Promising chemical barrier substance applied to ichthyofauna in hydroelectric plants

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SUMMARY

In hydroelectric plants, there are, on a regular or untimely basis, shutdowns of the generating units in order to carry out some maneuvers for tests and mechanical maintenance. The low operating flow increases the probability of accumulation of ichthyofauna in the draft tube. As a result, the variables that determine the quality of water can change, which requires a broad and coordinated human effort to rescue trapped fish. In addition to the risks related to work safety, there are large economic liabilities as a result of the downtime of the generating units. To minimize this problem, it is necessary to improve techniques for repelling fish from risk areas. The detection of chemical substances in water is one of the most efficient methods of communication between fish. Thus, this study presents a brief review of alarm substances, which are released by the fish epidermis as a sign of defensive response to a dangerous situation and are promising for use as a chemical barrier in the hydroelectric sector.

KEYWORDS: Imprisoned fish. Fish repulsion. Alarm substances.

1 INTRODUCTION

Hydroelectricity is one of the main sources of energy in the world, with China being the country with the largest production. In Brazil, approximately 65% of the energy mix corresponds to hydroelectric energy (IEA, 2017).

Currently, hydropower plants are the most efficient way of converting primary to secondary energy, reaching 90% efficiency (ANEEL, 2005). The low cost of supply compared to other sources – such as oil, natural gas, coal, and uranium – in addition to the fact that the operation of hydropower plants causes low emission of gases that cause the greenhouse effect, give hydroelectric energy the world label of renewable and clean (ANEEL, 2008).

During mechanical maintenance, either scheduled or untimely, in Francis or Kaplan-type generating units, the low operating flow rate can lead to the accumulation of ichthyofauna inside the spiral casing of the draft tube and the drainage well (PERRY et al., 2014). It is important to emphasize that maintenance of the generating units requires a complex operation, both in terms of workforce and machine stoppage hours, in addition to the issue of work safety for the ichthyofauna rescue teams.

In this way, the risks to ichthyofauna in the operation and maintenance of hydroelectric plants are an integral part of the energy generation process, requiring the improvement of techniques for repulsing fish from risk areas.

There are studies that show proven effectiveness of physical methods such as grids and screens (Andrade et al., 2012); the “divert fish” method, popularly known as “fool fish” method, which consists of opening the spillway and/or stopping the turbine adjacent to the one to be dewatered (CEMIG, 2016); the bypass method, which consists in creating an alternative way to return the fish to its natural environment; the elevator method, which consists of a collecting bucket which is raised to the level of the reservoir and releases the fish into a channel that takes them upstream from the dam; locks, which are systems that raise the water level to the level of the upstream river, with sluices that open to release fish; and tank trucks, which capture fish downstream and release them upstream (SILVA, 2010).

Although unusual, there are also steel chain curtains that are used to repel fish through visual and acoustic stimuli (SILVA, 2010). Behavioral barriers (bubbles, sounds, strobe lights and electrical currents) are still sparsely used due to the uncertainties of its effectiveness, which depends on the species and size of the fish, and on environmental conditions, such as turbidity and flow (BOWEN, 2004; PERRY et al., 2014; WISENDEN & SMITH, 1997).

Due to the great sensitivity of fish in detecting and reacting to biochemical substances in water, chemical barriers, still little discussed, are also candidates for minimizing the extensive social and environmental impacts of hydroelectric power plants.

Alarm substances, which are released by physical damage to the skin of fish in a defensive response to a dangerous situation, are one of the main and most efficient chemical repulsion systems in fish. However, there is no detailed characterization of these substances, and the behavior of the aquatic community is still unpredictable. In this context, the present study aims to present a review of alarm substances.

2 **OBJECTIVES**

Review the alarm substances extracted from the skin of fish to support research on chemical methods for repulsing fish, especially in the hydroelectric sector.

3 **METHODOLOGY**

Publications in ScienceDirect, Scientific Electronic Library Online (SciELO), Springer Link and Medical Literature Analysis and Retrieval System Online (MedLine) portals were searched using the descriptors “alarm response in fish”, “alarm behavior in fish”, “chemical alarm”, “repulse skin extract in fish” and “hydroelectric fish repulsion”. The inclusion criteria were articles published in English and Portuguese, in full, in journals indexed in the virtual databases described above. Exclusion criteria were letters, editorials, abstracts in event proceedings or journals. The search for articles was not limited by period, the studies were searched until the year 2020. In total, 37 studies were selected according to their relevance to the evidence to be analyzed.

4 **RESULTS**

Habitat and environmental conditions can shape fish behavior and morphology. Chemoreception or the ability to detect chemical substances can increase survival, allowing for greater awareness of threatening environmental disturbances (ABREU et al., 2016; TOA et al., 2004).

Chemical signals are important in cases in which fish have limited sight, such as dark regions, with many visual obstacles or with high turbidity (TOA et al., 2004). Chemical communication overcomes physical barriers and reaches distances where lights, bubbles and sounds do not reach and cannot be sensed by fish (JORDÃO & VOLPATO, 2000).

Stressors in fish can come from physical contact with other species, visual or auditory stimuli, memory of a stressful condition and cues released by a previously stressed conspecific (BARCELLOS et al., 2011; BROWN & SMITH, 1997). The latter is the only one that involves chemical communication between specimens. These alarm signals are stored by epidermal cells and can be released into water as a result of injury, i.e., alarm substance. The chemical signals released into the water by uninjured fish exposed to stressful situations can be called disruptive substances (BROWN & GODIN, 1997; TOA et al., 2004; BARCELLOS et al., 2011; ABREU et al., 2016).

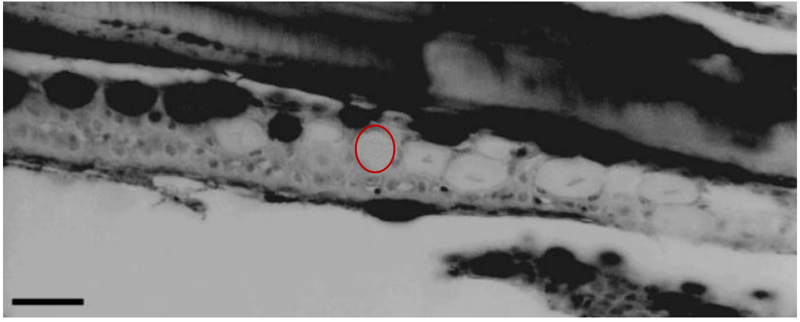
Several studies have evaluated the behavior change of fish that have received water conditioned by chemical signals from uninjured fish, that have only been exposed to disturbing situations, such as sighting of predators, induction of long-term fasting, changes in the physicochemical characteristics of the water, fishing nets, among others. All authors identified defense reactions of recipient fish, indicating the existence of chemical communication (ABREU et al., 2016; BARCELLOS et al., 2011; JORDÃO & VOLPATO, 2000; OLIVOTTO et al., 2002).

Ammonia (NH3) was pointed out by Jordão & Volpato (2000) and Vavrek et al. (2008) as the disruptive substance released by fish. Toa et al. (2004) state that this substance is possibly released in through urine.

Alarm substances, which are synthesized and stored in specialized epidermal cells, are released only after physical injuries (MAJER et al., 2009).

Club cells, where alarm substances are stored, are incorporated into the epithelium (**Figure 1**). Depending on the fish species, cells can be located in different regions of the epidermis (center or inferior), without direct access to the body surface through pores (IDE et al., 2003; CHIA et al., 2019).

Figure 1 – Club cell where the alarm substance is stored in fish.



Source: Ide et al., 2003.

Verheijen (1962) and Heczko & Seghers (1981) state, through studies with fish exposed to alarm signals, that the primary defense mechanism is searching for cover and the secondary mechanism is agglomeration. However, Frisch (1938), Ide et al. (2003) and Mathuru et al. (2012) state that the main reaction is fast swimming followed by immobility. All these reactions can persist for up to four hours and be transmitted visually to other fish, composing an efficient communication between prey (BROWN & GODIN, 1997; HINTZ et al., 2017).

It is important to emphasize that the recognition of predators and non-predators is an innate response, as during experiments carried out by Jordão & Volpato (2000), fish showed a fear reaction against a predator they had never had contact with. However, greater exposure to dangerous situations determines better responses to alarm signals (MAJER et al., 2009).

Animals belonging to other taxa, such as gastropods, echinoderms, and amphibians, also identify and react to conspecific alarm substances (CHIVERS & SMITH, 1994). Echinoderms have greater chemoreception potential, both in variability and in distance from the source, due to their greater susceptibility to predation (MAJER et al., 2009).

Studies targeting chemical alarm signals among fish date back decades. Frisch (1938) showed the existence of chemical alarm signals among fish of the order *ostariofisios*, which includes carp, pacus and catfish, which represent approximately 72% of fish species found in freshwater. In scientific literature, it is common to find the term “Schreckstoff” (in German) referring to alarm substances, as that was how Frisch, the pioneer in this theme, named these substances.

Other older studies have also analyzed the behavior of fish exposed to alarm substances (PFEIFFER, 1962; VERHEIJEN, 1962; SMITH, 1977). Lawrence & Smith (1989) found, through experiments with extracts obtained from the epidermis of the *Pimephales promelas* species at different dilutions, that the extract of 1 cm^2 of epidermis is capable of causing effects in 58 m^3 of water, showing the extreme sensitivity and effectiveness of alarm substances.

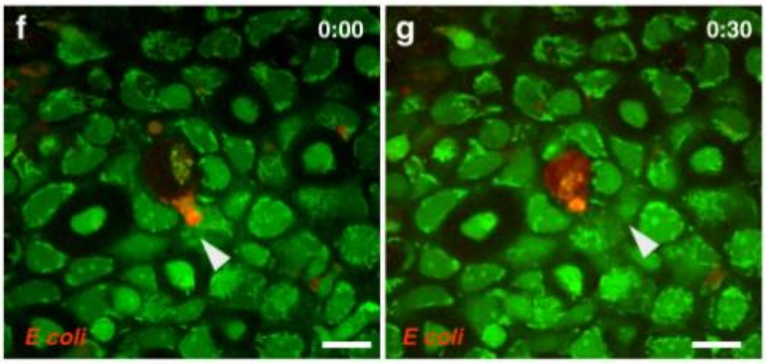
For the extraction of alarm substances, other authors use very similar methods. Ide et al. (2003) used 133 juvenile fish of the *Brycon amazonicus* species. The epidermis donor fish were sacrificed by immersion in a benzocaine solution (350 mg L-1, ethyl-p-amino and benzoate). About 13.5 cm^2 of epidermis, on both sides of the fish, were removed and homogenized in 100 mL of distilled water. Then, this solution was filtered, and distilled water was added to it until the final volume reached 200 mL. The extract was stored at -20 ºC until the time of the experiments. Toa et al. (2004) removed 5 g of epidermis from juvenile Rainbow Trout from the dorsal section along the lateral line and placed in 100 mL of distilled water, the extract was homogenized and filtered in two meshes (1 mm and 20 µM). Then, 400 mL of fish tank water was added to the extract solution. Brown & Godin et al. (1999) initially tested the dispersion of alarm substances with dye in an aquarium 35 cm wide, 22 cm long and 23 cm high and concluded that uniform distribution of the substance was reached in approximately 20 seconds. For the experiments, they removed 5.2 cm^2 of skin from fish of the species *Poecilia reticulata* and macerated it with 50 mL of distilled water, the resulting extract was filtered and made up to a final volume of 100 mL with distilled water. Then, 5 mL of this solution was inserted into the aquarium, and fish agglomeration, rapid swimming and freezing were observed in response to alarm signals.

The molecular structure of alarm substances is not yet fully known, but it has been assumed, since the study by Frisch (1938), that one of the active components is hypoxanthine 3-N-oxide (Ide et al., 2003). Experiments performed by Mathuru et al. (2012) indicate that proteins and most lipids are not active components of alarm substances. However, glycosaminoglycans, present in the mucosa, were correlated with fish behavior. Data also indicate that alarm substances include chondroitin oligosaccharides, with a minimum size of one tetrasaccharide (~1,000 Daltons). Other studies also suggest the presence of chondroitin and other additional components, possibly compounded with nitrogen oxide (BROWN et al., 2000; HINTZ et al., 2017).

Chia et al. (2019) further state that there may be a bacterial component in fish alarm substances. These authors indicate that this substance can originate in a skin compartment directly linked to immunity and be only stored in club cells in the epidermis. Biochemical characterization indicates that alarm substances are a composition that can be extracted by wheat germ agglutinin (Mathuru et al., 2012), which binds mucus or an antibody to chondroitin sulfate, a component of mucus. However, mucus evokes a strong response only after being heated, implying the need for some form of decomposition. Mucus, which contains a diverse population of bacteria, has multiple functions on fish skin, including defending against infectious agents. Mathuru et al. (2012) state that there is mild alarm behavior in fish exposed to mucus after epidermal desquamation.

Bacteria are known to produce substances that can stimulate vertebrate chemosensory systems. They are found in the mucus of the epidermis and, once inside the fish, they can be absorbed by neutrophils. Chia et al. (2019) inserted zebrafish into water containing chemically killed *Escherichia coli*, which was stained red. Figure 2 shows the transport of the bacteria into the club cell, the same cell that stores the alarm substances.

Figure 2 – Introduction of *Escherichia coli* in A club cell, where alarm substances are also stored.



Source: Chia et al., 2019.

Furthermore, Chia et al. (2019) also raise the hypothesis that immobility can be a behavior of fish exposed to alarm substances constituted by bacteria, while for substances without these microorganisms, fish's behavioral effect consists of continuous movement.

It has long been known about the effectiveness of the substance between different species (PFEIFFER, 1963; SMITH 1977). Mirza & Chivers (2001) and Majer et al. (2009) reinforce this statement, adding that the intensity of the response is stronger among the same species. In short, the signals to act are not identical when it comes to heterospecifics, but they are similar enough to be recognized.

Fish defensive reactions are induced by different chemical signals emanating from sense-related stresses. These animals use the combination of information and the context of the situation to determine their avoidance strategy (ABREU et al., 2016).

The sense that identifies the substance is the smell (PFEIFFER, 1963; BROWN & GODIN, 1997). Chivers & Smith (1994) reaffirm this fact with *Astyanax fasciatus*, a sightless fish that had alert reactions when exposed to alarm substances. It is important to emphasize that the olfactory system is more developed depending on the species, which influences the perception sensitivity of the alarm substance. Size and age of the fish are also determinants in the intensity of the response to the substance (MAJER et al., 2009).

Furthermore, when fish are exposed to stress-inducing stimuli, their physiologies change. Overall, stress affects metabolism and increases cortisol and blood glucose levels (BARCELLOS et al., 2011; TOA et al., 2004; VAVREK et al., 2008). Cortisol regulates metabolic energy, hydromineral balance and oxygen uptake (OLIVOTTO et al., 2002).

Exposure to stressful situations can also release heat shock proteins (HSP). There was an increase in the expression of HSP70 protein in the brain and liver of fish in the presence of a predator, indicating the possibility of a psychological stressor, perceived through vision. However, HSP70 is usually induced when animals are exposed to pollutants, heavy metals, industrial effluents, pesticides, and hydrocarbons. Other types of stress, such as capture, confinement and handling may affect some indicators of the physiological stress response (cortisol and glucose), but may not induce a cellular stress response in fish (TOA et al., 2004).

Hormonal or metabolic plasmatic changes depend on the nature and intensity of the stimulus, on the experimental design and on the studied species. These factors may be responsible for the lack of a clear stress response (IDE et al., 2003).

Another factor that should be taken into consideration is that continuous exposure to any stress stimulus can have physiological consequences. Habituation to predators can reduce the degree of stress on prey by up to 40%, significantly reducing defense response (JÄRVI, 1990). Environmental conditions, such as a decrease in pH, can result in a structural change in alarm substances, reducing their efficiency in repulsing ichthyofauna (BROWN et al., 2000; MATHURU et al., 2012). In addition, mating behavior suppresses fish responses to the skin extract, as the pathways that mediate reproduction and defense are anatomically segregated (CHOI et al., 2005; VERDUGO et al., 2019).

Given the above, it can be said that alarm substances are promising in the application of chemical barriers in numerous situations that expose the ichthyofauna to the risk of death.

However, the introduction of any substance into aquatic life must be previously subsidized by rigorous laboratory tests. It is necessary to build more knowledge about these substances, to quantify the concentrations of toxic substances and then apply toxicological tests to assess their effects on organisms (ANDRADE & ARAÚJO, 2011; COSTA et al., 2008; DAMATO & BARBIERI, 2012).

5 CONCLUSION

Considering the perspective of sustainability, which has characterized and guided the current hydroelectric sector against other forms of electricity generation, the environmental issue is of great importance, as it is one of the pillars of the principle of sustainability. Thus, the creation of more robust ichthyofauna repulsion mechanisms in hydropower plants is of great importance and highly desirable.

Alarm substances such as chemical barriers have the potential to mitigate the risks that ichthyofauna are often exposed to. However, it is necessary to characterize the nature of these substances in more detail from a biochemical point of view. In addition, toxicological and behavioral tests on fish in a laboratory, and analysis of applicability in the field and on a large scale are needed. It is also necessary to evaluate the possibility of synthesizing alarm substances, since their acquisition depends on the sacrifice of some organisms. Otherwise, the main objective of minimizing environmental impacts will not be achieved.

6 ACKNOWLEDGEMENTS

We would like to thank the Sustainable Energy in Brazil R&D Program (ANEEL/PD-06631-0009/2019) and the Jirau Energia team, which has provided all the necessary support for the development of this research.

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