Environmental Technology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tent20

Weakly electric fish for biomonitoring water quality

Juergen Clausen [a], Roel van Wijk [b] & Henning Albrecht [a]

[a] Karl und Veronica Carstens-Stiftung, Am Deimelsberg 36, 45276, Essen, Germany

[b] Faculty of Biology, Utrecht University, Padualaan 8, 3584CH, Utrecht, The Netherlands

Accepted author version posted online: 26 Aug 2011.Published online: 10 Nov 2011.

To cite this article: Juergen Clausen, Roel van Wijk & Henning Albrecht (2012) Weakly electric fish for biomonitoring water quality, Environmental Technology, 33:10, 1089-1099, DOI: 10.1080/09593330.2011.610827

To link to this article: http://dx.doi.org/10.1080/09593330.2011.610827

Environmental Technology
Vol. 33, No. 10, May 2012, 1089–1099

Weakly electric fish for biomonitoring water quality

Juergen Clausen[a*], Roeland van Wijk[b] and Henning Albrecht[a]

[a]*Karl und Veronica Carstens-Stiftung, Am Deimelsberg 36,45276 Essen, Germany;*
[b]*Faculty of Biology, Utrecht University, Padualaan 8,3584CH Utrecht, The Netherlands*

(*Received 7 February 2011; Accepted 1 August 2011*)

Environmental pollution is a major issue that calls for suitable monitoring systems. The number of possible pollutants of municipal and industrial water grows annually as new chemicals are developed. Technical devices for pollutant detection are constructed in a way to detect a specific and known array of pollutants. Biological systems react to lethal or non-lethal environmental changes without pre-adjustment, and a wide variety have been employed as broad-range monitors for water quality. Weakly electric fish have proven particularly useful for the purpose of biomonitoring municipal and industrial waters. The frequency of their electric organ discharges directly correlates with the quality of the surrounding water and, in this way, concentrations of toxicants down to the nanomolar range have been successfully detected by these organisms. We have reviewed the literature on biomonitoring studies to date, comparing advantages and disadvantages of this test system and summarizing the lowest concentrations of various toxicants tested. Eighteen publications were identified investigating 35 different chemical substances and using six different species of weakly electric fish.

Keywords: weakly electric fish; detection limits; electric organ discharge; biomonitoring; biosensor

1. Introduction

Population growth and higher living standards will cause ever-increasing demands for good quality municipal and industrial water. In many ways, the spectrum of pollutants is increasing. Where sewage flow is used for irrigation, the drainage water could contain disinfection by-products (DBPs) that were formed in the drinking water when it was chlorinated for public water supply and the chlorine reacted with natural dissolved organic carbon [1–3]. In that respect, there is great concern about adverse long-term health effects, for instance, cancer [4]. In addition to containing DBPs, the treated sewage effluent and, hence, the waters into which it is discharged, can also be expected to contain chemicals from pharmaceutical and other industries, hospitals and other medical facilities, households where unused medicines are flushed down the toilets, and human excreta containing incompletely metabolized medicines, which may have biological effects [5,6]. While not directly toxic or carcinogenic, these chemicals may produce adverse health effects by interfering with hormone production (endocrine disruptors), by weakening immune systems, and by other biological responses [7,8]. Other potential contaminants in the drainage water from sewage-irrigated crops and plants are substances like humic and fulvic acids. These are known precursors of DBPs when the water is chlorinated. The humic substances are formed as stable end products wherever organic matter is biodegraded [9].

It is evident that a broad spectrum of methods is necessary for evaluations of the condition of waste bodies using direct measurements of biological organisms (macroinvertebrates, fish and plants). This article will focus on a special category: the use of weakly electric fish for the purpose of biomonitoring water quality. It summarizes the available information on detection limits in these types of fish.

2. Weakly electric fish

In the late nineteenth century, several fish with weakly electric organs were discovered. In contrast to the well known electric eel (*Electrophorus electricus*), which can deliver electric shocks of several hundred volts, the discharges of weakly electric fish are comparably low ranging from mV to a few volts [10,11]. These fish belong to the orders of Osteoglossiformes and Gymnotiformes (Figure 1) and are found in the fresh waters of Africa and South America. The order of Osteoglossiformes falls into seven families, where only the Mormyridae (*c*.200 species) and Gymnarchidae (one recent species) represent electric fish. By contrast, all fish in the five families of Gymnotiformes (*c*.200 species) posses electric organs (Gymnotidae, Rhamphichthyidae, Hypopomidae, Sternopygidae and Apteronotidae) [11].

Weakly electric fish are mainly nocturnal animals that live and hunt in muddy, disturbed waters [11]. The fish emit series of brief electric signals, termed electric organ

*Corresponding author. Email: info@Carstens-Stiftung.de

ISSN 0959-3330 print/ISSN 1479-487X online

http://dx.doi.org/10.1080/09593330.2011.610827
http://www.tandfonline.com

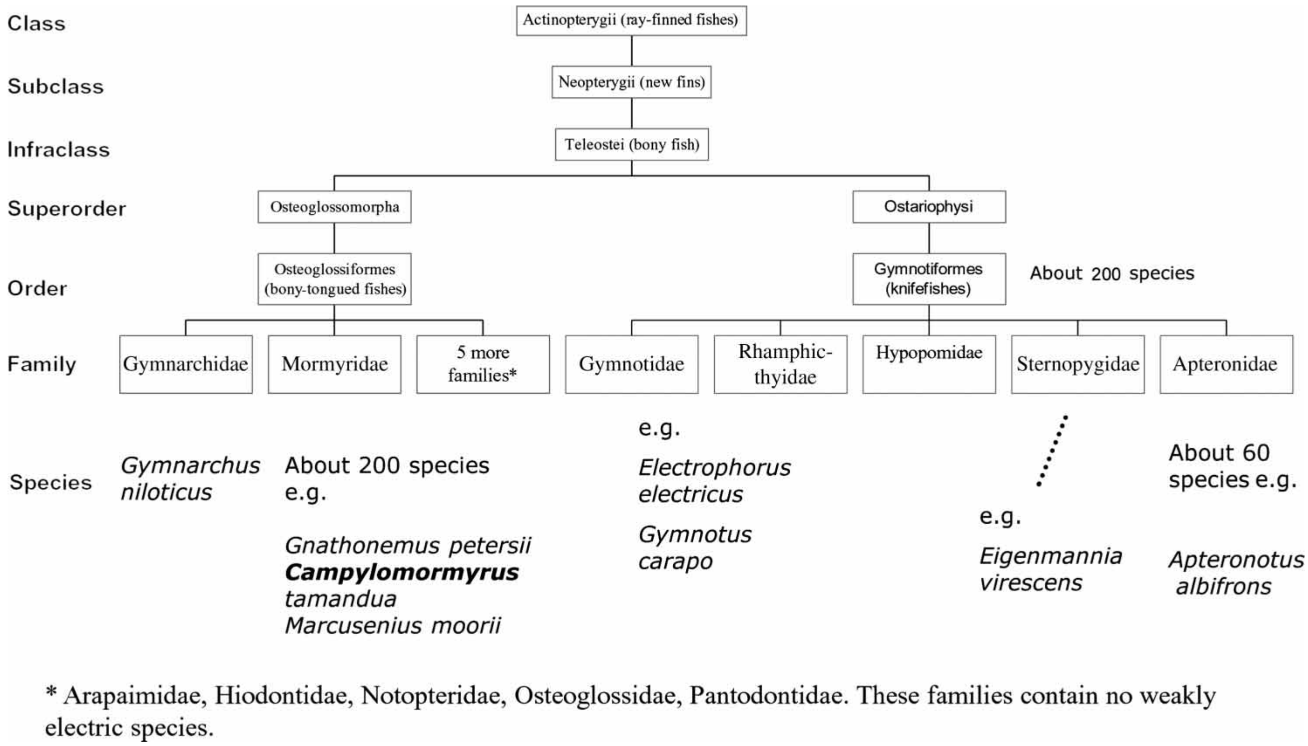

Figure 1. Simplified taxonomic tree of weakly electric fish. In the order of Osteoglossiformes, only members of the families Gymnarchidae and Mormyridae possess electric organs. In contrast, all species in the order of Gymnotiformes feature electric organs.

discharges (EOD), that are emitted by a specialized electric organ, which is made up of modified muscle or nerve cells [12]. In Mormyridae and Gymnarchidae, the electric organ is located in a relatively small area at the tail base [13], whereas it disperses through a large part of the body in Gymnotiformes [11]. The simplest type of an EOD is a monophasic pulse. However, several species produce multiphasic discharges of two or more phases [14].

The African Mormyridae and the South American Gymnotidae, Rhamphichthyidae and Hypopomidae (all Gymnotiformes) often produce multiphasic pulses at very irregular rates the pulses are relatively brief in duration compared with their interpulse interval (ratio usually less than 5%) resulting in a low EOD frequency (*c*.50–100 Hz). These fish are called 'pulse' fish [11,12,15]. The Sternopygidae and Apteronotidae (Gymnotiformes) and the one recent species of the Gymnarchidae (*Gymnarchus niloticus*) continuously produce very regularly spaced mono- or multiphasic pulses where the duration of the EOD is about the same as the duration of the interpulse interval, resulting in an almost sinusoidal EOD. The discharges mostly occur at high frequency (*c*.1–2 kHz). Because of the wavelike EOD appearance, these fish are called 'wave' fish [11,12,16].

The EODs build up stable, three-dimensional electric fields around the fish, resembling electric dipoles. The fish can detect distortions of this field caused by any nearby object (e.g. other fish) with a conductivity different from water. The distortions are detected by electroreceptors which are located all over the fish's skin [15]. Through the pioneering work of Hans Lissmann and Ken Machin it became clear that these fish had evolved a formerly unknown electric sense that allowed them to locate objects (active electrolocation) and hunt in their turbid environment [17]. The sudden appearance and detection of a novel object cause transient increases in the frequency of the EODs of the 'pulse' fish, enabling the fish to gain additional detailed information about that object. Thereby, these fish not only are able to detect the mere presence of certain objects but also can determine their distance and discriminate between different shapes, volumes and the composition of those objects, even in complete darkness [18–20]. If the object stays close to the fish, the fish gets habituated and does not exhibit behavioural responses anymore (adaptation) [17].

Besides navigation and electrolocation, further research revealed that these fish also use their electric sense to communicate to and interact with other electric fish, intraspecifically or interspecifically [21–33]. In some species, the pulse duration increases with body size over the lifetime and might play a role in male–male competition (intrasexual selection) and female choice (intersexual selection) [33–42].

In addition, the EODs vary with changes in temperature, pH and water quality [11,43]. An increase in EOD frequency is, however, not necessarily connected to changes in locomotion. The correlation between EOD frequency and water quality has been exploited for several biomonitoring studies [44–53].

3. Detection of environmental changes by weakly electric fish

Today, environmental pollution is a major issue, calling for suitable monitoring systems. The number of possible pollutants of drinking water grows annually as new chemicals are developed. Technical devices for pollutant detection are constructed in a way so that a specific and known array of pollutants is detected. Some of the disadvantages of those devices are that (1) new pollutants may slip through the technical warning system unless readjustment takes place or new devices are installed, (2) it is technologically, financially and operationally not feasible to detect over four million chemicals known to date, and (3) samples are taken on a discontinuous basis. Because biological systems are affected by pollutants and react in distinct ways to environmental (lethal or non-lethal) changes without pre-adjustment, they have been employed as broad-range monitors for water quality. Here, the lack of specificity is advantageous. A comprehensive review of known biological test systems by Cairns and van der Schalie summarizes advantages and disadvantages [54]. In addition to bacteria and other microorganisms, fish have also been used as biosensors. Among these, the use of weakly electric fish has been proven especially useful in various experiments, because the electric discharges are particularly sensitive to water quality and are easy to detect and process (see section 4).

Changes in the frequency, the amplitude, the waveform or the pulse duration of weakly electric fish and the duration of these changes can be used to identify a response of the fish to the presence of certain chemicals in the body of water.

4. Laboratory set-up for detection of EODs

The very basic set-up to visualise the EODs is a tank including the fish and two submerged electrodes attached to an oscilloscope. The electrodes detect the voltage variations and should be placed in vicinity to the fish. It is generally useful to offer a tube, in which the fish can hide during daytime, as a concession to its natural environment (muddy, disturbed waters). Moreover, the fish will normally not leave this hiding place during daylight, facilitating the anchoring of the electrodes in permanent close vicinity to the fish. For documentation and evaluation, it is necessary to amplify and digitize the signals and store them on a computer (Figure 2). In this way, the frequency, the intervals, the amplitudes and the waveform of the signals can be evaluated. Whereas first experiments started with single measurements at a time [44,55], later improvements allowed measurements of four [45] and even eight [47,48,56] individuals at the same time (including control fish). Acclimatization of the fish to the tank is crucial to get a stable baseline [48,52,57]. For detecting the dependence of the EODs on chemical and physical influences (pH, temperature, dissolved substances) a constant water flow has

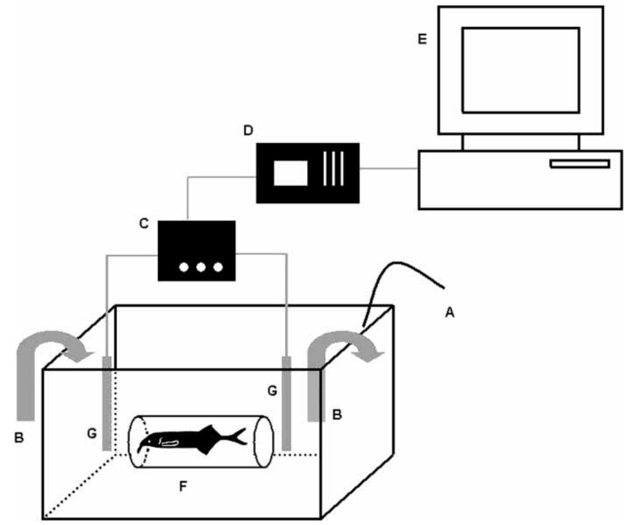

Figure 2. General scheme of the laboratory set-up for measuring the EODs of weakly electric fish. A: air supply; B: water supply (in and out); C: AC amplifier; D: converter (digitization of the EODs; E: computer (storage, analysis); F: fish in tube; G: electrodes. For clarity, control of temperature and pH has been omitted.

to be provided, as displayed in Figure 1 in [45]. Individual differences and diurnal irregularities have to be taken into consideration by statistical analysis of variance. The most advanced and sophisticated set-up so far has been employed by Thomas *et al.* [47], which even provided the opportunity to analyse, by Fourier analysis, the single harmonics of the recorded signals as a function of the toxicant, thereby granting higher reliability.

5. Detection limits of weakly electric fish

Most of the biomonitoring studies solely determined the change in the frequency of EODs. Only Thomas *et al*. additionally investigated the waveform after drug application [47,48]. The biomonitoring studies have paid little or no attention to behavioural modifications after drug exposure [44–46,49–52]. Again, only Thomas *et al*. have touched on eventual changes in locomotor behaviour, which have always been preceded by changes in the EOD characteristics [47,48].

In addition to these biomonitoring studies, further information on EOD sensitivity and detection limits comes from the field of neurophysiology, where the modes of action of stimulants and depressants of the central nervous system have been investigated [55,56,58–63]. Here again, the main parameter investigated was the modulation of the EOD frequency (all but one study – [60]); however, some studies also report on visual observations of slightly changed behaviour after drug exposure [55,56,62,63]. Only two studies directly measured the movement of the fish as a function of drug concentrations using electrodes [58,60], but here the data obtained were composite signals, not exclusively attributable to movement. In general, no strict correlation between EOD modifications and behaviour has been found in these studies. In most cases, the EOD was modified but no changes in behaviour were seen [55,56,60,62,63]. Four of these studies also investigated the signal amplitude. In two studies, the signal amplitude was unchanged [61,62], whereas Krivoy *et al*. [58,60] found amplitude variations for three out of five substances (up to 90% amplitude reduction). However, the informative value of the estimated amplitude changes is limited because of the set-up limitations mentioned by the authors [58].

Altogether, six different species of weakly electric fish were used in the experiments investigating modifications of the EODs after exposure to various environmental conditions. Thirty-five different substances were investigated (reported in 18 publications). The lowest detected concentrations are summarized in Tables 1 and 2 (Mormyridae) and Table 3 (Gymnotiformes).

5.1. Mormyridae

Most experiments have been performed with *Gnathonemus petersii* ('elephantnose fish'; see Figure 1a in the online supplementary material) and *Campylomormyrus tamandua* ('blunt-jaw elephantnose fish'; synonym *G. tamandua*). Both species have been exposed to various toxicants in several studies [44–46,49–53,55,56]. Lowest detected concentrations were 20 nm bis[tri-n-butyl tin]oxide (TBTO) and 78 nm copper. Cyanide was detected down to 0.2 µm. All detected concentrations are summarized in Tables 1 and 2. The usual period under observation has been up to 60 min. The response and adaptation times after exposure vary greatly (minutes to hours) depending on the nature and concentration of the applied toxicant and the type of fish. In general, it has been found that lower concentrations result in longer periods before the fish responds [44,46,49–52]. The sensitivity appears to be almost similar for the toxicants, but slightly higher for ammonium and phosphate in *G. petersii* compared with *C. tamandua.* Besides this interspecific variation, individuals of one species also exhibit a highly variant and inconsistent pattern of response (increase or decrease or no response in frequency/change of variance prior to and after exposure) to toxicant exposure (intraspecies variation) [50,51]. Therefore, it is the change in the pulsing pattern of each fish itself that characterizes a response to a toxicant rather than the quality of the variation between individuals or species. Temperature and pH have great influence on the frequency of EODs in Mormyridae [64–66]. Moreover, the EODs greatly alter during the diurnal cycle according to the general behaviour of the fish or exogenous factors such as visual disturbances or noise [46,56,57,67], even in the absence of any chemical stimulations. Generally, the frequency of EODs is lower during the day, but

Table 1. Detection limits of *Gnathonemus petersii* (Mormyridae) determined by EOD output.

Substance	Lowest concentration for detecting effect	Failed to detect	Remarks	Source
Apomorphine	307 µM	n.d.[2]		[55]
Haloperidol	109 µM	n.d.		[55]
Mercury	0.5 µM	n.d.	Response after 20 h	[44]
Sodium arsenite	46 µM	d.m.[3]	d.m. [3]	[53]
Ammonium	28 µM	n.d.		[52]
Phosphate	0.21 µM	0.15 µM	No response within 60 min at 0.15 µM	[52]
Nitrate	n.a.[4]	n.a.	No response to 161–1.2 mM	[52]
Bis[tri-n-butyl tin]oxide; TBTO	0.02 µM	n.d.		[52]
Cadmium	0.89 µM	n.d.		[50]
Chromium(III) sulphate	0.51 µM	n.d.		[50]
Trivalent chromium	0.25 M	n.d.	Presumably chromium(III) sulphate; preliminary results	[46]
Potassium dichromate	1.4 µM	0.7 µM		[50]
Potassium dichromate	0.34 µM	n.d.	Preliminary results	[46]
Copper	7.9 µM	n.d.	After 4 h	[44]
Copper	0.078 µM	0.016 µM	Response to 0.016 µM not significant within 60 min	[50]
Cyanide	3.8 µM	n.d.	After 4 h	[44]
Cyanide	0.2 µM	n.d.		[51]
Cyanide	0.2 µM	n.d.	Preliminary results	[46]
Phenol	9.6 µM	5.3 µM	5.3 µM only tested for 30 min	[45]
Lindane	0.17 µM	n.d.		[49]
Atrazine	0.15 µM	n.d.		[49]
Atrazine	0.12 µM	n.d.		[56]

[1]In general, there was no systematic analysis of detection thresholds. The values reported in this column just represent concentrations, where no change in the fish's behaviour was detected after exposure.
[2]n.d. = not determined.
[3]d.m. = data missing; original literature not available – data taken from [44].
[4]n.a. = not applicable.

Table 2. Detection limits of *Campylomormyrus tamandua* and *Marcusenius moorii* (both Mormyridae) determined by EOD output.

Species	Substance	Lowest detected concentration	Failed to detect[1]	Remarks	Source
Campylomormyrus tamandua	Ammonium	55 μM	28 μM		[52]
	Phosphate	0.53 μM	0.21 μM		[52]
	Nitrate	n.a.[2]	n.a.	No response to 161–1.2 mM	[52]
	Cadmium	0.89 μM	n.d.[3]		[50]
	Chromium(III) sulphate	1 μM	0.5 μM	Response to 0.5 μM not significant within 60 min	[50]
	Potassium dichromate	n.a.	0.7 and 1.4 μM		[50]
	Copper	0.078 μM	0.016 μM	Response to 0.016 μM not significant within 60 min	[50]
	Cyanide	0.2 μM	n.d.		[51]
	Bis[tri-n-butyl tin]oxide; TBTO	0.02 μM	n.d.		[52]
	Atrazine	0.15 μM	n.d.		[49]
	Lindane	0.17	n.d.		[49]
Marcusenius moorii	Benzocaine and isomers	0.1 mM	n.d.		[61,62]
	MS-222 Sandoz®	0.1 mM	n.d.	= Methanesulphonate of ethyl-m-aminobenzoate	[62]
	Methane sulphonic acid	n.a.	0.1 mM		[62]
	Ethyl benzoate	0.1 mM	n.d.		[62]
	Aniline	n.a.	0.1 mM		[62]
	Benzaldehyde	0.1 mM	n.d.	Weak signal	[62]
	p-Aminobenzoic acid	n.a.	0.1 mM		[62]
	Propiophenone	n.a.	0.1 mM		[62]
	n-Butyl acetate	n.a.	0.1 mM		[62]

[1]In general, there was no systematic analysis of detection thresholds. The values reported in this column just represent concentrations, where no change in the fish's behaviour was detected after exposure.
[2]n.a. = not applicable.
[3]n.d. = not determined.

Table 3. Detection limits of Gymnotiformes determined by EOD output.

Species	Substance	Lowest detected concentration	Failed to detect[1]	Remarks	Source
Apteronotus albifrons	Cyanide	0.5 μM	n.d.[2]		[47,48]
	Chlorpromazine	0.39 μM	n.d.		[63]
Gymnotus carapo	Chlorpromazine	2 μM	0.39 μM	Set-up limitations	[58]
	Chlorpromazine	0.039 μM	n.d.		[59]
	Methylphenidate	0.11 μM	n.d.		[59]
Eigenmannia virescens	Methylphenidate	0.02 μM	n.d.	Set-up limitations	[60]
	Nalorphine	0.16 μM	n.d.	Set-up limitations	[60]
	Phenobarbital	Slightly higher than 0.11 μM	0.11 μM	Set-up limitations	[60]
	Morphine	0.91 μM	n.d.	Set-up limitations	[60]
	Pentylenetetrazole	n.a.	18 μM	Set-up limitations	[60]
	Benzocaine	50 μM	n.d.		[61]
	Aniline	n.a.	0.1 mM		[61]
	Ethyl benzoate	0.1 mM	n.d.		[61]
	Caffeine	n.a.	0.1 mM	Preliminary results	[61]
	Mephenesin	0.75 mM	n.d.	Preliminary results	[61]
	Mescaline	1 mM	n.d.	Preliminary results	[61]

[1]In general, there was no systematic analysis of detection thresholds. The values reported in this column just represent concentrations, where no change in the fish's behaviour was detected after exposure.
[2]n.d. = not determined.
[3]n.a. = not applicable.

higher during night-time [53,67]. However, in the absence of exogenous stress factors, at constant temperature and pH and after acclimatization of the fish, the EOD frequency remains stable enough for reliable measurements [57,64].

5.2. Gymnotiformes

Thomas *et al*. have employed *Apteronotus albifrons* (Apteronotidae; 'black ghost knifefish'; see Figure 1b in the online supplementary material) for real-time detection of cyanide [47,48]. Concomitant analysis of EOD frequency and EOD waveform results in a higher reliability of the detection system [48]. *Apteronotus albifrons* responds to concentrations of potassium cyanide in the range of 0.5–2.8 μm, usually within the first 30 min (Table 3) [47,48]. The order of magnitude of Δf was about 5–15 Hz (information sparse). At 2.8 μm the fish already showed signs of suffocation [48]. The response times to detect cyanide pollution present a global decreasing trend as cyanide concentrations increase. The sensitivity of both parameters was identical, but waveform changes usually appeared somewhat later (20–560 min). The modality of response (increase/decrease of frequency) to increasing cyanide concentrations and the response time vary individually, but, in the single individual, the time variations of the EOD frequency and the waveform are remarkably stable under constant ambient conditions in this species [63,68–70], and no diurnal oscillations have been observed by Thomas *et al*. [47], though there are inconsistent statements on this matter [71]. Some other gymnotiform fish display significant day–night changes in their EODs [72–75]. Variations in the pH significantly decrease the EOD frequency (but have no effect on the EOD waveform) [76,77]. In addition, temperature is known to highly influence the EOD frequency in Gymnotiformes [17,68,70,78–80] with a Q_{10} around 1.5 in different species [68,70,78,80] ($\Delta f \approx 40$–65 Hz [70,80]). Again, no temperature effect on the EOD waveform has been observed in the range of 22–32°C in *A. albifrons* [70]. Adaptation to chemicals occurs within 60 min in Gymnotiformes [48,59,60], though data are partly conflicting [47]. Further experiments have been performed with *Eigenmannia virescens* (Sternopygidae) and *Gymnotus carapo* (Gymnotidae), though not in the context of biomonitoring [58–61] but for evaluation of the mode of action of stimulants and depressants of the central nervous system (Table 3).

6. Discussion and perspective

6.1. Characteristics of experiments employing weakly electric fish

Various aspects of the behaviour or physiological responses of fish have been studied for monitoring water quality [81], e.g. avoidance or rheotactic reactions [82] or ventilation frequency [83–85]. In all these systems either complex mechanical sensors or highly sensitive electrodes had to be employed to preprocess the behavioural signal to the data analysis system. Moreover, the relatively high rate of false alarms in these systems diminishes the reliability. Weakly electric fish, on the contrary, emit rather strong electric signals that are directly generated by the fish and comparatively easy to detect and process.

Several studies have shown the direct correlation between the EOD frequency and the quality of the surrounding water in weakly electric fish. The biological relevance of these modifications of the EODs caused by toxicants is unknown. Because these fish communicate with each other via their EOD output [21–31,33–37], it is possible that the EOD sensitivity to changes in water quality represents some kind of intraspecific early-warning system to inform other fish of the detected threat. However, such a kind of social behaviour has not been reported in the literature so far, and most experiments do not suggest a correlation between modified EODs and changed locomotion [55,56,60,62,63]. Therefore, the biological relevance has, to date, remained unsolved.

Generally, sensitivity of weakly electric fish is very high and has so far not been tested to its limits in most studies (Tables 1, 2 and 3). Where limits have been determined, longer observation times might lower the given limits in future experiments. On the other hand, adaptation to environmental changes (attenuation) has been observed and will limit the validity of longer observations (sections 2, 5.1 and 5.2). The differences in the reported response times of *G. petersii* towards copper (4 h [44] vs 15 min [50]) might be due to the improved set-up of Lewis *et al.* [50]. The most appropriate laboratory set-up has been introduced by Thomas *et al*. [47], measuring eight individuals at the same time. More importantly, their advanced data analysis (the increase in the number of measured parameters) provides a better way for coping with intraspecific sensitivity variations. In addition, selection of an appropriate temperature also reduces the variance ratio of the EODs [46].

For comparing sensitivity of species, criteria for identifying a response are also important. It has to be noted that the criteria for a positive response were more strict in Thomas *et al*. [47,48] compared with the experiments performed by Lewis *et al.* (e.g. [49–51]). Thomas *et al*. considered a change in the EOD frequency or waveform below or above a certain threshold as a positive response if at least 50% of *A. albifrons* reacted significantly. In contrast, a response was considered positive by Lewis and co-workers, if at least one of three individuals exhibited a significantly modified reaction pattern in a set of experiments after Student t-test and variance analysis.

It remains open as to whether the combination of the highly developed set-up (eight individuals measured at the same time, multiple biological responses evaluated) of Thomas *et al*. [47,48] with *G. petersii* (drawback: inter-individual and intra-individual variability; see section 5.1) would end up with better results, i.e. a higher sensitivity, than the presented combination of this set-up with *A. albifrons* (drawback: 0.2 μm cyanide not tested – no direct comparison possible) [47,48], whose natural advantage is the greater (compared with the tested Mormyridae) inter-individual response uniformity (increase/decrease of frequency) to increasing cyanide concentrations (see section 5.2) and temporal stability of the EODs. This has to be elucidated.

In addition, there are a huge number of species in these two orders of fish that have not yet been employed in monitoring measurements to date. Therefore, fish with even higher sensitivity and stability might be discovered in expanded screening experiments.

6.2. Stimulus detection and response

Two aspects in the electrosensory system have to be distinguished:

1) The detection of **distortions of the self-generated electric field** via changes in the potential distribution over the skin surface. The electroreceptor cells in the skin surface constantly measure the local electric voltage between the outside and the inside of the fish, which is proportional to the transepidermal currents passing through the electroreceptor cells. An object with a resistance different from water distorts the self generated electric field and thereby changes the voltage pattern at certain skin areas during discharges of the electric organ. This local modulation of the electric field is called the 'electric image' of an object [19,20,86–88]. The electroreceptor cells synapse on afferent fibres, and the afferent nerve fibres terminate centrally in the electrosensory line lobe in the hindbrain, where the signals are processed [89,90]. The synaptic transmission is mediated either chemically or by means of electrotonic synapses, depending on the kind of receptor cell [90,91]. Local modulations of the electric field are also produced when the fish is exposed to EODs of other fish or externally applied electrical currents [17,92]. Under these conditions, the perceived stimulus is of **electro-physical origin** and modifies the discharges of the electric organ. The fish might react with, for example, an attack (prey, intruding fish), evasive manoeuvres (obstacles, predators) or mere variation (frequency, duration etc.) of the EODs (communication) [17,28].
2) The reaction of the fish upon environmental **changes in water quality**, by modification of their EODs (e.g. frequency, waveform, duration). As mentioned above, the biological relevance of this phenomenon is unknown. The few authors that have commented on the mode of action assumed that the stimulus is of **chemical origin** and they predict a direct chemical interaction between the dissolved substances and cells. However, the mechanism by which the various chemical substances (Tables 1–3) might cause the EOD variations has not been investigated in these experiments. Walsh and Schopp [61,62] propose a mechanism of depressive action on the electromotor system of *Eigenmannia virescens* and *Marcusenius moorii*. The electromotor system consists of pacemaker and relay neurons in the medulla and electromotor neurons in the spinal cord. The axons of the electromotor neurons form the electric organ that generates the EODs. Benzocaine (a local anaesthetic) and its isomers are thought to depress the pacemaker cells directly and to block the synaptic transmissions of the downstream components, resulting in the observed decrease in the discharge frequency [61,62]. Similarly, Thomas *et al*. propose a depressing action of cyanide on the central nervous system due to anoxia and subsequent deterioration of the pacemaker cells, but an endocrine action is also conceivable according to the authors [47]. Kunze interprets the EOD variations after atrazine exposure as a response to general stress caused by physiological deteriorations [56]. The proposed modification of EODs as a response to toxin stress, mediated by the endocrine system, is generally plausible, because the influence of the endocrine system on the regulation and modulation of the EODs in weakly electric fish has been shown for cortisol [93], androgens [13,94–106] and estrogens [95,96,105]. However, these modulations of the EODs in living fish occurred within days, not within minutes and hours as reported in the biomonitoring experiments. Short-term modifications of the EODs (within minutes and hours) have, so far, only been reported for the melanocortins, a group of peptide hormones [107] and the monoamine neurotransmitter serotonin [108]. Various mechanisms for the interaction of the endocrine system and the EOD modulation in gymnotiform [15,32,93,102] and mormyrid [13,15,101] fish have been proposed (e.g. modification of the ion channel properties in the cells of the electric organ or manipulation of the medullary pacemaker nucleus).

Another target of chemical interactions may be the receptor cells themselves. *In vitro* experiments with excised skin of *G. petersii* have shown that divalent ions and L-glutamate affect the impulse generation and transmission in artificially electro-stimulated electroreceptors [91].

As mentioned above, the mode of action has not been investigated in detail in the presented biomonitoring studies. The proposed mechanism of direct pacemaker depression/stimulation is plausible for analgesics, depressants and stimulants, but has also not been proven.

In addition, it is more than questionable that this mechanism applies to the whole array of substances tested (Tables 1–3). As outlined above, there is good reason to assume that some of the EOD modifications observed were a response to toxin-induced stress mediated by the endocrine system. It also cannot be ruled out that the modified EODs, at least in some cases, directly reflect electro-physical changes in the medium (altered water conductivity [43,76]).

7. Summary

Weakly electric fish comprise a substantial set of unique and interesting characteristics: (1) the signals are, depending on the experimental conditions, rather large and easy to detect and process; (2) the observed parameter does not represent a passive, eventually long-lasting reaction, which is not under active control of the fish (like wound-healing, response to infections/intoxications), but an immediate active reply to a stimulus (section 5); (3) as long as high toxic concentrations are avoided, an individual fish can be tested more than once (sections 5.1 and 5.2) and each experiment is repeatable with one fish (keeping adaptation times in mind), avoiding intraspecific differences; finally, (4), the repeated investigation of several fish at the same time and the evaluation of several parameters from the recorded EOD transients (section 4) provide a stable system with a good signal-to-noise ratio. All these points render weakly electric fish an especially valuable tool for water monitoring. In addition, these characteristics suggest that weakly electric fish might be a helpful tool in basic research experiments not exclusively related to water monitoring (e.g. pharmacology).

Altogether, 18 publications have been identified, which employed six different species of weakly electric fish and measured the change in their EOD after exposure to dissolved chemicals. Thirty-five chemical substances were investigated. The lowest concentration detected was 20 nm bis[tri-n-butyl tin]oxide (TBTO) using *Gnathonemus petersii* and *Campylomormyrus tamandua*.

The most reliable data have been collected using a set-up that measured eight individuals at the same time and evaluated multiple biological parameters—a set-up mandatory for future experiments. In addition, higher sensitivities might be retrieved by screening the numerous types of weakly electric fish not investigated to date.

Acknowledgements

We thank Sönke von den Berg (http://www.fsbio-hannover.de/oftheweek/63.htm) for his permission to use his pictures (supplementary online material) and Robert Mathie for his assistance.

References

[1] H. Bouwer, *Integrated water management: Emerging issues and challenges*, Agric. Water Manage. 45 (2000), pp. 217–228.

[2] H. Bouwer, P. Fox, P. Westerhoff, and J.E. Drewes, *Integrating water management and re-use: Causes for concern?* Water Qual. Int. January–February (1999), pp. 19–22.

[3] B. McCann, *By-product blues*, Water21, July–August (1999), pp. 15–18.

[4] G.A. Boorman, V. Dellarco, J.K. Dunnick, R.E. Chapin, S. Hunter, F. Hauchman, H. Gardner, M. Cox ,and R.C. Sill, *Drinking water disinfection byproducts: Rreview and approach to toxicity evaluation*, Environ. Health Perspect. 107 Suppl. 1 (1999), pp. 207–217.

[5] K.H. Langford, M. Reid, and K.V. Thomas, *Multi-residue screening of prioritised human pharmaceuticals, illicit drugs and bactericides in sediments and sludge*, J. Environ. Monit. 13 (2011), pp. 2284–2291.

[6] A. Ziylan and N.H. Ince, *The occurrence and fate of anti-inflammatory and analgesic pharmaceuticals in sewage and fresh water: Treatability by conventional and non-conventional processes*, J. Hazard. Mater. 187 (2011), pp. 24–36.

[7] C.G. Daughton and T.A. Ternes, *Pharmaceuticals and personal care products in the environment: Agents of subtle change?* Environ. Health Perspect. 107 Suppl. 6 (1999), pp. 907–938.

[8] M.L. Richardson and J.M. Bowron, *The fate of pharmaceutical chemicals in the aquatic environment*, J. Pharm. Pharmacol. 37 (1985), pp. 1–12.

[9] D.O. Hessen and L.J. Tranvik, *Aquatic Humic Substances: Ecology and Biogeochemistry*, Springer, Berlin, 1998.

[10] M.V.L. Bennett and H. Grundfest, *Studies on the morphology and electrophysiology of electric organs. III. Electrophysiology of the electric organs in mormyrids*, in *Bioelectrogenesis*, C. Chagas and A. Paes de Carvalho, eds., Elsevier, Amsterdam, 1961, pp. 113–135.

[11] W.G.R. Crampton and J.S. Albert, *Evolution of electric signal diversity in Gymnotiform fishes. I. Phylogenetic systematics, ecology and biogeography*, in *Communication in Fishes*, F. Ladich, S.P. Collin, P. Moller, and B.G. Kapoor, eds., Science Publishers, Enfield, NH, USA,2006, pp. 647–696.

[12] H.H. Zakon, D.J. Zwickl, Y. Lu, and D.M. Hillis, *Molecular evolution of communication signals in electric fish*, J. Exp. Biol. 211 (2008), pp. 1814–1818.

[13] A.H. Bass and S.F. Volman, *From behavior to membranes: Testosterone-induced changes in action potential duration in electric organs*, Proc. Natl. Acad. Sci. U.S.A. 84 (1987), pp. 9295–9298.

[14] C.D. Hopkins, *Neuroethology of electric communication*, Annu. Rev. Neurosci. 11 (1988), pp. 497–535.

[15] W. Heiligenberg and J. Bastian, *The electric sense of weakly electric fish*, Annu. Rev. Physiol. 46 (1984), pp. 561–583.

[16] M. Kawasaki and Y.X. Guo, *Neuronal circuitry for comparison of timing in the electrosensory lateral line lobe of the African wave-type electric fish* Gymnarchus niloticus, J. Neurosci. 16 (1996), pp. 380–391.

[17] H.W. Lissmann, *On the function and evolution of electric organs in fish*, J. Exp. Biol. 35 (1958), pp. 156–191.

[18] G. von der Emde and S. Schwarz, *Imaging of objects through active electrolocation in* Gnathonemus petersii, J. Physiol. Paris 96 (2002), pp. 431–444.

[19] G. von der Emde and S. Fetz, *Distance, shape and more: Recognition of object features during active electrolocation in a weakly electric fish*, J. Exp. Biol. 210 (2007), pp. 3082–3095.

[20] A.A. Caputi and R. Budelli, *Peripheral electrosensory imaging by weakly electric fish*, J. Comp. Physiol. A 192 (2006), pp. 587–600.

[21] P. Möhres, *Elektrische Organe im Dienste der Revierabgrenzung*, Naturwissenschaften 44 (1957), pp. 431–432.

[22] P. Black-Cleworth, *The role of electrical discharges in the non-reproductive social behaviour of* Gymnotus carapo *(Gymnotidae, Pisces)*, Anim. Behav. Monogr. 3 (1970), pp. 1–77.

[23] G.W.M. Westby and H.O. Box, *Prediction of dominance in social groups of electric fish,* Gymnotus carapo, Psychonomic Sci. 21 (1970), pp. 181–183.

[24] H.O. Box and G.W.M. Westby, *Behavior of electric fish (*Gymnotus-carapo*) in a group membership experiment*, Psychonomic Sci. 21 (1970), pp. 27–28.

[25] J.A. Valone, *Electrical emissions in* Gymnotus carapo *and their relation to social behavior*, Behaviour 37 (1970), pp. 1–14.

[26] C.D. Hopkins, *Sex differences in electric signaling in an electric fish*, Science 176 (1972), pp. 1035–1037.

[27] P. Moller and R. Bauer, *'Communication' in weakly electric fish,* Gnathonemus petersii *(mormyridae). 2. Interaction of electric organ discharge activities of two fish*, Anim. Behav. 21 (1973), pp. 501–512.

[28] T. Szabo, R. Bauer, and P. Moller, *Electric perception and behavior in electric fish*, Naturwissenschaften 60 (1973), pp. 10–18.

[29] C.C. Bell, J.P. Myers, and C.J. Russell, *Electric organ discharge patterns during dominance related behavioral displays in* Gnathonemus petersii *(Mormyridae)*, J. Comp. Physiol. 92 (1974), pp. 201–228.

[30] A. Scheffel and B. Kramer, *Electric signals in the social behavior of sympatric elephantfish (Mormyridae, Teleostei) from the upper Zambezi river*, Naturwissenschaften 87 (2000), pp. 142–147.

[31] K.D. Dunlap, B.T. DiBenedictis and S.R. Banever, *Chirping response of weakly electric knife fish* (Apteronotus leptorhynchus) *to low-frequency electric signals and to heterospecific electric fish*, J. Exp. Biol. 213 (2010), pp. 2234–2242.

[32] P.K. Stoddard, H.H. Zakon, M.R. Markham ,and L. McAnelly, *Regulation and modulation of electric waveforms in gymnotiform electric fish*, J. Comp. Physiol. A 192 (2006), pp. 613–624.

[33] R. Perrone, O. Macadar, and A. Silva, *Social electric signals in freely moving dyads of* Brachyhypopomus pinnicaudatus, J. Comp. Physiol. A 195 (2009), pp. 501–514.

[34] P. Serrano-Fernandez, *Gradual frequency rises in interacting black ghost knifefish,* Apteronotus albifrons, J. Comp. Physiol. A 189 (2003), pp. 685–692.

[35] H. Zakon, J. Oestreich, S. Tallarovic, and F. Triefenbach, *EOD modulations of brown ghost electric fish: JARs, chirps, rises, and dips*, J. Physiol. Paris 96 (2002), pp. 451–458.

[36] P. Machnik and B. Kramer, *Female choice by electric pulse duration: Attractiveness of the males' communication signal assessed by female bulldog fish,* Marcusenius pongolensis *(Mormyridae, Teleostei)*, J. Exp. Biol. 211 (2008), pp. 1969–1977.

[37] L. Maler and W.G. Ellis, *Inter-male aggressive signals in weakly electric fish are modulated by monoamines*, Behav. Brain Res. 25 (1987), pp. 75–81.

[38] B.A. Carlson, C.D. Hopkins, and P. Thomas, *Androgen correlates of socially induced changes in the electric organ discharge waveform of a mormyrid fish*, Horm. Behav. 38 (2000), pp. 177–186.

[39] P.G. Feulner, M. Plath, J. Engelmann, F. Kirschbaum, and R. Tiedemann, *Electrifying love: Electric fish use species-specific discharge for mate recognition*, Biol. Lett. 5 (2009), pp. 225–228.

[40] G.J. Hupe, J.E. Lewis, and J. Benda, *The effect of difference frequency on electrocommunication: Chirp production and encoding in a species of weakly electric fish,* Apteronotus leptorhynchus, J. Physiol. Paris 102 (2008), pp. 164–172.

[41] G.J. Hupe and J.E. Lewis, *Electrocommunication signals in free swimming brown ghost knifefish,* Apteronotus leptorhynchus, J. Exp. Biol. 211 (2008), pp. 1657–1667.

[42] M. Hagedorn and R. Zelick, *Relative dominance among males is expressed in the electric organ discharge characteristics of a weakly electric fish*, Anim. Behav. 38 (1989), pp. 520–525.

[43] A.A. Caputi, A.C. Silva, and O. Macadar, *The electric organ discharge of* Brachyhypopomus pinnicaudatus*. The effects of environmental variables on waveform generation*, Brain Behav. Evol. 52 (1998), pp. 148–158.

[44] W. Geller, *A toxicity warning monitor using the weakly electric fish,* Gnathonemus-petersi, Water Res. 18 (1984), pp. 1285–1290.

[45] J.W. Lewis, P.R. Campbell, and I.P. Toms, *Microcomputer-based monitoring of the effect of phenol on electric organ activity in the mormyrid fish* Gnathonemus petersi, Environ. Technol. 11 (1990), pp. 571–584.

[46] A.N. Kay and J.W. Lewis, *Fish monitors and the role of electric fish as potential indicators of water quality*, J. Appl. Ichthyol. 9 (1993), pp. 110–114.

[47] M. Thomas, A. Florion, D. Chretien, and D. Terver, *Real-time biomonitoring of water contamination by cyanide based on analysis of the continuous electric signal emitted by a tropical fish:* Apteronotus albifrons, Water Res. 30 (1996), pp. 3083–3091.

[48] M. Thomas, D. Chretien, A. Florion, and D. Terver, *Real-time detection of potassium cyanide pollution in surface waters using electric organ discharges wave emitted by the tropical fish,* Apteronotus albifrons, Environ. Technol. 17 (1996), pp. 561–574.

[49] J.W. Lewis, A.N. Kay, and N.S. Hanna, *Responses of electric fish (family mormyridae) to chemical changes in water quality. 2. Pesticides*, Environ. Technol. 14 (1993), pp. 1171–1178.

[50] J.W. Lewis, A.N. Kay, and N.S. Hanna, *Responses of electric fish (family mormyridae) to chemical-changes in water quality. 3. Heavy metals*, Environ. Technol. 15 (1994), pp. 969–978.

[51] J.W. Lewis, A.N. Kay, and N.S. Hanna, *Responses of electric fish (family mormyridae) to chemical-changes in water quality. 1. Cyanide*, Environ. Technol. 13 (1992), pp. 1169–1174.

[52] J.W. Lewis, A.N. Kay, and N.S. Hanna, *Responses of electric fish (family mormyridae) to inorganic nutrients and tributyltin oxide*, Chemosphere 31 (1995), pp. 3753–3769.

[53] H.D. Reinhardt, *Untersuchungen zu Funktion und Empfindlichkeit eines Biotestsystems für die automatischkontinuierliche Wasserüberwachung mit* Gnathonemus petersii *und* Daphnia pulex, Ph.D. diss., Universität Freiburg, 1978 (In German).

[54] J. Cairns and W.H. van der Schalie, *Biological monitoring. 1. Early warning systems*, Water Res. 14 (1980), pp. 1179–1196.

[55] P. Kunze and H.U. Wezstein, *Apomorphine and haloperidol influence electric behavior of a mormyrid fish*, Z. Naturforsch. C 43 (1988), pp. 105–107.

[56] P. Kunze, *Change of behavior with atrazine in an electric fish*, Z. Wasser Abwasserforsch. 22 (1989), pp. 108–111.

[57] P.R. Campbell, J.W. Lewis, and I.P. Toms, *Microcomputer-based detection and analysis of the electric organ discharges in the mormyrid fish* Gnathonemus petersi, Environ. Technol. 11 (1990), pp. 41–50.

[58] W.A. Krivoy, R.A. Huggins, D.C. Kroeger, B.E. Moberley, and J.R. Porter, *A technique for measuring the actions of drugs by using electric fish*, Med. Res. Eng. 6 (1967), pp. 16–20.
[59] B. Holmstedt, W. Krivoy, and D. Kroeger, *Electric fish (*Gymnotus carapo*) as a tool for bioassay of methylphenidate and chlorpromazine*, Acta Physiol. Scand. 61 (1964), pp. 177–181.
[60] W.A. Krivoy, M. Lane, and D.C. Kroeger, *Effects of drugs on the electric knife fish,* Eigenmannia virescens, Proc. Soc. Exp. Biol. Med. 114 (1963), pp. 640–644.
[61] R.R. Walsh and R.T. Schopp, *Modification of electric organ activity of the transparent knifefish by benzocaine*, Comp. Gen. Pharmacol. 2 (1971), pp. 341–346.
[62] R.R. Walsh and R.T. Schopp, *Frequency-depressing action of ethylaminobenzoate isomers on a mormyrid electric fish*, Am. J. Physiol. 211 (1966), pp. 1–5.
[63] B. Kramer, *Tranquilizer reduces electric organ discharge frequency in a teleost fish*, Naturwissenschaften 71 (1984), pp. 99–100.
[64] J.W. Lewis and A.N. Kay, *The effect of temperature on electric organ activity in 2 species of* Gnathonemus *(family mormyridae)*, Environ. Technol. 12 (1991), pp. 815–819.
[65] J.W. Lewis, A.N. Kay and N.S. Hanna, *Responses of the electric fish* Gnathonemus-tamandua *(family mormyridae) to changes in pH*, Environ. Technol. 13 (1992), pp. 701–705.
[66] B. Baier, *Effect of conductivity changes on the stability of electric signal waveforms in dwarf stonebashers (Mormyridae;* Pollimyrus castelnaui,P. marianne), J. Comp. Physiol. A 194 (2008), pp. 915–919.
[67] P. Moller, *'Communication' in weakly electric fish,* Gnathonemus niger *(Mormyridae). 1. Variation of electric organ discharge (EOD) frequency elicited by controlled electric stimuli*, Anim. Behav. 18 (1970), pp. 768–786.
[68] F.T. Erskine, D.W. Howe, and B.C. Weed, *The discharge period of the weakly electric fish* Sternachus albifrons, Am. Zool. 6 (1966), p. 521.
[69] T.H. Bullock, *Species differences in effect of electroreceptor input on electric organ pacemakers and other aspects of behavior in electric fish*, Brain Behav. Evol. 2 (1969), pp. 85–118.
[70] M. Thomas, A. Florion, and D. Chretien, *A new warning biomonitor using a weakly electric fish,* Apteronotus albifrons *(gymnotiformes), and the effect of temperature on the bioelectric responses*, Environ. Monit. Assess. 51 (1998), pp. 605–620.
[71] J.L. Larimer and J.A. MacDonald, *Sensory feedback from electroreceptors to electromotor pacemaker centers in gymnotids*, Am. J. Physiol. 214 (1968), pp. 1253–1261.
[72] M. Hagedorn, *The electric fish* Hypopomus occidentalis *can rapidly modulate the amplitude and duration of its electric organ discharges*, Anim. Behav. 49 (1995), pp. 1409–1413.
[73] C.R. Franchina and P.K. Stoddard, *Plasticity of the electric organ discharge waveform of the electric fish* Brachyhypopomus pinnicaudatus – *I. Quantification of day–night changes*, J. Comp. Physiol. A 183 (1998), pp. 759–768.
[74] P.K. Stoddard, M.R. Markham, V.L. Salazar, and S. Allee, *Circadian rhythms in electric waveform structure and rate in the electric fish* Brachyhypopomus pinnicaudatus, Physiol. Behav. 90 (2007), pp. 11–20.
[75] M.R. Markham, M.L. McAnelly, P.K. Stoddard, and H.H. Zakon, *Circadian and social cues regulate ion channel trafficking*, PLoS Biol. 7 (2009), e1000203. doi:10.1371/journal.pbio.1000203.
[76] M. Thomas, A. Florion, and D. Chretien, *The effect of pH and conductivity on the electric behaviour of* Apteronotus albifrons *(family apteronotidae) in a pollution monitoring system*, Environ. Technol. 18 (1997), pp. 1069–1083.
[77] F. Pimentel-Souza, *Electric organ discharge frequencies from two weakly electric gymnotiform fish exposed to carbon dioxide, conductivity and pH changes*, Braz. J. Med. Biol. Res. 21 (1988), pp. 119–121.
[78] C.W. Coates, M. Altamirano, and H. Grundfest, *Activity in electrogenic organs of knifefishes*, Science 120 (1954), pp. 845–846.
[79] H. Grundfest, *The mechanisms of discharge of the electric organs in relation to general and comparative electrophysiology*, Prog. Biophys. Biophys. Chem. 7 (1957), pp. 1–85.
[80] P.S. Enger and T. Szabo, *Effect of temperature on the discharge rates of the electric organ of some gymnotids*, Comp. Biochem. Physiol. 27 (1968), pp. 625–627.
[81] W.S.G.Morgan, *Bio-monitoring with fish – aid to industrial effluent and surface-water quality control*, Prog. Water Technol. 9 (1977), pp. 703–711.
[82] C.L.M. Poels, *Continuous and automatic monitoring of surface waters with fish*, Effluent Water Treat. J. 15 (1975), pp. 622–625.
[83] W.S.G. Morgan and P.C. Kuhn, *Method to monitor effects of toxicants upon breathing rate of largemouth bass (*Micropterus-salmoides *Lacépède)*, Water Res. 8 (1974), pp. 67–77.
[84] M.A. Cairns, R.R. Garton, and R.A. Tubb, *Use of fish ventilation frequency to estimate chronically safe toxicant concentrations*, Trans. Am. Fish. Soc. 111 (1982), pp. 70–77.
[85] G.P. Evans and D. Johnson, *Detection of pollution at drinking water intakes*, in *Environmental Protection: Standards, Comliance and Costs*, T.J. Lack, ed., Ellis Horwood, Chichester,1984, pp. 239–254.
[86] A.A. Caputi, R. Budelli, K. Grant, and C.C. Bell, *The electric image in weakly electric fish: Physical images of resistive objects* in Gnathonemus petersii, J. Exp. Biol. 201 (1998), pp. 2115–2128.
[87] G. der Emde, S. Schwarz, L. Gomez, R. Budelli, and K. Grant, *Electric fish measure distance in the dark*, Nature 395 (1998), pp. 890–894.
[88] C. Assad, B. Rasnow, and P.K. Stoddard, *Electric organ discharges and electric images during electrolocation*, J. Exp. Biol. 202 (1999), pp. 1185–1193.
[89] C.C. Bell, *Sensory coding and corollary discharge effects in mormyrid electric fish*, J. Exp. Biol. 146 (1989), pp. 229–253.
[90] N.J. Berman and L. Maler, *Neural architecture of the electrosensory lateral line lobe: Adaptations for coincidence detection, a sensory searchlight and frequency-dependent adaptive filtering*, J. Exp. Biol. 202 (1999), pp. 1243–1253.
[91] A.B. Steinbach and M.V.L. Bennett, *Effects of divalent ions and drugs on synaptic transmission in phasic electroreceptors in a mormyrid fish*, J. Gen. Physiol. 58 (1971), pp. 580–598.
[92] A. Watanabe and K. Takeda, *The change of discharge frequency by A.C. stimulus in a weak electric fish*, J. Exp. Biol. 40 (1963), pp. 57–66.
[93] K.D. Dunlap, P.L. Pelczar, and R. Knapp, *Social interactions and cortisol treatment increase the production of aggressive electrocommunication signals in male electric fish,* Apteronotus leptorhynchus, Horm. Behav. 42 (2002), pp. 97–108.

[94] J.H. Meyer and H.H. Zakon, *Androgens alter the tuning of electroreceptors*, Science 217 (1982), pp. 635–637.

[95] J.H. Meyer, *Steroid influences upon the discharge frequencies of a weakly electric fish*, J. Comp. Physiol. A 153 (1983), pp. 29–37.

[96] M. Hagedorn and C. Carr, *Single electrocytes produce a sexually dimorphic signal in South American electric fish,* Hypopomus occidentalis *(Gymnotiformes, Hypopomidae)*, J. Comp. Physiol. A 156 (1985), pp. 511–523.

[97] A.H. Bass and C.D. Hopkins, *Hormonal control of sex differences in the electric organ discharge (EOD) of mormyrid fishes*, J. Comp. Physiol. A 156 (1985), pp. 587–604.

[98] A.H. Bass, *A hormone-sensitive communication system in an electric fish*, J. Neurobiol. 17 (1986), pp. 131–155.

[99] A. Mills and H.H. Zakon, *Coordination of EOD frequency and pulse duration in a weakly electric wave fish: The influence of androgens*, J. Comp. Physiol. A 161 (1987), pp. 417–430.

[100] R.E. Landsman and P. Moller, *Testosterone changes the electric organ discharge and external morphology of the mormyrid fish,* Gnathonemus petersii *(Mormyriformes)*, Experientia 44 (1988), pp. 900–903.

[101] A.H. Bass and C.D. Hopkins, *Hormonal control of sexual differentiation: Changes in electric organ discharge waveform*, Science 220 (1983), pp. 971–974.

[102] A. Mills and H.H. Zakon, *Chronic androgen treatment increases action potential duration in the electric organ of* Sternopygus, J. Neurosci. 11 (1991), pp. 2349–2361.

[103] S. Herfeld and P. Moller, *Effects of 17alpha-methyltestosterone on sexually dimorphic characters in the weakly discharging electric fish,* Brienomyrus niger *(Gunther, 1866) (Mormyridae): Electric organ discharge, ventral body wall indentation, and anal-fin ray bone expansion*, Horm. Behav. 34 (1998), pp. 303–319.

[104] K.D. Dunlap, P. Thomas, and H.H. Zakon, *Diversity of sexual dimorphism in electrocommunication signals and its androgen regulation in a genus of electric fish,* Apteronotus, J. Comp. Physiol. A 183 (1998), pp. 77–86.

[105] J. Schaefer and H.H. Zakon, *Opposing actions of androgen and estrogen on* in vitro *firing frequency of neuronal oscillators in the electromotor system*, J. Neurosci. 16 (1996), pp. 2860–2868.

[106] W.P. Few and H.H. Zakon, *Androgens alter electric organ discharge pulse duration despite stability in electric organ discharge frequency*, Horm. Behav. 40 (2001), pp. 434–442.

[107] M.R. Markham and P.K. Stoddard, *Adrenocorticotropic hormone enhances the masculinity of an electric communication signal by modulating the waveform and timing of action potentials within individual cells*, J. Neurosci. 25 (2005), pp. 8746–8754.

[108] P.K. Stoddard, M.R. Markham. and V.L. Salazar, *Serotonin modulates the electric waveform of the gymnotiform electric fish* Brachyhypopomus pinnicaudatus, J. Exp. Biol. 206 (2003), pp. 1353–1362.