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Review

Increasing the reliability and reproducibility of aquatic ecotoxicology: Learn lessons from aquaculture research

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ABSTRACT

Regulatory ecotoxicology highly relies on aquatic toxicity studies carried out under controlled conditions. Researchers recently expressed increasing concern about their possible lack of repeatability/reproducibility in many cases. Poor experimental designs, inappropriate statistics and lack of accurate reporting are often pointed out. However, I believe that there is also insufficient attention paid to the various experimental conditions under which fish studies are conducted. These conditions encompass numerous factors (temperature, photoperiod, food, stressors…) which modulate fish response to chemicals. Their effects are poorly studied in ecotoxicology but have been investigated for decades in aquaculture research. It is therefore proposed herein to consider experimental ecotoxicology from an aquaculture perspective. An overview of modulating factors and plausible associated experimental flaws is presented, with emphasis to fish health, growth and reproduction which are the most common regulatory endpoints. Photoperiod and temperature mainly determine growth/reproductive status for which fish also have species and stage-specific nutritional requirements. Stressors, sex ratio, density, water quality and factorial interactions may induce experimental bias. Modulating factors can strongly limit findings applicability and might explain the lack of reproducibility in some cases. Aquaculture knowledge/experience can already allow avoiding some experimental flaws (e.g., stress) while further research is warranted for some other aspects (e.g., nutrition). Detailed reporting of fish husbandry and experimental conditions is of utmost importance for study quality assessment.

1. Introduction

Environmental aquatic hazard identification and risk assessment heavily rely on experimental ecotoxicology whose findings may thus have worldwide implications (regulations, conventions…) with sometimes important economic consequences (Harris et al., 2014). This includes academic research whose findings, although not necessarily intended to be used for regulatory purposes in the first place, might be used by regulators years later because the findings would become of regulatory interest (Moermond et al., 2016). With these possible impacts in mind, scientists should ensure that their experimental findings have a sufficient degree of certainty and conclusions have a sufficient degree of relevance before moving forward and publishing them, as advocated by Harris et al. (2014). Several researchers have thus expressed worries about the reliability and reproducibility of a significant amount of studies published in peer-review journals (Agerstrand et al., 2011; Harris et al., 2014; Hanson et al., 2016). In order to reconnect with high quality research, some of them posited principles on methods and strategies for more sound ecotoxicology (Harris et al., 2014; Hanson et al., 2016). Additionally, OECD (2012) issued a guidance on how to perform good quality regulatory studies, yet applicable to any ecotoxicology experiment. This guidance gathered and implemented the scientific and technical input of ecotoxicology experts from member states of the Organization for Economic Co-operation and Development (OECD). More recently, US EPA (2016) updated its OPPTS 850 series of test guidelines on ecological effects by including much more scientific and technical advice. An expert group also proposed a new set of criteria to assess the value of both regulatory testing and academic studies in ecotoxicology, implying that good quality reporting should include all these criteria (Moermond et al., 2016). This international work namely implemented the input of 75 scientists from 35 different countries. The authors strongly advocated for improving reporting in peer-reviewed scientific journals. All these recent contributions aimed at improving the quality of ecotoxicological research. However, to my opinion, none of them sufficiently addressed the case of modulating factors. Modulating factors are each single controlled parameter of an experimental setup which, although applied the same way to all experimental units, can modulate the fish biological response to chemicals under laboratory conditions. By "fish biological response", it is understood any measured variable, from gene expression to apical endpoints

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like health, growth or reproduction. So far, hazard and risk assessment mainly rely on apical endpoints, considered as more robust endpoints than gene expression, enzyme activity or hormonal synthesis which are more sensitive to modulating factors and biological variability. However, -omics and adverse outcome pathways, which assume that adverse effects on apical endpoints can be precociously predicted, are considered as promising tools for risk assessment (van Aggelen et al., 2010; Groh et al., 2015). In addition, upcoming regulations on endocrine disruption will address modes of action, bringing more and more importance for mechanistic endpoints like enzyme activities and hormone synthesis. Regulatory ecotoxicology would therefore rely on physiological endpoints in the future. It is of utmost importance to recall that these endpoints are very sensitive to many modulating factors furthermore probably not all identified yet. Modulating factors encompass environment (temperature, photoperiod, light spectrum/intensity, handling, water quality), nutrition (feed type, feeding rate, feed proximate composition, feed fatty acid profile), populations (sex-ratio, density) and individuals (genetics, life-history). In fieldwork, they are often called "confounding factors" (i.e. confounding with the chemical (s) effects), which would obviously be an improper naming for laboratory experiments where those factors are supposed to be controlled and fixed, i.e. applied the same way to every experimental units. In an ideal experiment on toxicity of chemical A on species B, modulating factors should be set within relevant ranges that (i) do not affect fish physical integrity and potential performances more than the chemical could (may generate false negative) and (ii) do not generate a response to the chemical that normally would not happen (may generate false positive). However, many modulating factors are insufficiently considered in the framework of experimental aquatic ecotoxicology. A good illustration for this is the insufficient amount of accurate guidance on how to deal with many of these factors in most fish OECD and US EPA testing guidelines and guidance, yet considered as the gold standard in regulatory ecotoxicology. Regarding the published literature, there is little to no specific reporting requirement on these aspects in peer-reviewed journals in the ecotoxicology area (Moermond et al., 2016) and the level of reported information is therefore highly variable from one article to another (personal observation).

Modulating factors have been deeply investigated in aquaculture research for decades because of the obvious socio-economic implications. This encompassed research on biology (stress, growth, reproduction, health, welfare…) and husbandry techniques which improved fish performances and minimized adverse effects of modulating factors. It encompassed a wide variety of findings which - it is my firm belief - could be used by ecotoxicologists to improve their experimental designs, results interpretation and reporting.

In the first part of this overview, I provide sourced evidence that these factors probably affect most ecotoxicological findings since they highly influence fish performance and physiological status. Advice on possible methods to limit adverse or biasing effects is given where relevant. In a second part, major teachings of aquaculture research are drawn for results interpretation and reporting. The needs for future research on ecotoxicological species is also addressed, with emphasis to zebrafish (*Danio rerio*). It is beyond the scope of this overview to explain by which physiological mechanisms this or that specific modulating factor affects the fish biological response. Recent mechanistic reviews are given to the reader where possible. However, given the very multiplicity of modulating factors, fine mechanistic links of transmission between factor and effects are often poorly understood in the framework of aquaculture research (Migaud et al., 2013). This should not prevent us from taking into account any factor identified as "modulating" when designing, executing and reporting a study. Although modulating factors can affect virtually any biological function, this paper will focus on fish health, growth and reproduction, which include the most common endpoints encountered in regulatory ecotoxicology. The following databases were used for the literature search: Sciencedirect, Pubmed, Wiley-online and Springer as well as Google search engine. I used a number of keywords like “fish”, “temperature”, “photoperiod”, “light”, “growth”, “growth rate”, “stress”, “stressor”, “handling”, “reproduction”, “vitellogenin”, “egg”, “feeding”, “food”, “nutrition”, “fatty acid”, “chemical”, “toxicant”, “aquatic toxicity”, “ecotoxicology” (list not exhaustive). These keywords were crossed in different ways. Because this method did not yield many articles dealing with the cross-effects of laboratory-relevant modulating factors with toxicants, I completed this search by using combined keywords like “fish and stressor”, “fish and handling” or “fish and feeding” followed by a screening of all article titles in ecotoxicology journals like Chemosphere, Science of the Total Environment, Aquatic Toxicology and Environmental Ecotoxicology and Safety. This resulted in the screening of thousands of article titles and hundreds of abstracts. An article was considered relevant when it dealt with one or several laboratory-relevant modulating factors whose tested levels were within the range of fish husbandry and experimental practices that can reasonably be assumed to be encountered in ecotoxicology laboratories.

2. Modulating factors: what they do and what we should do

2.1. Photoperiod and temperature

Photoperiod and temperature are the most influential environmental factors on fish growth (Gardeur et al., 2007; Volkoff et al., 2010). Fish exhibit a wide range of species-specific thermal optima which have to be determined experimentally for each species. In general, within each species, growth optimal temperature is higher for juveniles and tends to decrease alongside with growth (Volkoff et al., 2010). Difference of temperature as low as 2–3 °C can lead to significant growth rate differences within a few weeks (e.g., Degani et al., 1989; Person-Le Ruyet et al., 2004; Kling et al., 2007). In addition, water temperature range allowing normal embryo and larval development and growth are often narrow and species-specific. Réalis-Doyelle et al. (2016) investigated the effects of egg incubation temperature on hatching and survival of brown trout (*Salmo trutta*) until first feeding. They observed the highest hatching, survival and lowest deformity rates at 6 and 8 °C. Adverse effects (lower hatching, lower survival or higher deformity rates) were observed both below and above these temperatures (4 °C and 10 °C respectively). This study illustrates the sometimes very narrow range of temperature setting allowed for a study to be of significant relevance.

Regarding photoperiod, growth generally increases with increasing daylength, due to both an increase of feed intake and a better efficiency of conversion into body mass (Boeuf and Le Bail, 1999; Boeuf and Falcon, 2001; Volkoff et al., 2010). Thus, differences of photoperiod (8 L:16D against 16 L:8D) resulted in high differences of growth rate in Eurasian perch (*Perca fluviatilis*), (Gardeur et al., 2007). The reader may refer to regulatory guidelines (e.g. OECD TG 203, 1992; OECD TG 210, 2013) and general guidance documents like OECD (2012) which give recommendations on adequate thermal and photoperiod ranges for development and growth of some fish species like zebrafish. These guidelines and guidance documents however do not inform about the biological and ecotoxicological consequences of being below or above the recommended ranges.

Photoperiod and temperature are also species-specific environmental drivers of the reproductive cycle in temperate fish species (Migaud et al., 2010; Wang et al., 2010). For most tropical fish species, the cueing factors are less known and certainly very diverse, although temperature and photoperiod are effective, at least in some species. Temperature and photoperiod actually play dual roles. Their variations synchronize gametogenesis and the reproductive events, while their absolute values affect gamete quality (Bobe and Labbé, 2010; Taranger et al., 2010; Wang et al., 2010; Migaud et al., 2013). It is probable that fish have narrow ranges of temperature below and above which gametogenesis is impaired, especially oogenesis. According to Hokanson et al. (1973), brook trout (*Salvelinus alpinus*) egg viability would