individuals in a group exposed to a carcinogen fail to develop detectable cancer because the mutations were not sufficient to induce it or because the anti-cancer defences of the species prevented or repaired the damage to the DNA. Potential examples are the placozoan *Trichoplax adhaerens*, which can tolerate levels of radiation of 248.6 Gy by overexpressing genes involved in DNA repair (Fortunato et al., 2021), or the planarians *Girardia (Dugesia) tigrina* and *Polycelis feline* which recover quickly from an acute sublethal exposure to ultraviolet radiation without developing cancer (Kalafatić et al., 2006). Such results are important to consider because they make it possible to explore trade-offs between the ability of a species to repair its DNA, other damages leading to cancer, and the maintenance of other important functions such as movement or reproduction (Boutry et al., 2020; Dujon et al., 2022). The use of a positive control during such experiments can help identify such situations. A positive control can be achieved by exposing a group of animals to a known risk factor (usually causing a large cancer prevalence) during the experiment and then comparing the effect sizes of interest of this positive control to those of the experimental treatment and negative control groups. This approach allows for the comparison of the relative ability of a risk factor to induce cancer and how detrimental it is to a species, but it also allows for the detection of potential situations in which the cancer induction experiment abnormally failed, which is not possible solely with a negative control (Gross & Mantel, 1967; Harris et al., 2014). Using a negative control is also of key importance to estimate the background rate at which a species may naturally develop tumours (e.g. Hartung, 1942; Stephan, 1962), a parameter that is important to take into account when establishing dose–response relationships (Lipsitch et al., 2010; Zeise et al., 1987). The effect of the specific vehicle (often a solvent) that is used to expose or inject animals with the carcinogenic substance should also be investigated as some carcinogenic substances may require an organic solvent in which to be dissolved (Harris et al., 2014). For example, Foster (1963) used alcohol as a vehicle to induce cancer in *Dugesia dorotocephala* with a range of polycyclic aromatic hydrocarbons. Foster included an experimental treatment in this study in which planarians were exposed to alcohol without polycyclic aromatic hydrocarbons to ensure it was not inducing tumours.

While performing a cancer induction experiment, confounding variables must be carefully considered. In the laboratory, the nutritional status of planarians is known to influence the incidence



of spontaneous tumours (Chandebois, 1975; Lange, 1966) and it is, therefore, important that the subjects are starved prior to starting a laboratory experiment to achieve standardized results. In hydras, overfeeding increases the budding rate of cancerous individuals, leading to a dilution of the tumour cells between the buds. As a consequence, it is common for overfed cancerous hydras to progressively reach a similar external aspect as healthy individuals despite the proliferation of tumour cells (Alexander Klimovich, Personal Communication). However, as soon as the level of food abundance is reduced, the budding rate decreases, and the tumorous phenotype reappears in polyps. In the field it can be difficult to define what a negative control would be, and it is, therefore, important to carefully consider potential confounding variables and pseudoreplication (e.g. spatial autocorrelation) to ensure that the study is replicable (Dutilleul, 1993; Hurlbert, 1984). To alleviate some of those limitations, mesocosm experiments are a promising avenue. The idea is to enclose animals in a miniature ecosystem in the laboratory or in the wild, replicating some of the key functions observed in the natural ecosystem but in a way that is much easier to manipulate and observe (Odum, 1984). Mesocosms are often designed to conduct complex experiments that account for physical processes (tidal currents, turbulence, cycling of nutrients, thermal stratification and mixing) or biological complexities (population, community and ecosystem) (Sharma et al., 2021). To the best of our knowledge there have been no mesocosm experiments involving cancerous invertebrates conducted so far, thus studies on invertebrate models can be used as a basis for future work (e.g. planarians, Bordalo et al., 2018; bivalves, Buffet et al., 2014). The use of genetically identical animals (e.g. clones in hydras and planarians) can also help reduce the source of variabilities in the experiment, allowing for fine tuning and rigorous manipulative experiments, particularly when the aim is to identify gene expression differences between groups or how epigenetic differences can lead to large phenotypic variance (Laskowski et al., 2019), including in behaviour (Bierbach et al., 2017).

Designing and performing advanced studies that investigate the effect of cancer on the behaviour or fitness of invertebrates (but also more broadly on any animal model) is likely to be time-consuming and labour intensive, requiring expert knowledge in both oncology and ecology. To reduce these costs, efforts should be made to establish collaborations that develop and use automation methods to reduce the workload of scientists involved in such studies (Dujon, Vittecoq, et al., 2021). Such tools have been developed for annotating the behaviour of *Drosophila* (Kain et al., 2013; Pereira et al., 2022) and partially for hydras (Han et al., 2018). Overall the development of such automation methods in invertebrates is easier compared to a range of vertebrate species (Pereira et al., 2022; Valletta et al., 2017; Weinstein, 2018). In addition, sensitivity and power analyses should be conducted prior to running an experiment to ensure that it is realistically possible to detect potential effect size differences between control and treatment groups, particularly if the prevalence of cancer is low (see examples in Bramwell et al., 2021). Furthermore, factorial design experiments should be considered to reduce the number of animals involved (Shaw et al., 2002), especially for species

that may require ethics approval or a sampling permit (e.g. certain bivalve species) and for which the number of individuals that can be used is limited (Drinkwater et al., 2019).

4 | EXAMPLES OF KEY QUESTIONS FOR WHICH INSIGHTS COULD BE GAINED USING INVERTEBRATE MODELS

4.1 | How does cancer progression affect behaviour and life-history traits in host species?

It is currently not known, for the vast majority of animal species, how cancer progression affects the behaviour of the host (Vittecoq et al., 2015). Cancer is predicted to induce in the host a suite of effects that are similar to those observed when hosts are infected with parasites. When emerging cancers rapidly influence the host condition and/or behaviour, it is likely that the host will die from the tumour burden or from increased predation/parasitism risk with a fitness close to zero. If the effects of cancer progression on host condition/behaviour are more gradual, then certain species may more easily modify their resource allocation, allowing them to reproduce earlier and at least partially mitigate the effect of cancer on their fitness (Dawson et al., 2018; Perret et al., 2020; Vittecoq et al., 2013). This is the case for *Drosophila*, for example, in which cancerous females reproduce earlier compared to healthy females (Arnal et al., 2017) but also social interactions between males change to mediate cancer progression (Dawson et al., 2018). Further examples of how cancer can alter the host behaviour are well documented in Tasmanian devils. Indeed females with Devil Facial Tumour Disease (DFTD) reproduce at an earlier age compared to 20 years ago, at the beginning of the outbreak (Jones et al., 2008) and the progression of DFTD negatively influences devils' likelihood of interaction within their social network (Hamilton et al., 2020).

At the tumour scale, cancer cells are well known to manipulate healthy cells to their own benefit, for example during angiogenesis. It is also possible that cancers are able to alter the behaviour of the whole host, probably in subtle but significant ways (as observed for some parasitic species, Poulin, 2010; Tissot et al., 2016; Ujvari, Beckmann, et al., 2016; Ujvari, Gatenby, & Thomas, 2016). A potential initial point to study host manipulation by cancer would be to investigate potential changes in the behavioural syndromes of affected individuals. Using well-designed experiments and robust statistical analyses, which are made easier by the relative ease of manipulating invertebrates, subtle changes in behaviour can be detected (O'Dea et al., 2021; Sih et al., 2004). It may be especially interesting to investigate how transmissible cancers manipulate their host to enhance their transmission rate (Burioli et al., 2021; Dujon, Gatenby, et al., 2020). Because cancer progression is often influenced by diet parameters, cancerous individuals could potentially have some control on their tumour progression by adjusting their feeding behaviour in a way that prevents/limits cell proliferation (self-medication hypothesis) (Tissot et al., 2016). Alternatively,



cancer cells could also increase host appetite in a quantitative/qualitative way that favours their growth (cancer manipulation hypothesis). *Drosophila* would be good model systems to explore these questions. For instance, to test the self-medication hypothesis and the cancer manipulation hypothesis, healthy and cancerous flies could be provided with the same amount of different diets (see for instance Ponton et al., 2015) and the percentage of consumed food could be estimated before and after feeding. If differences exist then it could be relevant to explore if changes in cancerous individuals favour host survival or conversely tumour progression, or both. Hypothetically, it could be possible to observe an increase in food consumption because it accelerates reproduction and/or facilitates cancer progression, both of which can be independently quantified.

Understanding the role of sleep in cancer processes is increasingly considered as an important line of investigation. Questions on sleep and cancer remain unanswered since most sleep disorders in tumour-bearing individuals are only reported once cancer is diagnosed. As sleep monitoring is possible in *Drosophila* (Cirelli & Bushey, 2008), it is then possible to compare cancerous and healthy groups to detect putative differences which can then be further explored to determine whether sleep changes in cancerous individuals are favourable to host survival and/or to tumour progression. Addressing questions related to sexual selection are also possible with this model, exploring for instance whether cancer alters the host's sexual attractiveness and/or competitiveness. Knowing that courtship is costly for male *Drosophila* (Cordts & Partridge, 1996) and also that they adaptively allocate their mating effort in response to variation in female quality (see Byrne & Rice, 2006), it is also possible to test if cancer-bearing flies (due to depleted energy reserves and/or reduced survival) invest less time and energy into choosing mates.

4.2 | What are the optimal conditions for a transmissible cancer to spread in a bivalve population?

Transmissible cancers in marine bivalves have the potential to be dispersed by oceanic currents and human activities since their host species are also often invasive species (Caza et al., 2020; Yonemitsu et al., 2019). This situation is likely to worsen due to climate change (Bramwell et al., 2021) and by environmental degradation facilitating the emergence of cancer in wildlife populations (Dujon, Brown, et al., 2021; Dujon, Ujvari, & Thomas, 2021). Only a handful of studies have so far investigated the effect of environmental parameters on the survival of transmissible cancer cells in the soft-shell clam *Mya arenaria* or the blue mussel *Mytilus edulis* (Burioli et al., 2019; Giersch et al., 2022; Sunila & Farley, 1989). The ability to induce tumours in bivalves by injecting them with transmissible cancer cells should allow for the design of manipulative experiments to investigate which environmental factors may lead to bivalve transmissible neoplasia outbreaks in marine bivalve populations (Walker

et al., 2009) and aid in the development of epidemiological models. This is particularly important as the number of transmissible cancers in marine bivalves is likely underestimated (Dujon, Bramwell, et al., 2021; Ujvari, Gatenby, & Thomas, 2016).

4.3 | To what extent does immunosuppression caused by cancer boost pathogen communities in animal populations?

Animals with cancer are, overall, predicted to be more susceptible to infectious diseases primarily because malignancies reduce the ability of species to fight pathogens and parasites (Kareva, 2020; Pollock & Roth, 1989; Vittecoq et al., 2013) and modify their holobiont. For example cancerous individuals in *Hydra oligactis* have an altered microbiota, and they also harbour a higher number of commensal ciliates on their surface compared to healthy individuals, (Boutry, Tissot, et al., 2022). However it may not systematically be the case as evidenced for the Tasmanian devil for which the presence of DFTD does not affect the parasitic tick load (Belkhir et al., 2022). Therefore, further investigations are required. Invertebrate models, which lack an adaptive immune system, offer the possibility to test for interactions between exposure to pollution, cancer progression and infection by pathogens, with the aim to quantify the contribution of each component but also their synergies, for example, using the synzootic theory which describes co-occurring enzootic or epizootic processes that produce worse health outcomes in wild animals (Sweeny et al., 2021). This is especially important as human activities disturb the evolutionary equilibrium reached over evolutionary times by hosts and pathogens (Hassell et al., 2021). Animal populations which develop cancers after being exposed to pollution, habitat degradation and reduction of genetic diversity (Dujon, Ujvari, & Thomas, 2021; Giraudeau et al., 2018; Ujvari et al., 2018) are likely to promote the emergence of new pathogens. These strains would further disturb ecosystems, accelerate biodiversity loss and impact human populations, thus cancer needs to be integrated into the One Health approach (Dujon, Brown, et al., 2021). *Drosophila* could be suitable models to explore the extent to which the presence of cancerous individuals in a population facilitates the establishment of novel pathogens, and/or contribute to the abundance/diversity of an established pathogen community. There is a long list of parasitic organisms known to attack or infect *Drosophila*, both in natural and laboratory conditions (Kraaijeveld & Wertheim, 2009). *Drosophila* infected with pathogens could, for instance, be released into cages containing healthy populations of *Drosophila* (as control) and cages containing various percentages (from 10% to 90%) of flies with cancer. Such experimental set-ups will help explore how these different situations (prevalence or frequency of cancer observed in a population) can contribute to and facilitate the spread of pathogen infections within a population. It will also help



to understand if the presence of cancerous individuals permits a stable pathogen community to maintain itself over time.

4.4 | How does cancer alter competitive interactions and predation rate?

Using hydras, Boutry, Mistral, et al. (2022) recently explored the novel interactions between tumorous individuals and other species like pathogens and predators. However, this pioneer study should be repeated with other species before generalizations can be made. Again, *Drosophila* could be suitable models. It would be relevant, for instance, to explore the extent to which, all things being equal, the presence of individuals with cancer reduces the intensity of intraspecific competition for resources. Also, in addition to adult *D. melanogaster* individuals, other *Drosophila* species could be considered, such as *D. simulans* and/or *D. ananassae* which are known to be at a selective disadvantage when in interspecific competition with *D. melanogaster* at 25°C (Singh & Kumar, 2013; Tantawy & Soliman, 1967). This context makes it possible to explore the extent to which the presence of cancerous individuals attenuates, or even reverses, the outcome of interspecific competition.

To explore links between cancer progression, predation risk and environmental factors, previous cancerous flies' models and predators like spiders and mantis could be suitable. Experiments could, for instance, determine if the presence of refuges may modify the vulnerability of cancerous individuals to predators, and/or determine how the modality of food distribution for flies (i.e. concentrated in one or few areas or equally distributed everywhere in the cage) influences the predation risks of health and tumorous flies.

5 | CONCLUSION

In conclusion, invertebrate species have the potential to provide novel and unprecedented information on the importance of oncogenic processes in animal evolutionary ecology. They can also allow scientists to move beyond descriptive aspects and provide a conceptual and theoretical framework that explains and predicts the ecology of host-tumour interactions and their consequences for ecosystems. The findings of such studies should attract attention not only from ecologists and evolutionary biologists, but also from researchers in a broad range of disciplines such as cancer and cell biology, immunology, conservation biology and wildlife management.

AUTHORS' CONTRIBUTIONS

A.M.D. designed and wrote the original manuscript; J.B. and S.T. wrote the section on hydras; J.M., L.G. and O.R. provided expertise on the husbandry and induction of cancer in hydras and provided photographs and videos; B.U. and F.T. provided their expertise on the ecology and evolution of cancer. All authors read and edited the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The sources of the data used in Figure 1 are cited in the legend.

ORCID

Antoine M. Dujon  <https://orcid.org/0000-0002-1579-9156>

Beata Ujvari  <https://orcid.org/0000-0003-2391-2988>

Frédéric Thomas  <https://orcid.org/0000-0003-2238-1978>

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