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Hydra, a model system for environmental studies

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ABSTRACT ***Hydra*** **have been extensively used for studying the teratogenic and toxic potential of numerous toxins throughout the years and are more recently growing in popularity to assess the impacts of environmental pollutants.** ***Hydra*** **are an appropriate bioindicator species for use in environmental assessment owing to their easily measurable physical (morphology), biochemical (xenobiotic biotransformation; oxidative stress), behavioural (feeding) and reproductive (sexual and asexual) endpoints.** ***Hydra*** **also possess an unparalleled ability to regenerate, allowing the assessment of teratogenic compounds and the impact of contaminants on stem cells. Importantly,** ***Hydra*** **are ubiquitous throughout freshwater environments and relatively easy to culture making them appropriate for use in small scale bioassay systems.** ***Hydra*** **have been used to assess the environmental impacts of numerous environmental pollutants including metals, organic toxicants (including pharmaceuticals and endocrine disrupting compounds), nanomaterials and industrial and municipal effluents. They have been found to be among the most sensitive animals tested for metals and certain effluents, comparing favourably with more standardised toxicity tests. Despite their lack of use in formalised monitoring programmes,** ***Hydra*** **have been extensively used and are regarded as a model organism in aquatic toxicology.**

KEY WORDS: *Hydra, toxicity, bioassay, metal, regeneration*

Introduction

Aquatic ecosystems are subjected to the release of numerous contaminants contributing multiple stresses on organisms living in this environment. The study of the effects of these anthropogenic inputs of contaminants as well as natural sources of chemicals on aquatic organisms represents an important endeavour in the protection of aquatic ecosystems. It is generally agreed that measuring only the chemical and physical attributes of water cannot provide the sole assessment of the health of an aquatic ecosystem (Ten Brink and Woudstra, 1991). The concept of biological monitoring or biomonitoring is a product of the assumption that the measurement of the condition or health of biota can be used to assess the health of an ecosystem (Herricks and Cairns, 1982). The toxic effects of these stressors could be measured at all levels of biological organisation (from molecular to individuals to population and to communities). The cnidarian, *Hydra spp*, is an ancestral metazoan that has recently gained increased attention in aquatic toxicology as a sensitive and possible target species of the benthic community (Pascoe *et al.*, 2003, Segner *et al.*, 2003) and has become an increasingly popular bioindicator species. One attractive feature of this benthic representative involves the multiple physiological targets that can be assessed while conducting these tests. For example, xenobiotic biotransformation, oxidative stress, growth, asexual reproduction, morphological changes and feeding behaviour could be conveniently determined in these organisms. These endpoints represent key targets that underlie survival, growth and reproduction which form the basis of environmental risk assessment strategies. Their widespread prevalence in freshwater ecosystems makes it a demonstrative bio-indicator. *Hydra* also have a fast reproductive rate, are cost-effective, easily cultured and cared for in the laboratory (Arkhipchuk *et al.*, 2006a). These points shall be discussed in more detail below:

Abbreviations used in this paper: AChE, acetylcholinesterase; EC_{50}, effective concentration needed to effect 50% of the exposed population; HO heme oxidase; LC_{50}, lethal concentration needed to kill 50% of the exposed population; LOEC, lowest observable effect concentration; MAC, minimal effective concentration; MEC, minimal effect concentration; NOEC, no observable effect concentration; NM, nanomaterial; SOD, superoxide dismutase; TT, toxicity threshold; WEC, whole embryo culture bioassay.

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History of Hydra in environmental studies

Toxicity tests using the *Hydra* have been increasingly used over the years. Since *Hydra* were first described in the scientific literature in the early 1700s (Campbell, 1989), they have been used to advance knowledge in many areas of biological research (Slobodkin and Bossert, 2001), particularly focusing on developmental studies. *Hydra* have the astonishing capacity to regenerate which makes them immortal organisms (Bosch, 2009). *Hydra* have a long history as model systems in developmental biology resulting from the remarkable plasticity in their differentiation capacity and their ability to regenerate missing body parts (Bode, 2003; Holstein *et al.*, 2003; Hemmrich *et al.*, 2007). *Hydra* have also become an established and valuable indicator species for use in toxicity testing. Since the early eighties, *Hydra* have been used in developmental toxicity studies to detect the teratogenic potential of chemicals as they are the highest animal life capable of whole body regeneration (Johnson *et al.*, 1982). Initially, this was done by the use of dissociated cells to make an artificial embryo (Johnson *et al.*, 1982) and later Wilby (1988) recommended the use of dissected gastric sections to study regeneration. Toxicity was initially based on morphological changes that occurred in the animals as described by Johnson *et al.*, (1980). These morphological changes were later used as the bases of a morphology and regenerative scale from 10 to 0 devised by Wilby (1988). This scale provides the most commonly used morphological and regenerative endpoints used in *Hydra* toxicity testing today. As *Hydra* reproduce asexually under normal conditions, population growth has also been used as a toxicity endpoint in the *Hydra* population reproduction toxicity test method (Stebbing and Pomroy, 1978). Testing protocols have been developed for survival and morphology (Blaise and Kusui, 1997, Trottier *et al.*, 1997), population growth (Holdway, 2005), and teratogenicity (Johnson *et al.*, 1982, Quinn *et al.*, 2008b). Traditionally *Hydra* were used to assess the acute and regenerative toxicity of metals but organic compounds including more recently pharmaceuticals and nanomaterials have also been investigated (see section 4 below). Initially *Hydra* were used in toxicity tests on their own (Johnson *et al.*, 1982; Blaise and Kusui, 1997; Beach and Pascoe, 1998; Pachura-Bouchet *et al.*, 2006) and later as part of a bioassay battery of organisms (Arkhipchuk and Malinovskaya, 2002). The use of a battery of bioassays for the evaluation of complex environmental samples has been widely recommended as superior to a single bioassay, since it is unlikely that a single bioassay will be responsive to all possible toxicants (Clarke and Barrick, 1990). The sensitivity of *Hydra* morphology as an indicator of sub-lethal toxicity and the rapidity with which population growth rate effects can be observed make *Hydra* a uniquely useful toxicology test species.

Hydra anatomy

Hydra are one of the simplest multi-cellular organisms known and consist of a tube made up of two connected epithelial cell layers (Steele, 2002). There is an opening or mouth (hypostome) at the top end of the tube, enclosed by tentacles that contain stinging cells (nematocysts), allowing the *Hydra* to catch prey (Steele, 2002). The mouth and tentacles are called the hydranth (Holdway, 2005). The rest of this organism is known as the column and has four distinctive sections: the gastric section located between the tentacles and the first (apical) bud; the budding section which produces the buds; the peduncle which is located between the lowest bud and basal disc and the basal disc which is the foot-like formation (Holdway, 2005; see also in this issue Böttger and Hassel, 2012). This structural complexity, simpler than vertebrates with central nervous system and specialized organs, but more complex than cultured cells, makes *Hydra* comparable to a living tissue whose cells and distant regions are physiologically connected (Galliot *et al.*, 2006).

Like other cnidarians, *Hydra* are diploblastic organisms with 2 tissue layers: the outer ectoderm and inner endoderm, separated by an acellular mesoglea layer (Slobodkin and Bossert, 2001; Hoffmeister-Ullerich, 2007; see also in this issue Sarras, 2012). The endoderm lines the gastrovascular cavity, a water-filled sac, which acts both as a hydrostatic skeleton and the site for food digestion and nutrient absorption (Slobodkin and Bossert, 2001). Having a simple tubular body and being diploblastic, all of the epithelial cells of the *Hydra* are in constant contact with the aqueous environment, allowing toxic substances to be exposed to all body surfaces of the animal (Beach and Pascoe, 1998; Karntanut and Pascoe, 2000). *Hydra* possess a simple nervous system which include a nerve net that stretches throughout the body (Sakaguchi *et al.*, 1996). Its structure and the simple anatomy of *Hydra* allow it to be a valuable and sensitive indicator of pollution or other pressures in the outside environment (Beach and Pascoe, 1998; Holdway *et al.*, 2001).

Fast reproductive rate

Hydra have the ability to reproduce both sexually and asexually. Under favourable conditions and for the majority of the time, *Hydra* typically reproduce asexually by budding which results in the rapid production of a large numbers of genetically identical organisms (Pollino and Holdway, 1999). This has the advantage for toxicity testing of producing *Hydra* that are genetically similar (Beach and Pascoe, 1998). This lack of genetic variation allows experimental results to be reproduced more easily with decreased coefficient of variation (Beach and Pascoe, 1998). This method of reproduction produces a high reproductive rate allowing large numbers of *Hydra* to be cultured in a short period of time (Holdway, 2005). *Hydra* can also go through sexual reproduction, where they will make male and/or female gonads and stimulate a sexual cycle (Littlefield *et al.*, 1991; Martin *et al.*, 1997). They tend to reproduce sexually under stressful conditions such as variations in water temperature or other environmental stimuli that precede declines in population density (Brien, 1953; Ribi *et al.*, 1985; Martin *et al.*, 1997; Yoshida *et al.*, 2006).

Ease of culture

Hydra are relatively easy organisms to culture and maintain in the laboratory, forming large colonies that normally reproduce asexually by budding, offering a cost effective animal for toxicity testing. Mass laboratory culture procedures were first developed and published for *Hydra* in the 1950s (Loomis, 1953). To culture large amounts of *Hydra*, an abundance of food, commonly live brine shrimp nauplii (*Artemia salina*) or any other appropriate food such as *Daphnia*, clean culture solution and daily care is required allowing the population to double every 1 to 4 days (Lenhoff and Brown, 1970). Given optimal conditions, *Hydra* populations can continue to reproduce asexually and grow logarithmically for an indefinite period of time (Loomis, 1953). Various factors can contribute to the growth of *Hydra* including the water used, temperature, pH, dissolved oxygen, amount of food and ionic balance. Laboratory

tests using *H. littoralis* and *H. vulgaris* have demonstrated that *Hydra* may require a minimum of 6 mg/L of dissolved oxygen, a maximum water hardness of 750 mg CaCO3/L, a pH range of 6 - 8, temperatures of 20 - 30°C and daily feeding of *Artemia* in order to achieve logarithmic growth (Loomis, 1953; Fu *et al*., 1991). Studies have shown that calcium ions and potassium ions are required for *Hydra* stock cultures, especially *H. littoralis* and *H. viridissima* (Lenhoff and Brown, 1970). Other ions which are often added to *Hydra* culture medium include: chloride, magnesium, sodium and bicarbonate (Muscatine and Lenhoff, 1965).

Widespread prevalence in freshwater ecosystems

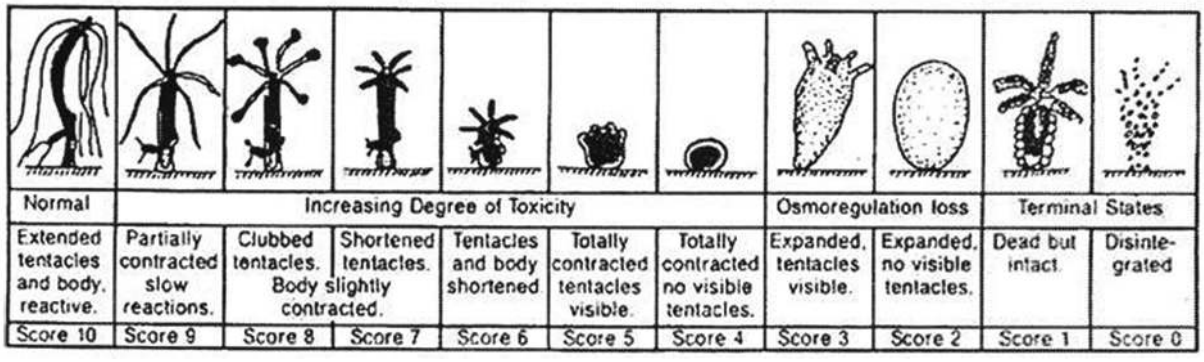
Hydra are ubiquitous inhabitants of freshwater environments generally found attached to natural submerged substrates such as sticks, rocks and plants (Slobodkin and Bossert, 2001) where they remain as sessile polyps or when food is scarce as floating polyps carried passively by water currents (Lomnicki and Slobodkin, 1966). The Green *H. viridissima* are usually found in clear waters while pink *H. vulgaris* are usually found in more turbid waters (Holdway *et al*., 2001). As *Hydra* occupy one of the lower trophic levels within freshwater foodwebs, changes in their population could have an indirect but significant effect on the rest of the freshwater community. Ecologically, *Hydra* play the role of both predators and prey in aquatic ecosystems (Slobodkin and Bossert, 2001). As predators, *Hydra* have been shown to ingest cladocerans (Schwartz and Hebert, 1989), copepods (Link and Keen, 1995), rotifers (Walsh, 1995), and larval fish (Elliott *et al*., 1997), as well as their standard laboratory food, brine shrimp, *Artemia* sp. (Loomis and Lenhoff, 1956). They can be prey themselves for flatworms (Slobodkin and Bossert, 2001). *Hydra* are ecologically important and play an important role in structuring the planktonic make-up of ponds (Schwartz *et al*., 1983) and are therefore a valuable indicator species in ecotoxicology.

Endpoints

Morphology

Toxicity in *Hydra* is typically measured by drastic changes in morphology assessed using a binocular microscope (Bossert and Galliot, 2012). As the amount of toxicant increases *Hydra* progressively exhibit morphological changes that were conventionally measured and expressed based on the observed changes in the animal with normal, bulbed or clubbed tentacles, shortened tentacles, tulip phase and disintegration as endpoints (Table 1). More recently, progressive changes in animal morphology have been measured and scored on a scale from 10 (normal, elongated tentacles and body), 8 (clubbed or bulbed tentacles), 6 (shortened tentacles), 5 (tulip phase), 2 (loss of osmoregulation) to score 0 (disintegrated) devised by Wilby (1988) (Table 1 and Fig. 1A). Scores 10–6 are reversible, sub-lethal indicators while the tulip phase (score 5 and below) is considered irreversible and used as the endpoint for lethality (Blaise and Kusui, 1997). The progres-

A Hydra morphology – Toxicity to polyp phase



B Hydra Regeneration – Inhibition of regeneration phase

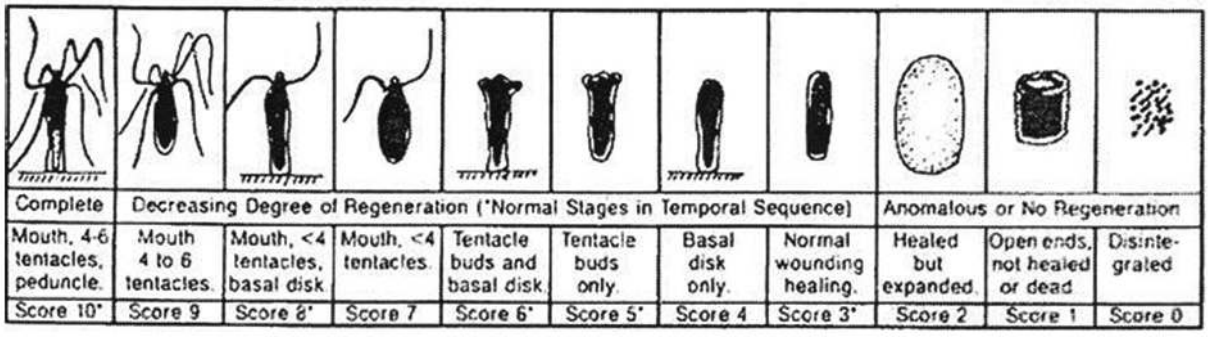


Fig. 1. Toxicity in *Hydra* based on morphological changes (A) and inhibition of regeneration (B) based on the scale from 10 to 0 devised by Wilby (1988) *(reproduced with kind permission of Wilby, Tesh and Shore, 1989). Pictures of morphological changes in* Hydra *at various stages of the health index are shown in Bossert and Galliot (2012, this issue).*

sive changes observed using the Wilby scale have the advantage over the conventional method of being more sensitive, reveal more detail on the pattern of response and provide a means of studying the ability of the animals to recover after exposure (Karntanut and Pascoe, 2000). Today this scale forms the basis of most *Hydra* toxicity tests and has been used extensively to assess toxicity of numerous compounds and effluents.

Regeneration

Hydra have an unsurpassed capability of regeneration and can be considered a perpetual embryo as it permanently renews its inventory of differentiated cells (including nerve cells) from pluripotent stem cells (Müller, 1996). When *Hydra* polyps are cut into pieces they are able to regenerate the absent structures entirely (Bode, 2003; Holstein *et al.*, 2003; Galliot *et al.*, 2006; Hoffmeister-Ullerich, 2007). But regeneration can also occur after dissociation of the tissues, from cells that form reaggregates (Noda, 1971; Gierer *et al.*, 1972). In initial toxicological studies, regeneration was measured by the use of dissociated cells to make an artificial embryo (Johnson *et al.*, 1982) and later Wilby (1988) recommended the use of dissected gastric sections (located below the hypostome (mouth) and above the budding region) consisting of mitotically active multipotent stem cells to study regeneration. In both cases the developmental toxicity (D) was compared to adult survival toxicity (A) to produce a toxicity index (TI) based on the A/D ratio, that specifically evaluates the ability of a toxicant to alter development and unambiguously ranks substances according to their hazard potential (Johnson *et al.*, 1982; Wilby, 1988; Quinn *et al.*, 2008b). Morphological changes are observed using a binocular microscope and the degree of regeneration assessed. Today this is most commonly done using Wilby's (1988) classification (Table 1 and Fig. 1B), with a score ≤5 corresponding to lethality (Pachura-Bouchet *et al.*, 2006).

Hydra have been successfully used to examine the teratogenic potential of several chemicals including effluents and water samples (Fu *et al.*, 1991, Fu *et al.*, 1994) and various chemicals (Johnson *et al.*, 1986; Mayura *et al.*, 1991; Yang *et al.*, 1993; Bowden *et al.*, 1995,) including endocrine disrupting compounds (Pascoe *et al.*, 2002; Pachura-Bouchet *et al.*, 2006) and pharmaceuticals (Pascoe *et al.*, 2003; Quinn *et al.*, 2008b; Quinn *et al.*, 2009). Good correlation was found between the *in vitro Hydra* regeneration assay and teratogenicity *in vivo*, as reported by Bowden *et al.*, (1995) and Wilby and Tesh (1990), who proposed it as a screening tool for teratogenicity. Furthermore the combination of *Hydra* developmental hazard index (A/D ratio) and rat whole embryo culture test have been recommended for use together to facilitate the rapid detection and ranking of hazardous chemicals associated with complex mixtures of chemical waste (Mayura *et al.*, 1991; Yang *et al.*, 1993). In many studies the regenerated animal's ability to feed was also observed as a further sub-lethal behavioural endpoint (Pascoe *et al.*, 2003; Quinn *et al.*, 2008b; Quinn *et al.*, 2009).

TABLE 1

***HYDRA* TOXICITY CLASSIFICATION SCHEME BASED ON MORPHOLOGY AND REGENERATION SCORES (10-1) TAKEN FROM WILBY (1988)**

Morhpology	Score	Morphology (Wilby, 1988)	Regeneration (Wilby, 1988)
Normal	10	Extended tentacles, body reactive	Mouth, 4-6 tentacles, peduncle
	9	Partially contracted, slow reactions	Mouth, 4-6 tentacles
Clubbed / bulbed tentacles	8	Clubbed tentacles, body slightly contracted	Mouth, <4 tentacles, basal disc
	7	Shortened tentacles, body slightly contracted	Mouth, <4 tentacles
Shortened tentacles	6	Tentacles and body shortened	Tentacle buds & basal disc
Tulip	5	Totally contracted, tentacles visible	Tentacle buds only
	4	Totally contracted, no visible tentacles	Basal disc only
	3	Expanded, tentacles visible	Normal wounding healing
Loss of Regulation	2	Expanded, no visible tentacles	Healing but expanded
	1	Dead but intact	Open ends, not healed or dead
Disintegration	0	Disintegrated	Disintegrated

Scores 6 are reversible and sub-lethal, while scores 5 are considered lethal.

Reproduction

As mentioned above *Hydra* have the ability to reproduce sexually under stressful conditions or most commonly asexually under favourable conditions. *Hydra* have the capacity to sense their environment such as food availability and temperatures, which determine the outcome of asexual reproduction and longevity (Schaible *et al.*, 2011). Indeed, a clear trade-off was found between asexual reproduction and maintenance with food intake favouring budding, while starvation limits the budding process and maintains survival. This gives the opportunity to examine the outcomes of energy allocation upon various environmental stresses on the fitness of *Hydra*. Under asexual reproduction *Hydra* that are well fed use the excess of cells that are produced by forming buds in the middle of their body (Müller, 1996; Böttger and Hassel, 2012). The ability of *Hydra* to regenerate is due to the constantly proliferating epithelial and interstitial cells in its body column, in the absence of bisection this constant cell renewal allows the animal to bud at a rapid rate (Hoffmeister-Ullerich, 2007). This asexual mode of reproduction involves a tissue consisting of stem cells with continuous renewal potential. *Hydra* represent a key organism to study the effects of pollution on stem cell activity, integrity and capacity to differentiate into many other tissues. Indeed, the potential impacts of pollution on stem cell regeneration could compromise the future of offspring.

Hydra have a high asexual reproductive rate resulting in large numbers being cultured in a short period of time (Holdway, 2005). This allows the reproductive effects of a possible toxicant to be determined (Mitchell and Holdway, 2000). The *Hydra* population reproduction toxicity test method determines the maximum concentration at which a chemical or wastewater has no statistically significant effect over 7-days of exposure on the population growth as measured by changes in the number of intact hydroids (one hydroid equals one animal plus any attached buds) with the no observable effect concentration (NOEC) and lowest observable effect concentration (LOEC) determined (Stebbing and Pomroy, 1978; Holdway, 2005) . It measures the biological effects of low levels of contaminants using the rate of asexual reproduction of *Hydra*. The mean relative population growth rate (K) is calculated and is defined as:

$$K = \frac{\ln (ny) - \ln (nx)}{T}$$

where nx is the number of *Hydra* at the beginning of the first day (tx), ny is the number of *Hydra* after y - x days (ty) and *T* is the length of the test period in days (ty – tx).

The *Hydra* reproduction toxicity test is a useful, cost effective and relatively easy test to assess the population reproductive toxicity of both pure chemicals (Pollino and Holdway, 1999; Holdway *et al*., 2001) and effluents and environmental samples (Mitchell and Holdway, 2000; Rosenkrantz *et al*., 2008) in a relatively short time. This test method assesses population reproduction attributes which are extremely difficult and or expensive to do in many other tests or with many other types of test organisms. An alternative technique for measuring asexual reproduction is simply to count the number of hydranths in each well before and after a 96 hour exposure (Quinn *et al*., 2007). Although not as sensitive as the morphology or feeding tests, hydranth numbers potentially offer another end-point of interest to assess *Hydra* sub-lethality. *Hydra* can also undergo energy demanding sexual reproduction, where they will make male and/or female gonads and stimulate a sexual cycle. *Hydra* are generally dioecious (i.e. male and female organs are kept on separate individuals), but hermaphroditism and sex reversals can occur (Lenhoff, 1983). Moreover, the appearance of sexual dimorphism does stop asexual budding activity i.e., both processes occur at the same time. Sexual reproduction occurs more frequently in larger individuals that reached full adult size where growth is stopped. The size of individuals was related to bud numbers while no clear trend was found between either male or female gonad sizes. They tend to reproduce sexually under stressful conditions (Holdway, 2005). Temperature was found to regulate sexual reproduction in *H. oligactis* with sexual reproduction occurring at lower temperatures (10-12 °C) (Littlefield *et al*., 1991). However there is a cost for sexual reproduction. Sexually reproducing organisms undergo aging (Brien, 1953; Yoshida *et al*., 2006). The price of aging is the result of facilitating reproduction in the early life stage of organisms. Indeed, signs of aging were observed in sexually differentiated *Hydra* such as reduced food capture, contractile movements and reproduction with a higher rate of mortality in these populations. Moreover, the number of germ cells increased with a concomitant drop in the number of somatic cells. Interestingly, the *Chlorella* algae symbiosis with *H. viridis* was also shown to influence regeneration and sexual differentiation in polyps (Habetha *et al*., 2003; see in this issue Kovacevic, 2012). Under a low feeding regime, asexual growth was reduced in polyps lacking the algae, suggesting increased food assimilation. According to Habetha *et al.,*2002) in most cases, female gonads were produced only when symbiotic algae were present but had no effects of spermatogenesis. During oogenesis, symbionts were actively transferred from endodermal epithelial cells to the ectodermal oocytes indicating the involvement of green algae in the control of sexual differentiation in the green *Hydra*. The possible outcome for this association involves the potential effects of biotoxins and cyanobacterial blooms on the integrity of algae-*Hydra* symbiosis.

Feeding

Feeding behaviour is ecologically significant because of its direct effects on reproduction, population growth and abundance and composition of species it predates and is therefore a useful endpoint to study toxicity in aquatic ecosystems (Juchelka and Snell, 1994). Toxicant modification of feeding behaviour could eventually lead to reduced survival and reproduction, resulting in adverse consequences at the population level (Halbach, 1984; Kooijman and Metz, 1984). Feeding is one of an organism's most basic interactions with the environment and is a function of many physiological parameters (Lasker *et al*., 1982). Feeding rates are particularly important in regulating *Hydra* population densities. Laboratory studies have shown that *Hydra* population growth rates are directly related to frequency of feeding (Muscatine and Lenhoff, 1965; Otto and Campbell, 1977) and in the field, *Hydra* population densities were shown to closely follow increases in zooplankton abundance (Cuker and Mozley, 1981). The feeding process is divided into a series of discrete steps: capture of prey with nematocysts, transport of prey to mouth, mouth opening, ingestion, digestion and ejection of exoskeleton. The steps between capture and ingestion are termed feeding reaction or response (Lehhoff, 1961). Feeding behaviour in *Hydra* is initiated by the association of glutathione (GSH) with a putative external chemoreceptor (Bellis *et al*., 1992) and a wide variety of environmental parameters are know to affect this response (Lehhoff, 1961; see in this issue Pierobon, 2012). It is possible that the occurrence of oxidative stress and/or diminished amounts of GSH by its extensive conjugation to xenobiotics could potentially hamper feeding activity in *Hydra* (Quinn, 2004). Animal feeding behaviour has already been identified as a potential endpoint for the study of the more subtle effects of pharmaceuticals (Fent *et al*., 2006). The feeding reaction has been successfully used in several studies to examine the sub-lethal toxicity of several compounds including heavy metals (Beach and Pascoe, 1998; Quinn *et al*., 2007) and pharmaceuticals (Pascoe *et al*., 2003; Quinn *et al*., 2008a; Quinn *et al*., 2009) with the sub-lethal response investigated using feeding bioassay proved to be considerably more sensitive than that recorded in lethal studies (Beach and Pascoe, 1998).

Attachment

Attachment to a substrate is needed in order to feed, grow and reproduce and is therefore essential to *Hydra*. In a study by Quinn *et al*., (2007) a clear relationship between increased toxicity to a reference chemical ($CdCl_2$) based on morphology and a decrease in attachment was evident with *Hydra* attachment significantly reduced by a concentration as low as 0.02 mg/L when compared to the control. To our knowledge this is the first time attachment has been reported as a toxicity endpoint. However an *in vitro* study by Lomnicki and Slobodkin, (1966) observed the secretion of a 'bubble' relating to food intake resulting in effectively turning the animal upside down and in *Hydra* detachment. *Hydra* attachment may be a sensitive toxicity endpoint that merits further study.

Biochemistry and biotransformation of xenobiotics

Metals

The biochemistry and detoxification processes in *Hydra* are generally not well documented with only a handful of studies having been published. It is possible that owing to its diploblastic morphology resulting in constant contact with the aquatic environment, diffusion is the main method for toxicant accumulation and detoxification in *Hydra* (Walker *et al*., 2006). This may be true for metals as an analogue of the metal binding protein metallothionein (a common biomarker of metal exposure) or other metal binding proteins which serve an important role in homeostatic control and sequestration of metal ions, has not yet been discovered in *Hydra* (Andersen *et al*., 1988; Karntanut and Pascoe, 2002). This might

form the biochemical basis of their high sensitivity towards heavy metals (for more detail on metals see Section 4.1). Despite this, metals may be sequestered and expelled by *Hydra* following exposure, as has been observed with uranium accumulated in discharged nematocyst cells which are routinely discarded as new cells replace them (Hyne *et al.*, 1992a). *Hydra* have some capacity to express heat shock proteins of 70 kDa family which can constitute a protection mechanism against heavy metals since there is a cross protection mechanism between thermo tolerance and metal tolerance (Brennecke *et al.*, 1998). However, there were some reports on the inability of *Hydra oligactis* to acquire thermo tolerance due to the low levels of heat shock protein expression in this species (Gellner *et al.*, 1992). The relative inability of *Hydra oligactis* to protect against temperature increases and heavy metal contamination makes this test species useful to study the interaction of temperature changes and metal pollution in the context of climate changes.

Organics

However both cellular stress responses and phase I and II detoxification enzymes have been identified and characterised in *Hydra* (Fig. 2). *Hydra* were found to posses glutathione S-transferase (GST) activity (Stenersen *et al.*, 1987) an enzyme that plays an important role during phase II biotransformation in the detoxification and metabolism of many xenobiotic and endogenous compounds (Hoarau *et al.*, 2004). Moreover, GST activity was induced in *Hydra* following exposure to the antiepileptic drug and common environmental pollutant carbamazapine, at environmentally relevant concentrations (Quinn, 2004), indicating a phase II biotransformation response. Carbamazepine exposure also led to the induction of oxidative metabolism as shown by a significant increase in both heme oxidase (HO) and lipid peroxidation, indicating that *Hydra* appear to possess both mixed function oxidase and conjugation capabilities that are inducible upon exposure to this xenobiotic (Quinn, 2004; Vernouillet *et al.*, 2010). This was corroborated in a separate study where no significant bioaccumulation of carbamazepine by *H. attenuata* fed with carbamazepine contaminated *T. platyurus* was found, indicating that either uptake of the drug was weak or there is a high detoxification activity as revealed by the increased HO and cytochrome P450 3A4-like activity observed (Vernouillet *et al.*, 2010).

Acetylcholinesterase

The gene encoding for acetylcholinesterase (AChE) in *H. magnipapillata* was isolated (Takahashi and Hamaue, 2010) which makes it susceptible to organochlorine and organophosphorus pesticides. However, the sequences of peripheral anionic and choline binding sites differed from the other known sequenced taxa. This could lead to changes in susceptibility to these pesticides which are for the moment unknown. Moreover, the gene was expressed in both the ectodermal and endodermal epithelial cells except for the tentacles and the basal disk. The absence of AchE activity in regenerating tips and either the head or foot suggests that the cholinergic neural system is not involved in regeneration.

Oxidative stress

On the other hand, *Hydra* have the capacity to protect against oxidative stress since they contain two superoxide dismutase (SOD) genes (Dash *et al.*, 2007). The SOD are of the type MnSOD which are principally found in the mitochondria and of the type CuZnSOD which is cytosolic in cells. *Hydra* subjected to thermal, starvation, metal and oxidative stresses responded by regulating both forms of SOD. The same group of investigators also identified a glutathione peroxidase family using phospholipid hydroperoxide as a co-substrate (Dash *et al.*, 2006). As above, *Hydra* exposed to starvation, metal and oxidative stresses responded by regulating upward their glutathione peroxidase transcripts, making them a useful test species to examine the impact of pollution-induced oxidative stress. For example, exposure of *Hydra* to increasing concentrations of carbamazepine, a persistent drug commonly found in municipal effluents, led to the induction of cytochrome P450-3A-like, heme oxidase activity and lipid peroxidation suggesting that biotransformation potentiates the toxicity of this drug by oxidative stress (Quinn, 2004).

DNA integrity

The genotoxicity potential of contaminants can also be investigated using *Hydra*. In a study investigating the toxic effects of aspiron and metamizole sodium, the latter caused nucleolar structural damage in 90 % of the *Hydra* cells as early as 30 min of exposure (Arkhipchuk *et al.*, 2004). Exposure of brown and green *Hydra* to increasing concentration of aluminium was genotoxic as determined by the Comet assay (Kovačević, 2007). The brown were more sensitive to aluminium than green *Hydra*, showing again a protective role of symbiosis to metals and the evolutionary advantage provided by symbiosis (see in this issue Kovacevic, 2012). Indeed, DNA tail length and intensity changes were stronger in brown than in green *Hydra*. However, behavioural responses to the presence of aluminium ions were observed more rapidly in green *Hydra*.

Toxins

Metals

Hydra are generally sensitive to metal salts and other cations (Holdway *et al.*, 2001). For example, the reported 7-day toxicity

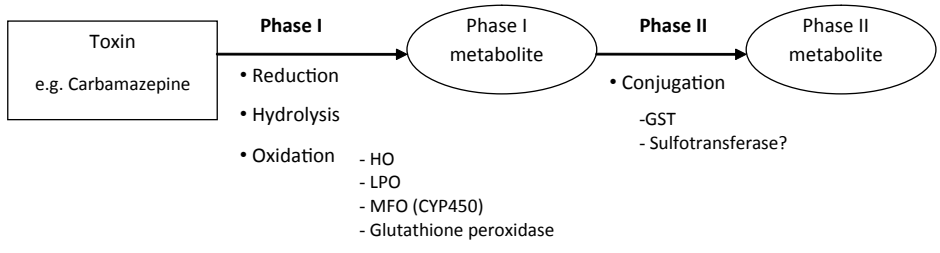


Fig. 2. Evidence of biotransformation of xenobiotics in *Hydra* in a two-phase process. *The first-phase reactions include oxidation, reduction and hydrolysis with the greatest importance ascribed to oxidation enzymes involved in the metabolism of the majority of xenobiotics, giving rise to more polar compounds. In second-phase reactions, the metabolites produced in the first phase are conjugated with products of the endogenous metabolism (e.g. GST) to give rise to polar compounds subsequently eliminated from the body.*

threshold for the green *Hydra* was 0.56 and 250 μg/L for cadmium and zinc respectively. This makes them excellent freshwater invertebrates for testing for the presence of dissolved metals based on their sensitivity and to rapidly determine population reproduction in the laboratory. Copper has been regularly found to be the most toxic heavy metal in comparative acute toxicity studies between *Hydra* species (*H. vulgaris, H. oligactis* and *H. viridissima*), followed by cadmium and zinc (Beach and Pascoe, 1998; Pollino and Holdway, 1999; Karntanut and Pascoe, 2000; Holdway *et al*., 2001; Karntanut and Pascoe, 2002; Karntanut and Pascoe, 2005). The green *Hydra* (*H. viridissima*) containing stable algal symbiotes has been routinely observed as the most sensitive *Hydra* species with a 96 hour LC_{50} (the lethal concentration need to kill 50% of the exposed population) range of 8.5 - 28 μg/L for copper, 3 - 210 μg/L for cadmium and 935 - 11,000 μg/L for zinc (Pollino and Holdway, 1999; Holdway *et al*., 2001; Karntanut and Pascoe, 2002; Karntanut and Pascoe, 2005). The increased acute sensitivity of this species to copper may result from copper affecting the zoochlorellae as it is a potent algaecide (Pollino and Holdway, 1999). However in an interesting study, Karntanut and Pascoe (2005) compared the toxicity of heavy metals to both symbiotic and aposymbiotic (free of their endosymbiotic algae) *H. viridissima*. Although the toxicity was similar for both groups, at the lower Cu concentrations the symbiotic *Hydra* was better able to tolerate the toxicant. They hypothesized that at low concentrations the copper taken up by symbiotic *Hydra* may be sequestered by the algae, providing a degree of protection for the polyp itself. However at the higher concentrations it is probable that any defence systems are overwhelmed by the toxicant effect and any slight benefit derived from the endosymbiotic algae is of little consequence. Indeed in some cases hormetic effects of metals on *Hydra* at lower concentrations have been observed (Pollino and Holdway, 1999; Karntanut and Pascoe, 2002).

Sensitivity to metals is thought to be partially due to the inability of *Hydra* to conjugate and expel metals owing to the lack of the metal binding protein metallothionein responsible for the uptake, transport and regulation of metals. This makes it a particularly sensitive species to metal contamination and a very effective bioindicator species for exposure of metals in the environment. Indeed toxicity endpoints for copper exposure were in the range of dissolved copper concentrations that one would expect to find in many contaminated environmental sites (Pollino and Holdway, 1999). *Hydra* have also been shown to accumulate metals, potentially leading to exposure to higher concentrations in the environment. Deposits were observed in the discharged nematocysts of *H. viridissima* after a 24 hour exposure to 200 - 3900 μg/L uranium in a single compound mixture as well as in an effluent (Hyne *et al*., 1992a) and were presumed to be responsible for the reduced post-exposure ability of the *Hydra* to capture live *Artemia* sp. (Hyne *et al*., 1992a). In this study aluminium, magnesium and zinc were also found within the symbiotic algal cells of the *Hydra* (Hyne *et al*., 1992a). In another study, copper, cadmium and zinc were demonstrated to accumulate in *H. vulgaris* through both waterborne and food-borne exposure routes (Karntanut and Pascoe, 2007).

The acute effects of metals on *Hydra* are based on lethality established by the morphological effects on the animal e.g. > stage 5 on the Wilby (1988) scale (Table 1 and Fig 1A). However a reduction in population growth measured by reduced asexual budding is another sensitive and environmentally relevant endpoint. Effects on population growth were observed at 8 - 16 μg/L for copper (Stebbing and Pomroy, 1978; Pollino and Holdway, 1999; Karntanut and Pascoe, 2005), 0.8 μg/L for cadmium (Holdway *et al*., 2001), 75 μg/L for zinc (Holdway *et al*., 2001) and 50 μg/L for lead (Browne and Davis, 1977) on *H. viridissima* and 60 μg/L for nickel on *H. littoralis* (Santiago-Fandiño, 1983). However as highlighted by Holdway *et al*., (2001) metal toxicity is greatly modified by the abiotic factors such as water hardness, pH and temperature used by the investigators rather than just species differences. High water hardness tends to increase complexation of metals as well as provide competing cations (Ca^{2+} and Mg^{2+}) which can decrease the effects of toxic divalent metals (Riethmuller *et al*., 2001). The toxicity of uranium to *H. viridissima,* as measured by population growth, was significantly reduced when water hardness was increased from 6.6 mg $CaCO_3$/L to levels of both 165 and 330 mg $CaCO_3$/L (Riethmuller *et al*., 2001). Lower pH values may also increase the concentration of soluble and bioavailable free metal ions, which are more capable of causing internal toxic effects (Riethmuller *et al*., 2001, Walker *et al*., 2006). For example, based on population growth uranium was determined to be more toxic to *H. viridissima* at a pH of 6.6 than a pH of 8.6 (Hyne *et al*., 1992b). The toxicity of magnesium sulfate ($MgSO_4$), and the influence of calcium (Ca), were assessed in very soft freshwater where Ca was shown to have an ameliorative effect on Mg toxicity (van Dam *et al*., 2010). It was concluded that magnesium can be toxic at concentrations approaching natural background levels, but toxicity is dependent on Ca concentrations, with exposure in very low ionic concentration, Ca-deficient waters posing the greatest risk to aquatic life (van Dam *et al*., 2010). Therefore, when comparing the toxicity of metals or the potential impact of metals on the environment, exposure conditions and abiotic factors should also be taken into account.

Organic toxicants

Hydra reportedly have a lower sensitivity to organic compounds than to metals and therefore a limited application for toxicity testing of organic toxicants. When selected organic toxicants were tested, the acute and sub-chronic toxicity endpoints for *Hydra* were higher than literature values for most species with toxicity endpoints beyond the range that one would expect to find in the environment (Pollino and Holdway, 1999). Toxicity tests on *H. oligactis* using the polychlorinated biphenyls (PCBs) Aroclor 1016 and Aroclor 1254 resulted in a 72 hour LC_{50} range of 5,000 - 20,000 μg/L, although sub-lethal inhibitory effects on reproduction and regeneration were seen at levels of 1,000 μg/L - 4,000 μg/L (Adams and Haileselassie, 1984). A low sensitivity for the reference toxicant 4-chlorophenol was reported by Mitchell and Holdway (2000) with a 96 hour LC_{50} of 34,000 μg/L similar to the value of 32,000 μg/L for *H. vulgaris* reported by Pollino and Holdway (1999). These findings are consistent with those of previous studies, using toxicants such as the organochlorine pesticide lindane (Taylor *et al*., 1995), the chlorinated hydrocarbon insecticide mirex (Lue and de la Cruz, 1978), ethylene dibromide (Herring *et al*., 1987), PCBs, atrazine, and DDT (Benson and Boush, 1983). In each case, *Hydra* species were less sensitive to the toxicant compared with other invertebrate species. Therefore, *Hydra* may have only limited application for the toxicity testing of organic toxicants and cannot be considered a sensitive model for invertebrates (Pollino and Holdway, 1999).

However, exposures of *H. attenuata* to several organophosphates demonstrated that *Hydra* may be sensitive to some organics

with a minimal affective concentration (MAC) range from 0.003 – 100,000 μg/L and toxicity correlated with increasing compound hydrophobicity (Lum *et al.*, 2003). When a suite of chlorophenols were tested with *H. vulgaris*, the 92 hour MAC range was 40 – 500,000 μg/L with the more chlorine-substituted compounds generally being the most toxic (Mayura *et al.*, 1991). *Hydra* have also been successfully used as part of a battery of test organisms to investigate the ecological risks for aquatic ecosystems posed by the toxicity of pesticides used in banana production (Castillo *et al.*, 2006) and to investigate the effect of insecticides (Dimiline WP 25, Torak EC 24 and Gamacide 20) (Kalafatic *et al.*, 1991).

Endocrine disrupting compounds (EDCs)

H. vulgaris (previously named *H. attenuata*) is reported to be one of the most sensitive species to acute and chronic toxicity of the endocrine disrupting compound 4-nonylphenol compared to several freshwater invertebrates (Pachura-Bouchet *et al.*, 2006). The toxicity recorded for *Hydra* with a 96 hour LC_{50} of 97.5 μg/L and a "no observed effect concentration" (NOEC) for tentacle morphology of <25 μg/L, occurred at concentrations that are representative of those at polluted sites (Pachura *et al.*, 2005). The reproductive toxicity of bisphenol A (BPA) on both asexual and sexual reproduction was investigated in *H. oligactis* (Fukuhori *et al.*, 2005). Exposure to BPA at mg/L range (1-10 mg/L) had adverse effects on sexual reproduction where the asexual method was favoured. It was not clear whether the estrogenic properties of BPA accounted for the observed toxicity since the concentrations required were high compared to reported environmental concentrations and the low dose required for producing hormonal effects in fish. In another study, exposure of *H. vulgaris* to BPA and 17α-ethynylestradiol (EE2) led to changes in the structure of polyps at concentrations above 58 and 42 μg/L BPA and EE2 respectively (Pascoe *et al.*, 2002). Regeneration was inhibited at 460 and 150 μg/L of BPA and EE2 respectively. The effect of exposure to EE2 was found to impair sexual reproduction in *H. vulgaris*, but at such high, environmentally unrealistic concentrations (500 μg/L) it was felt that the response was probably the result of general toxicity rather than disruption of hormonal / signalling processes (Pascoe *et al.*, 2002). This has led to the conclusion that these primitive cnidaria are not subject to disruption by estrogens or estrogen mimics (Pascoe *et al.*, 2002).

Notwithstanding this, the presence of indolamines, steroids and neuropeptides have been identified in cnidarian tissues, which indicates that endocrine disrupters might also disrupt the signalling pathways in *Hydra* (Tarrant, 2005). Evidence suggests that both classical fast (acetylcholine, glutamate, GABA, glycine) and slow (catecholamines and serotonin) transmitters as well as neuropeptides are involved in cnidarian neurotransmission, although cumulative data are incomplete (Kass-Simon and Pierobon, 2007). However, it remains unknown whether these compounds are involved in larger signal cascades comparable to the vertebrate hypothalamic-pituitary-gonadal axis. Regeneration of the apical, gastral and basal fragments was inhibited by dopamine synthesis inhibitors but did not produce morphological abnormalities (Ostroumova and Markova, 2002). In another study, head specific differentiation was influenced by specific protein kinase inhibitors (Cardenas *et al.*, 2000). Head cellular proliferation was seemingly under the control of protein kinase C pathway while head cellular differentiation involved tyrosine protein-kinase Src signalling which is considered a proto-oncogene in vertebrates.

Pharmaceuticals

The addition of novel contaminants, particularly pharmaceutical drugs into the environment, primarily by municipal effluents, is causing much environmental concern. Recent research has indicated that *H. vulgaris* are sensitive to pharmaceuticals typically found in wastewater effluents (Pascoe *et al.*, 2003; Quinn *et al.*, 2008a; Quinn *et al.*, 2008b; Quinn *et al.*, 2009) as reviewed by Blaise *et al.*, (2006). Pharmaceuticals are continuously present at low concentrations so chronic effects are thought to be more relevant (Fent *et al.*, 2006). Although lethality based on morphological effects was observed at high environmentally unrealistic concentrations (mg/L) (Pascoe *et al.*, 2003; Quinn *et al.*, 2008a, Quinn *et al.*, 2008b; Quinn *et al.*, 2009), the toxicity threshold (TT) value (TT=(NOEC×LOEC)1/2) for ibuprofen was 320 μg/L, (Quinn *et al.*, 2008a) only a factor of 10 higher than the concentration found in Canadian effluents (22 μg/L (Brun *et al.*, 2006). When exposed as a mixture, pharmaceuticals were 2 to 3 orders of magnitude lower for the equivalent toxicity (EC_{50} and TT) for the individual pharmaceutical, indicating they may act additively in a mixture, having sub-lethal effects at environmentally relevant (μg/L–ng/L) concentrations (Quinn *et al.*, 2009). Similarly Pascoe *et al.*, (2003) found that *Hydra* regeneration was inhibited by 3 of the 10 drugs examined (diazepam, digoxin and amlodipine) at 10 μg/L after an extended exposure time (17 days). When subjected to tier two toxicity assessment under EU regulatory guidance using environmentally relevant concentrations, a MEC/PNEC value >1 was calculated for gemfibrozil, ibuprofen and naproxen indicating teratogenic potential (Quinn *et al.*, 2008b). Biochemical biomarker responses have also been observed in *Hydra* following exposure to pharmaceuticals. Despite not being bioaccumulated (Vernouillet *et al.*, 2010) carbamazepine was found to affect global cytochrome and cytochrome P450 3A-like activity, lipid peroxidation (LPO), heme oxidase (HO) and GST activity (Quinn, 2004; Vernouillet *et al.*, 2010). Oxidative metabolism with an increase in LPO was found at a threshold concentration of 7.1 μg/L (Quinn, 2004), similar to maximum concentration of 6.3 μg/L reported in WWTPs (Ternes, 1998). These results indicate the potential for chronic effects to occur at environmentally relevant concentrations and the usefulness of *Hydra* to assess these potential effects.

Nanomaterials

We found only a handful of studies dealing with the toxicity of nanomaterials (NMs) to *Hydra*. The toxicity of 11 NMs were examined in a test battery of aquatic biotests (Blaise *et al.*, 2008) where *Hydra* proved to be the most sensitive species with 96 hour EC_{50} in the 0.1-1 mg/L range for inorganic NMs: copper and zinc iron oxides, indium tin oxides and holmium oxides nanoparticles. *Hydra* were also seemingly sensitive to single-wall carbon nanotubes with an EC_{50} in the 1-10 mg/L range. Interestingly rod-shaped nanocrystals proved excitatory to cnidarians (Malvindi *et al.*, 2008). Exposure of *Hydra* to rod-shaped semiconductor quantum dots resulted in an unexpected tentacle-writhing behaviour suggesting the involvement of tentacle neurons and depends on Ca^{2+} ions. Moreover, this effect was not produced when spherical quantum dots were used instead of the rod-shaped ones. It was suggested that the electrical properties of rod-shaped of the quantum rods accounted to the observed neuronal stimulation. The bioavailability of cadmium-based quantum dots could be enhanced by the presence of positive charges at the surface of the nanoparticles

(Tortiglione *et al*., 2009). At acidic pH, amino-pegylated coated cadmium quantum dots were actively internalized by tentacle and body ectodermal cells in *Hydra vulgaris*. Negatively charged quantum dots were not bioavailable to the *Hydra*. The uptake of positively charged nanoparticles involved annexin proteins in the cell membranes which are involved in pro-apoptotic mechanisms of cell death. In another study, the toxicity of uncapped titanium dioxide and zinc-capped titanium dioxide was also examined in the *Hydra* to determine the resulting effects of capping in the *Hydra* (Yeo and Kang, 2010). Although there was no marked overall toxicity of these nanoparticles as determined by the absence of tissue necrosis or apoptotic cells, some damage was found with zinc capped TiO_2 and uncapped TiO_2 under UV-A photo conditions. However it was shown that it was the photo conditions that produced effects on morphology and cytotoxicity.

Industrial and municipal effluents

Hydra have also been used to test the environmental impact of both municipal and industrial effluents, most commonly as a member of a battery of test organisms in bioassay studies. In a study of acute toxicity to ten industrial effluents on *H. vulgaris*, four were found to be lethal and eight sub-lethal with a 96 hour LC_{50} varying from 18.8 - 100% effluent (Blaise and Kusui, 1997). From this study the simple and cost-effective *Hydra* microassay appears to be a valuable (sub)lethal toxicity screening tool for effluents. Another study investigating the developmental hazards of industrial wastewater samples tested on *H. vulgaris* resulted in a minimal effect concentration (MEC) (based on both adult morphology and artificial embryo toxicity) of 6-31% effluent (Fu *et al*., 1991). These authors also reported 72 and 96 hour LC_{50} ranging from 2.7 to >100% for industrial wastewaters, which closely matched the LC_{50} values of fathead minnow (*Pimephales promelas*) bioassays run in parallel (Fu *et al*., 1994). As part of a battery of bioassays, *Hydra* species have been used to assess the toxicity of vinasse (a byproduct of the sugar industry) (Ferreira *et al*., 2011), animal feed additives (Marroquin-Cardona *et al*., 2009) and pesticides (Castillo *et al*., 2006). When exposed to retention pond water containing gold mine effluent, population reproduction in *H. viridissima* was reduced by 80 to 100% by a treatment of 0.1% pond water (pH 6.5), in which copper and zinc were the most likely toxic components (Dam *et al*., 2008). A significant decrease in population growth, in *H. viridissima*, was also noted when exposed to 100% retention pond water (pH 7.5 - 8.0) from a uranium mine (Hyne *et al*., 1992a) although additional work showed the retention pond water to be toxic at 32% with a reduced pH of 6.6 (Hyne *et al*., 1992b).

Municipal effluents are an important and highly complex source of pollution into the environment. Due to this complexity biological assessment with the use of bioassays rather than chemical analysis is often favoured. In a toxicity test of municipal sewage on the morphology of *H. vulgaris* the 96 hour LC_{50} varied from 16.7 - >98% effluent and the 96 h EC_{50} for tentacle clubbing from 4.9 - >98% effluent making the *Hydra* assay a more sensitive indicator of toxicity than the Microtox® test (Pardos *et al*., 1999). *H. hexactinella* has also been used to test the toxicity of urban runoff water collecting in stormwater basins where only one out of the three basin samples tested were toxic to *Hydra* with a 96 hour LC_{50} of 61% stormwater (Rosenkrantz *et al*., 2008). However, the basin water found to be toxic had the highest levels of copper, cadmium, lead, nickel and zinc (Rosenkrantz *et al*., 2008). This supports the conclusion that, like the metal-laden mine effluents tested, effluents are most likely to be toxic to *Hydra* if they contain metals. The *H. vulgaris* bioassay was also included in a battery of tests used to appraise the detoxification capacity of the three strains of *Pleurotus* fungi on municipal effluent (Dellamatrice *et al*., 2005) and the impact of sewage effluent on the deterioration of wetlands (Oberholster *et al*., 2008).

Comparison of *hydra* sensitivity with other test organisms

Hydra are often used as part of a battery of test organisms representing different levels of biological organization used to assess the toxicity of a chemical or effluent. Bioassays with other invertebrates, such as daphnia (*Daphnia magna*) and C*eriodaphnia* (*C. affinis* or *C. dubia*), are widely used to detect chronic toxicity (after 21-days exposure) of chemical solutions, environmental and waste waters (Viganò *et al*., 1996). In several studies the *Hydra* bioassay, particularly when chronic changes in morphology and reproduction rate were measured, was more sensitive than other bioassays undertaken with invertebrate, vertebrate and plant test organisms (Diaz-Baez *et al*., 2002; Ronco *et al*., 2002a; Arkhipchuk *et al*., 2006b; Oberholster *et al*., 2008). In particular, sub-lethal effects on *Hydra* morphology were found to be considerably more sensitive than lethal effects (Blaise and Kusui, 1997), closely matching the toxicity response of the fathead minnow (Fu *et al*., 1994) and are generally more sensitive than the Microtox test (Pardos *et al*., 1999). *H. vulgaris* is particularly sensitive to Cd and Cu, responding at similar concentrations to *G. pulex*, an indicator species of freshwater quality and with 48 and 72 hours LC_{50} values showing similar sensitivity to *Daphnia magna* (Beach and Pascoe, 1998). *H. vulgaris* have also been shown to be more sensitive than *G. pulex* for variety of toxicants including benzene, trichloroethylene and allylamine (Slooff *et al*., 1983).

Hydra was seen as a suitable animal model in biotesting strategies designed for toxicity assessment of all types of water media and incorporating diverse toxicity including cytotoxicity and genotoxicity endpoints (Arkhipchuk and Malinovskaya, 2002; Arkhipchuk and Garanko, 2002). For specific objectives, the use of bioassays with *Hydra* and mammalian cell culture in tandem is recommended to facilitate the rapid detection and ranking of the developmental hazards of different toxins (Mayura *et al*., 1991, Yang *et al*., 1993). When used in parallel with the rat whole embryo culture (WEC) bioassay the *Hydra* regeneration assay was found to support the *in vivo* potential teratogenic hazard data (Yang *et al*., 1993; Bowden *et al*., 1995). *Hydra* thus stands out as a useful animal model for comprehensive and comparative assessment of different types of toxicity.

Use in monitoring

Hydra are a very popular model organism in aquatic toxicity and are increasingly used for numerous ecotoxicological studies, but have not been extensively used in formal monitoring programmes. Despite this, *Hydra* are used for the biological toxicity testing of mine waste waters using freshwater organisms (Hyne *et al*., 1996) and as part of an ecotoxicological testing protocol for Australian tropical freshwater ecosystems (Riethmuller *et al*., 2003) developed by Environment Australia under the Environmental Research

Institute of the Supervising Scientist (eriss). In these monitoring programmes biological toxicity tests were developed using local aquatic species from different trophic levels and phyla, and with various endpoints. In assessing the toxicity of mine waste (Supervising Scientist Report 110), the tests use two species of *Hydra* (*H. viridissima* and *H. vulgaris*) using either survival or reproduction as the endpoint, a water flea (*Moinodaphnia macleayi*) using either survival or reproduction as the endpoint, and a fish embryo/larva (*Mogurnda mogurnda*) with hatchability and survival as endpoints (Hyne *et al*., 1996). In the more recent ecotoxicological testing protocols for Australian tropical freshwater ecosystems (Supervising Scientist Report, 173) the method is based on the *Hydra* population growth test as described by Hyne *et al*., (1996) using *H. viridissima* (Riethmuller *et al*., 2003).

Hydra were also used in the WaterTox network, an international network of laboratories from eight participating countries, that examined the applicability of a battery of simple, inexpensive bioassays in environmental management and the relevance of the test results in establishing the toxicological quality of water sources and drinking water. The core battery of tests consisted of 2 animal assays (48 hour acute toxicity to *D. magna* (Dutka, 1989) and 96 hour exposure lethality to *H. vulgaris*, (Trottier *et al*., 1997) and a vascular plant bioassay (*L. sativa* 120 hour exposure inhibition of root elongation (Dutka, 1989). This program initially established quality control mechanisms for the bioassays used (Ronco *et al*., 2002b) followed by examining the sensitivity, applicability and reproducibility of selected battery to screen drinking water and drinking water sources of the presence of toxicants (Diaz-Baez *et al*., 2002). These bioassays have been subsequently used in several other studies (Arkhipchuk and Garanko, 2002; Oberholster *et al*., 2008) where *Hydra* was found to show good sensitivity.

Strengths and weaknesses of the *Hydra* model system

Standardised and relatively easy culture methods, consistent rapid asexual reproduction leading to a population of genetically identical clones, a diploblastic structure allowing exposure of cells directly to the environment, easily observable and quantifiable morphological changes and a widespread prevalence in freshwater ecosystems make *Hydra* a model test species for use in aquatic toxicology. For these reasons, *Hydra* have been used in numerous small scale tests and bioassays to study the toxicity of various pollutants. *Hydra* have been shown to regenerate into a healthy adult polyp from either a seriously injured intact individual (Loomis and Lenhoff, 1956), a ball of disassociated cells known as an artificial embryo (Johnson *et al*., 1982) or a dissected section of their gastric region (Quinn *et al*., 2008a). Due to this tremendous regenerative capacity, *Hydra* have been widely used in developmental biology and to assess the teratogenic effects of many environmental pollutants. Their rapid rate of asexual reproduction by budding allows the population reproduction effects of a potential toxicant to be determined in the laboratory, which is another enormous benefit of the *Hydra* model system.

However the main weakness of using *Hydra* as a toxicity test organism lies in its reported lack of sensitivity to organic toxicants. It has been reported that for organic toxicants acute and sub-chronic toxicity endpoints for *Hydra* were higher than literature values for most species and beyond the range that could be found in environment (Pollino and Holdway, 1999). Despite *Hydra* having a large potential for use in bioassays to test inorganic toxicants (particularly metals), it is felt that *Hydra* have a limited application for toxicity testing of organic toxicants. However as we have highlighted in section 4 there are several cases where *Hydra* were reported to have good sensitivity, particularly to the more novel contaminants nonylphenol and pharmaceuticals. Another apparent drawback for the use of *Hydra* in toxicity testing lies in its reported inability to metabolize xenobiotics (Fu *et al*., 1994) particularly the lack of metallothionein. However it appears that the more we investigate the biochemistry of these animals the more biochemical / biomarker stress responses are being expressed (Section 3 and 4). One important drawback to the use of *Hydra* is the ability of the exposure parameters (water hardness, pH, conductivity, temperature and feeding) to affect the toxicity endpoints potentially leading to both false positive and negative results. Nevertheless, this can be overcome by careful experimental planning and the measurement of these parameters during exposures involving *Hydra*.

Conclusion

Part of the success of the use of *Hydra* in aquatic toxicology and in bioassays for toxicity testing is due to the wide array of lethal and non-lethal endpoints that can be used in both acute and chronic studies. For lethality, morphology and regeneration are appropriate endpoints. However the non-lethal endpoints which also include morphology and regeneration along with reproduction, feeding, various biochemical endpoints and attachment are particularly sensitive and have been found by several authors to be more sensitive than other bioassays undertaken with invertebrate, vertebrate and plant test organisms. *Hydra* are commonly used in acute toxicity tests with a 96 hour exposure time. However several studies have found increased sensitivity of the hydra bioassay using the standard toxicity endpoints (morphology and reproductive capacity) following extended chronic exposure periods of up to 21 days. Arkhipchuk *et al*., (2006b) reported 3-5 times increased sensitivity for chronic lethal effects, and up to 10-16 times for chronic sub-lethal effects over acute exposure effects, indicating exposure time should be carefully chosen. Clearly, the inclusion of numerous effects-based endpoints (morphology, regeneration, reproduction [hydranth number], biomarker expression, prey ingestion and attachment) can contribute to a better overall understanding of the chronic effects of contaminants, and *Hydra*, as a recognized animal model for aquatic studies, has a role to play in this respect. Appropriately designed, relatively simple and inexpensive laboratory toxicity tests using *Hydra* with a selection of acute and sub-lethal endpoints are generally adequate, with small application factors, for predicting the environmental risk of polluting chemicals to freshwater ecosystems.

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