

COMMUNICATION SIGNALS AND SOUND PRODUCTION MECHANISMS OF MORMYRID ELECTRIC FISH

JOHN D. CRAWFORD* AND XIAOFENG HUANG

Department of Psychology, University of Pennsylvania, 3815 Walnut Street, Philadelphia, PA 19104, USA

*e-mail: jud@psych.upenn.edu

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Summary

The African weakly electric fishes *Pollimyrus isidori* and *Pollimyrus adspersus* (Mormyridae) produce elaborate acoustic displays during social communication in addition to their electric organ discharges (EODs). In this paper, we provide new data on the EODs of these sound-producing mormyrids and on the mechanisms they use to generate species-typical sounds.

Although it is known that the EODs are usually species-specific and sexually dimorphic, the EODs of closely related sound-producing mormyrids have not previously been compared. The data presented demonstrate that there is a clear sexual dimorphism in the EOD waveform of *P. isidori*. Females have a multi-phasic EOD that is more complex than the male's biphasic EOD. In this respect, *P. isidori* is similar to its more thoroughly studied congener *P. adspersus*, which has a sexually dimorphic EOD. The new data also reveal that the EODs of these two species are distinct, thus showing for the first time that species-specificity in EODs is characteristic of these fishes, which also generate species-specific courtship sounds.

The sound-generating mechanism is based on a

drumming muscle coupled to the swimbladder. Transverse sections through decalcified male and female *P. adspersus* revealed a muscle that envelops the caudal pole of the swimbladder and that is composed of dorso-ventrally oriented fibers. The muscle is five times larger in males ($14.5 \pm 4.4 \mu\text{l}$, mean \pm S.D.) than in females ($3.2 \pm 1.8 \mu\text{l}$). The fibers are also of significantly larger diameter in males than in females. Males generate courtship sounds and females do not.

The function of the swimbladder muscle was tested using behavioral experiments. Male *P. adspersus* normally produce acoustic courtship displays when presented with female-like electrical stimuli. However, local anesthesia of the swimbladder muscle muted males. In control trials, males continued to produce sounds after injection of either lidocaine in the trunk muscles or saline in the swimbladder muscles.

Key words: electric fish, electric organ discharge, sonic muscle, swimbladder, sexual dimorphism, courtship display, communication, mormyrid, *Pollimyrus isidori*, *Pollimyrus adspersus*.

Introduction

Interest in the sensory physiology of the African mormyrid fish has focused on the unusual morphology of the ear and hearing (Heusinger, 1826; Stipetić, 1939; von Frisch, 1938; Crawford, 1997b) and the generation and detection of weak electric fields for object detection, orientation and communication (Lissman, 1963; Bullock and Heiligenberg 1986; Kramer, 1990; Kramer and Kuhn, 1994; von der Emde and Zelick, 1995; Cain et al., 1994; Moller, 1995; Hopkins et al., 1997). Although various kinds of connections between the swimbladder and the inner ear (otophysic connections) have evolved among other fishes (see Schellart and Popper, 1992), the mormyrids are the only fishes in which each ear has its own dedicated pressure transducer in the form of a separate tympanic ear-bladder. Behavioral (Diesselhorst, 1938; McCormick and Popper, 1984; Marvit and Crawford, 1999) and neurophysiological (Crawford, 1993, 1997a; Kozloski and Crawford, 1998) studies have shown that the mormyrid auditory system is among the most sensitive in

fishes, with hearing extending from approximately 100 Hz to above 1 kHz (Marvit and Crawford, 1999).

The analysis of the electromotor (e.g. Bass, 1986; Grant et al., 1986) and electrosensory systems of mormyrids (e.g. Hopkins, 1988; Amagai et al., 1998; Friedman and Hopkins, 1998; Bell, 1989; Bell and Grant, 1989; von der Emde and Bleckmann, 1997, 1998) has progressed relatively rapidly. The use of electrical signals for communication and orientation is well known, as are many of the anatomical and physiological substrates underlying these behaviors. The electric organ discharge (EOD) waveforms of mormyrids are usually species-specific (e.g. Lissmann, 1958; Moller et al., 1979; Hopkins, 1981; Crawford and Hopkins, 1989), and within species there is usually sexual dimorphism in the waveforms of breeding adults (e.g. Bass and Hopkins, 1983; Bratton and Kramer, 1988; Crawford, 1992).

Two members of the genus *Pollimyrus*, both indigenous to the Niger River basin in West Africa, have highly elaborated

acoustic courtship displays (Crawford et al., 1997a; Crawford, 1997b). Male *P. isidori* and *P. adspersus* expend considerable energy broadcasting species-specific sounds while coaxing females to deposit eggs in their territories (Crawford et al., 1997b). Electric signals also function in communication (see Hopkins, 1986; Kramer, 1990; Moller, 1995) and play a role in evoking sound production in *Pollimyrus* (Crawford, 1992). Our first goal in the current work was to determine whether the typical mormyrid pattern of species- and sex-specificity in EODs exists in these species in which acoustic signaling is so important. Our second goal was to elucidate the peripheral mechanism by which these mormyrids make their courtship sounds. Although much has been discovered about the mechanisms of electrogenesis in mormyrids, the mechanism of sound production has remained a mystery.

In this paper, we show clear species and sex differences in the EODs of *Pollimyrus* spp. and demonstrate the role of a sexually dimorphic swimbladder muscle in male sound production. Portions of this work have been communicated previously (Huang et al., 1996).

Materials and methods

Animals

The specimens of *Pollimyrus isidori* used in these studies were collected in the field by the first author in Mali, West Africa (see Crawford et al., 1997b). These wild-caught adults were used as breeding stock, and some of the data presented in this report are based on fish raised in our laboratory. *Pollimyrus adspersus* were obtained from commercial importers and were reported to have been collected near the Atlantic coast in Nigeria, West Africa. Representative specimens have been deposited in the Ichthyology Collection of the Academy of Natural Sciences, Philadelphia, PA, USA (ANSP 177610-177622).

The morphological differences between these two closely related species have been detailed by Bigorne (1990) and photographed by Crawford et al. (1997b). Briefly, the most definitive distinction between *P. isidori* and *P. adspersus* seems to be the number of scales around the caudal peduncle: 12 in *P. adspersus* and 14 or more in *P. isidori*. Adult *P. isidori* are smaller than adult *P. adspersus*, and they have a more elongate body shape. In males, the curvature (indentation) at the base of the anal fin may be more pronounced in *P. isidori*. Both species produce sounds, and these acoustic signals are also distinct (see Results). In both species, the EODs are only approximately 100 μ s in duration but, like the sounds, the EODs also distinguish these species. Live *P. isidori* tend to be very light in coloration on the sides, some individuals being distinctly silvery, and have a yellowish hue on the dorsal surface. In contrast, *P. adspersus* are often quite dark – sometimes red-brown in coloration. However, coloration is somewhat variable within a species and may change within an individual over time. Additional discussion of these *Pollimyrus* species, and their use in earlier published studies, has been

provided in previous papers (Crawford, 1997b; Crawford et al., 1997b).

Fish were maintained in laboratory aquaria and bred according to methods described previously (Crawford, 1992; Crawford et al., 1997a). Animals were brought into breeding condition by simulating a tropical rainy season in 500 l aquaria. The EODs and sounds for our study were recorded from adult fish that had been exposed to environmental conditions that induced reproductive behavior.

EOD and sound recording

EODs were recorded in water with a conductivity of $35 \pm 5 \mu\text{S cm}^{-1}$ and a temperature of $27 \pm 1 \text{ }^\circ\text{C}$ (means \pm s.d.). Three low-impedance carbon rods were used as electrodes for differential recording with a PAR 5115 amplifier. The recording bandwidth of the amplifier was 30 Hz to 300 kHz, and EODs were digitized at 5 MHz (200 ns point⁻¹) with a Tektronix 2221A oscilloscope. The sounds presented here were recorded from adult breeding males as described in earlier papers (Crawford et al., 1997a,b). Sounds were digitized at 44 kHz and analyzed using bioacoustics software (Canary version 1.2). The sonagrams in Fig. 2 are based on 2048-point fast Fourier transforms (FFTs), with a frame length of 1024 points, 87.5% overlap, those in Fig. 7 with 512-point FFTs, 256-point frame length, 50% overlap. In both cases a Hamming window was used.

Swimbladder volume

Fish were perfused with physiological saline followed by AFA fixative (Formol-Alcohol; Humason, 1967). Specimens were then immersion-fixed for 1 week in AFA. Swimbladder volume was determined by removing the intact bladder and filling it from a known calibrated volume of fluid. The bladders were first evacuated with a syringe and hypodermic needle under a dissecting microscope to remove any fixative that had penetrated the bladder. The length of the bladder was measured. With the long axis of the bladder inclined at 45° on a glass slide, the bladder was filled with a gelatin solution (10% in water) from a calibrated glass microsyringe (Hamilton; 1 ml). A second penetration of the swimbladder was made near the filling point to allow gas to escape as the bladder was filled. Bladders filled in this fashion assumed a normal appearance, typical of bladders observed in live fish. The length was remeasured to confirm that the bladders had not been distended by the filling procedure.

Swimbladder muscle analysis

The swimbladder and adjacent structures were examined in transverse sections of decalcified fish. AFA-fixed specimens were decalcified using an acidic decalcifying solution (Fisher Scientific; Calex, CS510-1D). Specimens were exposed to Calex for 16 h on a vibrating table, with a solution change after the first 4 h. Tissue was dehydrated through a series of alcohols (ethanol and *t*-butanol) and infiltrated with paraffin in a vacuum oven. Sections were cut at 30 μ m on a rotary microtome and mounted on glass slides. The sections were

deparaffinized with toluene and rehydrated before staining. Tissue sections were stained with a Mallory Trichrome staining procedure, employing acid fuchsin, Methyl Blue and Orange G stains (Humason, 1967; Gray, 1964).

The rostro-caudal length of the swimbladder muscle was measured by counting the sections that included the muscle. Muscle surface areas were measured from images digitized using a dissecting microscope (Leica) equipped with a CCD video camera (NEC T1-24A). Images were analyzed using JAVA (version 2.0) analysis software. Muscle volumes were estimated from the surface areas of a series of 10 transverse sections evenly spaced along the rostro-caudal extent of the muscle, using linear interpolation between sections. In Fig. 5, muscle volume (i.e. size) was normalized to body volume. Body volume was measured by fluid displacement in a graduated cylinder.

In addition to measuring muscle surface areas, the diameters of individual muscle fibers were estimated from fiber profiles in the same transverse sections. The width of each profile was measured perpendicular to the fiber's long axis. This was performed from digitized images made with a compound microscope (Leica) with a 40 \times objective; the CCD video camera and software were as described above. For each fish, 260 fiber profiles were measured. Sampling was as follows. Thirty fibers were measured from each of the 10 sections starting along the rostro-caudal length of the muscle. Ten fibers were sampled from each of three quadrants, dorsal, middle and ventral. Sections through the distal-most parts of the muscle (i.e. near the ends) were small in area and closer to circular in shape, and only 10 fiber profiles were measured from each of these sections. Thus, for each fish, the mean fiber diameter was estimated from an average of 260 measured profiles (i.e. $8 \times 30 + 2 \times 10$).

Results

Electric organ discharges

There has been extensive characterization of the EODs of adult *P. adspersus* under breeding conditions (Crawford, 1992) and under non-breeding conditions (Westby and Kirschbaum, 1982; Bratton and Kramer, 1988). However, the EODs of *P. adspersus*, and those of its close relative *P. isidori*, have not been compared because the EODs of *P. isidori* have not previously been recorded. This comparison is of particular interest since these are the only two species of electric fish so far known to use sounds during reproductive communication (Crawford et al., 1997a). The EODs of eight *P. isidori* originating from Mali were examined (Fig. 1). They were similar to those of *P. adspersus* (compare Fig. 1E here and with Crawford, 1992) in that (1) the most prominent component of the EOD was a large negative-going phase (N) of short duration (<50 μ s) and (2) the total duration of the EOD (D1) was approximately 100 μ s in both species: $102 \pm 25.5 \mu$ s for eight *P. isidori* (Fig. 1) compared with $88 \pm 20.8 \mu$ s ($N=14$) in *P. adspersus* (means \pm S.D.) (see also Table 2 in Crawford, 1992).

The differences between species in EOD waveform were sufficient to classify these species reliably with the aid of an

oscilloscope and might be exploited by fish for communication (see Hopkins and Bass, 1981; Graff and Kramer, 1992; von der Emde and Zelick, 1995; Amagai, 1998). Female *P. isidori* EODs had a small positive-going component (P0 in Fig. 1) that preceded the first larger positive peak (P1). This component (P0) was not present in *P. adspersus* of either sex. Male *P. isidori* EODs were biphasic (P1 and N). The male *P. isidori* EODs lacked a P2 component, and this distinguished them from the EODs of both male and female *P. adspersus*. As in *P. adspersus*, small differences in the EOD waveform of *P. isidori* distinguished males from females. The most obvious sexual dimorphism was the presence of the P0 component in females but not in males.

Acoustic communication signals

Male *P. isidori* and *P. adspersus* both produced conspicuous acoustic displays while courting females from their territories (Fig. 2; Crawford, 1997b; Bratton and Kramer, 1989). The

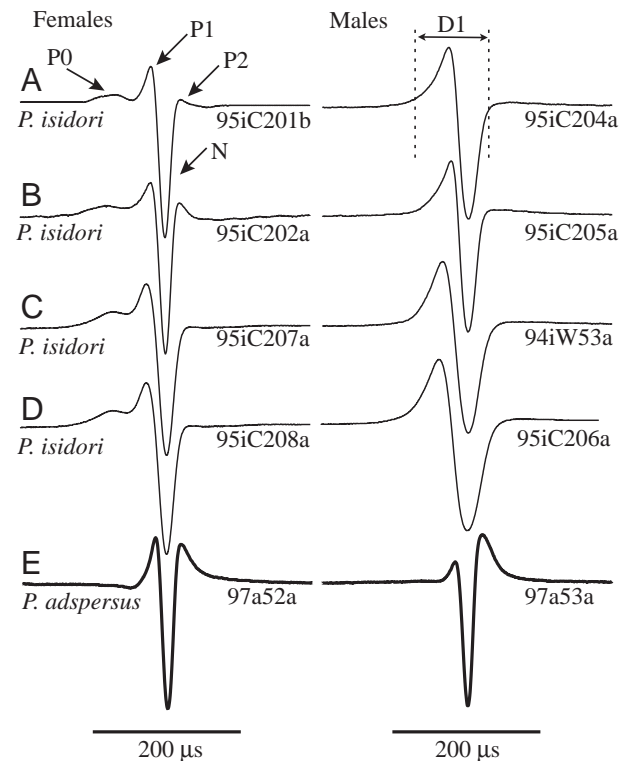


Fig. 1. Examples of electric organ discharges (EODs) recorded from *Pollimyrus isidori* (A–D) and *P. adspersus* (E). EODs of adult breeding females are shown on the left and of males on the right. The various components of the EOD are indicated for the first pair of EODs (A; see also Crawford, 1992). Three positive (P) components (P0, P1 and P2) and a single negative component (N) are indicated on the female EOD (left), and the total duration (D1), as measured with a criterion of 5%, is shown on the male EOD (right). EODs were normalized to equivalent peak-to-peak voltage. The magnitude of the *Pollimyrus* EOD field is approximately 5 mV cm^{-1} at a distance of 10 cm in water of conductivity $35 \mu\text{S cm}^{-1}$. Further details of the EODs of *P. adspersus* can be found in Crawford (1992). Fish identification numbers are given.

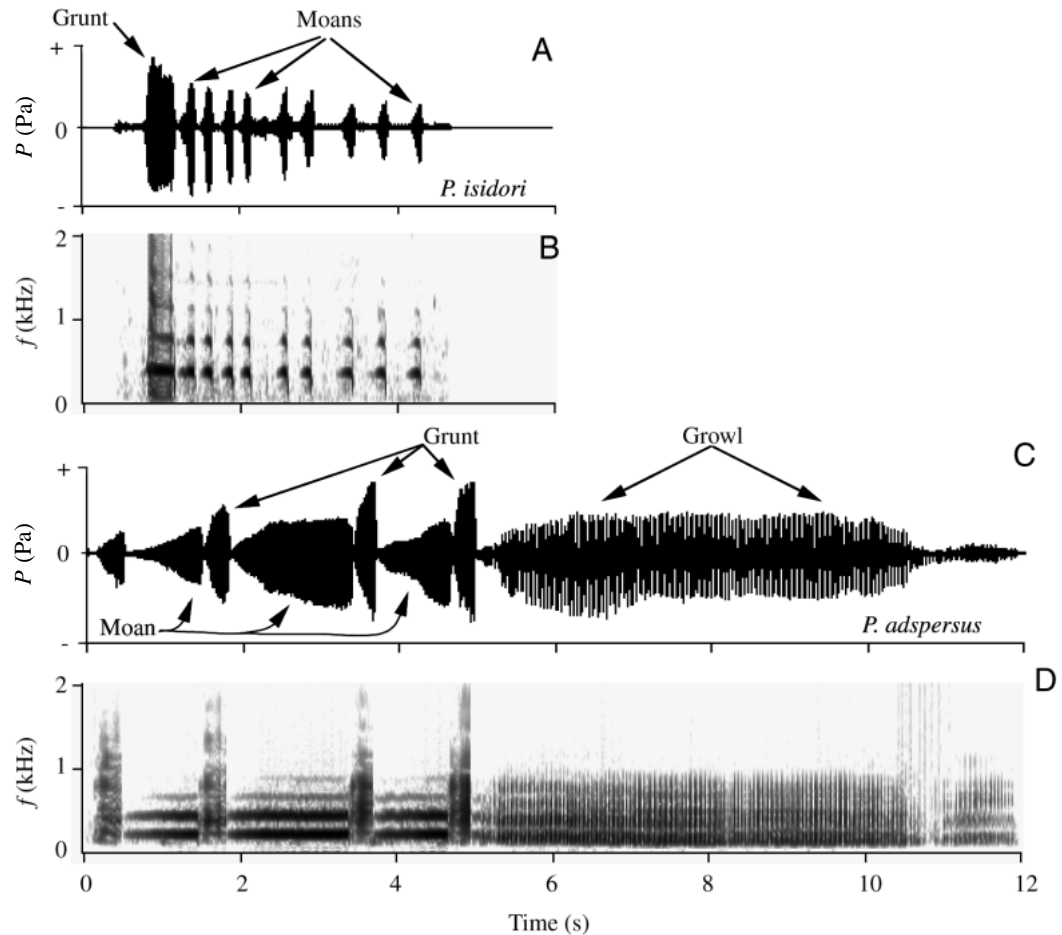


Fig. 2. Sonic displays of male *Pollimyrus isidori* (A,B) and *P. adspersus* (C,D). Each example is shown as an oscillographic display of the pressure waveform (P) (A,C) and a sonographic display of frequency (f) as a function of time (B,D). This figure has been reproduced from Crawford (1997b) with permission from Gordon and Breach Publishers, Lausanne.

sounds were of low frequency, with a bandwidth from approximately 100 Hz to 3 kHz, and with harmonically related peaks in the 200–1 kHz region. The displays of these species were rather stereotypic, consisting of two distinct types of sound. The first type, the grunt, consisted of a sequence of brief acoustic pulses (duration 5 ms). The pulses were regularly spaced at intervals of 16–25 ms. *P. isidori* grunted with a significantly lower pulse repetition rate (44 pulses s^{-1}) than did *P. adspersus* (56 pulses s^{-1}). The second type of courtship sound, the moan, was distinguished by their tonal quality. These sounds were complex tone bursts with a clear fundamental and harmonic (Fig. 2B,D). Moans appeared to be generated by producing acoustic pulses at high rates, so that the resulting sound pressure waveform was continuous. Male *P. isidori* (332 Hz) moaned at a higher peak frequency than *P. adspersus* (240 Hz) (Fig. 2B,D).

These two elements of the courtship display were used in different temporal patterns in the two species of *Pollimyrus*. In *P. isidori*, males typically produced a single grunt followed by a succession of brief moans (Fig. 2A). In *P. adspersus*, grunts and moans were produced in alternation (Fig. 2C). Thus, not only were there clear species differences in the structure of grunts and moans, but there were also differences in the overall temporal pattern formed by these two sounds during displays. Male *P. adspersus* often generated a third sound, a growl,

following a grunt–moan sequence (Fig. 2C). *P. isidori* did not produce growls after their grunt–moan sequences.

Swimbladder and swimbladder muscles

Like other mormyrids, *Pollimyrus* have a swimbladder that occupies a large portion of the body cavity (Fig. 3A; Orts, 1967). Since the swimbladder plays a critical role in coupling the mechanical energy to the water for sound production in many fishes (for reviews, see Schneider, 1967; Tavolga, 1971; Fine, 1997), we suspected that the swimbladder of *Pollimyrus* might be part of the sound-generating apparatus. Moreover, since there is behavioral dimorphism in sonic production, we expected a corresponding sexual dimorphism in the swimbladder or in other structures associated with it.

We found no sexual dimorphism in swimbladder volume when we examined adult breeding *P. adspersus*. The swimbladder scaled in proportion to body volume in both sexes, representing a fixed 5% of body volume (Fig. 3B). However, we discovered a marked dimorphism in the size of a muscle that envelops the caudal pole of the swimbladder (Fig. 3A). The muscle was extrinsic to the bladder wall and remained *in situ* when the swimbladder was removed from the body cavity. In males, this muscle formed a dense band of tissue composed of dorso-ventrally oriented muscle fibers (Fig. 4). The muscle was present in females (Fig. 4D), but was much

smaller than in males (Fig. 4B). The total volume of the muscle (left and right sides combined) was approximately five times larger in males ($14.5 \pm 4.4 \mu\text{l}$) than in females ($3.2 \pm 1.8 \mu\text{l}$; $N=7$, means \pm s.d.; $t=8.3$, d.f.=12, $P \leq 0.0001$; t -test after arcsine transformation of volumes normalized to body size; Fig. 5A). We also found that muscle size increased rapidly with male body size (slope $1.86 \mu\text{l ml}^{-1}$), but increased only marginally with body size in females (slope $0.28 \mu\text{l ml}^{-1}$; Fig. 5B). The individual fibers constituting these bladder muscles were larger in males than in females. The mean fiber diameter was approximately 2.5 times greater in males, and this difference was also highly significant ($t=5.35$; d.f.=12; $P \leq 0.0002$; Fig. 6). Thus, there was a pronounced sexual dimorphism in the *P. adspersus* swimbladder muscle, suggesting that males may use this muscle for sound production. By comparison, there were no significant differences between males and females in the diameters of either red or white muscle fibers sampled from within the trunk musculature.

Behavioral evaluation of swimbladder muscle function

The presentation of a female-like electric display is a well-known and potent releaser of male sonic courtship in *P. adspersus* (Crawford, 1991; Crawford et al., 1997a). This evoked-courtship technique was used to evaluate sound

production behavior under several different conditions, including unilateral anesthetic injections into the sonic muscle (0.2% lidocaine with 1 p.p.m. epinephrine; Abbot Laboratories, Chicago, USA). Males normally produced a strong courtship display consisting of an alternation of grunts and moans during an EOD stimulus (Fig. 7A). Even males that had been removed from their home territory and injected with lidocaine in the trunk muscles gave a sonic display within minutes of being replaced on their home territory (Fig. 7B). When lidocaine was injected into the swimbladder muscle, males continued to respond to the play-back electrode by rapidly circling it, but did not make any sounds (Fig. 7C). Thus, territorial males failed to generate sounds when their swimbladder muscles were immobilized with local anesthetic.

Five different males were tested under combinations of pre-test, control and injection of lidocaine into the swimbladder muscle (Fig. 8). All the males tested during a pre-test presentation of the electric stimulus (Fig. 8: left column) produced sounds. Two individuals (C and D) were injected in the sonic muscle with physiological saline ($30 \mu\text{l}$) prior to testing, and both responded to the electric stimulus with sound production. Two individuals (B and C) received an alternative control procedure in which lidocaine was injected into the trunk musculature ($30 \mu\text{l}$), and they also responded to the electric stimulus with sound production. In contrast, none of four individuals receiving injections ($30 \mu\text{l}$) of the same lidocaine solution into the swimbladder muscle (A, C, D and E) made any sounds during presentation of the electric stimulus. These results suggested that male sound production depends upon the sexually dimorphic swimbladder muscles in *Pollimyrus*.

Discussion

There are several hundred species of mormyrid fish and, so far as is known, all are weakly electric. Species-specificity and sexual dimorphism in the EOD waveform appear to be the rule, at least among sexually reproductive fish. The data presented here show for the first time that there are species and sex differences in EODs, even among closely related species in which the use of elaborate sounds has evolved. Thus, both EODs and sounds could potentially mediate mate choice in these species.

EOD-based behavioral decisions (e.g. Hopkins and Bass, 1981) may be difficult for *Pollimyrus* because the duration of the EOD is extremely short ($<100 \mu\text{s}$). Behavioral and neurophysiological studies (Hopkins and Bass, 1981; Amagai et al., 1998; Amagai, 1998) indicate that the salient features of EODs are time disparities between the major voltage transitions, transitions that are encoded by phase-locked spikes generated in the Knollenorgan pathway. The *Pollimyrus* EODs fall at one extreme in the roughly three orders of magnitude range of EOD durations known among mormyrids: approximately $80 \mu\text{s}$ to 20 ms (Hopkins, 1986). Moreover, the temporal differences between male and female EODs within a species are even smaller, representing only a few microseconds

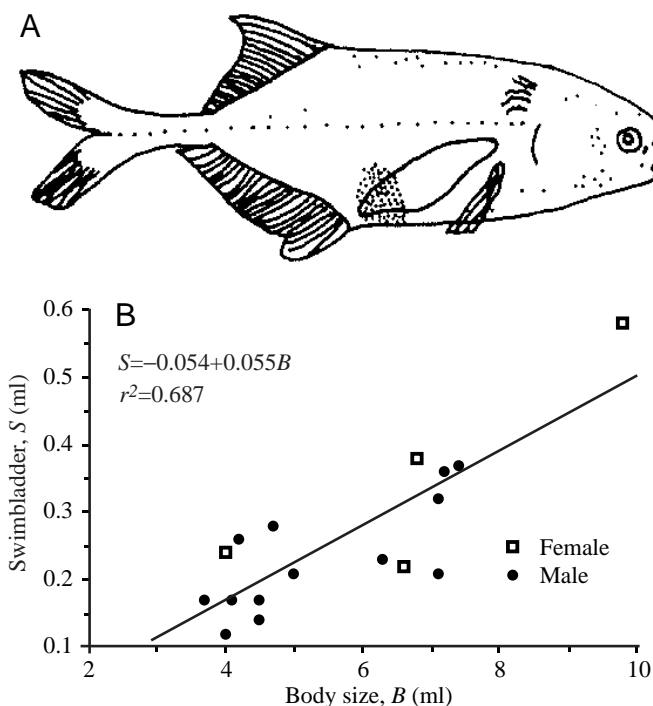
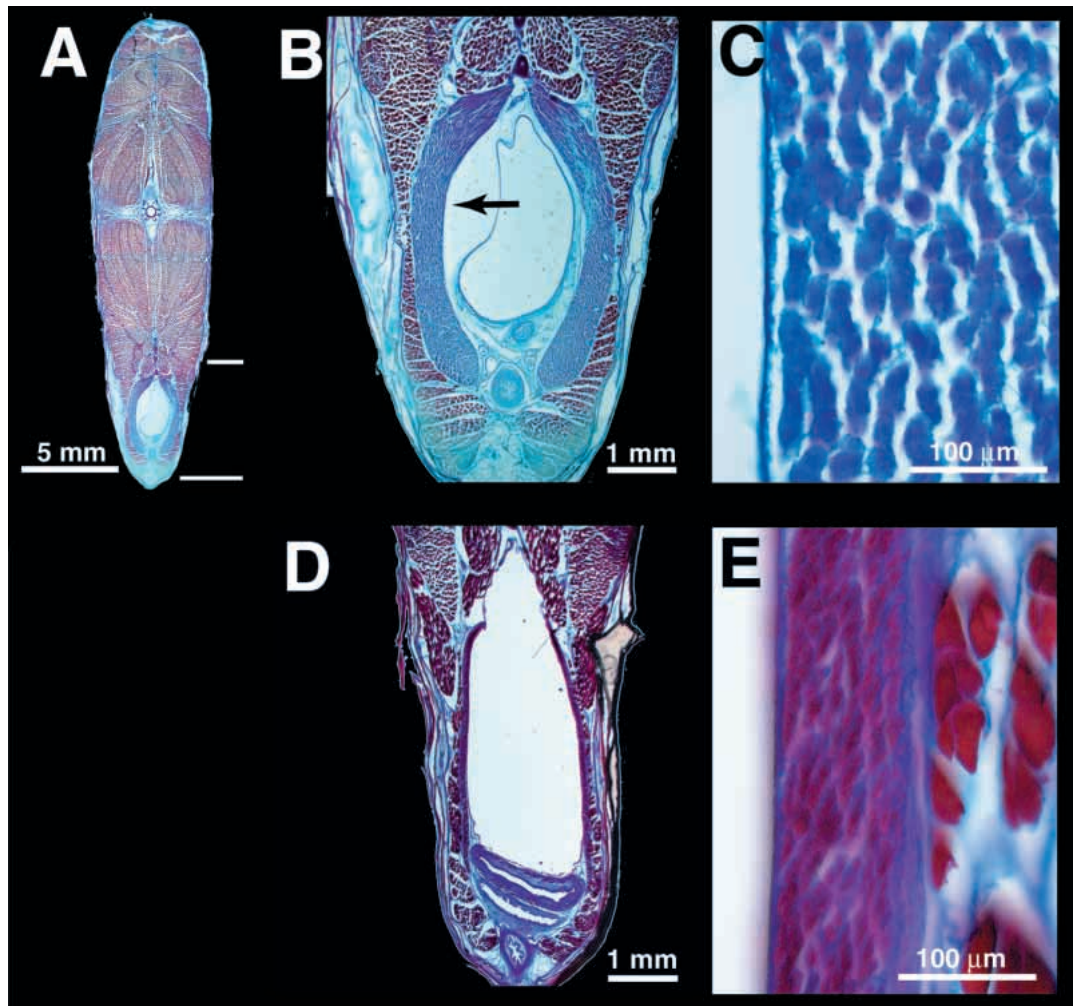


Fig. 3. (A) Drawing of a male *Pollimyrus adspersus* and (B) graph of swimbladder size (S) as a function of body size (B). The profile of the swimbladder is illustrated as it extends in a postero-ventral direction from just above the pectoral fin in A. The location of the swimbladder muscle is indicated by stippling at the posterior pole of the swimbladder. The graph (B) shows that the swimbladder volume increased linearly with body size in both males and females, and this correlation was significant ($r=0.83$, $P < 0.001$, d.f.=15).

Fig. 4. Transverse sections through the region of the swimbladder muscles in male (A–C) and female *Pollimyrus adspersus* (D,E). At low magnification (A,B), the large open area in the center of the body is occupied by the swimbladder lumen. The arrow in B shows the location of bands of dorso-ventrally oriented swimbladder muscle fibers. In the male (B,C) these muscles were quite large and stained fuchsia in the Mallory Trichrome procedure. The surrounding trunk muscles were oriented longitudinally and stained a darker red color. In females (D,E) the band of dorso-ventrally oriented swimbladder muscles was much smaller and stained more like the surrounding trunk muscles. C and E show the muscle fibers at high magnification, revealing differences in morphology between males and females (see Fig. 6).

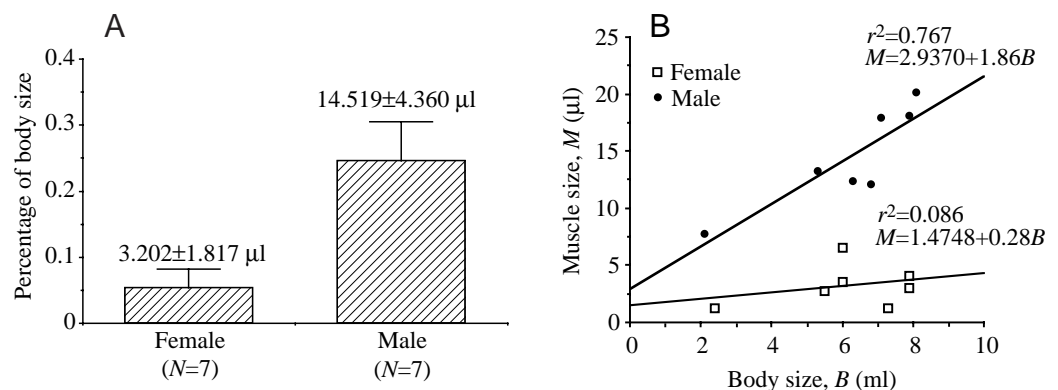


in *Pollimyrus* spp. In the mormyrid species for which there is experimental evidence for EOD-based sex recognition (*Brienomyrus brachyistius*), the duration of the EOD is approximately an order of magnitude longer than in *Pollimyrus*.

Behavioral studies with *P. adspersus* showed that males did not discriminate between male and female EOD waveforms

during sonic courtship. Rather, these studies demonstrated that the temporal pattern with which females presented their EODs to males was important and controlled the male's sonic behavior (Crawford, 1991). While the EODs of the two *Pollimyrus* species appear to be sufficient for reliable recognition of sex and species by human investigators, it remains to be seen whether the fish exploit the small

Fig. 5. Mean swimbladder muscle size as a percentage of body size (A) and the regression of actual muscle size on body size (B). Histogram columns in A show the mean value and the error bars indicate +1 S.D. The actual means (μl) with their standard deviations are provided above each bar. In B, an equation for the best-fitting line relating muscle size (M) to body size in ml (B) is provided at the right of each line. There was a highly significant difference in the slopes of the regressions for males and females ($t=9.54$, d.f.=10, $P<0.001$).



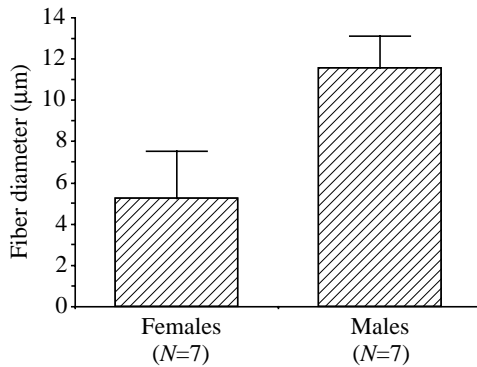


Fig. 6. Comparison of fiber diameters for male and female swimbladder muscles. Histogram columns indicate the mean value and the error bars show +1 s.d. Each individual is represented as the mean of 260 fiber diameters (see Materials and methods).

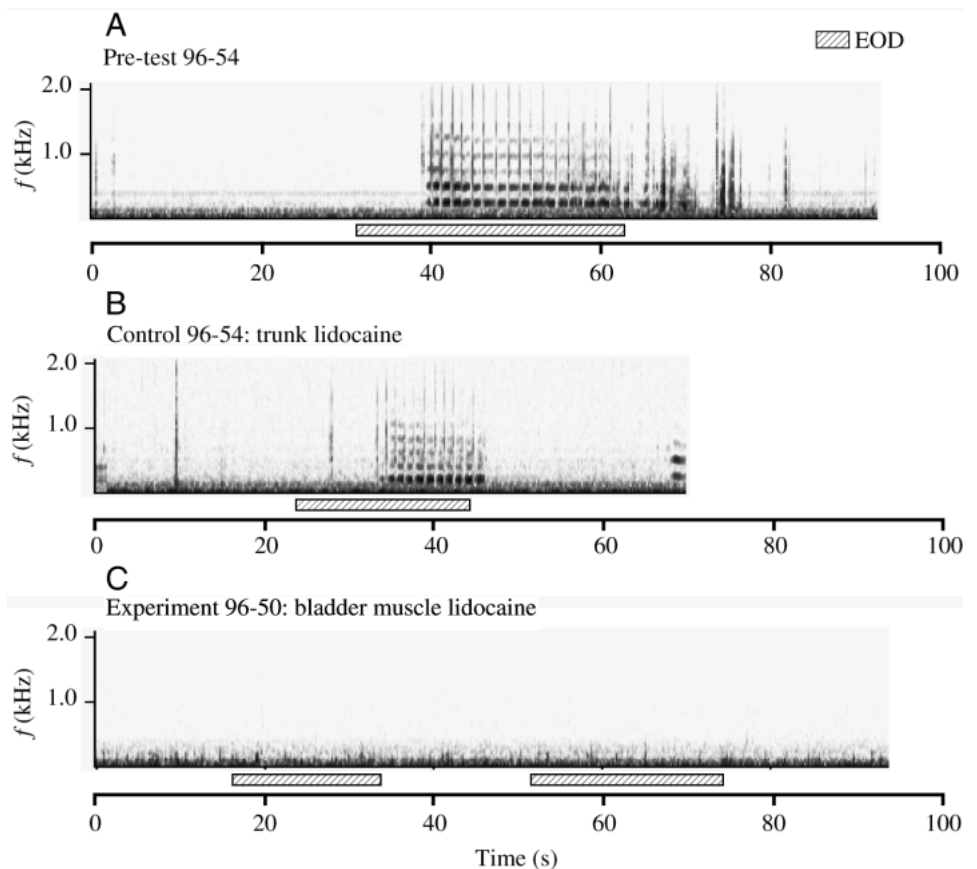
differences in EOD fine structure under natural circumstances. However, there is evidence that mormyrids with short-duration EODs, such as *Pollimyrus*, can be trained to discriminate between pairs of different conspecific EODs (Graff and Kramer, 1992), indicating that waveform discrimination may indeed be important under some circumstances. Nevertheless, it appears most likely that female *Pollimyrus* rely heavily on the robust acoustic cues presented by males for mate choice.

There are pronounced species and individual differences in the courtship sounds made by male *P. isidori* and *P. adspersus* (Crawford et al., 1997a). Male sound production is much more

energetically demanding than EOD production (Crawford et al., 1997b). Since these sounds have no known function other than communication, it is likely that female mate choice has been an important driving force in the evolution of these acoustic displays. Females may be able to make better choices on the basis of male sounds than on EODs because the sounds are produced mechanically and reveal information about potentially important physical attributes of the male sound producer (Crawford et al., 1997b). Because there is heavy investment in egg production by females, and a male-biased sex ratio of at least 2:1 (males to females) in natural breeding areas, individual females are likely to gain strong adaptive advantage by restricting their mating to robust mates of the appropriate species through informed choice (Andersson, 1994).

Given strong selection for discriminant mating by females, the relative scarcity of females and the comparatively small investment by males in gamete production, the best strategy for males could be to accept female mates in a relatively indiscriminant fashion (see Searcy and Brenowitz, 1988; Barlow, 1992; McLennan, 1995). There may even be some advantage for males gained by mixing viable eggs from matings with conspecific females with eggs from matings with heterospecifics (i.e. dilution effect; see Kynard, 1978; Colgan and Gross, 1977): decoy eggs could potentially increase the survival of the viable eggs in the event of egg predation in the male's nest (Hamilton, 1971; Sweeney and Vannote, 1982; Lank et al., 1991). Thus, the small species differences in EODs

Fig. 7. Behavioral experiments that demonstrated the role of the swimbladder muscle of *Pollimyrus adspersus* in male sound production. In these sonographic displays (A and B only), grunts appear as vertical lines and moans as horizontal stacks of harmonically related bands. The examples show acoustic responses to EOD-like stimuli presented to males under (A) normal pre-test conditions, (B) control conditions in which anesthetic was injected into the trunk muscles, and (C) experimental conditions in which anesthetic was injected into the putative sonic muscle. The electrosensory stimulus presentation is indicated by the horizontal bars under each sonagram. In all three cases, the males responded vigorously to the presented electrosensory stimulus with rapid swimming around the presentation electrode; however, no sounds were made after the swimbladder muscle had been injected with local anesthetic (C). In C, the electrosensory stimulus was presented twice. *f*, frequency.



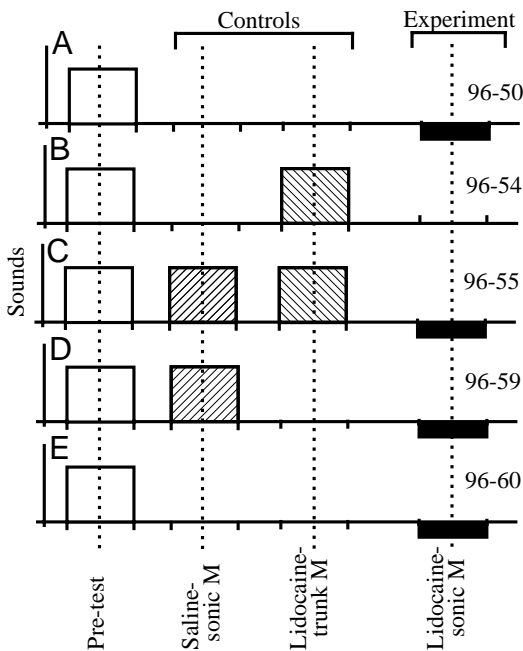


Fig. 8. Summary of behavioral trials evaluating sound production under the pre-test, control and experimental conditions. The presence of a column, up or down, indicates that the particular male (A–E) was tested under the condition listed along the bottom. As presented, the data are in binary form, simply indicating whether males made any sounds. All five males produced sounds when pre-tested (left column, open bars). The downward black bars indicate that the males were tested but made no sounds (experimental column, right side). Pre-test, stimulus presented with no manipulation to the test animal; Saline-sonic M, saline injection into the swimbladder muscle prior to testing; Lidocaine-trunk M, lidocaine injected into the trunk muscles; Lidocaine-sonic M, lidocaine injected into the swimbladder muscle. Fish identifications are provided in each panel (e.g. 96-50).

among female *Pollimyrus* may not actually pose an important discrimination problem for males.

The muscles enveloping the caudal pole of the *Pollimyrus* swimbladder are strongly dimorphic in both total size and muscle fiber diameter, and local anesthesia of these muscles renders males mute. We conclude that these muscles are sonic muscles used for generating grunts, moans and growls, and possibly the other sounds made by these animals (see Crawford et al., 1986, 1997a,b). The muscles appear to be drumming muscles used to drive the swimbladder into oscillation according to various temporal patterns determined by motor circuits in the brainstem (see Bass et al., 1986, 1994). In these respects, the sonic mechanisms of mormyrids appear to be like those of a phylogenetically diverse collection of fishes known to make sounds by drumming on the swimbladder with specialized muscles (for reviews, see Schneider, 1967; Tavalga, 1971; Bass et al., 1997; Fine, 1997).

In those fishes in which swimbladder-based sound-production mechanisms have been more completely investigated, the fundamental frequency of the sounds is determined by the

frequency at which the sonic muscles contract (Skoglund, 1961; Fine, 1978; Bass and Baker, 1991). Further, recent studies of toadfish (*Opsanus*) suggest that the individual muscle fibers are capable of contracting on each contraction cycle of the muscle (Rome et al., 1996). Thus, these studies indicate that, during sound production, each fiber contracts at the same frequency as the fundamental frequency of the emitted sound. In the oyster toadfish (*Opsanus tau*), the fundamental frequency is typically approximately 200 Hz (Fine, 1997) or one fiber contraction every 5 ms. In the light of these findings, the sonic muscles of *P. isidori* could be particularly interesting because of the unusually high fundamental frequency of their moan. The fundamental frequency of the moan in *P. isidori* is typically approximately 332 Hz (estimated population mean at 29 °C), and some individuals consistently achieve a moan fundamental in the range 375–400 Hz (Crawford et al., 1997a). If individual fibers are contracting at these high frequencies, they must be contracting faster than any of the vertebrate superfast twitch fibers described to date (Rome et al., 1996).

The mormyrids have evolved two mechanisms for broadcasting energy into their aquatic environment for communication at night. Both mechanisms used muscle tissue as starting material which was modified in opposing directions through selection. In the electromotor system, the contractile machinery was dispensed with to make an electric organ that generates a significant external electric field. In the sonic motor system, muscles were modified to contract at unusually high rates and to drive the gas-filled buoyancy-control system as a sound radiator. Female behavior has probably been the principal force driving the evolution of male sound production, and the electromotor system has probably been shaped through a complex of constraints including the requirements of the electrosensory orientation system and social interactions with other electric fish.

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References

- Amagai, S. (1998). Time coding in the midbrain of mormyrid electric fish. II. Stimulus selectivity in the nucleus extero-lateralis pars posterior. *J. Comp. Physiol. A* **82**, 131–143.
- Amagai, S., Friedman, M. A. and Hopkins, C. D. (1998). Time coding in the midbrain of mormyrid electric fish. I. Physiology and anatomy of cells in the nucleus extero-lateralis pars anterior. *J. Comp. Physiol. A* **82**, 115–130.

- Andersson, M.** (1994). *Sexual Selection*. Princeton: Princeton University Press.
- Barlow, G.** (1992). Is mating different in monogamous species? The midas cichlid fish as a case study. *Am. Zool.* **32**, 91–99.
- Bass, A. H.** (1986). Evolution of a vertebrate communication and orientation organ. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 13–70. New York: John Wiley & Sons.
- Bass, A. H. and Baker, R.** (1991). Evolution of homologous vocal control traits. *Brain Behav. Evol.* **42**, 336–349.
- Bass, A. H., Bodnar, D. A. and McKibben, J. R.** (1997). From neurons to behavior: vocal-acoustic communication in teleost fish. *Biol. Bull.* **192**, 158–160.
- Bass, A. H. and Hopkins, C. D.** (1983). Hormonal control of sexual differentiation: changes in electric organ discharge waveform. *Science* **220**, 971–974.
- Bass, A. H., Marchaterre, M. A. and Baker, R.** (1994). Vocal-acoustic pathways in a teleost fish. *J. Neurosci.* **14**, 4025–4039.
- Bass, A. H., Segil, N. and Kelley, D. B.** (1986). Androgen binding in the brain and electric organ of a mormyrid fish. *J. Comp. Physiol. A* **159**, 535–544.
- Bell, C. C.** (1989). Sensory coding and corollary discharge effects in mormyrid electric fish. *J. Exp. Biol.* **146**, 229–253.
- Bell, C. C. and Grant, K.** (1989). Corollary discharge inhibition and preservation of temporal information in a sensory nucleus of mormyrid electric fish. *J. Neurosci.* **9**, 1029–1044.
- Bigorne, R.** (1990). Revision systematique du genre *Pollimyrus* (Teleostei, Mormyridae) en Afrique de l'Ouest. *Rev. Hydrobiol.* **4**, 313–327.
- Bratton, B. O. and Kramer, B.** (1988). Intraspecific variability of the pulse-type discharges of the African electric fishes *Pollimyrus isidori* and *Petrocephalus bovei* (Mormyridae, Teleostei) and their dependence on water conductivity. *Exp. Biol.* **47**, 227–238.
- Bratton, B. O. and Kramer, B.** (1989). Patterns of the electric discharge during courtship and spawning in the mormyrid fish, *Pollimyrus isidori*. *Behav. Ecol. Sociobiol.* **24**, 349–368.
- Bullock, T. H. and Heiligenberg, W.** (1986). *Electroreception*. New York: John Wiley & Sons.
- Cain, P., Gerin, W. and Moller, P.** (1994). Short-range navigation of the weakly electric fish, *Gnathonemus petersii* (Mormyridae, Teleostei), in novel and familiar environments. *Ethology* **96**, 33–45.
- Colgan, P. and Gross, M.** (1977). Dynamics of aggression in male pumpkinseed sunfish (*Lepomis gibbosus*) over the reproductive phase. *Z. Tierpsychol.* **43**, 139–151.
- Crawford, J. D.** (1991). Sex recognition by electrical cues in a sound producing mormyrid fish, *Pollimyrus isidori*. *Brain Behav. Evol.* **38**, 20–38.
- Crawford, J. D.** (1992). Individual and sex specificity in the electric organ discharges of breeding mormyrid fish (*Pollimyrus isidori*). *J. Exp. Biol.* **164**, 79–102.
- Crawford, J. D.** (1993). Central auditory neurophysiology of a sound-producing mormyrid fish: the mesencephalon of *Pollimyrus isidori*. *J. Comp. Physiol. A* **172**, 1–14.
- Crawford, J. D.** (1997a). Feature-detecting auditory neurons in the brain of a sound-producing fish. *J. Comp. Physiol. A* **180**, 439–450.
- Crawford, J. D.** (1997b). Hearing and acoustic communication in the mormyrid electric fishes. *Mar. Freshwater Behav. Physiol.* **29**, 1–21.
- Crawford, J. D., Cook, A. P. and Herberlein, A. S.** (1997a). Bioacoustic behavior of African fishes (Mormyridae): potential cues for species and individual recognition in *Pollimyrus*. *J. Acoust. Soc. Am.* **102**, 1200–1212.
- Crawford, J. D., Hagedorn, M. M. and Hopkins, C. D.** (1986). Acoustic communication in an electric fish, *Pollimyrus isidori* (Mormyridae). *J. Comp. Physiol. A* **159**, 297–310.
- Crawford, J. D. and Hopkins, C. D.** (1989). Detection of a previously unrecognized mormyrid fish (*Mormyus subundulatus*) by electric discharge characters. *Cybiurn* **13**, 319–326.
- Crawford, J. D., Jacobe, P. and Benech, V.** (1997b). Field studies of a strongly acoustic fish in West Africa: reproductive ecology and acoustic behavior of *Pollimyrus isidori*, Mormyridae. *Behaviour* **134**, 677–725.
- Diesselhorst, G.** (1938). Horversuche an Fischen ohne Weberschen Apparat. *Z. Vergl. Physiol.* **25**, 748–783.
- Emlen, S. T. and Oring, L. W.** (1977). Ecology, sexual selection and the evolution of mating systems. *Science* **197**, 215–223.
- Fine, M. L.** (1978). Seasonal and geographical variation of the mating call of the oyster toadfish, *Opsanus tau* L. *Oecologia* **36**, 45–57.
- Fine, M. L.** (1997). Endocrinology of sound production in fishes. *Mar. Freshwater Behav. Physiol.* **29**, 23–45.
- Friedman, M. A. and Hopkins, C. D.** (1998). Neural substrates for species recognition in the time-coding electrosensory pathway of mormyrid electric fish. *J. Neurosci.* **18**, 1171–1185.
- Graff, C. and Kramer, B.** (1992). Trained weakly-electric fishes *Pollimyrus isidori* and *Gnathonemus petersii* (Mormyridae, Teleostei) discriminate between waveforms of electric pulse discharges. *Ethology* **90**, 279–292.
- Grant, K., Bell, C. C., Clause, S. and Ravaille, M.** (1986). Morphology and physiology of the brainstem nuclei controlling the electric organ discharge in mormyrid fish. *J. Comp. Neurol.* **245**, 514–530.
- Gray, P.** (1964). *Handbook of Basic Microtechnique*. New York: McGraw-Hill Book Company.
- Hamilton, W.** (1971). Geometry for the selfish herd. *J. Theor. Biol.* **31**, 295–311.
- Heusinger, C. F.** (1826). Bemerkungen über das Gehörwerkzeug des *Mormyrus cyprinoides*, *Gastroblecus compressus* und *Pimelodus synodontis*. *Arch. Anat. Physiol. Meckel.* **1**, 324–327.
- Hopkins, C. D.** (1981). On the diversity of electric signals in a community of mormyrid electric fish in West Africa. *Am. Zool.* **21**, 211–222.
- Hopkins, C. D.** (1986). Behavior of Mormyridae. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 527–576. New York: John Wiley & Sons.
- Hopkins, C. D.** (1988). Neuroethology of electric communication. *Annu. Rev. Neurosci.* **11**, 497–535.
- Hopkins, C. D. and Bass, A. H.** (1981). Temporal coding of species recognition signals in an electric fish. *Science* **212**, 85–87.
- Hopkins, C. D., Shieh, K. T., McBride, D. W. and Winslow, M. A.** (1997). Quantitative analysis of passive electrolocation behavior in electric fish. *Brain Behav. Evol.* **50** (Suppl. 1), 32–59.
- Huang, X., Kozloski, J. and Crawford, J. D.** (1996). Sexually dimorphic swimbladder muscles in the sonic fish *Pollimyrus isidori* (Mormyridae). *Soc. Neurosci. Abstr.* **22**, 178.
- Humason, G. L.** (1967). *Animal Tissue Techniques*, second edition. London: W. H. Freeman & Company.
- Kozloski, J. and Crawford, J. D.** (1998). Functional neuroanatomy of auditory pathways in the sound producing fish *Pollimyrus*. *J. Comp. Neurol.* **401**, 227–252.
- Kramer, B.** (1990). *Electrocommunication in Teleost Fishes*. Oxford: Blackwell Scientific Publications.

- Kramer, B. and Kuhn, B.** (1994). Species recognition by the sequence of discharge intervals in weakly electric fishes of the genus *Campylomormyrus* (Mormyridae, Teleostei). *Anim. Behav.* **48**, 435–445.
- Kynard, B.** (1978). Breeding behavior of a lacustrine population of three spine sticklebacks (*Gasterosteus aculeatus* L.). *Behaviour* **67**, 178–207.
- Lank, D., Bousfield, M. and Cooke, F.** (1991). Why do snow geese adopt eggs? *Behav. Ecol. Sociobiol.* **2**, 181–187.
- Lissmann, H. W.** (1958). On the function and evolution of electric organs in fish. *J. Exp. Biol.* **35**, 156–191.
- Lissmann, H. W.** (1963). Electric location by fishes. *Scient. Am.* **208**, 50–59.
- Marvit, P. M. and Crawford, J. D.** (1999). Behavioral measurements of auditory sensitivity in sound-producing electric fish: an audiogram for *Pollimyrus*. *Ass. Res. Otolaryngology* abstract 234.
- McCormick, C. A. and Popper, A. N.** (1984). Auditory sensitivity and psychophysical tuning curves in the elephant nose fish. *J. Comp. Physiol. A* **155**, 753–761.
- McLennan, D.** (1995). Male mate choice based on based upon female nuptial coloration in the brook stickleback, *Culaea inconstans* (Kirtland). *Anim. Behav.* **50**, 213–221.
- Moller, P.** (1995). *Electric Fishes: History and Behavior*. London: Chapman & Hall.
- Moller, P., Serrier, J., Belbenoit, P. and Push, S.** (1979). Notes on the ethology and ecology of the Swashi River mormyrids (Lake Kainji, Nigeria). *Behav. Ecol. Sociobiol.* **4**, 357–368.
- Orts, S.** (1967). Contribution a l'anatomie comparée et a la systematique des Mormyroïdes. *Mem. Acad. R. Sci. O-M.* **XVII**, 1–90.
- Rome, L. C., Syme, D. A., Hollingworth, S., Lindstedt, S. L. and Baylor, S. M.** (1996). The whistle and the rattle: The design of sound producing muscles. *Proc. Natl. Acad. Sci. USA* **93**, 8095–8100.
- Schellart, N. A. M. and Popper, A. N.** (1992). Functional aspects of the evolution of the auditory system of actinopterygian fish. In *The Evolutionary Biology of Hearing* (ed. D. B. Webster, R. R. Fay and A. N. Popper), pp. 295–322. New York: Springer-Verlag.
- Schneider, H.** (1967). Morphology and physiology of sound producing mechanisms in teleost fishes. In *Marine Bio-Acoustics*, vol. 2 (ed. W. Tavolga), pp. 135–158. New York: Pergamon Press.
- Searcy, W. and Brenowitz, E.** (1988). Sexual differences in species recognition of avian song. *Nature* **332**, 152–154.
- Skoglund, C. R.** (1961). Functional analysis of swimbladder muscles engaged in sound production of the toadfish. *J. Biophys. Cytol. (Suppl.)* **10**, 187–200.
- Stipetić, E.** (1939). Über das Gehörorgan der Mormyriden. *Z. Vergl. Physiol.* **26**, 740–752.
- Sweeney, B. W. and Vannote, R. L.** (1982). Population synchrony in mayflies: a predator satiation hypotheses. *Evolution* **36**, 810–821.
- Tavolga, W. N.** (1971). Sound production and detection. In *Fish Physiology*, vol. V (ed. W. S. Hoar and D. J. Randall), pp. 135–205. New York: Academic Press, Inc.
- von der Emde, G. and Bleckmann, H.** (1997). Waveform tuning of electroreceptor cells in the weakly electric fish, *Gnathonemus petersii*. *J. Comp. Physiol. A* **181**, 511–524.
- von der Emde, G. and Bleckmann, H.** (1998). Finding food: senses involved in foraging for insect larvae in the electric fish *Gnathonemus petersii*. *J. Exp. Biol.* **201**, 969–980.
- von der Emde, G. and Zelick, R.** (1995). Behavioral detection of electric signal wave-form distortion in the weakly electric fish, *Gnathonemus petersii*. *J. Comp. Physiol. A* **177**, 493–501.
- von Frisch, K.** (1938). The sense of hearing in fish. *Nature* **141**, 8–11.
- Westby, G. and Kirschbaum, F.** (1982). Sex differences in the waveform of the pulse-type electric fish, *Pollimyrus isidori* (Mormyridae). *J. Comp. Physiol. A* **145**, 399–401.