MURRAY–DARLING BASIN COMMISSION

Native Fish Strategy

Counting Murray-Darling fishes by validating sounds associated with spawning

A report to the Murray-Darling Basin Commission by
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Murray-Darling Basin Commission Native Fish Strategy on counting Murray-Darling fishes by validating sounds associated with spawning

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EXECUTIVE SUMMARY

Passive acoustics can identify the unique sounds made by fish species. These sounds can be useful in locating fish, deriving abundance estimates, and directing habitat management. To date, however, this technology has been little used in Australian rivers. Over 700 fish species worldwide produce sound including the Murray-Darling Basin native fish silver perch (*Bidyanus bidyanus*). Most sounds are produced during courtship, for communication or identification. Although passive bioacoustics is a standard technique for frog and some marine animal research disciplines, there have been few studies with freshwater fish.

This report details the first use of passive bioacoustics in a captive hatchery situation and develops a protocol and methodology to benchmark sound production in two key native fish species (golden perch and silver perch). This project also formed a scoping study for use of passive bioacoustics to provide a measure of the relative abundance of fish in key habitats and potentially migrating in fishways. A Soniferous Fish Research Team was formed to provide appropriate expertise to the project. The collaborative team included native fish research scientists, technicians, a bioacoustic expert, and a fish hatchery manager.

Several acoustic noises were isolated during the project and although these were absent from the controls they could not specifically be attributed to the fish. The results demonstrate some Murray-Darling Basin native fish potentially produce noises. Further research and replication is needed to clarify the mechanism of fish sound production, individual variation in vocalisation and the utility for research and management. The sonogram data did appear to include biological noise but the DIDSON camera proved unsuitable in hatchery tanks and further work with fixed cameras is needed to link sound production with fish behaviour.

Murray cod appear to provide a model species for further passive acoustic trials as these fish have complex social behaviour that can be observed in the semi-natural conditions of a hatchery pond. The suggested protocol is to set up a DIDSON camera or video recorder and hydrophones within the spawning drum (used by fish as an oviposition site). This method would allow visual confirmation of fish making noises, and the hydrophone would be placed in an optimal position to capture any vocalisations.

Passive bioacoustics is a powerful tool in the study of freshwater fish and can potentially enable scientists to identify and monitor fish populations. In future there may be applications for assessing the affects of noise pollution (primarily boats) on fish populations. Acoustic hydrophone arrays may also provide a useful long term method for more sophisticated monitoring of fish populations.

Key recommendations

- Verify sound production in Murray-Darling Basin fish.
- Continue to investigate which Murray-Darling Basin fish species make sounds and their frequency range.
- Correlate fish sound production and behaviour with visual equipment.
- Validate the environmental/physiological conditions when sounds are produced.
- Investigate Murray cod vocalisations in hatchery ponds incorporating DIDSON.
- Trial field application of the technology for key habitat identification and for population counts.

INTRODUCTION

At least 700 fish species make sounds (Katz 2002; Luczkovich et al. 2008). Sound is an ideal way for fish to communicate in rivers and floodplains because it attenuates little, is directional, and is useful where there is low visibility, high turbidity, or no light. To produce sounds fish have evolved the ability to drum their swim bladder with specialised muscles or bones as well as hydrodynamic tail slaps, fin flicks, fin spine extensions, and jumps. Some fish can hear through their lateral line while relatively large otoliths indicate species that specialise in sound production and hearing ability (Cruz and Lombarte 2004).

Silver perch (*Bidyanus bidyanus*) are part of the Grunter family and make a clearly audible grunting sound on capture. Striped bass (*Morone saxatilis*), a close relative of the Australian Percichthyids, golden perch (*Macquaria ambigua*) and Murray cod (*Maccullochella peelii peelii*), can also make sounds. While many fishes produce sound, this aspect of fish biology has not been specifically studied in the freshwater rivers of Australia. Identifying whether native fish species produce unique sounds may provide an innovative method of quantifying fish abundance independent of capture.

One innovative application of bioacoustics uses passive acoustics (monitoring fish sounds with a hydrophone) to count and record spatio-temporal patterns of fish reproduction by detecting sounds associated with spawning (Rountree *et al*. 2006). Spawning sounds may have evolved behaviourally to enable species recognition and also to synchronise gamete release in order to maximize external fertilisation. The applicability of this identification and counting tool depends on whether specific species produce reliably identifiable sounds during courtship and spawning. Monitoring courtship and spawning sounds can be used to identify and map important breeding habitats and to understand the relationships between fish reproduction and the fate of larvae (Hawkins *et al*. 2002).

Since many fish sounds are associated with spawning and reproduction, they can be used to measure the time and place of spawning and the abundance of spawning pairs. The location of calling fishes allows identification of essential fish habitat and could have important management implications (Sprague and Luczkovich 2001; Holt 2008). Studies of fish sound production in freshwater systems are little studied compared to marine environments (Anderson *et al*. 2008). The actual location of fish spawning sites remains unknown for most freshwater native fish and these essential habitats are in great need of protection.

Passive bioacoustics is economical, reliable, passive and time saving, and ultimately, may be used independently to count spawning aggregations and map the spawning areas of sound-producing (soniferous) fishes, thus enabling fishery managers to help protect critical stocks and habitats that have declined in recent years. Conversely, the technology might also determine zones of common carp (*Cyprinus carpio*) accumulation, which could lead to targeted control operations. Software now exists that with training, can automatically detect vocalisations by organisms. Accordingly, it is conceivable that completely automated fish monitoring systems could be developed using bioacoustics.

Passive bioacoustic studies are little reported in the fisheries literature prior to a special edition in the *Transactions of the American Fisheries Society* in early 2008. Hence, the technique is still progressing and has not yet been actively tested or used by researchers (Gannon 2008; Luczkovich *et al*. 2008).

Riverine bioacoustics offers many advantages over traditional sampling techniques, including:

1. Continuous monitoring allows counting or determination of daily and seasonal activity patterns.
2. Together with DIDSON sonar, enables detailed studies of fish behaviour without a need to handle the fish.
3. Can be used to passively count/monitor rehabilitation efforts (e.g. re-snagging).
4. Non-invasive and inexpensive hardware relative to other technologies (hydro-acoustic counters, DIDSON etc.) Inexpensive hardware relative to other technologies (hydro-acoustic counters, DIDSON etc.).
5. Long-term data acquisition capabilities.
6. Capability of remote monitoring.
7. Can be used as an educational tool.
8. Works at any time of day.

The specific objectives of this project were:

- To trial a bioacoustic technology to determine whether captive large-bodied native fish (Murray cod, golden perch, and silver perch) produce sounds associated with artificial or pond spawning, and whether spawning population counts can be obtained.
- To isolate individual spawning sounds produced by each fish species and its associated behaviours, for example, do individual species produce unique, distinguishable sounds for male dominance, courtship, spawning or distress?
- To test for soniferous sounds in wild collected adult carp spawned in captive conditions.
- To scope passive bioacoustics as a tool for measuring the relative abundance of fish in key habitats and potentially in fishways.

METHODS

Project inception and equipment

Riverine bioacoustics is a multidisciplinary field and required the formation of a Soniferous Fish Research Team to provide the appropriate expertise to carry out the project. Hence, the collaborative team comprised native fish research scientists/technicians, a bioacoustic expert, and a fish hatchery manager. These included Mr. Ivor Stuart (Kingfisher Research), Dr Lee Baumgartner (New South Wales Department of Primary Industries, Narrandera), Dr Mike Smith (Arthur Rylah Institute), Mr John McKenzie (ARI), Mr Karl Pomorin (ARI), Mr Stephen Thurstan (NSW DPI, Narrandera).

Research equipment was purchased after investigating the type of hardware and software needed for the project. This included equipment from Burns Electronics (Nelson Bay, NSW), a Terratec Aquaear Hydrophone MKII (frequency range 10Hz to 25KHz; sensitivity @3Khz, -164 dB re 1V/uPa) and amplifier (gain 0-15 dB). The hydrophone is essentially an underwater microphone that can convert sound pressure into an electronic signal.

Amplifier output was recorded to a portable battery operated Marantz digital recorder (PMD 660) or directly to a laptop computer. Recordings were viewed as sonograms with Cool Edit Pro (version 1.2). Sonograms are visual representations of sound along time, frequency and amplitude dimensions (see Figure 1 and 2). With continued recording and confirmation of the source of acoustic signals, a range of temporal and spectral attributes of the fish vocalisations can be quantified. This information can then be used to identify particular aspects of the vocalisations that are species and/or behaviour specific and appropriate as an identification/monitoring tool. In summary, the hydrophone recorded sounds which were ultimately stored (*.aif) and viewed and analysed on a laptop computer with specialist software.

A series of baseline bioacoustic experiments were conducted in 2007–08 during the spring/summer fish breeding season at the Narrandera Fisheries Centre (NFC) hatchery. As few of the team members were experienced in conducting sound trials and acoustic experiments the expert team member (Mike Smith) travelled to Narrandera on two occasions to train the other team members in the use of the equipment and software for recordings. To formalise the experiments and training sessions a standard data sheet and manual were produced (Appendix 1 & 2) and the transfer of expert skills was a major project outcome.

Study design

Hatchery tanks and reference trials

Before recording any potential fish noises, the project team decided upon a series of trials to benchmark or sound truth the ambient noises within the NFC Hatchery. There are often acoustic complications in captive conditions, hence the need to record benchmark noise within the 2000 litre spawning tanks. To achieve this, one tank was filled in the normal fashion for fish spawning and included flow through water and aeration but no fish ('No fish reference control'). The hydrophone was set up and a 24-hour sound recording taken with the filename 'GoldenP reference'.

The second part of the study design was to benchmark the noises from a solitary fish (male or female). This requires the same procedure as for the 'reference' trial but with one fish in the tank. The sound file was saved as 'GoldenP single'.

The final experimental phase was to benchmark the noises from a number of fish (mixed sexes), and this could include basic communication, aggression or a number of other non-spawning behaviours. Four fish that had been injected with hormones (two male and two female) were placed into a tank and recorded for 24 hours. The file was saved as 'GoldenPmix'. The 24-hour sample was used because some fish are more active at night or spawn during crepuscular or night time conditions. The DIDSON camera was set up to provide vision of the spawning fish.

Murray cod and common carp trials

It was originally envisaged that the hydrophone would also be used to record any sounds associated with the captive spawning of Murray cod and in the field with common carp. Unfortunately due to the project starting late (December 2007), the spawning season for Murray cod and common carp was completed before the initial training session for project staff held in Narrandera in December 2007. Unlike golden perch and silver perch, Murray cod are not spawned in the hatchery but in earthen ponds. Application of the bioacoustic technology needs further development before field deployment and this aspect of the project is addressed in the discussion.

RESULTS

Tank trials

Operating the acoustic equipment was an incremental learning experience for the team and necessitated two trips (December 2007 and January 2008) to NFC for the expert acoustic scientist to train the technical staff. A series of acoustic recordings were completed after the second training trip (Table 1). The DIDSON camera was trialled in the 2000 lt tanks but no vision could be recorded as the tank diameter was less than the minimum camera focus (5m+). Water temperature varied from 21.1 to 24.2°C throughout the trials.

Table 1: Biological details of the 24-hour sound recordings in hatchery tanks.

File name	No. fish	Hormone injected	Spawned
Reference trial	No fish		
SilverPMix	4 (2 F, 2 M)	Yes	1 F & 2 M spawned
GoldenPmix	1 F, 2 M	Yes	No
SilverPsingle	1	No	No

Data analyses and sonograms

Cool Edit Pro (version 1.2; now Adobe Audition) is dedicated sound analysis software that allows signals to be digitised and viewed either as waveforms (time/amplitude) or sonograms (frequency/time/amplitude). The software allows analysis of both the spectral and temporal properties of the signals (Figure 1 and 2). Any acoustic signal will vary in its temporal and spectral aspects and this variation is often species and behaviour specific (Gerhardt & Huber 2002). To use this information, it is important to assess variability (less variable components of signals are typically more useful for species specific information) and the underlying role the signal performs. For example, some signals attract the opposite sex (Gerhardt 1974) or are used in courtship (Balakrishnan & Pollack 1996), while others may display the sender's quality or fitness (Welch *et al*. 2001). The software was used to produce sonograms from the trials (Figure 1 to 4).

It was apparent there was little noise on the reference recordings taken from tanks without fish. In contrast, there were a number of signals on the recordings with fish. Some of these might have been fish touching the hydrophone but others show a different waveform. Without visual verification of fish behaviour it is difficult to attribute the sounds to a specific source or behaviour.

Figure 1: A sonogram of acoustic signals recorded in a tank with spawning golden perch. The noise could not be specifically isolated to the fish.

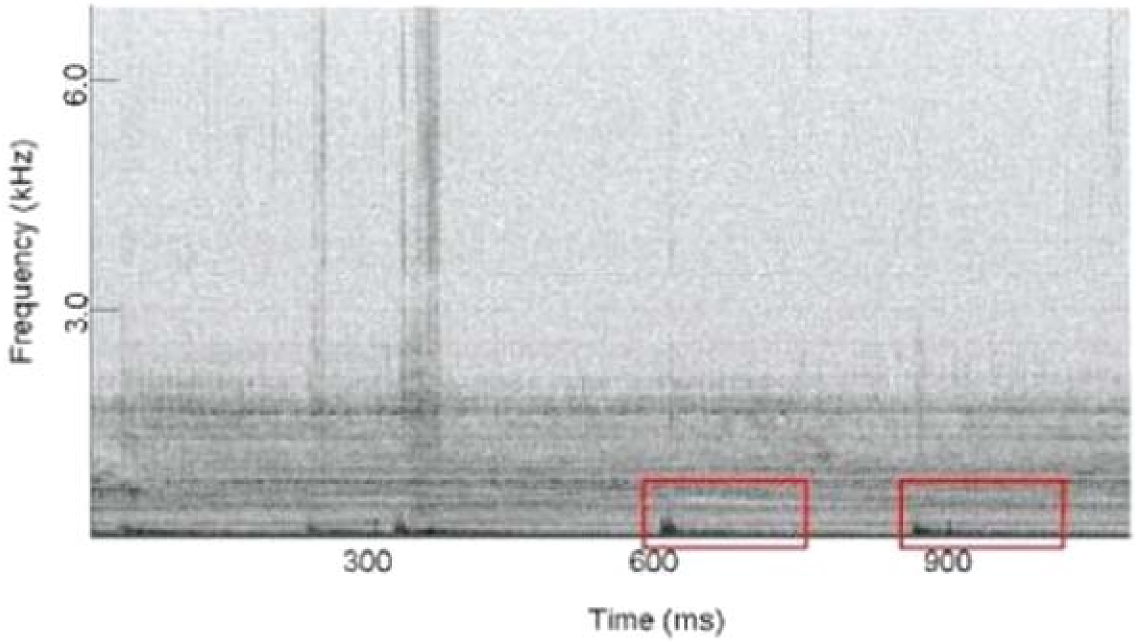


Figure 2: A sonogram of acoustic signals recorded in a tank with spawning silver perch. The noise could not be specifically isolated to the fish.

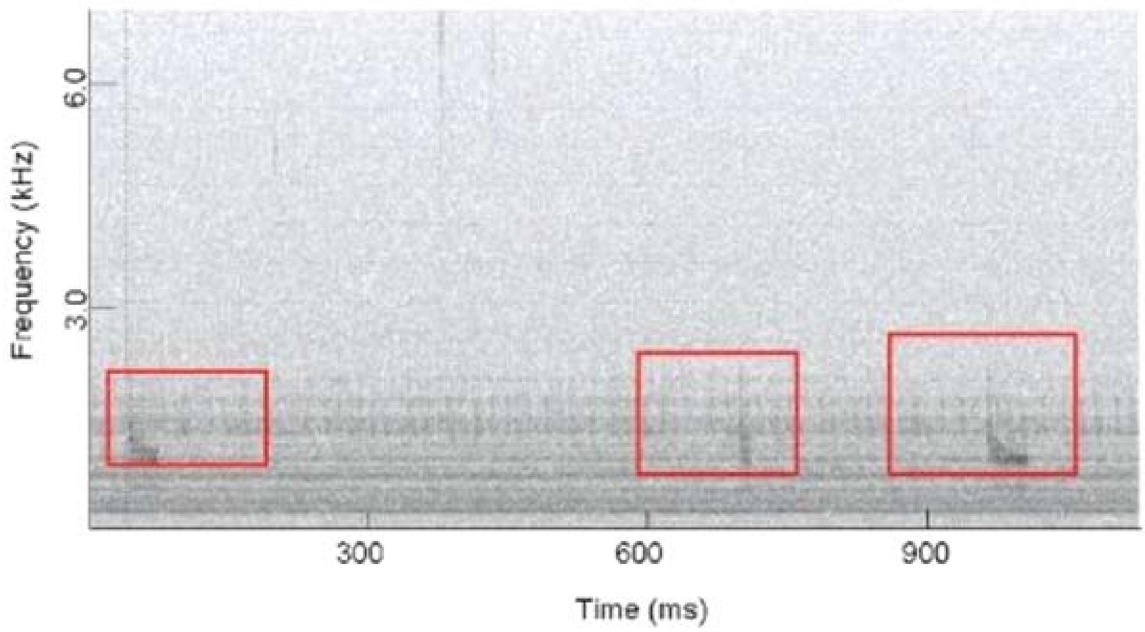


Figure 3: A sonogram of acoustic signals recorded in a tank with spawning golden perch. The noise could not be specifically isolated to the fish.

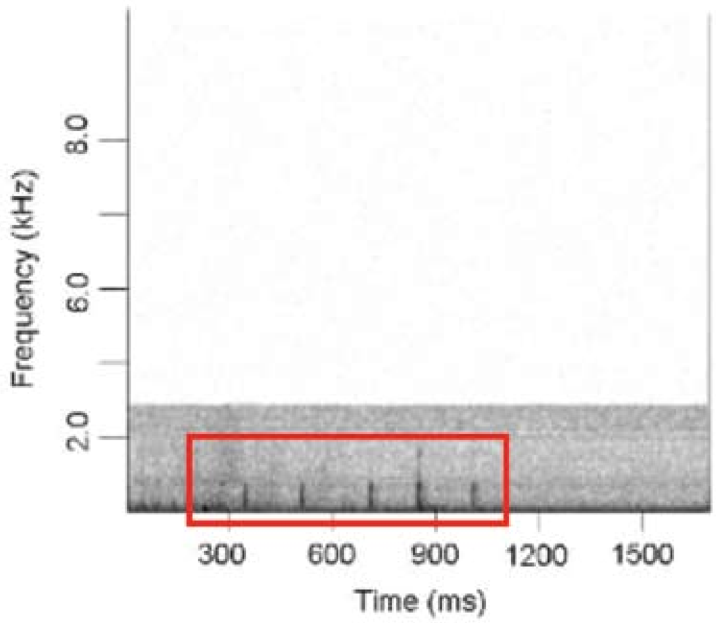
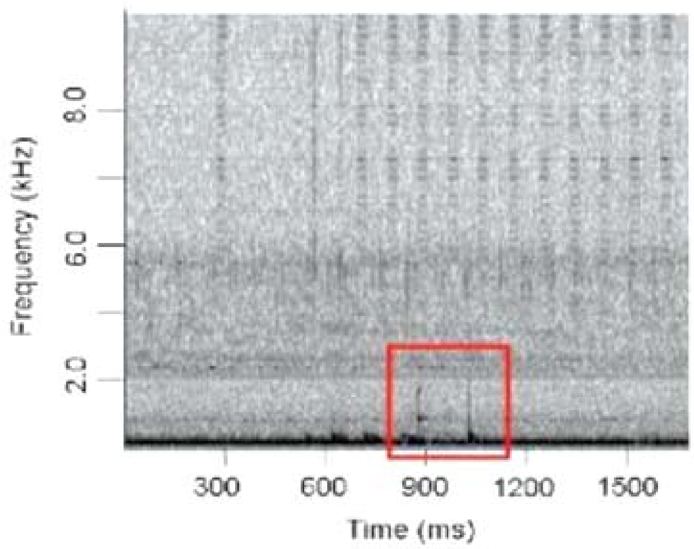


Figure 4: A sonogram of acoustic signals recorded in a tank with spawning golden perch. The noise could not be specifically isolated to the fish.



DISCUSSION

Several acoustic noises were isolated during the project but these could not be specifically attributed to the fish. Despite this, these results demonstrate some potential for MDB native fish to produce noises but further research is needed to clarify the mechanism of sound production and the utility for research and management. The sonogram data appears to include biological noise but because the DIDSON was not suitable for tank vision further work with high definition fixed cameras is needed to link sound production with fish behaviour (Aalbers and Drawbridge 2008).

Several bioacoustic research needs are required before this technique is adopted more generally for freshwater fish. These are: (1) determine which MDB fish species make sounds, (2) validate the sounds for each species, (3) correlate sound production and behaviour, and (4) validate the environmental/physiological conditions when sounds are produced (Rountree *et al.* 2006). Furthermore, researchers should consider the relationships between fish size/sex and sound production, and daily/seasonal patterns of sound production.

Considerable replication (both fish numbers and vocalisations) are still required to analyse the characteristics of the vocalisations. At this stage we do not have an adequate understanding of the degree of variability in the signals. Similarly, we need to develop a better understanding of the influence of key environmental factors. For example, rates of fish vocalisation may relate positively to season and water temperature (Connaughton *et al.* 2002).

The question of where fish produce sounds in their habitat is also important for identifying critical areas for management (Luczkovich *et al.* 1999). These questions might then lead to quantification of the number of fish in any given location. This could be achieved by correlating the amount of sound with the abundance of fish and this could be validated with DIDSON sonar coupled with passive acoustics.

Bioacoustic technology is commonly employed in the marine and terrestrial research disciplines (Luczkovich *et al.* 2000; Ferreira & Ferguson 2002; Gerhardt & Huber 2002; Hawkins *et al.* 2002; Tallamy *et al.* 2003), although similar work on freshwater systems is limited (Anderson *et al.* 2008) and there appears to be no precedent in Australian freshwater fish research and management. Hence, the hardware, software and recording protocols developed by the acoustic team are of use for future studies.

We were not able to examine sound production of Murray cod and non-native common carp; two key species for river managers. We suggest further work is needed to assess whether the technology could be useful for identifying key spawning habitats or for population surveys. Both species of fish might produce sound because Murray cod are long-lived and appear to have complicated courtship behaviour while for carp, other members of the Cyprinid family can produce sound (Johnston and Johnson 2000).

Murray cod in ponds should provide a model species for passive acoustic trials, including the DIDSON camera. The suggested protocol is to set up DIDSON or video recorder and hydrophones within the spawning drum (used by fish as an oviposition site). This method would allow visual confirmation of fish making noises, and the hydrophone would be placed in an optimal position to capture any vocalisations.

For applied research, particularly for estimating relative abundance in key habitats or fishways, we suggest the need for multidisciplinary team approaches to assess the applicability of riverine bioacoustics in the management and research of native fish in the Murray-Darling Basin. The team includes fish biologists and specialist acoustic experts. The techniques developed by this study will allow better determination of generic attributes important for fish to initiate spawning activities. This method has potential to identify critical spawning habitats, fish accumulations and eventually relative abundance in defined areas. This information can influence subsequent management interventions (Holt 2008).

Passive bioacoustics is a powerful tool in the study of freshwater fish and can potentially enable scientists to identify and monitor fish populations (Gannon 2008). In future there may be applications for assessing the affects of noise pollution (primarily boats) on fish populations. Acoustic hydrophone arrays may also provide a useful long term method for more sophisticated monitoring of fish populations. For accurate estimates of the relative abundance of fish in key habitats and potentially in fishways we suggest further progression of the bioacoustic technique (Luczkovich *et al.* 2008).

Recommendations

- Verify sound production in MDB fishes.
- Continue to investigate which MDB fish species make sounds and their frequency range.
- Correlate fish sound production and behaviour with visual equipment.
- Validate the environmental/physiological conditions when sounds are produced.
- Investigate Murray cod vocalisations in hatchery ponds incorporating DIDSON.
- Trial field application of the technology for key habitat identification and for population counts.

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APPENDIX 1: SONIFEROUS FISHES DATA SHEET

Species:	Date:	Location:

Tank no:	
Light:	Cond:
DO:	Secchi:
Ph:	°C:

No. females:	Size (mm):
No. males:	Size (mm):
Date & time into hatchery tank:	

Injection time:

Dosage female:

Dosage males:

Approx. spawn time:

Spawning occurred: Yes/No

Eggs viable: Yes/No

Courtship observed: Yes/No

Behavioural observations:

Tape on time:

Tape off time:

File name:

Video used: Yes/No

Comments:

APPENDIX 2: INSTRUCTIONS FOR RECORDING FISH

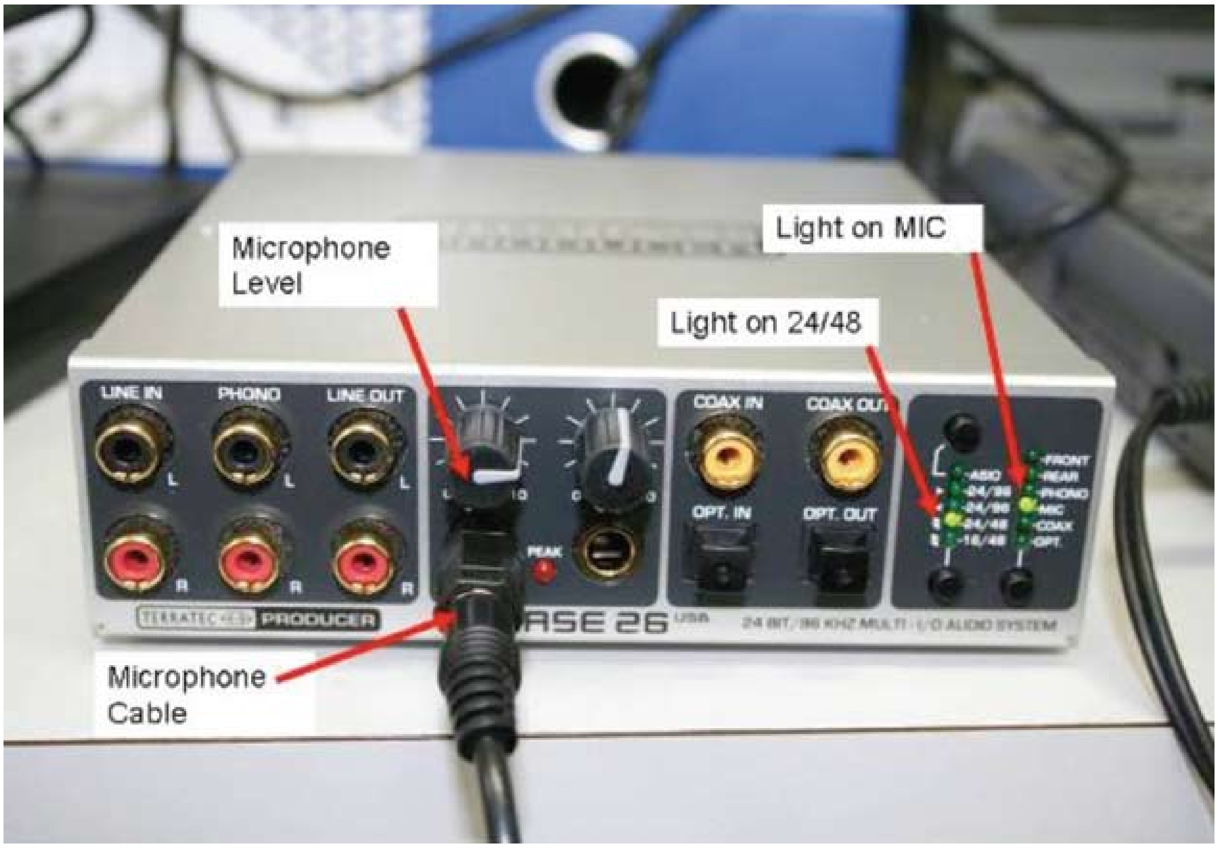
1) Place hydrophone in water. It needs to be freely floating in the water and not banging on the sides of the tank, the ground or any other solid objects. I would suggest that the hydrophone is as close as possible to where the eggs are dropped for cod and probably about 1 foot from bottom of tank for perch.
2) Make sure hydrophone is plugged into the hydrophone socket in the power supply unit (see figure below) and the cable to the digital recorder/computer is plugged into the “mic out” socket (see Figure 1 below).
3) The microphone power supply unit should have the power on (light will be on) and the filter switch in the downward position.

Figure 1: Hydrophone connection details.

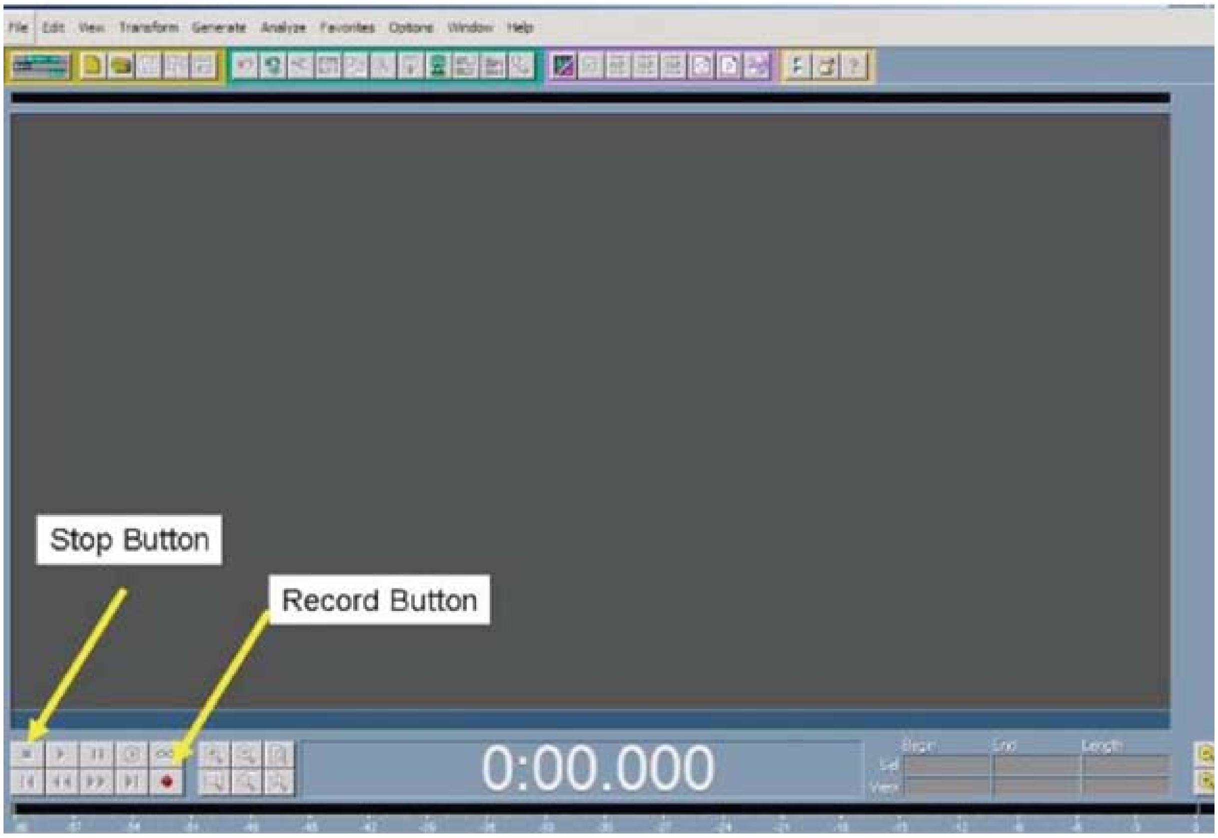
Computer Recorder

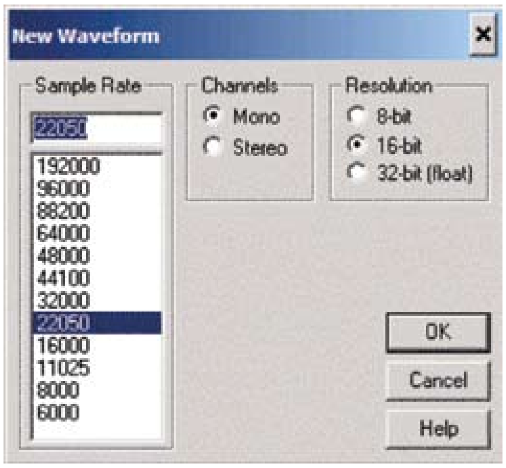
Plug the “mic out” cable into the “microphone socket on the front panel of Terratec unit (see Figure 2 below). The USB cable on the Terratec system (see Figure 3 below) plugs into the computer after the computer has been turned on. Make sure light is on 24/48 and MIC on the front panel of the Terratec (see figure below). They can be adjusted if necessary by pressing the buttons below, but you will probably need to unplug and replug the USB cable. Start Cool Edit Pro and click on the red dot (see figure below) to begin the recording process. This will bring up a box to set Sample Rate (22050), Channels (Mono) and Resolution (16-bit) (see Figure 4 below). Press OK and recording will begin. To end recording and save file (*.aif) press “Stop Button”. And follow software prompts.

Figures 2 and 3: Terratec microphone and USB cable details.



Figures 4 and 5: Cool Edit Pro recording software details.





Digital Recorder

Plug the "mic out" cable into the "mono" plug at the back of the digital recorder (see Figure 6 below). There is an attenuator at the end of the cable; this must be set to 15dB attenuation. Turn the unit on (power switch on right hand side) and press "REC PAUSE" to listen to the recording level with the headphones plugged into the digital recorder. There is a LED display on the front of the unit and a "REC LEVEL" knob on the front to adjust recording level. I suggest the level should be at about 80%. When you are satisfied the record level is fine – most important that it is not too loud that the recording is distorting (peaking on the led level display) – then press the "REC" button on the top left hand side. If it is working, the digital counter should start to count. The system should be able to record for a good 11 hours.

Once the recording is done, plug the system into the USB port of a computer (cable in the external drive boxes) and hold the button marked "COPY" and "USB" down while switching the unit on. The display will flash USB. You can then copy the file from the flash card to the hard drive of the computer or to one of the external drives. There is also a USB flash card unit can also be used.

Because the flash card will be full, you will need to format it after each recording session. To do this, hold the "SHIFT" button and press the "MENU/STORE" button. Then press the right arrow ("MARK+") until "FORMAT" comes up and press "ENTER" twice.

Figure 6: Details for digital recorder microphone connection.