Received: 13 September 2020 | Revised: 12 November 2020 | Accepted: 6 December 2020

DOI: 10.1111/are.15069

REVIEW ARTICLE

Aquaculture Research WILEY

Toxicity, physiological, histopathological and antiparasitic effects of the formalin, a chemotherapeutic of fish aquaculture

Marcos Tavares-Dias

Embrapa Amapá, Macapá, Brazil

Correspondence
Marcos Tavares-Dias, Embrapa Amapá, Macapá, Brazil.
Email: marcos.tavares@embrapa.br

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 303013/2015-0

Abstract

The search for more effective methods and drugs to control parasites in fish farming is constant. Several studies have evaluated the effects of formalin in freshwater and marine fish and their parasites. Thus, this paper focuses on the major toxicological aspects of this chemotherapeutic including growth performance, histopathological damages, haematological and biochemical alterations, and antiparasitic efficacy. Acute toxicity ($LC_{50-96\ h}$) to formalin sulphate vary widely among fish species (0.1–640.0 mg/L) depending on various factors, as many fish species are sensitive to concentrations near those required for controlling and treating parasites. The toxicity of formalin on some metabolic processes leads to disturbances and imbalances to various physiological functions, particularly respiration and haematological and biochemical processes, due to histopathological damages in gills and haematopoietic organs. Formalin often affects the gills, liver, kidney and spleen, and in the gills provokes an increase in mucus and chloride cells, hyperplasia and/or hypertrophy of lamellae and epithelial lifting. Formalin used in therapeutic baths is extremely effective against various protozoan and monogenean species, which are parasites that infect gills, skin and/or fins of farmed freshwater and marine fish.

KEYWORDS
antiparasitic, formaldehyde, immunity, parasites, toxicity, treatment

1 | INTRODUCTION

Global fish production continues to grow due to advancements in intensive production systems (Hoai, 2020). The growing demand of food fish combined with the scope of economic gain has favoured both horizontal and vertical expansion of aquaculture around the world. Freshwater and marine aquaculture and the aquarium industry also play an active role to conserve the global diversity of fish species. However, the rapid expansion of fish aquaculture and aquarium industries may lead to inadequate environmental conditions that induce stress in fish, particularly due to poor management (Andrade-Porto et al., 2017; Hoai, 2020). Diseases in the aquaculture of food fish and ornamentals have been responsible for economic losses around the world, and their management is second only to feed in terms of costs. The adequate management of diseases is crucial for the expansion of the aquaculture industry since parasites can cause severe morbidity and mortality of fish stocks (Hoai, 2020). The main objective of improving rearing methods is to ensure fish welfare through technological solutions while achieving maximum productivity.

Preventative and *in situ* treatment methods should have minimal negative impacts on the health of farmed fish (Andrade-Porto et al., 2017). Methods for preventing and treating fish diseases are aimed mainly at controlling and limiting the occurrence of parasites and pathogens. Such management regimes have only shown the capacity to limit the occurrence of parasites and pathogens rather than accomplish complete elimination. As a consequence, economic losses from diseases may still occur in fish culture

despite treatment. Nevertheless, water quality must be monitored since adverse changes in the environment may compromise the ability of fish to cope with stressors. The physiological and immune systems of fish are sensitive to environmental changes and they can react to lethal and sublethal levels of xenobiotics or their metabolites, which are used as chemotherapeutic agents to control and treat parasitic diseases (Nouh & Selim, 2013). Negative effects from these chemotherapeutic agents include increasing the susceptibility of fish to other diseases and mortality (Andrade-Porto et al., 2017; Fajer-Ávila et al., 2007; García-Magaña et al., 2019).

Parasitic diseases are common in fish farming (Andrade-Porto et al., 2017; Hoai, 2020). In general, parasites of fish are simple to diagnose within broad taxa, which allows selection of adequate control and treatment options using antiparasitic chemotherapeutics. Various chemotherapeutics are available in the market that can be used to control and treat ectoparasites in fish. These chemotherapeutic are often used for different fish species with different metabolisms and hence, their efficacy can vary according to fish and parasite species. Formalin has been recognized as an effective chemotherapeutant to control various ectoparasite diseases in fish (Allison, 1954; Andrade-Porto et al., 2017; Chmelova et al., 2016; Fish, 1940a, 1940b; Fish & Burrows, 1940; García-Magaña et al., 2019; Harms, 1996; Sandoval-Gío et al., 2019; Stoskopf, 1988; Subasinghe & Yusoff, 1993). However, further review is needed regarding the adequate use of formalin and its toxicological, histomorphological and physiological effects on fish.

Formalin $CH_2(OH)_2$ is a liquid formaldehyde solution in standard concentration of 37%–40%, which is heavily diluted for use in aquaculture mainly to treat freshwater and marine fish and fish eggs, and to disinfect hatcheries and other facilities (Akpoilih & Adebayo, 2010; Andrade-Porto et al., 2017; Beevi & Radhakrishnan, 1987; El-Gawad et al., 2015; Farmer et al., 2013; Fish, 1940a, 1940b; Fish & Burrows, 1940; Harms, 1996; Hodkovicova et al., 2019). Formalin has been used by researchers since the early 1900s to control parasites in rainbow trout (Fish, 1940a, 1940b). Formalin has since become the most used chemotherapeutic in the control and treatment against diseases in fish farming because of its versatility and effectiveness, particularly for controlling and treating diseases of skin, fins and gills. The use of this chemotherapeutic in aquaculture now requires registration and is used for different fish species around world. However, formalin is considered to be a potential carcinogen and thus, precautions must be taken when administering this chemotherapeutic agent (Andrade-Porto et al., 2017; Chmelova et al., 2016; Hodkovicova et al., 2019; Holladay et al., 2010; Lai et al., 2016).

The effective dose of formalin used to treat fish is determined by the time that the fish are subjected to the baths and the tolerance of the animals. It is noteworthy that keeping the fishes in their rearing system during treatment may mitigate added stress from handling. Furthermore, parasites are unable to infect other areas of fish farming when fish are maintained in the rearing system, thus reducing the potential for reinfection. Nevertheless, the adequate concentration of formalin for a prolonged or short-term bath depends on certain factors such as fish species and condition, water quality, and parasite species; factors that will be discussed here.

The aim of this review was to gather information from published research that focus on the use of formalin for controlling and treating ectoparasites in freshwater and marine fish farming. Focus was also given to acute toxicity and potential harm to the physiology, immunity and histomorphology of fish.

2 | ACUTE TOXICITY OF FORMALIN IN FRESHWATER AND MARINE FISH SPECIES

Acute toxicity of formalin has been determined for some freshwater and marine fish species, of which indicated that this chemotherapeutic has a relatively low toxicity for some fish species and high for other fish species. The toxic effects of formalin in fish include mortality and are directly proportional to the concentration (Table 1). However, tolerance to formalin varies among fish depending on the species, size (age), treatment conditions (Chmelova et al., 2016; Cruz et al., 2005; Cruz-Lacierda et al., 2012; Fajer-Ávila et al., 2003; Hoseini & Nodeh, 2013; Hoseini et al., 2013; Intorre et al., 2007; Neves et al., 2020; Tancredo et al., 2019), among other factors. Therefore, the toxicity of this chemotherapeutic to each fish species must be determined before application, and the data of toxicity and safe ranges of the dose of formalin can be extrapolated for fish farming. This is particularly important as the recommended concentrations and treatment times for formalin are near the lethal concentration for some farmed fish species.

The acclimation of fish to lethal formalin concentrations may increase tolerance to this chemotherapeutic agent. For *Paralichthys olivaceus* fry, the 24 h-LC_{50} was 209.0 mg/L of formalin and the 48 h-LC_{50} was 182.0 mg/L (Jung & Kim, 1998) and 141 mg/L (Park et al., 1994). For *Clarias gariepinus* fry, the 24 h-LC_{50} of formalin was 90.0 mg/L (Mbaru et al., 2011), and for *O. niloticus* fry, the 24 h-LC_{50} was 81.3 mg/L of formalin (Aly et al., 2020). For *Oncorhynchus mykiss* fingerlings, the 48h-LC_{50} of formalin was 50.0 mg/L (Tisler & Zagorc-Koncan, 1997). For *O. mykiss* fingerlings, the 48 h-LC_{50} of formalin was 168.0 mg/L, for *Salvelinus namaycush* fingerlings was 167.0 mg/L, for *Salvelinus fontinallis* fingerlings was 157.0 mg/L, for *Salmo trutta* fingerlings was 185.0 mg/L, for *Ictalurus puntactus* fingerlings was 96.0 mg/L and for *Lepomis macrochirus* fingerlings was 140.0 mg/L (Willford, 1967). For hybrid *Huso huso* × *Acipenser ruthenus* fingerlings, the 48 h-LC_{50} of formalin was 216.0 mg/L and for *O. mykis* fingerlings was 211.0 mg/L (Hung et al., 2019). Kouril and Prikryl (1988) reported 15 min-LC_{50} of formalin of 1.2 mg/L for *Tinca tinca*. For *Centropomus viridis* juveniles, the 12 h-LC_{50} of formalin was 495.4 mg/L (Morales-Serna et al., 2020).

For *Xiphophorus maculatus*, *Oreochromis niloticus*, *Danio rerio* and *Carassius auratus*, the 24 h-LC_{50} of formalin was 152.1, 191.3, 186.1 and 110.7 mg/L, respectively (Tancredo, Ferrarezi, et al., 2019). For *Clarias macrocephalus* fry, the 49h-LC_{50} of formalin was 82.5 mg/L of formalin, and for *Epinephelus coioides* fingerlings, the 24 h-LC_{50} of formalin was 214.0 mg/L (Cruz-Lacierda et al., 2012). For

TABLE 1 Lethal concentrations ($LC_{50-96\ h}$) of formalin for different fish in aquaculture

Fish species – Life phase	LC (mg/L)	References
Hoplias lacerdae (FW) – Larva	2.0	Cruz et al. (2005)
Chanos chanos (MW) – Fingerling	232.0	Cruz and Pitogo (1989)
Clarias gariepinus (FW) – Fingerling	42.5	Okomoda et al. (2010)
Clarias gariepinus (FW) – Fingerling	1.8	Andem et al. (2015)
Arapaima gigas (FW) – Fingerling	36.4	Andrade-Porto et al. (2018)
Ictalurus punctatus (FW) – Fingerling	69.0	Clemens and Sneed (1958)
Ictalurus punctatus (FW) – ND	0.1	Leteux and Meyer (1972)
Rutilus rutilus caspicus (MW) – Fingerling	49.0	Hoseini and Nodeh (2013)
Oreochromis niloticus (FW) – Fingerling	148.0	Mert et al. (2015)
Danio rerio – (FW) – Fingerling	30.0	Mohammed et al. (2012)
Danio rerio – (FW) – ND	45.7	Resendes et al. (2018)
Mugil liza (MW) – Fingerling	20.8	Pahor-Filho et al. (2012)
Morone saxatilis (MW) – Fingerling	4.9	Reardon and Harrell (1990)
Morone saxatilis (MW) – Fingerling	10.8	Reardon and Harrell (1990)
Morone saxatilis (MW) – Fingerling	13.5	Reardon and Harrell (1990)
Morone saxatilis (MW) – Fingerling	15.5	Reardon and Harrell (1990)
Morone saxatilis (MW) – Fingerling	18.0	Wellborn (1969)
Aequidens portalegrensis (FW) – Fingerling	40.7	Domitrovic (1997)
Rutilus rutilus caspicus (MW) – Fry	49.0	Hoseini et al. (2013)
Salmo salar (MW) – Fry	64.0	Bills et al. (1977)
Puntius gonionotus (FW) – Fry	67.4	Chinabut et al. (1988)
Puntius gonionotus (FW) – Fry	73.2	Chinabut et al. (1988)
Puntius gonionotus (FW) – Fry	75.2	Chinabut et al. (1988)
Cyprinus carpio (FW) – Fry	118.0	Chinabut et al. (1988)
Cyprinus carpio (FW) – Fry	122.9	Chinabut et al. (1988)
Cyprinus carpio (FW) – Fry	128.8	Chinabut et al. (1988)
Channa striatus (FW) – Fry	147.1	Chinabut et al. (1988)
Channa striatus (FW) – Fry	152.6	Chinabut et al. (1988)
Channa striatus (FW) – Fry	166.8	Chinabut et al. (1988)
Oncorhynchus mykiss (FW) – Fry	430.0	Bills et al. (1977)
Oncorhynchus mykiss (FW) – Fry	430.0	Howe et al. (1995)
Salvelinus namaycush (MW) – Fry	370.0	Bills et al. (1977)
Ictalurus punctatus (FW) – Fry	14.7	Howe et al. (1995)
Ictalurus punctatus (FW) – Fry	243.0	Bills et al. (1977)
Ameiurus melas (FW) – Fry	229.0	Bills et al. (1977)
Lepomis cyanellus (FW) – Fry	640.0	Bills et al. (1977)
Lepomis macrochirus (FW) – Fry	100.0	Bills et al. (1977)
Micropterus dolomieui (FW) – Fry	503.0	Bills et al. (1977)
Micropterus salmoides (FW) – Fry	529.0	Bills et al. (1977)
Paralichthys olivaceus (MW) – Fry	141.0	Jung and Kim (1998)
Oreochromis niloticus (FW) – Fry	2.9	Dureza (1995)
Clarias gariepinus (FW) – Juvenile	112.2	Ayuba et al. (2013)
Trachinotus carolinus (MW) – Juvenile	69.1	Birdsong and Avault (1971)
Trachinotus carolinus (MW) – Juvenile	71.6	Birdsong and Avault (1971)
Trachinotus carolinus (MW) – Juvenile	74.9	Birdsong and Avault (1971)
Paracheirodon axelrodi (FW) – Adult	67.9	Dias et al. (2018)
Corydoras melanistius (FW) – ND	50.7	Santos et al. (2012)
Anguilla rostrata (FW) – ND	329.6	Hinton and Eversole (1980)

FW, freshwater fish; MW, marine water fish; ND, Not informed.

Gymnocorymbus ternetzi, the 24 h-LC_{50} of formalin was 72.0 mg/L (Nam & Heo, 2004). For *Cyprinus carpio*, the 24 h-LC_{50} of formalin was 135.4 mg/L (Tancredo et al., 2019), and for *Sphoeroides annulatus*, the 48h and 72-LC_{50} of formalin was 87.0 and 79.0 mg/L, respectively (Fajer-Ávila et al., 2003). For *Danio rerio* adults, the 1h-LC_{50} of formalin was 648.0 mg/L, and for *Carassius auratus* adults was 272 mg/L (Intorre et al., 2007). For *Danio rerio* larvae, the 144 h-LC_{50} of formalin varied of 0.2 to 4.9 Mn (Meinelt et al., 2005), and for *Siganus rivulatus* juveniles, the 72 h-LC_{50} of formalin was 551.0 mg/L (Nasser et al., 2017). For *Lophiosilurus alexandri* larvae with 7 days post-hatch (ph), the 12 h-LC_{50} of formalin was 108.9 mg/L and for larvae of 45 days was 244.4 mg/L (Neves et al., 2020).

In *Rhamdia quelen* fingerlings, exposure to 0.2 ml/L of formalin caused high mortality (Carneiro et al., 2005). Rothen et al. (2002) demonstrated that *Hemigrammus caudovittatus* and *Trichogaster*

trichopterus exposed to 12.5 or 25.0 mg/L of formalin, for 24 or 48 h, were more sensitive than *Danio rerio*, which showed a higher survival (95%–100%). *Etroplus swatensis* exposed to 100–500 mg/L of formalin for 2–4 min died during 48 h after treatment (Wijeyaratine & Gunawardene, 1988). *Clarias gariepinus* fry exposed to 50.0–2000 mg/L of formalin for 1 h had a mortality of 57.3%–100% while exposure for 15 min caused mortality of 54.0%–92.3% (Mbaru et al., 2011). However, *C. gariepinus* fingerlings, exposed to 3700 mg/L of formalin, had 2.7%–8.7% of mortality (Yisa et al., 2014). In *R. quelen*, exposure to 740.0 mg/L for 24–120 h caused mortality of 2.0%–100% (Carneiro et al., 2006). *Micropterus salmoides* exposed to 630.0–1.420.0 mg/L of formalin for 24 h caused 90.0%–100% of mortality (Carmichael & Tomasso, 1983). In *Rutilus rutilus caspicus*, long-term exposure to 10, 15 and 25 mg/L of formalin affected survival at both 24 and 72 h, while no further change in survival was shown for 168 h of exposure. However, 12 and 24 h of exposure to 25 mg/L of formalin reduced the survival of fish when compared to 10 and 15 mg/L (Ghelichpour & Eagderi, 2012). The survival of *O. niloticus* exposed to 250.0 mg/L of formalin was 92.0% (Perera & Pathiratne, 2005). Sultana et al. (2013) reported that the mortality of *Channa punctatus* exposed to 925.0–3700 mg/L of formalin was of 66.7%–100%. *Lepomis macrochirus* exposed to 5 mg/L of formalin presented 100% of mortality within 48 h, while exposure to 10 and 15 mg/L was shown with mortality rates of 80% and 50%, respectively (Allison, 1957).

In embryos of *C. carpio* and *C. gariepinus*, exposure to 2000.0 mg/L formalin for 15–30 min had no effect on the survival up to 38 h post-fertilization (Theron et al., 1991a). In *Clarias gariepinus* larvae with an age of 4 days ph exposed to 200.0 mg/L formalin for 30–90 min showed survival of 97.0%–92.7% after treatment. *C. carpio* larvae (4 days ph) exposed to 200.0 mg/L formalin for 30–90 min showed survival of 0 to 80.7% after treatment. *C. gariepinus* larvae (12 days ph) exposed to 200.0 mg/L of formalin for 30–90 min showed survival of 44.0%–96.0%. *C. carpio* larvae (12 days ph) exposed to 200.0 mg/L of formalin for 30–90 min showed survival of 28.0%–71.3%. However, *C. carpio* larvae with 21 days ph exposed to 200.0 mg/L formalin for 30–90 min showed survival of 90.0 to 93.7%. *C. gariepinus* larvae with 20 days ph exposed to 200.0 mg/L formalin for 30–90 min showed survival of 39.0%–97.0%. (Theron et al., 1991b). *Ictalurus punctatus* exposed to 50.0 mg/L of formalin showed 0% mortality after administration of two doses (Tieman & Goodwin, 2001). For *Micropterus salmoides* fry (24 h ph) exposed to 1000–10,000 mg/L of formalin, the survival varied from 89.0%–97.0%. However, after 48 h of exposure to 10.0–250 mg/L survival varied from 1.0 to 92.0% (Wright, 1976).

The toxicity of formalin for fish in waters of different quality has been studied. Bills et al. (1977) observed that water hardness had no effect on the toxicity of formalin in fish, whereas in soft water with a pH of 9.5 the formalin was more toxic than with pH 6.5 and 8.5. However, no significant differences in the effects of formalin in water of different hardness and pH have been reported (Chinabut et al., 1988; Marking et al., 1972; Piper & Smith, 1973; Stoskopf, 1988). Temperature may also influence the toxicity of formalin (Bills et al., 1977; Bodensteiner et al., 1993; Chmelova et al., 2016; Clemens & Sneed, 1958; Fish, 1939; Forwood et al., 2014; Piper & Smith, 1973) because it increases toxicity of this chemotherapeutant. Concentrations of 30.0 or 40.0 mg/L of formalin in temperatures of 24.1–26.9°C also caused a decline in dissolved oxygen levels of water (Rowland et al., 2006).

Alterations in fish behaviour after exposure to formalin are due to endogenous and exogenous processes, and they may help to understand the health and viability of fish exposed to this chemotherapeutic. In general, alterations in behaviour are related to changes in physiological and histomorphological functions of fish exposed to formalin. Behavioural alterations reported for fish species exposed to lethal or sublethal concentration of formalin include: jumping due to skin irritation, agitation, respiratory distress, loss of balance, erratic swimming, lethargy, exophthalmia, crowding on the water surface, loss of hydrodynamic equilibrium, spasms, agonistic confrontation, darkening of the body, sudden and quick movement and excessive accumulation of mucus (Andem et al., 2015; Andrade et al., 2005; Andrade-Porto et al., 2018; Ayuba et al., 2013; Intorre et al., 2007; Jimmy et al., 2013; Mohammed et al., 2012; Morales-Serna et al., 2020; Nam & Heo, 2004; Nouh & Selim, 2013; Okomoda et al., 2010; Pahor-Filho et al., 2015; Perera & Pathiratne, 2005; Santos et al., 2012; Sultana et al., 2013; Tancredo, Ferrarezi, et al., 2019). Many of these behavioural problems are related to reduced oxygen consumption (Mohammed et al., 2012), perhaps due to damages in the gill epithelium from exposure to formalin. Treatment with formalin may cause a decrease in oxygen consumption and an increase in the energy expenditure for swimming. Hence, formalin may induce an increased metabolic rate while causing a decrease in swimming due to stress in exposed fish.

Dissolved oxygen also influences the toxicity of formalin in fish. Knight et al. (2016) analysed the degradation of formalin in brackish water recirculating systems and the response of the biological filter during 5 days with a 25 mg/L formalin treatment, and reported that degradation rates changed over time (0.69–7.55 formalin mg/L per h), resulting in periods below therapeutic concentrations during treatment. Meinelt et al. (2005) reported that the toxicity of formalin decreases with the presence of natural organic matter in water. Salinity was shown to have no influence on the toxicity of formalin in *Trachinotus carolinus* (Birdsong & Avault, 1971). However, salinity influenced the 96- LC_{50} of formalin for *Morone saxatilis*, perhaps due to damage in the gills of fish, leading to a disruption in osmoregulation or respiration. Furthermore, these alterations may be caused by osmotic stress in the absence of an acclimation period of fish to salinities (Reardon & Harrell, 1990).

The mechanism of toxicity to formalin is little understood in fish. Toxicity occurs when concentrations of formalin exceed physiological thresholds and interrupt physiological functions in fish. Formalin may accumulate in gills and interfere in osmoregulation by decreasing branchial Na^+ and K^+ adenosine triphosphate activity, but this has yet to be investigated. However, such accumulation of formalin causes severe damages to fish gills and affects energy metabolism,

which compromise the swimming performance and equilibrium of fish. Given that swimming is central to many aspects of fish biology, a decreased performance may have implications for interspecific and intraspecific interactions and may influence the fitness of individuals. Alterations in behaviour after exposure to formalin appear to be caused by the neurotoxic effects and by the irritation to the perception system of the fish. Therefore, strategies are needed to reduce this toxicity of formalin in fish aquaculture systems. Moreover, swimming performance of fish could be used as an indicator for measuring toxic effects of formalin, which include the impairing of transport or exchange of respiratory gases as well as alterations in energy transformations, or inhibition of functions of the nervous and muscular systems.

3 | HISTOMORPHOLOGICAL ALTERATIONS ON GILLS AND HAEMATOPOIETIC ORGANS OF FRESHWATER AND MARINE FISH EXPOSED TO FORMALIN

Gills of fish are in contact with the external environment and may be subject to morphological changes when exposed to chemical substances (Pahor-Filho et al., 2015). Gills and haematopoietic tissues of fish are key organs for metabolizing formalin, and gills are the first to be affected by exposure to formalin. Hence, the histological evaluation of these tissues is considered an effective tool for detecting the effects of exposure to this chemotherapeutic in different fish species (Andrade-Porto et al., 2018; Bodensteiner et al., 1993; Mert et al., 2015; Nouh & Selim, 2013; Pahor-Filho et al., 2015), since exposure to formalin causes severe damage in branchial epithelium and compromises respiration of fish (Table 2). The gills are directly in contact with water and are the first organ to respond when exposed to acute and sublethal concentrations of formalin. Furthermore, exposure of fish to formalin causes damages to the liver, kidney and spleen (Tables 3-5).

Pampus argenteus exposed to 80 mg/L of formalin increased the superoxide dismutase activity of the liver in the first 2 days and decreased in the third day. The superoxide dismutase activity of the kidney also increased in the first day and decreased in the third day after exposure. Catalase activity of the liver increased in the first 2 days and decreased in the third day, but no significant changes were observed in the kidney. Glutathione activity of the liver increased in the first day and decreased during the last 2 days of exposure to formalin. However, the kidneys showed no changes. Malondialdehyde content in the liver and kidney increased in the second and third days, and in the liver increased throughout the 3 days of treatment (Hu et al., 2019). In the same tissues of *O. mykiss* exposed for 24 h to 50–200 mg/L of formalin, there was an increase in malondialdehyde activity and a decrease in glutathione, catalase, glutathione and peroxidase activities (İspir et al., 2017). Exposure of *O. mykiss* to 200 ml/m^3 of formalin resulted in metabolic plasticity in the liver with decreased levels of oxidative stress biomarkers and aminotransferases activity (Tkachenko & Grudniewska, 2016). On the other hand, Smith et al. (1987) reported that *Oncorhynchust shawytscha* exposed to 618.0 mg/L of formalin showed no consistent effect on gill Na^+, K^+-ATPase activity.

Fish gills are morphologically and physiologically complex given that they perform several functions such as gas exchange, ion exchange, acid base balance, excretion of nitrogenous waste and other metabolic functions. Fish live in intimate contact with the water, of which the gills surface comprises over half of the body surface area and the delicate gill epithelium separates the internal environment from the external environment (Afaghi & Zare, 2020; Basirun et al., 2019; Kumar & Ram, 2015). The liver, spleen and kidney are pivotal organs of the body that maintain homeostasis. The liver is the centre of metabolism and detoxification, and the kidneys are involved in elimination of wastes from the body and selective reabsorption. The liver plays a central role in the metabolism of toxins (Jegede, 2013; Latif et al., 2013). The fish spleen is an erythro- and leukopoietic organ involved in the synthesis of new erythrocytes and lymphocytes, and this haematopoietic tissue is the only organ in fish to trap antigens (Balamurugan et al., 2012; Taheri et al., 2016). Hence, these haematopoietic organs show morphological alterations as responses to toxicity from formalin (Tables 3-5). However, the few available studies have shown that the spleen undergoes changes in its haematopoietic structure/function due to the toxic effects of formalin (Table 5).

It has been suggested that the reactions of formalin with functional groups occur by intra- and intermolecular cross-linking of macromolecules that modify the physical characteristics of tissues (Fox et al., 1985; McDonnell & Russell, 1999). Understanding the differences in the effects of formalin concentrations on tissues and biological mechanisms may provide essential information to manage the use of this chemotherapeutic in aquaculture. Although studies on different species often involve the repetition of the same experimental methodologies, this review attempts to demonstrate the advantage of such comparative studies for a thorough understanding of formalin bioaccumulation and its potential harm to tissues.

4 | PHYSIOLOGICAL AND IMMULOGICAL ALTERATIONS CAUSED BY FORMALIN IN FRESHWATER AND MARINE FISH

Haematological and biochemical variables have been widely used for clinical diagnoses related to the physiology of fish and for determining the effects of external stressors and diseases. Fish haematology and other biochemical variables may be used to indicate stress caused by toxic chemicals such as formalin (Chmelova et al., 2016; Şahan, 2020; Witeska et al., 2013). Chemical products such as formalin may impair the physiology and compromise the health condition of exposed fish. Thus, blood and biochemical parameters are useful in identifying the lethal, sublethal or chronic toxicity concentrations of formalin and their effects in target organs.

TABLE 2 Histopathological effects of exposure to formalin on gills of different freshwater and seawater fish species

Fish species	Dose (mg/L)	Exposure	Tissue alterations	References
Oncorhynchus tshawytsch	200	Sublethal	Epithelial hypertrophy and hypertrophic and degenerating epithelial cells. These cellular changes often gave an irregular, ragged appearance to the lamellar surface.	Wedemeyer and Yasutake (1974)
Cyprinus carpio	280.0	Lethal	Moderate epithelial hypertrophy with epithelia partially desquamated from the lamellae and gill epithelia often severely swollen. Hyperplasia and hypertrophy of epithelial cells at the base of secondary lamellae	Kakuta et al. (1991)
Chanos chanos	100.0–500.0	Lethal	Slight epithelial lifting, complete separation of the entire epithelial layer and necrosis of the lamellar epithelial cells. Respiratory epithelia were ruptured at various points, thereby exposing the gill ray and capillaries to water. Exhibition of hyperplastic and club-shaped lamella	Cruz and Pitogo (1989)
Arapaima gigas	66.0–110.0	Lethal	Lamellar epithelium hypertrophy and hyperplasia, proliferation of mucus cells and mitochondria-rich cells, partial and total fusion of the lamellae, oedema, capillary constriction, lamellar aneurism and epithelial rupture (haemorrhage)	Andrade-Porto et al. (2018)
Ictalurus punctatus	92.0	Subletal	Hypertrophy, hyperplasia and excess mucous secretion	Bodensteiner et al. (1993)
Oncorhynchus mykiss	200.0	Sublethal	Progressive damages in gill damage, whit degenerative changes and some necrosis Hypertrophy accompanied by pyknotic and karyorrhetic epithelial nuclei, as well as epithelial separation	Wedemeyer and Yasutake (1974)
Oncorhynchus mykiss	250.0–500.0	Lethal	Necrosis in the gill lamellae epithelial cells and haemorrhage	Bulut et al. (2015)
Paralichthys olivaceus	141.0	Lethal	Hypertrophy of mucous and epithelial in secondary gill lamella	Park et al. (1994)
Paralichthys olivaceus	150.0–300.0	Subletal	Mild oedema and winding of secondary gill lamellae. Separation of epithelial layer, hypertrophy and scattered necrosis of the lamellar epithelial cells, and thrombosis of secondary gill lamellae	Cho et al. (1997)
Rutilus rutilus caspicus		Subletal	Epithelial lifting and hyperplasia, aneurysm at different intensities, and lamellar fusion and shortening, but curling rarely was observed	Ghelichpour et al. (2016)
Cyprinus carpio	629.0	Subletal	Destruction of the lamellas and inflammatory necrotic masses on the gill and hyperaemia. Lamellas were deformed with a separated surface layer and numerous mucinous cells. An amount of mononuclear lysis was observed among the bases of the lamellas.	Chmelova et al. (2016)
Huso huso × *Acipenser ruthenus*	50.0–200.0	Subletal	Mucus production, a large number of gill filaments with epithelial shedding, engorgement on apical surfaces, and degeneration of column cells. Column cells degeneration, gill lamellae rupture, cell hypertrophy on gill filaments and the rupture, and lamellar fusion	Hung et al. (2019)
Oncorhynchus mykis	167.0–250.0	Lethal	Gill epithelium often severely swollen, and in some instances, had completely desquamated from the lamellae, leaving only the blood capillary and supportive pillar cells. Leukocyte infiltration, some epithelial cells showed cloudy swelling, and others had pyknotic and fragmented nuclei. Presence pf blood plasma sometimes accumulated between gill epithelium and blood capillaries and haemorrhage	Smith and Piper (1972)
Oncorhynchus mykis	50.0–200.0	Subletal	Mucus production on gill tissue and a large number of gill filaments showed epithelial shedding, engorgement of the top of gill filaments and the degeneration of column cells. Degeneration of column cells, the rupture of gill lamellae, hypertrophy of cells on top gill filaments and gill filament with signs of fusion	Hung et al. (2019)

(Continues)

TABLE 2 (Continued)

Fish species	Dose (mg/L)	Exposure	Tissue alterations	References
Oreochromis niloticus	250.0	Lethal	Hyperplasia, hypertrophy, epithelial separation and club-shaped deformities	Perera and Pathiratne (2005)
Oreochromis niloticus	30.0–75.0	Subletal	Epithelial lifting, branchitis, hyperaemia, telangiectasia and hyperplasia	Mert et al. (2014)
Oreochromis niloticus	25.0	Subletal	Congestion and hyperplasia in the epithelium of the secondary lamellae, with infiltration of mononuclear leukocytes in the gill arch and lamellae	Nouh and Selim (2013)
Gymnocorymbus ternetzi	7.2–15.0	Subletal	Clubbing and slight swelling of gill lamellae	Nam and Heo (2004)
Mugil liza	57.5–540.0	Lethal	Severe hyperplasia and soft detachment of respiratory epithelium, increase in chloride cells and detachment of the respiratory epithelium	Pahor-Filho et al. (2015)
Corydoras melanistius	50.7	Lethal	Cell hyperplasia and reaching levels of filling interlamellar	Santos et al. (2012)
Salmo salar	167.0–250.0	Subletal	Increase in frequency of lamellar fusion and numbers of lamellar mucous cells	Speare et al. (1997)
Lophiosilurus alexandri	54.0–648.0	Lethal	Lamellar hyperplasia ranging from mild to severe, epithelial detachments and telangiectasia	Neves et al. (2020)

Exposure to sublethal and lethal concentrations of formalin causes alterations in biochemical, erythrocyte and leukocyte parameters (Table 6). Elevated cortisol concentration has been considered as the major stress response, as it increases rapidly after stress. Hyperglycaemia is a secondary stress response, which is stimulated by primary stress responses (release of catecholamines and corticosteroids into blood circulation) to supply energy needed to cope with stress. However, when in prolonged stress conditions, secondary stress responses can change the tertiary stress responses. During the adaptive phase, energy is prioritized towards organs and other physiological functions related to survival such as breathing, swimming, osmoregulation and tissue repair, reducing the contribution of energy to long-term anabolic activities such as growth, reproductive process and immune functions (Barton, 2002; Urbinati et al., 2020; Wendelaar Bonga, 1997).

Changes in erythrocytic parameters may occur in fish exposed to formalin in function of the oxygen demand (Table 6). Elevations in these blood parameters are a response to the inefficient uptake of oxygen in the gill epithelium, and as a consequence, the haematopoietic tissues release more erythrocytes to supply the oxygen demand in fish (Perera & Pathiratne, 2005). However, reductions in erythrocytic parameters are due to damages in gills and haematopoietic tissues caused by the exposure to formalin. In *O. mykiss* exposed to 167.0 or 250.0 mg/L of formalin, an increase in haematocrit was shown with haemolysis of erythrocytes as caused by stress (Smith & Piper, 1972). Witeska et al. (2013) reported that therapeutic baths with 63.0 mg/L of formalin induced increases in haematopoietic cell proliferation and apoptosis in *C. carpio*.

The fish immune system consists of a set of cellular and humoral components that function as the defence of the organism against foreign substances, such as parasites, toxins, toxic substances and malignant cells. This system is the first line of defence against invading agents, whereas acquired immunity is related to the elimination of the pathogen in a late stage and the production of immunological memory. The innate system includes cellular components, which are defence cells, that is granulocytes, monocytes, macrophages and natural killer cells; and humoral components, that is the complement system, antimicrobial enzyme system, and nonspecific mediators such as interferon and interleukins (Urbinati et al., 2020). For *Arapaima gigas*, short-term exposures in baths (1 h) to 220–550 mg/L of formalin showed no alterations in erythrocytes and leukocyte parameters (Andrade-Porto et al., 2017). However, in *Oreochromis niloticus* exposed to 15.0–75.5 mg/L of formalin for 168 h, an increase of micronucleus in erythrocytes was shown after exposure (Mert et al., 2015). In *D. rerio* exposed to sublethal concentrations of formalin, the incidence of micronuclei increased with concentration, indicating that this chemical agent caused genotoxic effects in erythrocytes of fish (Resendes et al., 2018).

The biochemical analysis of blood of fish exposed to formalin is relevant because these physiological parameters may be a sensitive indicator of stressful agents. Hence, they are important for determining the influence of pathophysiological conditions on the homeostasis of fish exposed to formalin. However, in *Colossoma macropomum*, exposure to 100.0–250.0 mg/L of formalin had no influence on plasma levels of glucose, chloride, sodium and potassium (Araújo et al., 2004). Similarly, for *Arapaima gigas*, exposure through short-term baths (1 h) to 220–550 mg/L of formalin showed no alterations in plasma levels of glucose, cortisol, total protein, sodium, calcium, potassium, chloride and magnesium (Andrade-Porto et al., 2017).

Same had careful to avoid stressing the fish, we cannot be assured that immunity fish of is not affected by the exposure to formalin (Sandoval-Gío et al., 2008). Hodkovicova et al. (2019) reported that after 10 days of treatment with 185.3 mg/L of formalin, there was a decrease in the lysozyme in skin mucus, a decrease

TABLE 3 Histopathological effects of exposure to formalin on liver of different freshwater and seawater fish species

Fish species	Dose (mg/L)	Exposure	Tissue alterations	References
Chanos chanos	100.0–500.0	Lethal	Focal and diffuse haemorrhages with vacuolation of hepatocytes and pigment deposits occurred in the parenchyma. Pyknotic nuclei and severe necrosis of hepatocytes with thick layers of fibrin, and complete destruction of the cell outline. Cloudy swelling of the hepatocytes and hepatocytes and sinusoidal dilation	Cruz and Pitogo (1989)
Clarias gariepinus	370.0	Sublethal	Multifocal necrosis of hepatocytes	Adeyemo et al. (2012)
Oncorhynchus mykiss	200.0	Sublethal	Shrinkage of liver cells and cytoplasmic degeneration. Sinuses were packed with red blood cells and blood and vacuolization Widespread cytoplasmic degeneration and loss of architecture and degenerate red blood cells were evident in sinuses	Williams and Wootten (1981)
Oncorhynchus mykiss	250.0–500.0	Lethal	Increased dilatation, haemorrhage and damage in the blood vessels	Bulut et al. (2015)
Paralichthys olivaceus	141.0	Lethal	Focal or massive necrosis in hepatocyte	Park et al. (1994)
Hoplias lacerdae	1.0–2.0	Lethal	Cordon disarrangement of hepatocytes, nuclei such as condensed chromatin and decrease in size, in addition to cell fusion in some regions and congestion in sinusoids	Cruz et al. (2005)
Cyprinus carpio	629.0	Subletal	Congestive parenchyma with dissemination of siderin deposits and moderate glycogen content	Chmelova et al. (2016)
Oreochromis niloticus	250.0	Lethal	Elevated vacuolization in hepatocytes	Perera and Pathiratne (2005)
Oreochromis niloticus	30.0–75.0	Subletal	Hyperaemia, hydropic degeneration and fatty degeneration	Mert et al. (2015)
Oreochromis niloticus	25.0	Subletal	Swollen and vacuolated hepatocytes with basophilic cytoplasm, congestion of sinusoids, vacuolar degeneration, proliferation of melanomacrophages centre, oedema, hyperplasia in the bile ducts	Nouh and Selim (2013)
Gymnocorymbus ternetzi	7.2–15.0	Subletal	Lytic degeneration and cytoplasmic and mild vacuolization in hepatocytes.	Nam and Heo (2004)
Corydoras melanistius	50.7	Lethal	Sinusoids capillaries congested and hepatocytes presented hypertrophic with changes in cytoplasmatic granulation	Santos et al. (2012)

in the anti-inflammatory cytokine transforming growth factor beta in gill tissue and an increase in interleukin-10 in kidney tissue. The pro-inflammatory cytokine interleukin-1b increased in the gill tissue immediately after the bath, as well as glutathione peroxidase in gill tissue, 24 h and 10 days after treatment baths. Holladay et al. (2010) suggest that certain components of the fish immune system, specifically the lymphoid progenitor compartment, may be negatively affected by exposure to formalin exposure. Hence, studies have suggested that the fish should be kept in quarantine to allow their immune system to recover from the influence of formalin (Sandoval-Gío et al., 2008).

The physiological balance and homeostasis in fish is maintained by the proper functioning of innate or natural, and adaptive or acquired resistance mechanisms. Thus, the simultaneous and adequate function of these systems and ideal environmental conditions are necessary. This presents certain limitations, especially in intensive fish aquaculture when considering large stocks are kept at high densities, requiring antiparasitic treatments and thus generating stressful conditions.

5 | GROWTH PERFORMANCE ALTERATIONS OF FRESHWATER AND MARINE FISH EXPOSED TO FORMALIN

Increasing fish production depends on feeding, handling and the absence of stress, among other factors. The use of chemotherapeutics to control and treat parasites is one of the most important issues in all phases of fish aquaculture production. Exposure to formalin may interfere in fish performance. In *C. gariepinus*, exposure to 185.0–222.0 mg/L (Jimmy et al., 2013) and 3700 mg/L of formalin (Yisa et al., 2014) decreased weight gain (Jimmy et al., 2013), while 1850.0 mg/L of formalin (Yisa et al., 2014) and 100 mg/L (Jimoh et al., 2020) increased the weight gain. Similarly, exposure to 1.6–25.0 mg/L of formalin also decreased the weight gain of *O. niloticus* (Omoregie et al., 1998). In contrast, exposure of *Salmo salar* to 618.0–925.0 mg/L of formalin increased the weight gain (Powell et al., 1996). Rábago-Castro et al. (2014) reported that *Ictalurus punctatus* exposed to 250.0 mg/L of formalin increased the weight gain and specific growth rate.

TABLE 4 Histopathological effects of exposure to formalin on kidney of different freshwater and seawater fish species

Fish species	Dose (mg/L)	Exposure	Tissue alterations	References
Chanos chanos	100.0–500.0	Lethal	Extensive degeneration of the renal tubules and intertubular tissue and extensive pigment deposits to form. The disappearance of the renal tubules led to replacement of the tubules with interstitial lymphoid tissue. Tubular degeneration and deposition of pigments	Cruz and Pitogo (1989)
Paralichthys olivaceus	141.0	Lethal	Hyaline droplet degeneration of tubular epithelial cells in the proximal convoluted segment of tubules	Park et al. (1994)
Paralichthys olivaceus	150.0–300.0	Subletal	Mild oedema of real tubule, renal tubule epithelium with hyaline droplet degeneration. Extensive hydropic degeneration and nuclear swelling of renal tubule epithelium	Cho et al. (1997)
Cyprinus carpio	629.0	Subletal	Congestive parenchyma with dissemination of siderin deposits and moderate glycogen content	Chmelova et al. (2016)
Cyprinus carpio	280.0	Lethal	Abnormalities such as enlargement of glomerula, thickening and cloudy swelling of epithelial cells of renal tubules and formation of an eosinophilic cast in the renal tubules	Kakuta et al. (1991)
Gymnocorymbus ternetzi	7.2–15.0	Subletal	Epithelial cell oedema of renal tubules	Nam and Heo (2004)
Oreochromis niloticus	25.0	Subletal	Congestion of intertubular capillaries and perivascular oedema and tubular nephrosis. Vacuolization in the renal epithelium with focal depletion of haematopoietic tissue	Nouh and Selim (2013)
Oncorhynchus mykis	167.0–250.0	Lethal	Congestion of blood sinusoids and nuclear swelling and hydropic degeneration of renal tubule epithelium	Smith and Piper (1972)

TABLE 5 Histopathological effects of exposure to formalin on spleen of different freshwater fish species

Fish species	Dose (mg/L)	Exposure	Tissue alterations	References
Clarias gariepinus	370.0	3 days	Massive lymphoid depletion	Adeyemo et al. (2012)
Cyprinus carpio	629.0	Subletal	Congestion and siderin deposits	Chmelova et al. (2016)
Oreochromis niloticus	25.0	Subletal	Proliferation of melanomacrophages centre and focal depletion in the lymphoid tissue	Nouh and Selim (2013)
Oncorhynchus mykis	167.0–250.0	Lethal	Reduction of lymphoid tissue in spleen	Smith and Piper (1972)

If the stressor agent is acute, the homeostasis can be recovered without severe consequences to the fish. However, stressors of a chronic nature lead to compromises of the energy stores, with the onset of physiological exhaustion and a reduced growth rate. The impairment of the fish defence system affects its health, welfare and survival (Barton, 2002; Urbinati et al., 2020; Wendelaar Bonga, 1997). The suppression on growth may be due to adverse effects of formalin on metabolism of the exposed fish (Omoregie et al., 1998). The suppressive effect of formalin may be due to a reduction of food and nutrient uptake. However, an exposure of 48 h with 222.0 mg/L of formalin had no influence on the weight of *Poecilia reticulata* (Andrade et al., 2005). *Oncorhynchus mykiss* exposed to intermittent baths with 200.0 mg/L of formalin for 1 h showed no alterations in growth rates, appetite, feed conversion and body condition index (Speare & Macnair, 1996). Exposure of *Oncorhynchust shawytscha* to 618.0 mg/L of formalin also showed no alterations in growth performance (Smith et al., 1987).

Few studies have investigated the effects of formalin on growth performance parameters despite the frequent use of this chemotherapeutic in fish aquaculture and aquaria. Further research is necessary to investigate whether exposure to formalin may lead to negative effects on the obtaining of food, as well as if swimming behaviour and avoidance of adverse conditions affect the fish appetite, as both activities can influence fish survival

TABLE 6 Haematological and biochemical effects of acute and subletal exposure to formalin for different fish species after

Fish species	Doses (mg/L)	Alterations	References
Oncorhynchus tshawytscha	200.0	Increase in plasma chloride, cholesterol and cortisol	Wedemeyer and Yasutake (1974)
Salmo salar	250.0	Increase in plasma glucose and total protein	Nieminen et al. (1983)
Oncorhynchus mykiss	200.0	Decrease in plasma calcium levels and in plasma chloride	Wedemeyer (1971)
Oncorhynchus mykiss	200.0	Increase in plasma chloride and cholesterol	Wedemeyer and Yasutake (1974)
Oncorhynchus mykiss	250.0	Decrease in haematocrit, haemoglobin, plasma lactate and glucose, and increase in total protein levels	Nieminen et al. (1983)
Oncorhynchus mykiss	1250	Decrease in haematocrit, haemoglobin, plasma lactate and glucose, and increase in total protein levels	Nieminen et al. (1983)
Oncorhynchus mykiss	200.0	Increase in haematocrit and plasma glucose, and increase in plasma glutamate oxaloacetic transaminase	Williams and Wootten (1981)
Salmo salar	1250	Increase in haematocrit and haemoglobin	Nieminen et al. (1983)
Salmo salar	618.0–925.0	Increase in plasma sodium, chloride and calcium	Powell et al. (1996)
Clarias gariepinus	370.0–480.0	Decrease in total erythrocyte number, and increase in haemoglobin, haematocrit and mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC), and increase in total leukocyte number	Okomoda et al. (2010)
Clarias gariepinus	100	Decrease in total erythrocyte number and haemoglobin	Jimoh et al. (2020)
Hemigrammus sp.	370.0	Increase in perceptual of lymphocytes, and decrease in perceptual of monocytes	Paixão et al. (2013)
Sarotherodon massambicus	80.0	Decrease in total erythrocyte number, and increase in haemoglobin and haematocrit and mean corpuscular volume (MCV)	Beevi and Radhakrishnan (1987)
Arapima gigas	55.0–66.0	Increase in plasma levels of glucose and cortisol, and decrease haematocrit, plasma levels of chloride and calcium	Andrade-Porto et al. (2017)
Colossoma macropomum	200.0–250.0	Increase in plasma calcium levels	Araújo et al. (2004)
Oreochromis niloticus	1.6–25.0	Decrease in total erythrocyte number and increase in plasma glucose levels	Omoregie et al. (1994)
Oreochromis niloticus	20.0	Decrease in total erythrocyte number, haemoglobin, haematocrit and serum level of aspartate aminotransferase (AST), and increase in serum level of alanine aminotransferase (ALT), urea, sodium and potassium	El- Deen et al. (2010)
Oreochromis niloticus	150.0	Decrease in perceptual of lymphocytes	Holladay et al. (2010)
Oreochromis niloticus	25.0	Decrease in total erythrocytes and leukocyte number, haemoglobin, haematocrit, plasma total protein and albumin, and increase in plasma glucose, urea, creatinine and alanine aminotransferase (ALT)	Nouh and Selim (2013)
Oreochromis niloticus	150.0–250.0	Decrease in total erythrocyte number, haematocrit and haemoglobin	Perera and Pathiratne (2005)
Oreochromis niloticus	5550	Increase in serum cortisol and glucose	Sandoval-Gío et al. (2019)
Cyprinus carpio	280.0	Increase in haematocrit, plasma glucose, total protein and lactic acid, and decrease in mean corpuscular haemoglobin concentration (MCHC)	Kakuta et al. (1991)
Cyprinus carpio	629.0	Increase in total erythrocyte number and basophils, haemoglobin, haematocrit, plasma glucose, lactate, calcium, and decrease in plasma level of alanine aminotransferase	Chmelova et al. (2016)
Cyprinus carpio	150.0	Decrease in total erythrocyte number, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), increase in serum cortisol and glucose, total leukocytes, lymphocytes and monocytes	Şahan (2020)

(Continues)

TABLE 6 (Continued)

Fish species	Doses (mg/L)	Alterations	References
Cyprinus carpio	63.0	Increase in haematocrit and perceptual of erythroblasts, and decrease in total thrombocytes	Witeska et al. (2013)
Carassius auratus	250.0	Increase in serum level of cortisol, glucose, calcium, albumin, and total protein, and decrease in serum level of chloride	Hoseini and Tarkhani (2013)
Oncorhynchus mykiss	120.0	Increase in plasma cortisol	Jørgensen and Buchmann (2007)
Oncorhynchus mykiss	200.0	Increase in plasma cortisol	Sanchez et al. (1997)
Paralichthys olivaceus	300.0	Increase in total erythrocyte number, immature erythrocyte number, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration (MCHC), alanine aminotransferase, serum magnesium, potassium, chloride and lactate dehydrogenase, and decrease in serum level of total protein, aspartate aminotransferase	Jung et al. (2003)
Paralichthys olivaceus	212.00	Increase in haemoglobin, haematocrit, immature erythrocyte number, and inorganic phosphorus, and decrease in serum level of total protein, magnesium, chloride, alkaline phosphatase and lactate dehydrogenase	Jung et al. (2003)
Heteropneustes fossilis	925.0	Decrease in total erythrocyte number, haemoglobin, haematocritic and mean corpuscular haemoglobin concentration (MCHC), and increase in mean corpuscular volume (MCV), total leukocyte number and clotting time	Srivastava et al. (2009)
Sparus aurata	150.0	Increase in plasma level of glucose, cortisol, calcium, sodium, chloride and sodium	Yildiz and Ergonul (2010)
Dicentrarchus labrax	150.0	Increase in plasma level of glucose, cortisol, calcium, potassium, phosphorus, magnesium, and decrease in haematocrit	Yildiz and Ergonul (2010)

6 | ANTIPARASITIC EFFICACY OF FORMALIN IN TREATED FRESHWATER AND MARINE FISH SPECIES

Successful aquaculture depends on numerous biological and environment factors. However, prevention and control of diseases is given priority for all production types and phases. Studies on the parasites in fish farming are important in food production and industrial processes. Infestation in fish farming increases with intensification because a high density causes an increase in the parasite populations, which may cause ideal conditions for epizooties for ectoparasites with a direct life cycle. Hence, researchers and fish farmers have experimented with various chemical products and methods of application to control and treat parasites in fish farming. Rucker et al. (1963) stated that in 1909, Leger already recommended the use of a 1300.0–1480.0 mg/L of formalin in baths of 15 min to control *Ichthyobodo necator* in *O. mykiss*. Since then, formalin has been recommended for controlling ectoparasites of farmed fish (Fish, 1940 a,b; Allison, 1954).

The control of parasitic diseases in fish is important for the success of the aquaculture and aquaria industries. Nevertheless, treatment for controlling and treating ectoparasites in fish farming involves the use of chemotherapeutic agents such as formalin. Infection by ectoparasites causes significant economic losses of billions of dollars for freshwater and marine fish farming industries worldwide. Hence, formalin has been used since the beginning of the 20th century to eradicate species of ectoparasites (Fish, 1939; Rucker et al., 1963), given that these infections are considered as a major problem in fish farming (Farmer et al., 2013; Jørgensen & Buchmann, 2007; Jørgensen et al., 2009; Katharios et al., 2006; Pironet & Jones, 2000). Furthermore, severe parasitic infestations can compromise the quality of fish destined for human consumption (Pahor-Filho et al., 2012).

The following types of baths of formalin and time of exposure may be used for administering treatments against ectoparasites: (i) Dip—fish are dipped into a concentrated solution of formalin for 1 min. (ii) Short-term baths—water flow is shut off and formalin is added to the tank at high concentrations, relative to toxicity and allowed to remain up to one hour. The flow is then turned on, and the chemical flushed out (iii) Long-term baths—low concentrations of formalin are added to tanks or aquaria and allowed to dissipate naturally. (iv) Flush—the entire dose of formalin is added at the flowing inlet and allowed to spread through and out of the container. Water flow is continuous, and concentrations are about the same as for short baths. (v) Constant flow—a metering device is used to introduce concentrated formalin into the water flow to give a constant, continuous dose.

Vigorous aeration must be provided in recirculating aquaculture systems or in cultivation tanks whenever formalin is applied. When using formalin as a prolonged bath in a flow-through tank system, it is recommended that the water flow be turned off for at least 12 h or up to 24 h to ensure enough exposure time with the parasite. Moreover, water quality (e.g. ammonia, nitrite, pH, dissolved oxygen and temperature) must be optimal before stopping

TABLE 7 Management strategies of therapeutic baths with formalin to control and treatment of freshwater and marine ectoparasite species

Parasite species	Doses (mg/L)	Exposure time	Results	References
Trichodina sp.	370.0–1850.0	1–2 h	High efficacy	Fish and Burrows (1940)
Trichodina sp.	620.0–925.0	24 h	High efficacy	Fish (1940a)
Trichodina sp.	10.0–15.0	72 h	High efficacy	Allison (1957)
Trichodina sp.	610.0–970.0	0.5–1 h	High efficacy	Ceccarelli et al. (1993)
Trichodina sp.	200.0	1 h	High efficacy	Diggles (2000)
Trichodina sp.	92.5	72 h	Low efficacy	Alcântrara-Rocha et al. (1993)
Trichodina sp.	46.0–92.5	–	High efficacy	Silva et al. (2009)
Trichodina sp.	250	1h	High efficacy	Vargas et al. (2003)
Trichodina spp.	370.0–740.0	1 h	High efficacy	Balta et al. (2008)
Trichodina sp.	20.0	24 h	High efficacy	El-Deen et al. (2010)
Trichodina spp.	925.0	10 min	High efficacy	García-Magaña et al. (2019)
Trichodina sp.	150.0	1 h each 2 days	High efficacy	Xu et al. (2015)
Trichodina epizootica	629.0	1 h	High efficacy	Chmelova et al. (2016)
Trichodina jadranica	75.0	1 h	High efficacy	Madsen et al. (2000)
Trichodina fultoni and *Trichodina salmincola*	25.0	72 h	High efficacy	Aly et al. (2020)
Ichthyophthirius multifiliis	100.0	240 h	No efficacy	Farmer et al. (2013)
Ichthyophthirius multifiliis	740.0	24–120	No efficacy	Carneiro et al. (2005), Carneiro et al. (2006)
Ichthyophthirius multifiliis	370.0–740.0	1 h	High efficacy	Balta et al. (2008)
Ichthyophthirius multifiliis	37.0	72 h	No efficacy	Alcântrara-Rocha et al. (1994)
Ichthyophthirius multifiliis	15.0	–	High efficacy	Allison (1957)
Ichthyophthirius multifiliis	25.0	7 days	High efficacy	Klein et al. (2004)
Ichthyophthirius multifiliis	400.0	1–2	High efficacy	Lahnsteiner and Weismann (2007)
Ichthyophthirius multifiliis	10.0–20.0	22 days	No efficacy	Rowland et al. (2009)
Ichthyophthirius multifiliis	30.0	22 days	High efficacy	Rowland et al. (2009)
Ichthyophthirius multifiliis	25.0	21 days	High efficacy	Tieman and Goodwin (2001)
Amyloodinium ocellatum	51.0	1–7 h	High efficacy	Fajer-Ávila et al. (2003)
Cryptocaryon irritans	35–50	5 h per day	High efficacy	Rasheed (1989)
Philasterides dicentrarchi	25.0–30.0	24–72 h	High efficacy	Budiño et al. (2012)
Cichlidogyrus tubicirrus and *Cichlidogyrus* sp.	25.0	72 h	High efficacy	Aly et al. (2020)
Cichlidogyrus sp.	20.0	24 h	High efficacy	El-Deen et al. (2010)
Cichlidogyrus sp.	5500.0	40 min	High efficacy	Sandoval-Gío et al. (2019)
Gyrodactylus sp.	5.0–15.0	72 h	High efficacy	Allison (1957)
Gyrodactylus sp.	620.0–925.0	24 h	High efficacy	Fish (1940a)
Gyrodactylus sp.	50.0–250	1 h	High efficacy	Vargas et al. (2003)
Dawestrema cycloancistrium	440.0–550.0	1 h	High efficacy	Andrade-Porto et al. (2017)
Diplectanum aequans	370.0–1100.0	1 h	No efficacy	Cecchini and Cognetti-Varriale (2003)
Gyrodactylus derjavini	20.0	18 h	High efficacy	Buchmann and Kristensson (2003)
Gyrodactylus corti	150.0–200.0	5 days	High efficacy	Jones et al. (2015)
Haliotrema sp. and *Euryhaliotrema* sp.	51.0	1 h	High efficacy	Fajer-Ávila et al. (2007)
Pseudorhabdosynochus sp. and *Haliotrema* sp.	30.0	24 h	High efficacy	Zafran et al. (1998)
Haliotrema sp.	150.0	1 h	No efficacy	Pironet and Jones (2000)
Microcotyle sp.	200.0	1 h	High efficacy	Katharios et al. (2006)

(Continues)

TABLE 7 (Continued)

Parasite species	Doses (mg/L)	Exposure time	Results	References
Urocleidoides sp.	370.0	1h per 3 days	High efficacy	Paixão et al. (2013)
Pseudorhabdosynochus lantauensis	250.0	1 h	High efficacy	Cruz-Lacierda et al. (2012)
Pseudorhabdosynochus lantauensis	100.0	1 h	No efficacy	Cruz-Lacierda et al. (2012)
Onchocleidus mimus	100.0	20 h	High efficacy	Farmer et al. (2013)
Lepidotrema bidyana	150–250	30 min	High efficacy	Forwood et al. (2013)
Lepidotrema bidyana and *Gyrodactylus* sp.	30.0–40.0	120 days	High efficacy	Rowland et al. (2006)
Gyrodactylus sp.	46.0–92.5	–	High efficacy	Silva et al. (2009)
Gyrodactylus sprostonae	629.0	1 h	High efficacy	Chmelova et al. (2016)
Gyrodactyluse legans	50.0	14	High efficacy	William and Lewis (1963)
Monogenea gen. sp.	15.0	24 h	High efficacy	Fujimoto et al. (2006)
Monogenea gen. sp.	925.0	20 min	High efficacy	Sanches (2008)
Haliotrema johni	300.0	30 min	High efficacy	Liang and Leong (1992)
Polylabroides mutispinosus	400.0	25 min	High efficacy	Diggles et al. (1993)
Haliotrema abaddon	25.0	24 h	Low efficacy	Stephens et al. (2003)
Entobdella hippoglossi	200.0	160 min	No efficacy	Svendsen and Haug (1991)
Ligophorus uruguayens	135.0–540.0	1 h	High efficacy	Pahor-Filho et al. (2012)
Solostamenides platyorchis	135.0–540.0	1 h	High efficacy	Pahor-Filho et al. (2012)
Ligictaluridus floridanus	250.0	7 days	High efficacy	Rábago-Castro et al. (2014)
Neobenedenia melleni	1850.0	10 min	High efficacy	Sanches et al. (2007)
Neobenedenia melleni	250.0	1 h	High efficacy	Thoney and Hargis (1991)
Microcotyle hiatulae	250.0	1 h	No efficacy	Thoney and Hargis (1991)
Benedenia seriolae and *Zeuxapta seriolae*	250.0–400.0	1 h	High efficacy	Sharp et al. (2004)
Linguadactyloides sp.	610.0–970.0	0.5–1 h	High efficacy	Ceccarelli et al. (1993)
Linguadactyloides brinkmanni	925.0	10–15 min	High efficacy	Thatcher and Kritsky (1983)
Ichthyobodo necator	925.0	30 h	High efficacy	Fish (1940b)
Ichthyobodo necator	600.0	1 h per 9 days	High efficacy	Ostland et al. (1995)
Ichthyobodo necator	370.0–740.0	1 h	High efficacy	Balta et al. (2008)
Ichthyobodo necator	80.0	2 h	High efficacy	Jaafar et al. (2013)
Zeylanicobdella arugamensis	50.0	1 h	High efficacy	Cruz-Lacierda et al. (2000)
Myzobdella lugubris	150.0	1 h	No efficacy	Morrison et al. (1993)
Ergasilus ceylonensis	50.0	20 min	No efficacy	Wijeyaratine and Gunawardene (1988)
Caligus elongatus	10.0–250.0	0.5–18 h	No efficacy	Landsberg et al. (1991)
Argulus coregoni	0.002	16 h	No efficacy	Hakalahti-Sirén et al. (2008)
Lepeophtheirus kabatai	200.0	30 min	High efficacy	Ranjan et al. (2018)
Caecognathia coralliophila	100–200	24 h	High efficacy	Thing et al. (2016)
Caecognathia coralliophila	6.2–50.0	24 h	No efficacy	Thing et al. (2016)
Myxobolus sp.	0.004	15 min	High efficacy	Singhal et al. (1986)

water flow and beginning the therapeutic treatment. Heinen et al. (1995) reported that nitrification in the biofilter was not impaired by 1 h formalin treatments of up to 167.0 mg/L. However, indefinite treatment of 70.0 mg/L showed high nitrite levels for 9 days. Formalin remained detectable in the biofilter system for 11 h during indefinite treatment at 120 mg/L of formalin. In stable biofilters of recirculating aquaculture systems when exposed once to 9.25, 18.5, 37 or 55.5 mg/L formalin, the formalin concentration 55.5 mg/L also increased total ammonia nitrogen and nitrite nitrogen concentrations, and nitrification did not recover to pre-exposure concentrations during up to 8 days postexposure (Fredricks et al., 2018).

Formalin has been used extensively in freshwater and marine fish species to control and treat monogenean and protozoan species (Table 7). Nevertheless, the effective management of parasites often relies on the concentrations of formalin and treatment strategies.

Formalin is generally used therapeutically by fish farmers in treatment baths at 550.0–9.25 mg/L for up 1 h for consecutive days, for a maximum of three treatments (Adeyemo et al., 2012). Martins et al. (1999) recommended the application of 10 ml/m^3 of formalin against *Henneguya leporinicola* in cultivation ponds of *Leporinus macrocephalus*.

The use of low concentrations of formalin requires prolonged treatments for a satisfactory efficacy in cultivation ponds and recirculation aquaculture systems. In contrast, the use of high concentrations of formalin requires short-term baths for survival of fish and obtaining of satisfactory efficacy (Allison, 1954; Farmer et al., 2013; Fish, 1940a, 1940b; Fish & Burrows, 1940; Harms, 1996; Perera & Pathiratne, 2005; Rábago-Castro et al., 2014; Rowland et al., 2006; Stoskopf, 1988). Formalin has also been tested as a chemotherapeutic in fish farming ponds due to low cost (Jaafar et al., 2013; Jørgensen et al., 2009; Pahor-Filho et al., 2012; Rowland et al., 2006, 2009; Thorburn & Moccia, 1993; William & Lewis, 1963). For example, a treatment regime involving daily applications of formalin for controlling ichthyophthiriosis in earthen ponds had an average cost of US$ 466.37 per hectare/day (Rowland et al., 2009).

After *in vitro* exposure to 64.0 and 128.0 mg/L of formalin, the viability of *Ichthyophthirius multifiliis* stages was 0%. Formalin is currently used in trout farms at 200 mg/L for 1 h because this treatment has been effective against all free-living stages of this ectoparasite (Forwood et al., 2014). *In vitro* exposure to 25–100 mg/L of formalin was effective against *Tetrahymena pyriformis* (Gilbert et al., 1979). *In vitro* exposure to 100–200 mg/L of formalin was also effective against *Uronema nigricans* (Crosbie & Munday, 1999). Similarly, *in vitro* exposure with 200.0 mg/L for 6 or 12 h was effective against *Amyloodinium ocellatum* (Paperna, 1984). Jee et al. (2002) reported that *in vitro* exposure to 50.0 mg/L of formalin for 1.5 h was also effective against *Uronema marinum*. *In vitro* exposure of *Rhabdosynochus* sp. to 218 and 327 mg/L of formalin caused 100% of mortality within 1 h, while 110 mg/L required 150 min to eliminate all parasites (Morales-Serna et al., 2020), showing that the effect was dose dependent.

In vitro exposure with 62.0 mg/L of formalin was effective against *Philasterides dicentrarchi* (Iglesias et al., 2002). *In vitro* treatment with 300 mg/L of formalin for 30 min caused 100% of mortality in larvae and adults of *Sparicotyle chrysophrii* (Sitjà-Bobadilla et al., 2006). However, Jin et al. (2010) demonstrated that *in vitro* exposure to 50 mg/L for 3 h was moderately effective against *P. dicentrarchi*. Harikrishnan et al. (2010) also reported that 100–400 mg/L for 30 min was moderately effective against *P. dicentrarchi*. For *Dactylogyrus minutus*, 200 mg/L of formalin had a rapid *in vitro* efficacy, killing all parasites in 16 min (Tancredo, Marchiori, et al., 2019). *In vitro* exposure of *Haliotrema* sp. and *Euryhaliotrema* sp. adults to 83.0 mg/L of formalin for 1h reduced the number of parasites by 72.0%. However, formalin treatment was ineffective in preventing the hatching of monogeneans from eggs (Fajer-Ávila et al., 2007). Treatments with 200.0 mg/L of formalin for 6–35 days had no effect on the hatching of *Entobdella hippoglossi* eggs (Svendsen & Haug, 1991). Diggles et al. (1993) demonstrated that 200.0 mg/L of formalin decreased the viability of eggs and survival of oncomiracidia of *Polylabroides mutispinosus*.

The formalin EC_{50} for *Heterobothrium ecuadori* at 30 min of exposure was 225.0 mg/L, decreasing to 87.0 mg/L at 1 h of exposure and 47.0 mg/L at 105 min of exposure (Fajer-Ávila et al., 2003). Studies showed that the lethal concentrations (24-LC^{50}) of formalin for *Tetrahymena thermophila* were 37.5 mg/L, for *Tetrahymena pyriformis* was 37.5 mg/L, for *Tetrahymena* sp. was 50.0 mg/L. 24-LC_{50} of formalin, and for *Ichthyophthirius multifiliis* was 20.3 mg/L (Xu et al., 2016). Exposure of *A. gigas* to low concentration of formalin (33.0–66.0 mg/L) for 12 h showed no influence on the control of *Dawestrema cycloancistrium* in gills (Andrade-Porto et al., 2017).

The appropriate concentration of formalin for a prolonged bath is lower than 25 mg/L. Concentrations higher than 25 mg/L are appropriate for short-term baths. The lower concentration of 12.5 mg/L is recommended for more sensitive fish species or extremely sick fish that may have low tolerance to treatment using a higher concentration. The lower concentration may also be adequate for indefinite baths in cultivation ponds in some situations, but the use of formalin in ponds should be parsimonious.

7 | CONCLUSIONS

To control and treat ectoparasites in fish farming, fish producers and researchers in the past have experimented with various chemicals and methods of application. Formalin has a long history in the treatment against fish ectoparasites and is extremely effective, particularly against protozoan and monogenean species in freshwater and marine environments. Formalin is an excellent ectoparasiticide for use in small tanks and aquaria, where the depletion of dissolved oxygen can be easily avoided by using an aeration system. At lower concentrations, formalin must be applied as a prolonged bath and is put directly into the system water with the fish and left indefinitely. At higher concentrations, formalin must be applied as a short-term bath. However, caution is recommended for the successful use of formalin to control ectoparasites in fish. Furthermore, after treatment with formalin, the residues should be treated for before its discharge into the environment.

The acute toxicity of formalin varies among fish species due to some factors such as age and species. However, studies in marine fish species need to be carried out given the lack of information regarding their tolerance to treatment with formalin. Erythrocytic parameters of fish as a response to exposure of formalin occur with changes in the oxygen consumed by the organism and possibly the activation of haematopoietic organs. Formalin has toxic effects on homeostasis and immune system of exposed fish. Innate immunity plays a role in the defence against ectoparasites in farmed fish as well.

Caution must be taken when applying formalin in high temperatures to avoid increasing the toxicity, which may disrupt homeostasis

in fish. Therefore, careful measures of this water quality parameter in cultivation tanks must be taken to ensure fish survival when using this therapeutic. Further investigations are required to optimize treatments and to establish protocols to minimize the quantity of formalin applied while ensuring the best efficacy. More research is needed regarding the pharmacological aspects of formalin and management strategies, including the use of safe interventions focusing on the removal of all phases of ectoparasites in cultivation systems. The use of such strategies may provide more environmentally friendly alternatives when using formalin for the control ectoparasitic infections.

We are unable to suggest definitively that applications of formalin in freshwater aquaculture, fish mariculture and aquarium systems must be stopped under any circumstances, despite having negative impacts on the environment or presenting potential risks to food security. Formaldehyde has been classified as a human carcinogen, because the exposure to this chemical can lead to the development of human haematopoietic cancers, such as leukaemia. However, there appears to be no valid reasons for prohibiting the use of formalin in aquaculture when following basic precautions. Nevertheless, applications of formalin in fish farming should be made only when strictly necessary.

ACKNOWLEDGMENTS

The authors thank the National Council for Scientific and Technological Development (CNPq, Brazil) for the research grant awarded to Dr. M. Tavares-Dias (Grant 303013/2015-0).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Marcos Tavares-Dias https://orcid.org/0000-0002-8376-1846

REFERENCES

Adeyemo, O. K., Akano, O. F., & Emikpe, B. O. (2012). Effects of formalin on spawning success and organ histology in *Clarias gariepinus*. *Research Journal of Environmental Toxicology*, *6*, 42–50.

Afaghi, A., & Zare, S. (2020). Effects of exposure to sub-lethal concentrations of copper on hematological and histopathological alterations in common carp, *Cyprinus Carpio*. *Archives of Advances in Biosciences*, *11*(1), 26–36. https://doi.org/10.22037/aab.v11i1.28891

Akpoilih, B. U., & Adebayo, O. T. (2010). Effect of formalin on the hatching rate of eggs and survival of larvae of the African catish (*Clarias gariepinus*). *Journal of Applied Science and Environmental Management*, *14*, 31–34.

Alcântrara-Rocha, R. C. G., Ceccarelli, P. S., Melo, J. S. C., & Souza-Filho, V. M. (1993). Eficiência de produtos químicos no combate as infestações do parasite Trichodina sp. em lambari, Astyanax bimaculatus (Lenneus, 1758). *Boletim Técnico do Cepta*, *6*(2), 31–39.

Alcântrara-Rocha, R. C. G., Ceccarelli, P. S., Santos-Neto, J. C., Rodrigues, A., Cervi, R. C., & Ribeiro, P. (1994). Eficácia de diferentes produtos químicos no controle de *Ichthyophthirius multifiliis* Fouquet (1876) em alevinos de pacu *Piaractus mesopotamis* Holmberg (1887). *Boletim Técnico do Cepta*, *7*, 1–8.

Allison, L. N. (1954). Advancements in prevention and treatment of parasitic diseases of fish. *Transactions of the American Fisheries Society*, *83*(1), 221–228.

Allison, R. (1957). Some new results in the treatment of ponds to control some external parasites of fish. *The Progressive Fish-Culturist*, *19*(2), 58–63.

Aly, S., Fathi, M., Youssef, E. M., & Mabrok, M. (2020). Trichodinids and monogeneans infestation among Nile tilapia hatcheries in Egypt: prevalence, therapeutic and prophylactic treatments. *Aquaculture International*, *28*(4), 1459–1471. https://doi.org/10.1007/s10499-020-00537-w

Andem, A. B., Esenowo, I. K., Ibor, O. R., & Abosi, A. (2015). Toxicological effects of formaldehyde concentrations on African catfish, *Clarias gariepinus* fingerlings. *International Journal of Fisheries and Aquatic Studies*, *2*(5), 75–79.

Andrade, R. L. B., Andrade, L. S., Boscolo, W. R., & Soares, C. M. (2005). Comportamento, sobrevivência e desenvolvimento de lebistes, *Poecillia reticulata*, submetidos a agentes utilizados na profilaxia de doenças. *Acta Scientiarum Animal Sciences*, *27*, 523–528.

Andrade-Porto, S. M., Affonso, E. G., Kochhann, G., Malta, J. C. O., Roque, R., Ono, E. A., Araújo, C. S. O., & Tavares-Dias, M. (2017). Antiparasitic efficacy and blood effects of formalin on *Arapaima gigas* (Pisces: Arapaimidae). *Aquaculture*, *479*, 38–44. https://doi.org/10.1016/j.aquaculture.2017.05.009

Andrade-Porto, S. M., Ramos, C. A., Tavares-Dias, M., Roque, R., Barcellos, J. F. M., Souza, H. E. M., Araújo, C. S. O., & Malta, J. C. O. (2018). Histopathological evaluation of formalin toxicity in *Arapaima gigas* (Arapaimidae), the giant fish from Amazon. *Pesquisa Veterinária Brasileira*, *38*(6), 1015–1025. https://doi.org/10.1590/1678-5150-PVB-5106

Araújo, L. D., Chagas, E. C., Gomes, L. C., & Brandão, F. R. (2004). Efeito de banhos terapêuticos com formalina sobre indicadores de estresse em tambaqui. *Pesquisa Agropecuária Brasileira*, *39*(3), 217–221.

Ayuba, V. O., Iyakwari, S. P., & Oyeniyi, M. E. (2013). Acute toxicity of formalin on *Clarias gariepinus* Juveniles. *Production Agriculture and Technology Journal*, *9*(1), 21–28.

Balamurugan, S., Deivasigamani, B., Kumaran, S., Kumaran, M., Rajsekar, T., & Priyadharsini, P. (2012). Melanomacrophage centers aggregation in *P. lineatus* spleen as bioindicators of environmental change. *Asian Pacific Journal of Tropical Disease*, S635–S638.

Balta, F., Kayis, S., & Altinok, I. (2008). External protozoan parasites in three trout species in the eastern Black Sea region of the Turkey: Intensity, seasonality, and their treatments. *Bulletin of the European Association of Fish Pathologists*, *28*(4), 157–162.

Barton, B. A. (2002). Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, *42*, 517–525.

Basirun, A. A., Ahmad, S. A., Yasid, N. A., Sabullah, M. K., Daud, H. M., Sha'arani, S., Khalid, A., & Shukor, M. Y. (2019). Toxicological effects and behavioural and biochemical responses of *Oreochromis mossambicus* gills and its cholinesterase to copper: a biomarker application. *International Journal of Environmental Science and Technology*, *16*(2), 887–898. https://doi.org/10.1007/s13762-018-1711-1

Beevi, R. M., & Radhakrishnan, S. (1987). Haematological effects of sublethal concentration of formalin on *Sarotherodon mossambicus* (Peter). *Indian Academy of Science (Animal Sciences)*, *96*(6), 721–725.

Bills, T. D., Marking, L. L., & Chandler, J. H. (1977). Formalin: its toxicity to non-target aquatic organisms, persistence, and counteraction. *Investigations in Fish Control*, *73*, 1–7.

Birdsong, C. L., & Avault, W. Jr (1971). Toxicity of certain chemicals to juvenile pompano. *The Progressive Fish-Culturist*, *33*(2), 76–80.

Bodensteiner, L. R., Sheehan, R. J., Lewis, W. M., Wills, P. S., & Herman, L. (1993). Effects of repetitive formalin treatments on channel catfish

juveniles. *Journal of Aquatic Animal Health*, *5*(1), 59–63. https://doi.org/10.1577/1548-8667(1993)005<0059:EORFTO>2.3.CO;2

Buchmann, K., & Kristensson, R. T. (2003). Efficacy of sodium percarbonate and formaldehyde bath treatments against *Gyrodactylus derjavini* infestations of rainbow trout. *North American Journal of Aquaculture*, *65*, 25–27.

Budiño, B., Pata, M. P., Leiro, J., & Lamas, J. (2012). Differences in the *in vitro* susceptibility to resveratrol and other chemical compounds among several *Philasterides dicentrarchi* isolates from turbot. *Parasitology Research*, *110*, 1573–1578. https://doi.org/10.1007/s00436-011-2664-1

Bulut, C., Kubilay, A., Bektaş, Z. H., & Birden, B. (2015). Histopathological effects of formaldehyde (CH_2O) on rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). *Journal of Limnology and Freshwater Fisheries Research*, *1*(1), 43–48.

Carmichael, G. J., & Tomasso, J. R. (1983). Use of formalin to separate tadpoles from largemouth bass fingerlings after harvesting. *The Progressive Fish-Culturist*, *45*(2), 105–106.

Carneiro, P. C. F., Cirio, S. M., & Schorer, M. (2006). Estudo anatomopatológico de alevinos de jundiá infectados experimentalmente por *Ichthyophtirius multifiliis* e submetidos a tratamentos convencionais. *Archives of Veterinary Science*, *11*(1), 33–38.

Carneiro, P. C. F., Schorer, M., & Mikos, J. D. (2005). Tratamentos terapêuticos convencionais no controle do ectoparasita *Ichthyophthirius multifiliis* em jundiá (*Rhamdia quelen*). *Pesquisa Agropecuária Brasileira*, *40*(1), 99–102.

Ceccarelli, P.S., Alcântrara-Rocha, R.C.G., & Melo, J.S.C. (1993). Efeitos do formaldeído sobre a Trichodina sp. e Linguadactyloides sp. em alevinos de pacu Piaractus mesopotamis Holmberg (1887). Boletim Técnico do Cepta, 6(2), 23–30.

Cecchini, S., & Cognetti-Varriale, A. M. (2003). Dehydration is more effective for the control of embryonic development and larval hatching of *Diplectanum aequans* (Monogenea, Diplectanidae) than formalin and trichlorfon. *Aquaculture International*, *11*, 261–265.

Chinabut, S., Limsuwan, C., Tonguthai, K., & Pungkachonboon, T. (1988). *Toxic and sublethal effect of formalin on freshwater fishes*. http://www.fao.org/3/AC278E/AC278E00.htm

Chmelova, L., Sehonova, P., Doubkova, V., Plhalova, L., Blahova, J., Tichy, F., Modra, H., & Svobodova, Z. (2016). Effects of therapeutic formalin bath on selected indices in fish. *Neuroendocrinology Letters*, *37*(1), 67–77.

Cho, J. K., Chun, M. N., & Yang, H. C. (1997). Histopathological response of flounder, *Paralichthys olivaceus*, treated with formalin and neutral-formalin. *Journal of Fish Pathology*, *10*(1), 53–63.

Clemens, H. P., & Sneed, K. E. (1958). The chemical control of some diseases and parasites of channel catfish. *The Progressive Fish-Culturist*, *20*(1), 8–15.

Crosbie, P. B. B., & Munday, B. L. (1999). Environmental factors and chemical agents affecting the growth of the pathogenic marine ciliate *Uronema nigricans*. *Diseases of Aquatic Organisms*, *36*, 213–219.

Cruz, C., Fujimoto, R. Y., Luz, R. K., Portella, M. C., & Martins, M. L. (2005). Toxicidade aguda e histopatologia do fígado de larvas de trairão (*Hoplias lacerdae*) expostas à solução aquosa de formaldeído a 10%. *Pesticidas: Ecotoxicologia E Meio Ambiente*, *15*, 21–28.

Cruz, E. R., & Pitogo, C. L. (1989). Tolerance level and histopathological response of milkfish (*Chanos chanos*) fingerlings to formalin. *Aquaculture*, *78*(2), 135–145. https://doi.org/10.1016/0044-8486(89)90027-6.

Cruz-Lacierda, E. R., Pineda, A. J. T., & Nagasawa, K. (2012). *In vivo* treatment of the gill monogenean *Pseudorhabdosynochus lantauensis* (Monogenea, Diplectanidae) on orange-spotted grouper (*Epinephelus coioides*) cultured in the Philippines. *Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society*, *5*(5), 330–336.

Cruz-Lacierda, E. R., Toledo, J. D., Tan-Fermin, J. D., & Burreson, E. M. (2000). Marine leech (*Zeylanicobdella arugamensis*) infestation in cultured orange-spotted grouper, *Epinephelus coioides*. *Aquaculture*, *185*, 191–196.

Dias, H. M., Santos, R. F. B., Pereira, N. L., Sousa, N. C., Couto, M. V. S., Abe, H. A., & Fujimoto, R. Y. (2018). Toxicological effects of four chemicals used for prophylaxis of Amazonian ornamental fish. *Biota Amazônia*, *8*(4), 13–16. https://doi.org/10.18561/2179-5746/biotaamazonia.v8n4p13-16

Diggles, B. K. (2000). Chemotherapy of the ciliate *Trichodina* sp. on juvenile turbot {*Colistium nudipinnis*) with notes on the susceptibility of fish with abnormal pigmentation. *New Zealand Journal of Marine and Freshwater Research*, *34*, 645–652.

Diggles, B. K., Roubal, F. R., & Lester, R. J. G. (1993). The influence of formalin, benzocaine and hyposalinity on the fecundity and viability of *Polylabroides mutispinosus* (Monogenea: Microcotylidae) parasitic on the gills of *Acanthopagrus australis* (Pisces: Sparidae). *International Journal for Parasitology*, *23*(7), 877–884.

Domitrovic, H. A. (1997). El exempleo de peces autóctonos para la realización de ensayos de toxicidade: evaluación de la especie *Aequidens portalegrensis* (Hensel, 1970). *Revista De Ictiologia*, *5*(1), 37–42.

Dureza, L. A. (1995). Toxicity of formalin and potassium permanganate to *Oreochromis niloticus* fry and fingerlings and subsequent gill pathology. *Research Digest*, *1*(2), 357–358.

El-Deen, N. A. E., Ismaiel, M. M., Mohamed, A. E., & El-Ghany, O. A. A. (2010). Comparative studies on the impact of humic acid and formalin on ectoparasitic infestation in Nile tilapia *Oreochromis niloticus*. *Nature and Science*, *8*(2), 121–125.

El-Gawad, E. A., Shen, Z. G., & Wang, H. P. (2015). Efficacy of formalin, iodine and sodium chloride in improvement of egg hatching rate and fry survival prior to the onset of exogenous feeding in yellow perch. *Aquaculture Research*, *47*(8), 2461–2469. https://doi.org/10.1111/are.12694

Fajer-Ávila, E., Farra, I. A., Aguilar-Zarate, G., Contreras-Arce, R., Zaldivar-Ramirez, J., & Betancourt-Lozano, M. (2003). Toxicity of formalin to bullseye puffer fish (*Shoeroides annulatus* Jenyna 1843) and its effectiveness to control ectoparasites. *Aquaculture*, *223*, 41–50.

Fajer-Ávila, E. J., Velásquez-Medina, S. P., & Betancourt-Lozano, M. (2007). Effectiveness of treatments against eggs, and adults of *Haliotrema* sp. and *Euryhaliotrema* sp. (Monogenea: Ancyrocephalinae) infecting red snapper, *Lutjanus guttatus*. *Aquaculture*, *264*(1–4), 66–72. https://doi.org/10.1016/j.aquaculture.2006.12.035

Farmer, B. D., Fuller, S. A., Mitchell, A. J., & Straus, D. L. (2013). Efficacy of bath treatments of formalin and copper sulfate on cultured white bass, *Morone chrysops*, concurrently infected by *Onchocleidus mimus* and *Ichthyophthirius multifiliis*. *Journal of the World Aquaculture Society*, *44*(2), 305–310.

Fish, F. F. (1939). Simplified methods for the prolonged treatment of fish diseases. *Transactions of the American Fisheries Society*, *68*(1), 178–187. 10.1577/1548-8659(1938)68[178:SMFTPT]2.0.CO;2

Fish, F. F. (1940a). Formalin treatments pass new tests. *The Progressive Fish-Culturist*, *7*, 31–32. 10.1577/1548-8640(1940)7[31:FTPNT]2.0.CO;2

Fish, F. F. (1940b). Formalin for external protozoan parasites. *The Progressive Fish-Culturist*, *7*, 1–10. 10.1577/1548-8640(1940)7[1:FFEPP]2.0.CO;2

Fish, F. F., & Burrows, R. E. (1940). Experiments upon the control of trichodiniasis of salmonid fishes by the prolonged recirculation of formalin solutions. *Transactions of the American Fisheries Society*, *69*(1), 94–100. 10.1577/1548-8659(1939)69[94: EUTCOT]2.0.CO

Forwood, J. M., Harris, J. O., & Deveney, M. R. (2013). Efficacy of current and alternative bath treatments for *Lepidotrema bidyana* infecting silver perch, *Bidyanus bidyanus*. *Aquaculture*, *416–417*, 65–71. https://doi.org/10.1016/j.aquaculture.2013.08.034

Forwood, J. M., Harris, J. O., Landos, M., & Deveney, M. R. (2014). Minimum effective concentrations of formalin and sodium percarbonate on the free-living stages of an Australian isolate of

Ichthyophthirius multifiliis. *Parasitology Research*, *113*, 3251–3258. https://doi.org/10.1007/s00436-014-3987

Fox, C. H., Johnson, F. B., Whiting, J., & Roller, P. P. (1985). Formaldehyde fixation. *Journal of Histochemistry & Cytochemistry*, *33*(8), 845–853.

Fredricks, K. T., Cupp, A. R., Schleis, S. M., Erickson, R. A., & Gaikowski, M. P. (2018). Effects of formaldehyde on nitrification in biofilters of small - scale recirculating systems. *Aquaculture Research*, *49*, 3207–3217.

Fujimoto, R. Y., Vendruscolo, L., Schalch, S. H. C., & Moraes, F. R. (2006). Avaliação de três diferentes métodos para o controle de monogenéticos e Capillaria sp. (Nematoda: Capillariidae) parasitos de acará-bandeira Pterophyllum scalare Liechtenstein, 1823. *Boletim do Instituto De Pesca*, *32*(2), 183–190.

García-Magaña, L., Rodríguez-Santiago, M. A., Grano-Maldonado, M. I., Laura Jiménez-Vasconcelos, L., & Guerra-Santos, J. (2019). The effectiveness of sodium chloride and formalin in trichodiniasis of farmed freshwater tilapia *Oreochromis niloticus* (Linnaeus, 1758) in Southeastern Mexico. *Latin American Journal of Aquatic Research*, *47*(1), 164–174. https://doi.org/10.3856/vol47-issue1-fulltext-18

Ghelichpour, M., & Eagderi, S. (2012). Effect of formalin treatment on saltwater tolerance in Caspian roach (*Rutilus rutilus caspicus*). *International Research Journal of Applied and Basic Sciences*, *3*(5), 1027–1031.

Ghelichpour, M., Rajabiesterabadi, H., & Hoseini, S. M. (2016). Gill histopathological characteristics of Caspian roach (*Rutilus rutilus caspicus*) fingerlings treated with potassium permanganate and formalin. *Aquaculture Research*, *47*(1), 276–282. https://doi.org/10.1111/are.12489

Gilbert, J. P., Gratzek, J. B., & Brown, J. (1979). An *in vitro* method for testing synergistic action of parasiticides using malachite green and formalin as a model system. *Journal of Fish Diseases*, *2*, 191–196.

Hakalahti-Sirén, T., Mikheev, V. N., & Valtonen, E. T. (2008). Control of freshwater fish louse *Argulus coregoni*: a step towards an integrated management strategy. *Diseases of Aquatic Organisms*, *82*, 67–77. https://doi.org/10.3354/dao01971

Harikrishnan, R., Jin, C. N., Kim, M. C., Kim, J. S., Balasundaram, C., & Heo, M. S. (2010). Effectiveness and immunomodulation of chemotherapeutants against scuticociliate *Philasterides dicentrarchi* in olive flounder. *Experimental Parasitology*, *124*, 306–314.

Harms, C. A. (1996). Treatments for parasitic diseases of aquarium and ornamental fish. *Seminars in Avian and Exotic Pet Medicine*, *5*(2), 54–63.

Heinen, J. M., Weber, A. L., Noble, A. C., & Morton, J. D. (1995). Tolerance to formalin by a fluidized-bed biofilter and rainbow trout *Oncorhynchus mykiss* in a recirculating culture system. *Journal of the World Aquaculture Society*, *26*(1), 65–71.

Hinton, M. J., & Eversole, A. G. (1980). Toxicity and tolerance studies with yellow phase eels: Five chemicals. *The Progressive Fish-Culturist*, *42*(4), 201–203.

Hoai, T. D. (2020). Reproductive strategies of parasitic flatworms (Platyhelminthes, Monogenea): the impact on parasite management in aquaculture. *Aquaculture International*, *28*, 421–447. https://doi.org/10.1007/s10499-019-00471-6.

Hodkovicova, N., Chmelova, L., Sehonova, P., Blahova, J., Veronika Doubkova, V., Plhalova, L., Fiorino, E., Vojtek, L., Vicenova, M., Siroka, Z., Enevova, V., Dobsikova, R., Faldyna, M., Svobodova, Z., & Faggio, C. (2019). The effects of a therapeutic formalin bath on selected immunological and oxidative stress parameters in common carp (*Cyprinus carpio*). *Science of the Total Environment*, *653*, 1120–1127.

Holladay, S. D., Smith, B. J., & Gogal, R. M. (2010). Exposure to formaldehyde at therapeutic levels decreases peripheral blood lymphocytes and hematopoietic progenitors in the pronephros of tilapia *Oreochromis niloticus*. *Aquatic Biology*, *10*, 241–247.

Hoseini, S. M., & Nodeh, A. J. (2013). Susceptibility of Caspian roach *(Rutilus rutilus caspicus)* fingerlings to formalin. *Journal of Applied Aquaculture*, *25*(3), 191–197. https://doi.org/10.1080/10454438.2013.763516

Hoseini, S. M., Nodeh, A. J., & Hossein, S. A. (2013). Formalin toxicity to Caspian roach. *Rutilus Rutilus Caspicus Fry. Ecopersia*, *1*(2), 199–206.

Hoseini, S. M., & Tarkhani, R. (2013). Serum biochemical characteristics of *Carassius auratus* (L) following short-term formalin or NaCl treatment. *International Journal of Aquatic Biology*, *1*, 14–21.

Howe, G. E., Marking, L. L., Bills, T. D., & Schreier, T. M. (1995). Efficacy and toxicity of formalin solutions containing paraformaldehyde for fish and egg treatments. *The Progressive Fish-Culturist*, *57*(2), 147–152.

Hu, J., Le, Q., Wang, Y., Yu, N., Cao, X., Kuang, S., Zhang, M., Gu, W., Sun, Y., Yang, Y., Xu, S., & Ya, X. (2019). Effects of formaldehyde on detoxification and immune responses in silver pomfret (*Pampus argenteus*). *Fish and Shellfish Immunology*, *88*, 449–457. https://doi.org/10.1016/j.fsi.2019.03.019.

Hung, Y. W., Lin, Y. H., Huang, H. Y., Wang, W. S., Chiu, C. F., Chiu, C. C., Chiu, H. W., Tsai, W. H., & Hung, S. W. (2019). Acute toxicity and drug residue analysis of formalin in two cold water fishes: cultured hybrid bester sturgeon (*Huso huso* × *Acipenser ruthenus*) and rainbow trout (*Oncorhynchus mykiss*). *United Journal of Biomedical Engineering and Science*, *1*(1), 1–10.

Iglesias, R., Parama, A., Alvarez, M. F., Leiro, J., & Sanmartin, M. L. (2002). Antiprotozoals effective *in vitro* against the scuticociliate fish pathogen *Philasterides dicentrarchi*. *Disease of Aquatic Organisms*, *49*, 191–197.

Intorre, L., Meucci, V., Di Bello, D., Monni, G., Soldani, G., & Pretti, C. (2007). Tolerance of benzalkonium chloride, formalin, malachite green, and potassium permanganate in goldfish and zebrafish. *Journal of the American Veterinary Medical Association*, *231*(4), 590–595.

Ispir, U., Kirici, M., Yonar, M. E., & Yonar Mise, S. (2017). Response of antioxidant system to formalin in the whole body of rainbow trout, *Oncorhynchus mykiss*. *Cellular and Molecular Biology*, *63*, 13–16.

Jaafar, R. J., Kuhn, J. A., Chettri, J. K., & Buchmann, K. (2013). Comparative efficacies of sodium percarbonate, peracetic acid, and formaldehyde for control of Ichthyobodo necator—an ectoparasitic flagellate from rainbow trout. *Acta Ichthyologica Et Piscatoria*, *43*(2), 139–143.

Jee, B. Y., Jo, M. R., Kim, J. W., & Park, M. S. (2002). In vitro efficacy of formalin, hydrogen peroxide and copper sulfate on the scuticocilliate *Uronema marinum* at low salinity. *Journal of Fish Pathology*, *15*(3), 111–115.

Jegede, T. (2013). Histological alterations in organs of African giant catfish (*Heterobranchus bidorsalis*) fingerlings exposed to copper sulphate. *Journal of Agricultural Science*, *5*(3), 254–260.

Jimmy, U. U., Tsukwa, K., & Ayuba, V. O. (2013). *Effect of subletal concentration of formalin on the growth of Clarias gariepinus fingerlings*. Proceedings of 28th Fison Annual Conference, Nov. 25-30, 176–178.

Jimoh, J. O., Akinsorotan, A. M., Olasunkanmi, J. B., Omobepade, B. P., Babalola, T. O., & Fatoyinbo, O. V. (2020). Effects of prophylactics on growth, and hematology of African catfish, *Clarias gariepinus* (Burchell, 1822) fingerlings. *Egyptian Journal of Aquatic Biology & Fisheries*, *24*(1), 471–481.

Jin, C. N., Harikrishnan, R. H., Moon, Y. G., Kim, M. C., Kim, J. S., Balasundaram, C., & Heo, M. S. (2010). Effectiveness of chemotherapeutants against scuticociliate *Philasterides dicentrarchi*, a parasite of olive flounder. *Veterinary Parasitology*, *168*, 19–24.

Jones, S. R. M., Thoney, D. A., & Balfry, S. K. (2015). Comparative efficacy of formalin, freshwater and hydrogen peroxide against *Gyrodactylus corti* infestations on captive wolf-eels (*Anarrhichthys ocellatus*). *Bulletin European Associated Fish Pathology*, *35*(2), 64–72.

Jørgensen, T. R., & Buchmann, K. (2007). Stress response in rainbow trout during infection with *Ichthyophthirius multifiliis* and formalin bath treatment. *Acta Ichthyologica Et Piscatoria*, *37*(1), 25–28.

Jørgensen, T. R., Larsen, T. B., & Buchmann, K. (2009). Parasite infections in recirculated rainbow trout (*Oncorhynchus mykiss*) farms. *Aquaculture*, *289*, 91–94.

Jung, S. H., & Kim, J. W. (1998). The lethal concentration (LC_{50}) and lethal time (LT_{50}) of formalin on olive flounder fingerlings, *Paralichthy olivaceus*. *Bulletin of National Fisheries Research and Development Institute*, *54*, 125–129.

Jung, S. H., Sim, D. S., Park, M. S., Jo, Q. T., & Kim, Y. (2003). Effects of formalin on haematological and blood chemistry in olive flounder, *Paralichthys olivaceus* (Temminck et Schlegel). *Aquaculture Research*, *34*(14), 1269–1275. https://doi.org/10.1046/j.1365-2109.2003.00936.x

Kakuta, I., Namba, K., Uematsu, K., & Muraciu, S. (1991). Physiological response of the fish, *Cyprinus carpio*, to formalin exposure. I. effects of formalin on urine flow, heart rate, respiration. *Comparative Biochemistry Physiology*, *100C*(3), 405–411.

Katharios, P., Papandroulakis, N., & Divanach, P. (2006). Treatment of *Microcotyle* sp. (Monogenea) on the gills of cage-cultured red porgy, *Pagrus pagrus* following baths with formalin and mebendazole. *Aquaculture*, *251*, 167–171.

Klein, S., Feiden, A., Boscolo, W. R., Reidel, A., Signor, A., & Signor, A. A. (2004). Utilização de produtos químicos no controle de Ichthyophthirius multifiliis, Fouquet (1876) em alevinos de surubim do Iguaçu Steindachneridion sp., Garavello (1991). *Semina: Ciências Agrárias*, *25*(1), 51–58.

Knight, S. J., Boles, L., & Stamper, M. A. (2016). Response of recirculating saltwater aquariums to long-term formalin treatment. *Journal of Zoo and Aquarium Research*, *4*(2), 77–84.

Kouril, J., & Prikryl, I. (1988). Sensitivity of the larvae of tench (*Tinca tinca* L.) to short-term baths in NaCl and formalin solutions as depending on the time of exposure (in Czech). *Research Institute of Fish Culture and Hydrobiology at Vodnany*, *17*, 108–115.

Kumar, M., & Ram, M. (2015). Effect of copper and zinc on oxygen consumption of the fresh water fish, *Clarias batrachus* (Linn.). *Journal of Entomology and Zoology Studies*, *3*(6), 46–50.

Lahnsteiner, F., & Weismann, T. (2007). Treatment of Ichthyophthiriasis in rainbow trout and common carp with common and alternative therapeutics. *Journal of Aquatic Animal Health*, *19*(3), 186–194.

Lai, Y., Yu, R., Hartwell, H. J., Moeller, B. C., Bodnar, W. M., & Swenberg, J. A. (2016). Measurement of endogenous versus exogenous formaldehyde–induced DNA–protein crosslinks in animal tissues by stable isotope labeling and ultrasensitive mass spectrometry. *Cancer Research*, *76*(9), 2652–2661. https://doi.org/10.1158/0008-5472.CAN-15-2527

Landsberg, J. H., Vermeer, G. K., Richards, S. A., & Perry, N. (1991). Control of the parasitic copepod *Caligus elongatus* on pond-reared red drum. *Journal of Aquatic Animal Health*, *3*(3), 206–209. https://doi.org/10.1577/1548-8667(1991)003<0206:COTPCC>2.3.CO;2

Latif, A., Ali, M., Sayyed, A. H., Igbal, F., Usman, K., Rauf, M., & Kaoser, R. (2013). Effect of copper sulphate and lead nitrate, administered alone or in combination, on the histology of liver and kidney of *Labeo rohita*. *Pakistan Journal of Zoology*, *45*(4), 913–920.

Leteux, F., & Meyer, F. P. (1972). Mixtures of malachite green and formalin for controlling ichthyophthirius and other protozoan parasites of fish. *The Progressive Fish-Culturist*, *34*(1), 21–26.

Liang, K. S., & Leong, T. S. (1992). Treatment of cultured golden snapper, *Lutjanus johni* Bloch, infected with monogeneans. *Aquaculture*, *106*(1), 1–8. https://doi.org/10.1016/0044-8486(92)90244-f

Madsen, H. C. K., Buchmann, K., & Mellergaard, S. (2000). Treatment of trichodiniasis in eel (*Anguilla anguilla*) reared in recirculation systems in Denmark: Alternatives to formaldehyde. *Aquaculture*, *186*, 221–231.

Marking, L. L., King, E. L., & Chandler, J. H. Jr (1972). *Section of toxicology. Quarterly report of progress for July-September 1972*. Fish control laboratory, La Crosse, Wisconcin, pp. 3–8.

Martins, M. L., Souza, V. N., Moraes, J. R. E., & Moraes, F. R. (1999). Gill infection of Leporinus macrocephalus Garavello and Britski, 1988 (Osteichthyes: Anostomidae) by Henneguya leporinicola n. sp. (Myxozoa: Myxobolidae). Description, histopathology and treatment. *Revista Brasileira De Biologia*, *59*(3), 527–534.

Mbaru, E. K., Mkare, T. K., & Joseph, O. R. (2011). Tolerance of yolk sac and free swimming fry of African catfish (*Clarias gariepinus*, Burchell 1822) to chemotherapeutic doses of formalin. *African Journal of Agricultural Research*, *6*(2), 323–330.

McDonnell, G., & Russell, A. D. (1999). Antiseptics and disinfectants: activity, action, and resistance. *Clinical Microbiology Reviews*, *12*(1), 147–179.

Meinelt, T., Pietrock, M., Burnison, K., & Steinberg, C. (2005). Formaldehyde toxicity is altered by calcium and organic matter. *Journal of Applied Ichthyology*, *21*, 121–124.

Mert, R., Karasu Benli, A. Ç., & Arslan, G. (2015). Determination of histological and genotoxic effects of formalin on Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research*, *46*(11), 2798–2807. https://doi.org/10.1111/are.12434

Mohammed, S. V. N., Sheriff, M. A., Mohideen, A. K. S., & Azmathullah, N. M. (2012). Toxicity of formalin on behaviour and respiration in *Danio rerio*. *International Journal of Environmental Sciences*, *2*(4), 1904–1908.

Morales-Serna, F. N., López-Moreno, D. G., Medina-Guerrero, R. M., Abad-Rosales, S. M., Martínez-Brown, J. M., Leonardo Ibarra-Castro, L., & Fajer-Avila, E. J. (2020). Toxicity of formalin for juvenile Centropomus viridis and in vitro efficacy against the parasite Rhabdosynochus sp. (Monogenea: Diplectanidae). *Journal of Applied Ichthyology*, *26*(5), 740–744. https://doi.org/10.1111/jai.14077

Morrison, J. R., Fox, S. R., & Rogers, W. A. (1993). Control of an infestation of a fish leech (*Myzobdella lugubris*) on catfishes in tanks and earthen ponds. *Journal of Aquatic Animal Health*, *5*(2), 110–114.

Nam, Y. E., & Heo, G. J. (2004). Acute and subacute toxicity of formalin in blacktetra (*Gynnocorymbus ternetzi*). *Journal of Veterinary Medice and Biotecnology*, *5*(2), 45–52.

Nasser, N., Babikian, J. M., & Saoud, I. P. (2017). Toxicity of four chemotherapeutic agents to rabbitfish *Siganus rivulatus*. *Journal of the World Aquaculture Society*, *48*(6), 877–886.

Neves, L. C., Miranda Filho, K. C., Lorenzini, J. P. S., Nakayama, C. L., & Luz, R. K. (2020). Effect of formalin in early stages of the freshwater neotropical catfish, *Lophiosilurus alexandri*. *Environmental Toxicology and Pharmacology*, *73*, 103277. https://doi.org/10.1016/j.etap.2019.103277

Nieminen, M., Pasanen, P., & Laitinen, M. (1983). Effects of formalin treatment on the blood composition of salmon (*Salmo salar*) and rainbow trout (*Salmo gairdneri*). *Comparative Biochemistry and Physiology C*, *76*(2), 265–269.

Nouh, W. G., & Selim, A. G. (2013). Toxopathological studies on the effect of formalin and copper sulphate in tilapia as a commonly used disinfectant in aquaculture. *Journal of Applied Environmental and Biological Sciences*, *3*(6), 7–20.

Okomoda, J., Ayuba, V. O., & Omeji, S. (2010). Haematological changes of *Clarias gariepinus* (Burchell, 1822) fingerlings exposed to acute toxicity of formalin. *Production Agriculture and Technology Journal*, *6*(1), 92–101.

Omoregie, E., Eseyin, T. G., & Ofojekwu, P. C. (1994). Chronic effects of formalin on erythrocyte counts and plasma glucose of Nile tilapia, *Oreochromis niloticus*. *Asian Fisheries Science*, *7*, 1–6.

Omoregie, E., Eseyin, T. G., Ofojekwu, P. C., & Amali, E. I. (1998). Effects of sublethal concentrations of formalin on weight gain in Nile Tilapia *Oreochromis niloticus* (Trewavas). *Asian Fisheries Society*, *10*, 323–327.

Ostland, V. E., Byrne, P. J., Speare, D. J., Thorburn, M. A., Cook, A., Morrison, D., & Ferguson, H. W. (1995). Comparison of formalin and chloramine-t for control of a mixed gill infection (bacterial gill disease and ichthyobodiasis) in rainbow trout. *Journal of Aquatic*

Animal Health, *7*(2), 118–123. https://doi.org/10.1577/1548-8667(1995)007<0118:COFACT>2.3.CO;2

Pahor-Filho, E., Filho, K. C. M., Klosterhoff, M., Romano, L. A., & Pereira-Júnior, J. (2015). Histopathological and behaviour effects of formaldehyde treatment in juvenile mullet, *Mugil liza* (Valenciennes). *Aquaculture Reseach*, *46*, 3040–3045.

Pahor-Filho, E., Miranda-Filho, K. C., & Pereira-Júnior, J. (2012). Parasitology of juvenile mullet (*Mugil liza*) and effect of formaldehyde on parasites and host. *Aquaculture*, *354*, 111–116.

Paixão, L. F., Santos, R. F. B., Ramos, F. M., & Fujimoto, R. Y. (2013). Efeitos do tratamento com formalina e sulfato de cobre sobre os parâmetros hematológicos e parasitos monogenéticos em juvenis de *Hemigrammus* sp. (Osteichthyes: Characidae). *Acta Amazonica*, *43*, 211–216.

Paperna, I. (1984). Chemical control of Amyloodinium ocellatum (Brown 1931) (Dinoflagellida) infections: In vitro tests and treatment trials with infected fishes. *Aquaculture*, *38*(1), 1–18. https://doi.org/10.1016/0044-8486(84)90133-9

Park, I. S., Kim, H. B., Kim, M. S., & Park, C. W. (1994). Acute toxicity effects of formalin to flounder, *Paralichthys olivaceus*. *Journal of Fish Pathology*, *8*(1), 57–67.

Perera, H. A. C., & Pathiratne, A. (2005). Effects of short term exposure to therapeutic levels of formalin on health status of Nile tilapia, *Oreochromis niloticus*. *Journal of the National Science Foundation of Sri Lanka*, *33*(4), 239–245.

Piper, R. G., & Smith, C. E. (1973). Factors influencing formalin toxicity in trout. *The Progressive Fish-Culturist*, *35*(2), 78–81.

Pironet, F. N., & Jones, J. B. (2000). Treatments for ectoparasites and diseases in captive Western Australian huffish. *Aquaculture International*, *81*, 349–361.

Powell, M. D., Speare, D. J., Fulton, A. E., & Friars, G. W. (1996). Effects of Intermittent Formalin treatment of Atlantic salmon juveniles on growth, condition factor, plasma electrolytes, and hematocrit in freshwater and after transfer to seawater. *Journal of Aquatic Animal Health*, *8*(1), 64–69.

Rábago-Castro, J. L., Sánchez-Martínez, J. G., Pérez-Castañeda, R. P., Vázquez-Sauceda, M. L., & Ruiz-Orozco, G. (2014). Chronic effects of a monogenean *Ligictaluridus floridanus* (Ancyrocephalidae) infection on channel catfish (*Ictalurus punctatus*) growth performance. *Acta Veterinaria Brno*, *83*, 83–87. https://doi.org/10.2754/avb201483020083.

Ranjan, R., Xavier, B., Santos, B., Megarajan, S., & Ghosh, S. (2018). Copepod parasite *Lepeophtheirus kabatai* (Ho & Dojiri, 1977) infestation in orange spotted grouper, *Epinephelus coioides* (Hamilton, 1822) and its control in captivity. *Indian Journal of Fisheries*, *65*(3), 122–125. https://doi.org/10.21077/ijf.2018.65.3.76423-17

Rasheed, V. M. (1989). Diseases of cultured brown-spotted grouper *Epinephelus tauvina* and silvery black porgy *Acanthopagrus cuvieri* in Kuwait. *Journal of Aquatic Animal Health*, *1*(2), 102–107. https://doi.org/10.1577/1548-8667(1989)001<0102:DOCBSG>2.3.CO;2

Reardon, I. S., & Harrell, R. M. (1990). Acute toxicity of formalin and copper sulfate to striped bass fingerings held in varying salinities. *Aquaculture*, *87*, 255–270.

Resendes, A. S., Santos, D. S., França, F. M., Petesse, M. L., Badaró-Pedroso, C., & Ferreira, C. M. (2018). Acute toxic and genotoxic effects of formalin in *Danio rerio* (zebrafish). *Ecotoxicology*, *27*, 1379–1386.

Rothen, D. E., Curtis, E. W., & Yanong, R. P. E. (2002). Tolerance of yolk sac and free-swimming fry of the zebra danio *Brachydanio rerio*, black tetra *Gymnocorymbus ternetzi*, Buenos Aires tetra *Hemigrammus caudovittatus*, and blue gourami *Trichogaster trichopterus* to therapeutic doses of formalin and sodium chloride. *Journal of Aquatic Animal Health*, *14*(3), 204–208. 10.1577/1548-8667(2002)014<0204:TOYSAF>2.0.CO;2

Rowland, S. J., Mifsud, C., Nixon, M., Read, P., & Landos, M. (2009). Use of formalin and copper to control ichthyophthiriosis in the Australian freshwater fish silver perch (*Bidyanus bidyanus* Mitchell). *Aquaculture Research*, *40*, 44–54.

Rowland, S. J., Nixon, M., Landos, M., Mifsud, C., Read, P., & Boyd, P. (2006). Effects of formalin on water quality and parasitic monogeneans on silver perch (*Bidyanus bidyanus* Mitchell) in earthen ponds. *Aquaculture Research*, *37*, 869–876.

Rucker, R. R., Taylor, W. G., & Toney, D. P. (1963). Formalin in the hatchery. *The Progressive Fish-Culturist*, *25*(4), 203–207.

Şahan, A. (2020). The effects of formaldehyde, hydrogen peroxide and trichlorphon applications on some hematological stress indicators in mirror carp (*Cyprinus carpio* L.). *Acta Aquatica Turcica*, *16*(1), 71–81. https://doi.org/10.22392/actaquatr.594137

Sanches, E. G. (2008). Controle de *Neobenedenia melleni* (Maccallum, 1927) (Monogenea: Capsalidae) em garoupa-verdadeira, *Epinephelus marginatus* (Lowe, 1834), cultivada em tanques-rede. *Revista Brasileira De Parasitologia Veterinária*, *17*(3), 145–149.

Sanches, E. G., Ostini, S., & Rodrigues, C. D. S. (2007). Ocorrência e tratamento de monogenoides em alevinos de pampo (*Trachinotus carolinus*) cultivados experimentalmente na região norte do estado de São Paulo. *Revista Brasileira De Parasitologia Veterinária*, *16*(1), 1–4.

Sanchez, J. G., Speare, D. J., Johnson, G. J., & Horney, B. S. (1997). Evaluation of the stress response in healthy juvenile rainbow trout after repetitive intermittent treatments with chloramine-t or formalin. *Journal of Aquatic Animal Health*, *9*(4), 301–308.

Sandoval-Gío, J. J., Rodríguez-Canul, R. P., & Vidal-Martínez, V. M. (2008). Humoral antibody response of the tilapia *Oreochromis niloticus* against *Cichlidogyrus* spp. (Monogenea). *Journal of Parasitology*, *94*(2), 404–409.

Sandoval-Gío, J. J., Rodríguez-Canul, R. P., Vidal-Martínez, V. M., Fájer-Ávila, E. J., & Améndola-Pimenta, M. (2019). Formalin toxicity to *Oreochromis niloticus*; its effectiveness against *Cichlidogyrus* spp. and host stress response. *Latin American Journal of Aquatic Research*, *47*(1), 34–41. https://doi.org/10.3856/vol47-issue1-fulltext-5

Santos, R., Dias, H., & Fujimoto, R. Y. (2012). Acute toxicity and histopathology in ornamental fish Amazon bluespotted corydora (*Corydoras melanistius*) exposed to formalin. *Anais Da Academia Brasileira De Ciências*, *84*(4), 1001–1007.

Sharp, N. J., Diggles, B. K., Poortenaar, C. W., & Willis, T. J. (2004). Efficacy of Aqui-S, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi lalandi* in New Zealand. *Aquaculture*, *236*, 67–83.

Silva, A. L., Alves, F. C. M., Talmelli, E. F. A., Ishikawa, C. M., Nagata, M. K., & Rojas, N. E. T. (2009). Utilização de cloreto de sódio, formalina e a associação destes produtos no controle de ectoparasitas em larvas de tilápia (*Oreochromis niloticus*). *Boletim do Instituto De Pesca*, *35*(4), 597–608.

Singhal, R. N., Swam, J., & Davies, R. W. (1986). Chemotherapy of six ectoparasitic diseases of cultured fish. *Aquaculture*, *54*, 165–171.

Sitjà-Bobadilla, A., Felipe, M. C., & Alvarez-Pellitero, P. (2006). *In vivo* and *in vitro* treatments against *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) parasitizing the gills of gilthead sea bream (*Sparus aurata*). *Aquaculture*, *261*, 856–864.

Smith, C. E., & Piper, R. G. (1972). Pathological effects in formalin-treated rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, *29*, 328–329.

Smith, S. D., Gould, R. W., Zaugg, W. S., Harrell, L. W., & Mahnken, C. V. W. (1987). Safe prerelease disease treatment with formalin for fall chinook Salmon Smolts. *The Progressive Fish-Culturist*, *49*(2), 96–99.

Speare, D. J., Arsenault, G., MacNair, N., & Powell, M. D. (1997). Branchial lesions associated with intermittent formalin bath treatment of Atlantic salmon, *Salmo salar* L, and rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, *20*(1), 27–33.

Speare, D. J., & Macnair, N. (1996). Effects of intermittent exposure to therapeutic levels of formalin on growth characteristics and body condition of juvenile rainbow trout. *Journal of Aquatic Animal Health*, *8*, 58–63.

Srivastava, S. J., Gupta, A. K., Chaturvedi, S. K., Abhinavand, A., & Singh, R. (2009). Effects of short-term therapeutic bath of malachite green and formalin on certain hematological parameters of a freshwater catfish, (*Heteropneustes fossilis*) (Bloch). *Journal of Experimental Zoology*, *12*(1), 79–83.

Stephens, F., Cleary, J., Jenkins, G., Jones, J., Raidal, S., & Thomas, J. (2003). Treatments to control *Haliotrema abaddon* in the West Australian huffish. *Glaucosoma Hebraicum*. *Aquaculture*, *215*(1–4), 1–10. https://doi.org/10.1016/s0044-8486(01)00876-6

Stoskopf, M. K. (1988). Fish chemotherapeutics. *Veterinary Clinics of North America: Small Animal Practice*, *18*, 329–347.

Subasinghe, R. P., & Yusoff, F. M. (1993). Retention of formaldehyde in the tissues of two tropical fish species following exposure to therapeutic levels. *Aquaculture Research*, *24*(6), 693–697. https://doi.org/10.1111/j.1365-2109.1993.tb00647.x

Sultana, S., Asrafunnesa, M., & Alam, T. (2013). Effects of formalin and some agrochemicals on the behavior and mortality of *Channa Panctatus* (Bloch, 1793). *University Journal of Zoology, Rajshahi University*, *32*, 15–20.

Svendsen, Y. S., & Haug, T. (1991). Effectiveness of formalin, benzocaine and hypo- and hypersaline exposures against adults and eggs of *Entobdella hippoglossi* (Muller), an ectoparasite on Atlantic halibut (*Hippoglossus hippoglossus* L.). laboratory studies. *Aquaculture*, *94*, 279–289.

Taheri, R., Salamat, N., & Movahedinia, A. (2016). Evaluation of lymphoid tissue structure in Sole (*Euryglossa orientalis*) and yellowfin seabream (*Acanthopagus latus*) affected by environmental contaminants in the Persian Gulf. *Journal of Immunotoxicology*, *3*(3), 301–313. https://doi.org/10.3109/1547691X.2015.1074958.

Tancredo, K. R., Ferrarezi, J. V., Marchiori, N. C., & Martins, M. L. (2019). Ecotoxicological assays to determine the median lethal concentration (LC_{50}) of formalin for fish. *Aquaculture International*, *27*, 685–694. https://doi.org/10.1007/s10499-019-00354-w

Tancredo, K. R., Marchiori, N. C., Pereira, S. A., & Martins, M. L. (2019). Toxicity of formalin for fingerlings of Cyprinus carpio var. koi and in vitro efficacy against Dactylogyrus minutus Kulwie c, 1927 (Monogenea: Dactylogyridae). *Journal of Parasitic Diseases*, *43*(1), 46–53. https://doi.org/10.1007/s12639-018-1056-1

Thatcher, V. E., & Kritsky, D. C. (1983). Neotropical Monogenoidea. 4. *Linguadactyloides brinkmanni* gen. et sp. n. (Dactylogyridae: Linguadactyloidinae subfam. n.) with Observations on its pathology in a Brazilian freshwater fish, *Colossoma macropomum* (Cuvier). *Proceedings of the Helminthological Society of Washington*, *50*(2), 305–311.

Theron, J., Prinsloo, J. F., & Schoonbee, H. J. (1991a). Treatment of *Cyprinus carpio* L. and *Clarias gariepinus* (Burchell) embryos with formalin and malachite green: effect of concentration and length of treatment on their survival. *Onderstepoort Journal of Veterinary Research*, *58*, 239–243.

Theron, J., Prinsloo, J. F., & Schoonbee, H. J. (1991b). Investigations into the effects of concentration and duration of exposure to formalin and malachite green on the survival of the larvae and juveniles of the common carp *Cyprinus carpio* L. and the sharptooth catfish *Clarias gariepinus* (Burchell). *Onderstepoort Journal of Veterinary Research*, *58*, 245–251.

Thing, C. Y., Ransangan, J., & Hatai, K. (2016). Antiparasitic effect of formalin, trichlorfon, hydrogen peroxide, and copper sulfate the parasitic isopod *Caecognathia coralliophila*. *Fish Pathology*, *51*(3), 1125–1127.

Thoney, D. A., & Hargis, W. J. Jr (1991). Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases*, *1*, 133–153.

Thorburn, M. A., & Moccia, R. D. (1993). Use of chemotherapeutics on trout farms in Ontario. *Journal of Aquatic Animal Health*, *5*, 85–91.

Tieman, D. M., & Goodwin, A. E. (2001). Treatments for ich infestations in channel catfish evaluated under static and flow-through water conditions. *North American Journal of Aquaculture*, *63*(3), 293–299.

Tisler, T., & Zagorc-Koncan, J. (1997). Comparative assessment of toxicity of phenol, formaldehyde, and industrial wastewater to aquatic organisms. *Water Air and Soil Pollution*, *97*, 315–322.

Tkachenko, H., & Grudniewska, J. (2016). Evaluation of oxidative stress markers in the heart and liver of rainbow trout (*Oncorhynchus mykiss* Walbaum) exposed to the formalin. *Fish Physiology Biochemistry*, *42*, 819–1832. https://doi.org/10.1007/s10695-016-0260-0

Urbinati, E. C., Zanuzzo, F. S., & Biller, J. D. (2020). Stress and immune system in fish. In B. Baldisserotto, E. C. Urbinati, & J. E. P. Cyrino (eds), *Biology and physiology of freshwater neotropical fish* (pp. 93–114). Elsevier Inc.

Vargas, L., Povh, J. P., Ribeiro, R. P., Moreira, H. L. M., Loures, B. T. R. R., & Maroneze, M. S. (2003). Efeito do tratamento com cloreto de sódio e formalina na ocorrência de ectoparasitas em alevinos de tilápia do Nilo (*Oreochromis niloticus*) revertidos sexualmente. *Arquivo De Ciências Veterinária E Zoologia Da UNIPAR*, *6*(1), 39–48.

Wedemeyer, G. (1971). The stress of formalin treatments in rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*Oncorhynchus kisutch*). *Journal Fisheries Research Board of Canada*, *28*(12), 1899–1904. https://doi.org/10.1139/f71-285.

Wedemeyer, G., & Yasutake, W. T. (1974). Stress of formalin treatment in juveniles spring chinook salmon (*Oncorhynchus tshawytsha*) and steelhead trout (*Salmo gairdneri*). *Journal Fisheries Research Board of Canada*, *31*(2), 179–184. https://doi.org/10.1139/f74-029.

Wellborn, T. L. Jr (1969). The toxicity of nine therapeutic and herbicidal compounds to striped bass. *The Progressive Fish-Culturist*, *31*(1), 27–32.

Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews*, *77*, 591–625.

Wijeyaratine, J. S., & Gunawardene, R. S. (1988). Chemotherapy of ectoparasite, *Ergasilus ceylonensis* of Asian cichlid, *Etroplus suratensis*. *Journal of Applied Ichthyology*, *4*, 97–100.

Willford, W. A. (1967). *Toxicity of 22 therapeutic compounds to six fishes*. U.S. Fish-Wildl. Serv., Invest. Fish Control. Rep. No. 18. 10 pp.

William, M., & Lewis, S. D. (1963). Control of epizootics of *Gyrodactylus elegans* in golden shiner populations. *Transactions of the American Fisheries Society*, *92*(1), 60–62. 10.1577/1548-8659(1963)92[60:COEOGE]2.0.CO;2

Williams, H. A., & Wootten, R. (1981). Some effect of therapeutic levels of formalin and copper sulphate on blood parameters in rainbow trout. *Aquaculture*, *24*, 341–353. https://doi.org/10.1016/0044-8486(81)90068-5

Witeska, M., Kondera, E., & Belniak, N. (2013). Hematological and hematopoietic changes induced by formaldehyde and malachite green in common carp (*Cyprinus carpio* L.). *Zoology and Ecology*, *23*(3), 245–251. https://doi.org/10.1080/21658005.2013.821790

Wright, L. D. (1976). Effect of malachite green and formalin on the survival of largemouth bass eggs and fry. *The Progressive Fish-Culturist*, *38*(3), 155–157.

Xu, D. H., Shoemaker, C. A., & Zhang, D. (2015). Treatment of *Trichodina* sp reduced load of *Flavobacterium columnare* and improved survival of hybrid tilapia. *Aquaculture Reports*, *2*, 126–131. https://doi.org/10.1016/j.aqrep.2015.09.00

Xu, D. H., Zhang, Q. Z., & Zhan, D. (2016). Two *in vitro* methods for screening potential parasiticides against *Ichthyophthirius multifiliis* using *Tetrahymena thermophila*. *Journal of Fish Diseases*, *39*, 285–294. https://doi.org/10.1111/jfd.12361

Yildiz, H. Y., & Ergonul, M. B. (2010). Is prophylactic formalin exposure a stress source for gilthead seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*). *Ankara Üniversitesi Veterinerlik Fakültesi Dergisi*, *57*, 113–118.

Yisa, T. A., Tsadu, S. M., Aidoo, T., Ibrahim, A., Gana, E. S., & Adama, B. S. (2014). Effect of chemical disinfectant (formalin) on hatching of eggs of African catfish (*Clarias gariepinus*), survival and growth performance of fry. *International Journal Current Microbiology and Applied Sciences*, *3*(3), 1133–1138.

Zafran, R. D., Koesharyani, I., Johnny, F., & Yuasa, K. (1998). *Manual for fish diseases diagnosis: Marine fish and crustacean diseases in Indonesia* (p. 44). Gondol Research Station for Coastal Fisheries.

How to cite this article: Tavares-Dias M. Toxicity, physiological, histopathological and antiparasitic effects of the formalin, a chemotherapeutic of fish aquaculture. *Aquaculture Research*. 2021;52:1803–1823. https://doi.org/10.1111/are.15069