Anemone fishes as models in ecotoxicology

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24.1 INTRODUCTION

The in-depth study of the effects of biological and chemical agents on the biology of organisms strongly relies on suitable model species, for which husbandry methods and various resources such as genomes, proteomes, and transcriptomes are available. These resources allow for a comprehensive examination of the compound's ecotoxicity (e.g., at multiple scales of biological organization from the effects on genes to populations, and at multiple life stages) (Segner and Baumann 2016).

In fish, the zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), fathead minnow (*Pimephales promelas*), and three-spined stickleback (*Gasterosteus aculeatus*) are the most common model species used to investigate the ecotoxicity of hazardous biological and chemical agents (Norrgren 2012). Although responses to toxic agents can be evolutionary conserved across fish species (Gunnarsson et al. 2008; Villeneuve et al. 2014), the fact that these model fishes are all freshwater or euryhaline (i.e., able to tolerate a wide range of salinity) species most often prevent the knowledge gained from them to be applied on marine species (Hsu et al. 2014; Kong et al. 2008). Indeed, such species not only live in ecosystems with distinct abiotic and biotic characteristics but also present different life-history strategies and metabolisms, requiring system-specific model species.

This lack of diversity in fish model species in ecotoxicology is particularly striking with respect to coral reef fishes, for which there is no such model species while being one of the most diverse groups of vertebrate species, on which more than 500 million people depend on for subsistence (Hoegh-Guldberg et al. 2019). Coral reef fishes play a key role in fuelling the exceptional productivity of coral reefs (Brandl et al. 2019), but very little is known regarding the pollutant bioaccumulation and biomagnification processes within the coral reef fish food web (Briand et al. 2018), from which various trophic levels are traditionally consumed by human populations. Moreover, most coral reef fishes have a bipartite life cycle with larvae developing in the ocean before returning to settlement habitats such as seagrass beds and mangroves and eventually recruiting into coral reefs (Sale 2004), which makes them potential vector of pollutants between all these marine ecosystems. Altogether, these examples demonstrate the urgent need to draw research avenues on coral reef fish ecotoxicology, not only to investigate the effects of pollutants on these populations but also to better understand their impacts on ecosystem functioning and human health.

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Among coral reef fishes, anemonefishes or Amphiprioninae, which belong to the Pomacentridae family and live with anemones, a well-known example of mutualism (Feeney et al. 2019), have been extensively studied in the past decades as evidenced in the previous chapters of this book. Briefly, these species have been used as models in myriad ecology and evolution studies investigating, for example, the adaptation of the fish population to climate change (e.g., Jarrold et al. 2017; Lehmann et al. 2019; McLeod et al. 2013), the dispersal and connectivity patterns of fish population in coral reefs (e.g., Planes et al. 2009), the larval recruitment and habitat selection processes in coastal marine fishes (e.g., Scott and Dixson 2016), and the social organization and sex changes in fish hierarchical groups (e.g., Buston 2003; Wong et al. 2016).

In this chapter, we highlight how anemonefishes can also serve as relevant fish models to examine the ecotoxicology of hazardous biological and chemical agents in the marine environment. We then review the ecotoxicological effects of various hazardous compounds on anemonefishes and present how future research using them as models will promote our knowledge of fish ecotoxicology.

24.2 THE EMERGENCE OF ANEMONEFISHES AS MODEL ORGANISMS IN ECOTOXICOLOGY

The anemonefish *Amphiprion ocellaris* (Cuvier 1830), or false clownfish, is the most widely used coral reef fish in experimental studies because it is one of the rare coral reef fish species that can be entirely and easily reared in aquaria, and which is readily largely available in the aquaculture market (Pouil et al. 2020). *A. ocellaris* is not the only anemonefish in this case, since the life cycle of, at least, 25 anemonefish species has so far been controlled in aquaria (Pouil et al. 2020). This extensive expertise on the biology and life cycle of multiple anemonefish species makes them relevant coral reef fish models for eco-evo-devo sciences (Roux et al. 2020). With a relatively short embryonic and larval development, anemonefishes are relevant model species to assess the impact of contaminants on the postembryonic development at molecular and endocrinological levels (Roux et al. 2020). Rearing techniques for anemonefishes were relatively well documented in the literature (e.g., Calado et al. 2017; Divya et al. 2011; Kumar et al. 2012; Madhu et al. 2006). Recently, a husbandry detailed protocol has been published providing a detailed description of the anemonefish husbandry system as well as live prey culturing protocols (Roux et al. 2021). The authors built the anemonefish husbandry system from the ones developed for zebrafish with some adaptations. Briefly, the recirculating system of artificial seawater was composed of 30 63-L rearing tanks placed on three shelves while the treatment of the outlet water was done in an 800-L technical sump tank below the rearing tanks. The sump technical tank was equipped with filtering foams, a phosphate reactor filled with resin, a skimmer, a UV sterilizer, 100 kg of live rocks for biological filtration, and a lift pump allowing the return of filtered seawater to the rearing tanks. One of the advantages of such a system is to be flexible with the possibility to change the number of the tanks and/or their volumes. This first step makes it possible to envisage a standardized breeding protocol necessary for using anemonefish as model species. As rearing volume can be an issue for several days of ecotoxicology experiments, especially when the compound is either rare, toxic, and/or expensive, Roux et al. (2021) developed a protocol for larval rearing protocol in small glass beakers (less than 1 L), useful for toxicity assessment where chemicals can be rare, expensive, and/or very toxic. While mass breeding reproduction protocols exist in private companies, they are not disclosed outside them. Thus, Roux et al.'s open-access technical paper makes breeding techniques of *A. ocellaris* available to the whole scientific community.

The commercial availability of numerous, genetically and phenotypically diverse anemonefish strains, especially for *A. ocellari*s, is an asset for ecotoxicological studies where responses from laboratory tests are inferred for those of the more diverse wild populations. Furthermore, the fact that most anemonefish species have a comparable life history allows for the use of similar housing materials and husbandry protocols, which could facilitate experiments involving multiple species. Other coral reef fish species, such as benthic spawners from the Apogonidae, Gobiidae, and Pseudochromidae families (Calado et al. 2017), can also be successfully bred in captivity while having shorter life cycles than anemonefish. However, the availability of these species is much more limited (Pouil et al. 2020) and the limited knowledge and molecular tools available for these species are limiting factors for using them as biological models. The relative proximity of anemonefish with established fish models such as zebrafish and medaka, in which extensive chemical screenings have already been performed, is another asset for the use of anemonefishes in ecotoxicology in comparison to other coral reef fish species (Roux et al. 2020). For example, an inhibitor of tyrosine kinase receptors known to decrease iridophore number in zebrafish (Fadeev et al. 2016) has been successfully used to show that white bars in *A. ocellaris* are formed by iridophores (Salis et al. 2019).

24.3 CURRENT KNOWLEDGE OF ANEMONEFISHES' ECOTOXICOLOGY

24.3.1 Data Survey

The biological and ecological characteristics of anemonefishes make them promising fish model organisms for ecotoxicological research (see the previous section for details), which could promote our ability to evaluate the effects of pollutants on coral reefs as well as marine ecosystems more generally. However, ecotoxicological research on anemonefishes is still limited. A systematic review has been performed using Web of Science (WOS) covering > 12,000 scholarly journals and providing a satisfactory representation of international mainstream scientific research (Moed 2006). Only ten peer-review articles involving bioaccumulation or toxicity assays through exposure to chemicals in anemonefish were found (Figure 24.1).

Our finding highlights the current need to get a more comprehensive dataset regarding the sensitivity of anemonefish to different classes of chemicals. Such data are required to consider them as proper complementary fish models. In the following sections, we summarized the current knowledge available about toxicity for some classes of chemicals on anemonefish. For the sake of clarity, the information is presented in chronological order. The overall observed effects are summarized in Figure 24.2.

24.3.2 Nitrogen Compounds

Several studies investigated the toxicity of ammonia (NH_3), nitrites (NO_2^-), and nitrates (NO_3^-) in anemonefish (Frakes and Hoff 1982; Medeiros et al. 2016; Rodrigues et al. 2014). The purpose of the aforementioned studies is mainly for ornamental fish production because nitrogen compounds are metabolic by-products excreted by fish and elevated concentrations of these products can have deleterious effects on fish production (Calado et al. 2017). Inventories of the major forms of nitrogen in the ocean revealed that the mean concentrations in the euphotic zone were 0.1, 0.3, and 7 mmol m^{-3} for NO_2^-, ammonium (NH_4^+) and NO_3^-, respectively

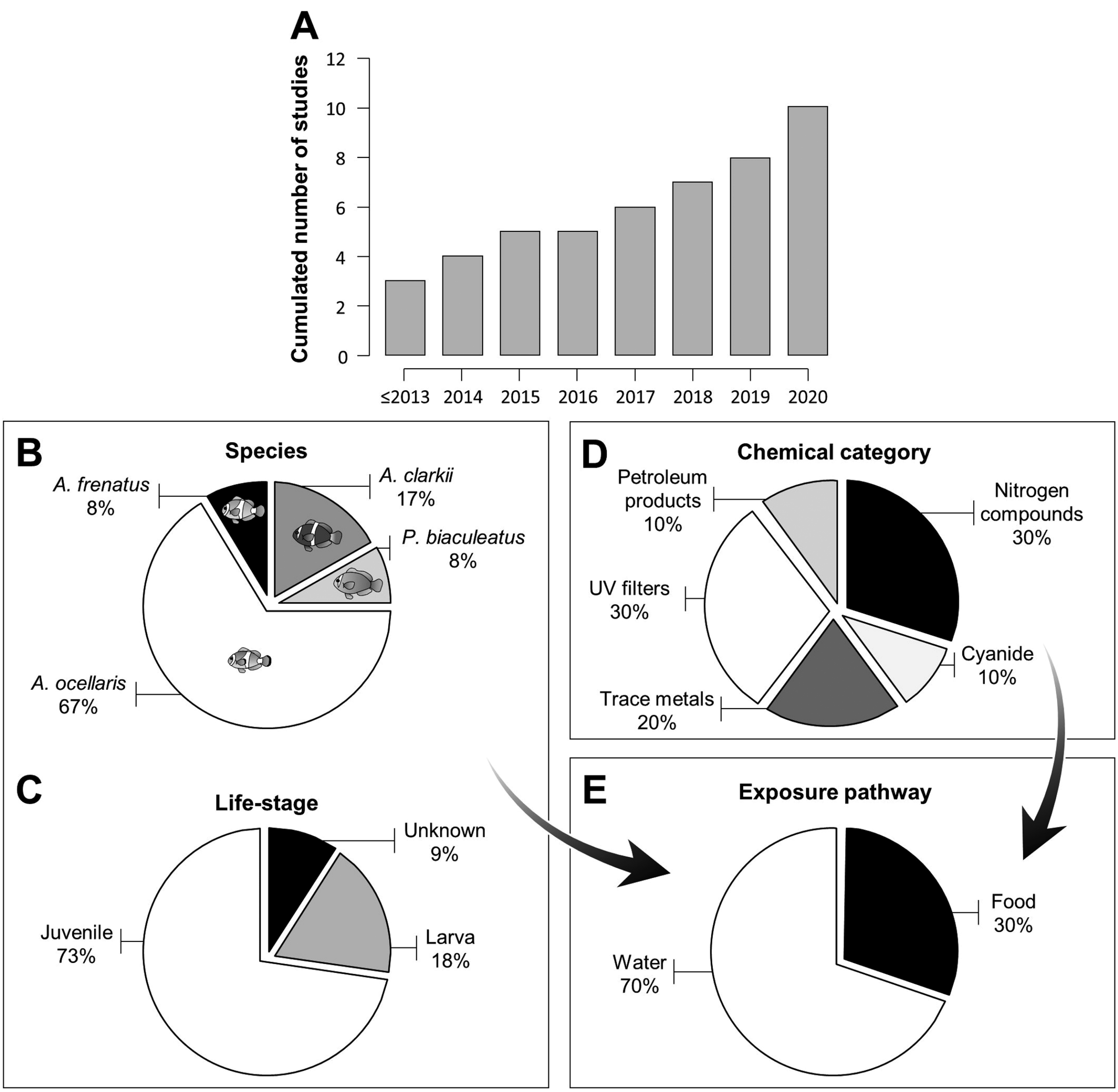

FIGURE 24.1 General trends in ecotoxicology research using anemonefish. (A) Cumulated number of studies published that experimentally examined the effects of chemicals on anemonefish fish species. Respective proportion of (B) anemonefish species, (C) life stages, (D) chemical category, and (E) exposure pathways.

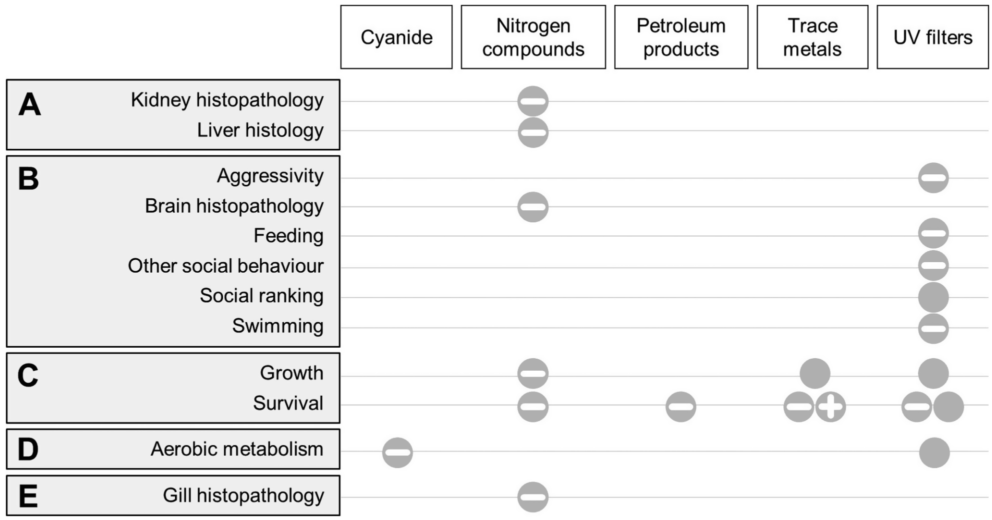

FIGURE 24.2 Ecotoxicological effects of different chemicals on anemonefish biological functions and systems: (A) excretory functions, (B) behavioral and sensory functions, (C) fitness indicators, (D) metabolism indicators, and (E) respiration functions. Solid circles indicate no effect while the symbols "–" and "+" indicate negative and positive effects, respectively.

(Gruber 2008). These compounds can also be found in the coastal tropical environment due to human activities (de Barros Marangoni et al. 2020). Frakes and Hoff (1982) were among the first to investigate the effects of nitrogenous compounds in anemonefish. They observed reduced growth in juvenile *A. ocellaris* exposed to 100 mg L^{-1} of NO_3^- while survival of larvae was three times lower when they reared at this NO_3^- concentration. More recently, Rodrigues et al. (2014) evaluated the median lethal concentration values (LC_{50}) and the histopathological effects of NH_3 on juvenile maroon clownfish *Premnas biaculeatus* exposed for 96 h to six concentrations (0.39–1.93 mg L^{-1} of NH_3-N). The 24 and 96 h LC_{50} values of NH_3-N were 1.68 and 0.89 mg L^{-1} (Table 24.1) respectively while fish exposed to different ammonia concentrations displayed histopathological alterations in the gills, kidney, liver, and brain. Such results have been confirmed in *A. ocellaris* juveniles by Medeiros et al. (2016) who exposed fish to six concentrations of NH_3 (0.23–1.63 mg L^{-1} of NH_3-N) and eight concentrations of NO_{2-} (26.3–202.2 mg L^{-1} of NO_2^--N). Authors found 24 h and 96 h LC_{50} of 1.06 and 0.75 mg L^{-1}, respectively, for NH_3-N, and 188.3 and 108.8 mg L^{-1}, respectively, for NO_2^--N. These results indicate that maroon clownfish are relatively sensitive to NH_3 and NO_2^- compared to other marine finfish (Medeiros et al. 2016; Rodrigues et al. 2014). Histological analysis showed that both nitrogenous compounds induced tissue lesions (Figure 24.2).

24.3.3 Petroleum Products

Exploitation of commercial quantities of oil and gas can impact coral reef ecosystems with the discovery of fields in shallow tropical seas (Neff et al. 2000). When crude or refined oil is accidentally spilt, these compounds are subject to several biological, chemical, and physical processes (i.e., weathering) that change the properties of oils. Neff et al. (2000) investigated the effects of weathering on the toxicity of three crude oils and a diesel fuel on marine organisms. Several tropical/subtropical and temperature model organisms including invertebrates (echinoderms and shrimps) and fish (silverside *Menidia beryllina* and anemonefish *A. clarkii*) were used. The water-accommodated fraction (WAF) of the four oils and their weathered fractions were prepared at a concentration of 28 g L^{-1} of oil in seawater. All the 96-h static-acute toxicity tests were performed with serial dilutions of 0, 8, 16, 64, or 75, and 100% of the WAF. The 96 h LC_{50} were ranging from 35% to > 100% of the WAF depending on the three crude oils and their weathered fractions (Table 24.1). Results were comparable between silverside and anemonefish fish showing similar sensitivity to the three oils. The 96 h LC_{50} values for diesel were ranging from 54 to 79% depending on the weathered fractions in silverside while 96 h LC_{50} was always > 100% in anemonefish suggesting a lesser sensitivity.

24.3.4 Trace Metals

Toxicity of trace metals has been poorly investigated in anemonefish. Interestingly, the only information on bioaccumulation and toxicity of metals is from two studies focused on essential elements. Furuta et al. (2005) examined the effect of Cu addition to rearing water on the survival *A. ocellaris*. The survival rates at 80 and 160 µg Cu L^{-1} were 65 and 80%, respectively while the survival rate was only 30% in the control conditions control in newly hatched larvae reared for 14 d. The positive effect of copper addition on the survival rate was confirmed with fish from seven different spawning events. The reason why the Cu supply in the rearing water improves the survival of larval anemonefish remains unclear (Furuta et al. 2005). Jacob et al. (2017)

TABLE 24.1
Median Lethal Doses of Different Chemicals in Anemonefish Species

Chemical category	Chemical	Species	LC_{50}	Exposure (h)	Reference
Nitrogen compounds	NH_3-N	*A. ocellaris*	1.68	24	Rodrigues et al. (2014)
		A. ocellaris	0.89	96	Rodrigues et al. (2014)
		A. ocellaris	1.06	24	Medeiros et al. (2016)
		A. ocellaris	0.83	48	Medeiros et al. (2016)
		A. ocellaris	0.75	72	Medeiros et al. (2016)
		A. ocellaris	0.75	96	Medeiros et al. (2016)
	NO_{2^-}-N	*A. ocellaris*	188.3	24	Medeiros et al. (2016)
		A. ocellaris	151	48	Medeiros et al. (2016)
		A. ocellaris	124.1	72	Medeiros et al. (2016)
		A. ocellaris	108.8	96	Medeiros et al. (2016)
Petroleum products	Wonnich crude	*A. clarkii*	35[a]	96	Neff et al. (2000)
	Wonnich 150°C+	*A. clarkii*	69[a]	96	Neff et al. (2000)
	Wonnich 200°C+	*A. clarkii*	> 100[a]	96	Neff et al. (2000)
	Campbell condensate	*A. clarkii*	39[a]	96	Neff et al. (2000)
	Campbell 150°C+	*A. clarkii*	81[a]	96	Neff et al. (2000)
	Campbell 200°C+	*A. clarkii*	> 100[a]	96	Neff et al. (2000)
	Agincourt crude	*A. clarkii*	> 100[a]	96	Neff et al. (2000)
	Agincourt 150°C+	*A. clarkii*	> 100[a]	96	Neff et al. (2000)
	Agincourt 200°C+	*A. clarkii*	> 100[a]	96	Neff et al. (2000)
	Agincourt 250°C+	*A. clarkii*	> 100[a]	96	Neff et al. (2000)
	Australian diesel	*A. clarkii*	> 100[a]	96	Neff et al. (2000)
	Diesel 200°C+	*A. clarkii*	> 100[a]	96	Neff et al. (2000)
	Diesel 250°C+	*A. clarkii*	> 100[a]	96	Neff et al. (2000)
Other	Cyanide	*A. ocellaris*[b]	28.45	96	Madeira et al. (2020)
		A. ocellaris[c]	50	96	Madeira et al. (2020)

Note: LC_{50}, expressed as mg L^{-1} excepted otherwise mentioned.
[a] LC_{50} expressed as per cent water-accommodated fraction (WAF).
[b] Small: 25.00 ± 0.03 mm, 0.30 ± 0.09 g.
[c] Medium: 38.00 ± 0.02 mm, 1.12 ± 0.21 g.

assessed the trophic transfer of three essential elements (Co, Mn, and Zn) in juvenile *A. ocellaris* exposed to two pH values simulating present-day conditions vs. acidified conditions (Δ pH = 0.5). Assimilation efficiencies (AEs) of three essential elements as well as other kinetic parameters, determined over a 20-d period following a single-feeding with radiolabelled pellets, were not affected by this experimental pH change although AEs were element dependent ($AE_{Zn} > AE_{Mn} > AE_{Co}$).

24.3.5 UV Filters

UV filters are recent anthropogenic pollutants encountered in the marine environment. Toxicity studies involving fish species have been mainly focused on UV filters used in sunscreens because of their potential ecological risk and due to their occurrence and persistence in aquatic ecosystems (Tovar-Sanchez et al. 2020). Anemonefish inhabiting shallow waters frequented by swimmers are relevant models for such assessments. We found three recent peer-reviewed studies assessing the effects of UV filters on anemonefish. Barone et al. (2019) investigated acute toxicity of TiO_2-based vs. oxybenzone-based sunscreens on *A. ocellaris*. Mortality, swimming, and feeding behavior were compared in juvenile fish exposed for 97 h to concentrations ranging from 0 to 100 mg L^{-1} of sunscreens mixed in seawater. They found that at the highest exposure concentration, oxybenzone-containing sunscreen had a negative impact on mortality, leading to 25% death over the 97-h exposure period. This concentration also impaired behavior with exposed fish showing abnormal swimming during the test while all of them stop to eat over the first 49 h. Mortality rate induced by TiO_2-based sunscreen exposure was much lower (< 7%) while normal swimming and feeding have been recovered at the end of the 97-h exposure period. Effects of another UV filter, the octocrylene (OC), on the physiology of *A. ocellaris* exposed through their food were assessed by Lucas et al. (2020). Juvenile fish were exposed for two months by feeding them using artificial dry food spikes with OC at a concentration of 10 μg g^{-1} dry weight and aerobic metabolic scope (AS) has been assessed as an indicator of the physiological state. The authors concluded that dietary exposure to OC at the tested concentration did not influence the aerobic metabolism of

A. ocellaris. Effects of chronic exposure to UV filter benzophenone-3 (BP-3) on social behaviors were investigated in juvenile *A. ocellaris* dietarily exposed to BP-3 (from 0 and 1,000 ng g^{-1}) over 90 d (Chen et al. 2018). Survival and growth were not affected by the BP-3 exposure except for a higher weight for the dominant fish while social rankings and intra-colonial social behaviors were not significantly affected by the BP-3 exposure (Figure 24.2).

24.3.6 Cyanide

Illegal cyanide fishing, mostly for marine ornamental trade, is one of the major anthropogenic threats to Indo-Pacific coral reefs, targeting a multitude of coral fish species (Calado et al. 2017; Madeira et al. 2020). In a recent study, Madeira et al. (2020) assessed the toxicity effects of cyanide in eight species of Pomacentridae including three *Amphiprion* species (*A. clarkii*, *A. frenatus*, and *A. ocellaris*). Juveniles of each species were exposed for 60 s to 50 mg L^{-1} of cyanide at 26°C. Only two species survived after 96 h with 50% survival for *A. ocellaris* and 20% for *Chromis cyanea*. In a second experiment, juveniles of *A. ocellaris* were exposed to different concentrations (0, 6.25, 12.5, 25, 50, and 100 mg L^{-1}) of cyanide for 96-h LC_{50} determination. 96-h LC_{50} were ranging from 20 to 53 mg L^{-1} depending on the fish size (Table 24.1).

24.4 HOW ANEMONEFISH MAY FUEL ADVANCEMENTS IN ECOTOXICOLOGY

24.4.1 Full Life-Cycle Fish Tests

Most of the ecotoxicological fish tests are primarily focused on acute or short-term exposure to chemicals providing toxicity values useful for regulatory decision making. Nevertheless, the long-term impacts of chemicals are still largely unknown while such information is important especially for persistent organic and inorganic pollutants. Some standardized chronic full life-cycle and multi-generational exposures have been implemented for fish such as the Fish Sexual Development Test (OECD Test No. 234) and the Extended One-Generation Reproduction Test (OECD Test No. 240) based on medaka and fathead minnow (OECD 2015). The implementation of such tests requires being able to carry out the complete life cycle of the model species in captivity, which remains complex today for most of the coral fish species. Anemonefish are among the only reef species whose rearing and reproduction can be relatively easily undertaken in captivity (Pouil et al. 2020). In contrast to many coral reef fishes that spawn in the open ocean, anemonefishes are benthic spawners and produce relatively big eggs (between 1.5 to 3.5 mm in length and 0.8 to 1.85 mm depending on species; Calado et al. 2017) glued to a support making them quite easy to handle. Most of the anemonefish embryos hatch, at 28°C, seven days post-fertilization (Calado et al. 2017). The spawning interval is short with reproduction events observed every two weeks in *A. ocellaris* in laboratory (Roux et al. 2021). Larvae can be easily fed on conventional zooplankton and show very fast growth rates (larval phase $\leqslant$ 15 d; Calado et al. 2017). Under optimal rearing conditions, juveniles up to 2.5 cm in length can be produced in five months in *A. percula* (Johnston et al. 2003). This reasonable rearing time is compatible to perform chronic exposure tests from eggs, or maternal transfer as suggested by Lucas et al. (2020), to juveniles. Nevertheless, sexual maturity is achieved late, often $>$ 1 year and spawning usually starts one to three months after the pair is established but sometimes it takes up to one year (Calado et al. 2017). In addition, mass rearing of anemonefish in laboratory facilities can be used to develop specific and reproducible strains well characterized both genetically and phenotypically, as it was done for zebrafish (Meyer et al. 2013) making a strong advantage to investigating bioaccumulation, organotropism, and the fate of contaminants. Nevertheless, managing genetic diversity in laboratory strains should be taken into consideration from the perspective of extrapolating the results of ecotoxicological laboratory tests to wild populations as we explained in section 24.2 of this chapter.

24.4.2 Single vs. Multi-Stressor Experiments in Laboratory

Past experimental studies demonstrated that climate change can affect many aspects of the biology and ecology of anemonefishes (see Chapter 23). For example, ocean acidification may lead to disruption of multiple sensory abilities in several species of anemonefishes (Dixson et al. 2010; Munday et al. 2009a; Simpson et al., 2011). While the veracity of these results is currently being debated (Clark et al. 2020), other studies also demonstrated that ocean acidification can affect anemonefishes' early life history (Munday et al. 2009b; Munday et al. 2011) and reproduction success (Kannan et al. 2020). Thus, anemonefishes appear to be particularly relevant models to investigate how the bioaccumulation and toxicity of a given chemical are affected by projected environmental conditions, and, conversely, whether the sensitivity of anemonefishes to climate change can be increased when co-exposed to a pollutant. However, very few studies have examined such multi-stressor scenarios combining ecotoxicology and environmental change in anemonefishes. As described in Section 24.3.4, Jacob et al. (2017) assessed the trophic transfer of three metals in juvenile *A. ocellaris* exposed to projected future pCO_2 levels over the next two centuries (pH 7.5) as well as present-day conditions (pH 8.0) and found no effect of acidification neither on the assimilation efficiency of the metals in anemonefish nor on the stomach pH. Interestingly, Madeira et al. (2020) found that cyanide exposure at increased temperatures (i.e., +3 and +6°C above present-day scenarios) led to higher mortality rates in juveniles *A. ocellaris*, when compared to the same exposure at control temperature (26°C) highlighting the potential synergistic effects of ocean warming and toxicity in anemonefish.

24.4.3 Laboratory and *In Situ* Models

Some aspects of clownfish ecology not only make them relevant as laboratory models as we have shown earlier but also open new horizons for their potential use in the field. In situ experiments, over the past decade, have received increased attention and acceptance as ways to complement traditional laboratory experiments by improving the connection between pollutant exposure (i.e., external bioavailable concentration) and the intrinsic sensitivity of the organisms (i.e., bioaccumulation, effects) under realistic conditions. Nevertheless, such an approach remains challenging to implement in fish. One of the most important considerations in performing in situ ecotoxicology studies is to examine the technique-related artefacts which can significantly influence test outcomes and the difficulties in establishing adequate controls to accurately interpret organism responses (Ferrari et al. 2013). In the saddleback clownfish *A. polymnus*, Jones et al. (2005), using parentage by DNA genotyping, found that one-third of settled juveniles had returned to a two-hectare natal area, with many settling < 100 m from their birth site, although another study found that self-recruitment can be highly variable (Nanninga et al. 2015). This represents the smallest scale of dispersal known for any marine fish species with a pelagic larval phase (Jones et al. 2005). This means that individuals can be tagged and tracked over time without altering their habitat and their life as well. Non-lethal recordings, samplings, and biometry can be performed over time on the same individuals depending on the objective of the study. Using anemonefish, in situ ecotoxicological studies could be performed with laboratory-reared organisms with a known life history, as well as indigenous organisms. In addition, Thorrold et al. (2006) described a new technique for transgenerational marking of embryonic otoliths in *A. melanopus*. The approach is based on the maternal transfer of ^{137}Ba from spawning females, exposed to the isotope, to egg material that is ultimately incorporated into the otoliths of embryos. The authors found that female *A. melanopus* continued to produce marked larvae over multiple clutches and for at least 90 days after a single injection. This technique can be extended by using different combinations of stable Ba isotopes, allowing marking fish from multiple populations and thus performing inter-population surveys over extended periods.

24.4.4 Availability of Genetic and Transcriptomic Data

Because of the increasing use of anemonefish species as fish models in a wide range of biological studies their genetic, physiological, and ecological backgrounds are relatively well characterized and multiple useful tools are available. These include the genomes of around a dozen anemonefish species (Marcionetti et al. 2019) as well as life-stage and tissue-specific transcriptomes in *A. ocellaris* and *A. percula* (Maytin et al. 2018; Salis et al. 2019, 2021b). A detailed description of the larval neuro-anatomy of *A. ocellaris* is also available (Jacob et al. 2016) as well as high-resolution time-lapse videos and descriptions of embryonic development in the same species (Salis et al. 2021a). The availability of such tools makes anemonefish relevant for assessing responses to contaminants at different scales: from molecular to individual endpoints and may help to cross the bridge from the individual to population levels.

24.5 REMAINING CHALLENGES AND FUTURE PERSPECTIVES

24.5.1 The Need for Standardized Husbandry

Although some recent advances have been made (see Roux et al. 2021), to date, the development of standardized husbandry protocols for anemonefish failed to keep pace with the increasing use of these species in laboratories for ecotoxicology studies. Some studies are still performed using purchased individuals from commercial suppliers without their genetic origin and their life-history traits being known (Chen et al. 2018; Jacob et al. 2017). A variety of husbandry methods are currently used based on artificial (e.g., Roux et al. 2021) or filtered natural seawater (e.g., Kumar et al. 2012) mostly in recirculating systems but also in flow-through systems. Such differences contribute to extensive variations in fish density, water chemistry, temperature and light conditions while feeding protocols are highly variable among the studies involving anemonefish. In addition, even in recent studies on husbandry protocols for anemonefish, growth, and survival performances, especially during the early stages of life are not always recorded (e.g., Roux et al. 2021) although this is an important aspect in ecotoxicology experiments requiring production of a sufficient number of healthy individuals on a regular basis. Since several species of clownfish have been produced for many years in private aquaculture farms for the ornamental fish market, it would be interesting to promote synergistic activities between academic research institutes and the private sector to optimize anemonefish husbandry protocols (Pouil et al. 2020).

24.5.2 From Individual to Population-Level Responses

A major challenge in ecotoxicology is to link responses highlighted at the individual level in the laboratory to population- and ecosystem-level responses in the field (Vighi and Villa 2013). Responses can be variable between natural populations of a given species. There is substantial evidence that genetic variation, at both the level of the individual and population, has a significant effect on behavior, fitness, and response to chemicals in fish. Coe et al. (2009) showed that the wild zebrafish were significantly more variable than the laboratory strains for several measures of genetic variability, including allelic richness and expected heterozygosity. While, to our knowledge, the genetic diversity of captive-bred anemonefish strains was never assessed, Madduppa et al. (2018) demonstrated that ornamental fishery contributes to a reduction in population size and genetic diversity in *A. ocellaris* populations in the Spermonde Archipelago, one of the main collection sites for ornamental fish in Indonesia. The potential lack of genetic variation in captive-bred anemonefish should be given due consideration for any study which attempts to extrapolate the results of ecotoxicological laboratory tests to wild populations. Therefore, the degree to which captive-bred strains are representative of wild anemonefish populations and congeneric species should be validated.

24.6 CONCLUSION

Although coral reefs are among the most sensitive aquatic ecosystems to anthropogenic threats, there is currently no clearly established model species of coral fish species used in ecotoxicology. Current standard fish-based tests in ecotoxicology are mostly limited to freshwater model species limiting knowledge gained to marine species. Due to their relatively easy husbandry, anemonefishes have been widely used in research examining the biology and ecology of coral reefs and coral reef fishes, leading to the development of various molecular tools and husbandry methods for these species. While our knowledge of the ecotoxicity of biotic and abiotic agents is still limited for this fish family, anemonefishes present many assets that make them promising species for examining fish ecotoxicology in future marine environments and coral reef ecosystems. However, key challenges remain, such as the standardization of husbandry protocols and the difficulty to extrapolate individual responses at population and community levels, which is true for any other fish model used in experimental work. We highlighted that anemonefish, especially *A. ocellaris*, have the potential to be a model in ecotoxicology. While they have barely been used in the past, they are now more and more considered for investigating the effects of some substances (e.g., sunscreens) and their use could be definitely extended as highlighted in this chapter. It is now clear that it is time to use an adequate fish model in order to properly assess the risk coral reefs are facing and will face with the increased number of contaminants or stressors in this high-diversity ecosystem. Therefore, the information provided here constitutes the first foundation to optimize ecotoxicology studies based on coral fish species using the promising anemonefishes as models.

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