Working towards the development of best practices in fish and fisheries research
or The troubles with fish and fish biologists!

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Abstract

Any institution that is active in the area of fish and fisheries research is likely to use very large numbers of animals in their projects. In this paper we explore some of the problems and ethical dilemmas faced by researchers and ethics committees, using our experiences at Murdoch University, Perth, Western Australia, as a guide. We have identified seven areas that have caused major debate during committee meetings, some of which are still under consideration. These areas are: 1) approval process; 2) sampling methods; 3) numbers; 4) euthanasia; 5) fish handling; 6) pain in fishes; 7) reporting. We suggest solutions to some of these problems and invite dialogue on others.

Introduction

What do we mean when we use the term "fish"? This may appear a strange question but in fact has different answers depending on who is using the term. For example, in Western Australia anything that is harvested from the water is considered a fish and researchers and managers at the Department of Fisheries work on shellfish, sharks and rays, bony fish, turtles and even algae. In contrast to this very broad view of what constitutes fish, an alternative view is that the term fish should only be used for the ray-finned fishes. However, the view taken by most fish biologists and taxonomists is that fish are those aquatic animals that have an internal skeleton, gills and limbs that if present are in the form of fins. Thus, the term covers animals from the jawless hagfishes and lampreys, to the cartilaginous sharks and rays to the bony fishes. The latter group includes the ray finned fishes such as snapper, salmon, goldfish and the lobe-finned fishes such as the lungfish and that "living fossil" the coelacanth. This definition would also most likely reflect the idea of fish held by most members of the public and ethics committee members. Although this is the group we will consider in this paper we acknowledge that ethics committees are increasingly called upon to consider projects utilising cephalopods (squid, cuttlefish and octopus)* and in future, possibly other invertebrates such as echinoderms (sea urchins, sea cucumbers, star fish etc) and crustaceans (crabs, lobster, prawns etc)*.

Using the above definition, fishes make up more than half of all of the species of living vertebrates and are represented by over 24,000 valid species, a number that is growing yearly (Nelson 1994). As would be expected in such a large group, fish exhibit huge morphological, physiological, behavioural and habitat diversity. For example, at 8 mm at maturity an Indo-Pacific goby is the smallest known vertebrate, while at 12 m long the whale shark is amongst the largest; most fish are "cold blooded" but some tunas, billfishes and sharks can regulate their internal temperature just like us; fish can live from less than one year to at least 120 years, the long-lived species may not even begin reproducing until 4–5 or even 20

*Footnote: In New Zealand and some states of Australia, research on crab, squid, octopus, lobster and crayfish have had AEC approval since 1 January 2000.

years of age; although most fish invest no parental care in their offspring others exhibit more care than some mammals; all known aquatic habitats from the deepest arctic oceans to equatorial mountain streams are inhabited by fish, with some fish like the mudskippers being able to spend extended periods foraging out of water (Nelson 1994; Moyle & Cech 2000). Yet, even for such a large and diverse group, we are tasked with developing best practice for welfare for the group as a whole.

At Murdoch University there is an active fish and fisheries research centre. In 2007, fish represented 25% of the animals used for teaching and 97.8% of the animals used for research—a total of 97.4% of the total animal use for the University (Murdoch University 2008). This represents over half a million fish in one calendar year. Any institution that is conducting research into fish and fisheries will no doubt be dealing with similarly hefty animal figures. The majority of research conducted by researchers at Murdoch University is field-based and includes surveys, community studies and biological studies of specific species or usually a combination of all. In this regard there are a number of similarities to wildlife studies that involve amphibians, reptiles, birds and/or mammal trapping. However, there are also a number of factors that make fish and fish welfare unique and care should be taken in extrapolating Animal Welfare guidelines for amphibians, reptiles, birds and mammals to fish.

It appears that research opportunities are increasing in fish and fisheries-related fields. Thus, we have identified this as being an area that our Research Ethics Office and the Animal Ethics Committee (AEC) must educate themselves about in more detail in order to face some of the challenges that arise. We have made some progress, but it seems that every question we answer raises another two or three. What follows is a discussion about seven main areas of focus.

Approval process

Initially when the committee started assessing applications for fish research there was a low approval rate. It was difficult to achieve a dialogue between the researchers and the committee and to achieve mutual understanding of goals and objectives. In this way fish research has a lot in common with other animal projects.

The main concerns concentrated on three areas:

Numbers

Impact—it is confronting for members of AECs that many animals will be impacted on to some degree. They also need to accept that for these research applications there are a large number of individuals that won't survive.

Accuracy—have researchers accurately captured the numbers so that they have included by-catch and all fish that will be captured not just those to be retained. What basis has been used for the estimation of the numbers and should non-capture techniques such as sonic surveys be included?

Methodologies

There are a number of methods used to capture fish. They all have the capacity to impact on fish welfare. They are often not completely understood by the committee, nor well explained by researchers.

Communication

No application forms are perfect, and, as with many other types of applications, there is often a difficulty in encouraging researchers to understand why the AEC needs to hear "the whole story" and how they should tell it. Common examples include the use of language which researchers do not even consider to be technical, such as:

- Poikilothermic—a reliance on external factors for temperature regulation. Most fish are poikilothermic. Exceptions include some sharks and tunas.
- Teleosts—the largest group of bony fish (compared to non-bony sharks);
- Demersal and Benthic—bottom dwellers;
- Pelagic—live higher in the water column.

Researchers may provide a list of species expected to be caught, but with little indication of the implications of the accepted status of the animals:

- Endemic—naturally occurring only in that region;
- Native—naturally occurring in that region and other regions;
- Feral/Exotic/Introduced—does not naturally occur in that area, but its existence is tolerated there;
- Noxious/Pest—an exotic that, by law, is not to be tolerated in that area.

Noxious fish are often not allowed to be returned alive to the water. Hence AECs must be mindful that

the consequence of capturing these animals, either intentionally or as by-catch will be their euthanasia. Differences in State legislation must also be considered.

Solutions

Application forms are continually under revision. However, no form can be perfect for every situation. A very successful change for the committee at Murdoch University has been to include fish biologists on the committee in Category B (a person with qualifications and experience in the use of animals in scientific or teaching activities). This has had numerous benefits:

- The committee's understanding of the complexities of fish management has improved.
- The fish research area has a greater understanding of the concerns and workings of the ethics committee, and therefore provides better information to the committee.
- The welfare of the fish is given greater scrutiny.
- The success rate for approval has increased.

It would be our strong recommendation that any specialised group (either an animal group such as wildlife or a research specialty area such as genetics) be represented on the committee. If that is not possible, you may be able to put in place a standing arrangement with a "consultant member".

Further education of both researchers and committee members in relation to legislation, welfare concerns and handling techniques continues.

Sampling methods

The commonly used sampling methods not only impact on researchers and their ability to practically deploy them. They have different welfare implications for both target species and by-catch. Committees may wish to place conditions on the use of specific nets or combinations of nets. Before they do so, it is important that they understand the techniques involved.

The techniques used currently under approval from the AEC at Murdoch University are discussed below:

Seine nets

These come in many sizes and involve an "active" trapping technique. They are useful for all types of studies. The net is anchored at one end on the shore and the free end brought round in a semi-circle. Each end is then brought together and the ends are then slowly pulled onto the shore. The middle section of the net is shaped into a pocket which is also called the bunt or cod end. Most fish are herded into the bunt, but there are often a number further out towards the periphery. The bunt should be kept in water while fish are handled, either *in situ* or in a bucket, with the fish around the periphery of the nets removed first. Seine nets can also be deployed as a large circle in open water with the bottom of the net being drawn together to stop fish escaping, while small seines can also be used as a haul or trawl net.

Gill nets

These are nets that are set and then left. The gills and spines of fish tend to get caught and tangled in the net when they swim straight into it. Some fish may be more tolerant than others, and by-catch, particularly tortoises/turtles, birds and mammals, may be adversely affected. In cases where this is predicted, researchers are required to monitor the nets more closely, sometimes "sitting" on them with continual observation. The gill nets are scanned routinely, and irregular movements are investigated further. Another factor determining frequency of observation is experience. It may be appropriate for the AEC to limit the number or type of nets until a high level of competency is reached. Is it practical then, for an AEC to impose set times on checking nets? Gill nets (as others) are often set at dusk and into the early evening. There may be practical concerns such as Occupational Health and Safety (OHS) risks that must be considered. In addition, frequent checking of nets that involve lifting and re-setting may impact on the fish number and species caught—which has repercussions for a comparative community study. In these cases AECs may need to accept that the net will be cleared less often, and therefore be prepared to accept a greater impact on by-catch, in an attempt to enhance data collection and scientific rigour or ensure investigator safety.

Fyke nets

These are another net type that is set and then left, and they are suited to smaller areas where there are lots of nooks and crannies for fish to hide in. At Murdoch University, and on advice from one of our researchers, we insist that the very last section of the net must be set so that it is out of the water; this

reduces the likelihood of tortoises, water birds and water rats drowning if they enter the net. Well set fyke nets have the potential to be left for up to 8 hours depending on targets, terrain and the knowledge of the operator on the likelihood of non fish by-catch. However, if set in freshwaters and it rains or if set in tidal areas, researchers must go back to re-set the net so that the top end always remains free of the water. A common problem if the nets are not checked often enough is consumption of target animals by means of by-catch such as ducks.

General recommendations for all three net types are:

- Use knotless or woven mesh whenever possible (although the resulting drag on large nets may make this impractical).
- Use suitable sized nets (e.g., if targeting small fish, use small nets that will minimise by-catch).
- Check at regular intervals or continually monitor whenever possible.
- Use the technique of sub-sampling as often as possible.
- Ensure there are enough people to enable work to proceed quickly. Often three people are the minimum. The third person is a useful scribe and can begin the counting and recording work, that otherwise gets left to the lab, when they are not assisting with the nets. Three people may also be necessary from an OHS point of view. Smaller nets and manual methods may be able to be competently undertaken by two people.

Electro fishing

This can be tuned differently to target specific fish. There are a number of OHS concerns and experience is essential. Settings must be changed for different water types (e.g., depending on salt content) and there must be a safety cut off switch in case of accidents. A backpack unit creates an approximate field diameter of between 2 and 5 metres. It usually runs with a DC setting. The current must be pulsed to avoid injury to fish. Fish are briefly stunned and the pulsed DC current often causes them to be attracted to the anode. They are easy to then count or capture. If removing them from the water, they can be netted and then either bagged or placed in an esky floating behind the researcher. The larger the size of the animal, the more unpleasant the effect is. For that reason electro fishing is used by Murdoch University researchers in freshwaters where salt water crocodiles are found. As the crocodiles find the effect objectionable they usually (hopefully) rapidly retreat away from the researchers. Water birds trying to land or prey on vulnerable fish also find it disagreeable.

Spear fishing

This is very selective and virtually eliminates by-catch, depending on the skill of the operator. Good operators also cause rapid death. This technique may be associated with otolith damage and therefore unless the operator is well skilled, it is not suitable for studies where otoliths are used for identifying the age of fish. A knife should be kept on hand for quick euthanasia in the event of any shots missing the target.

Line fishing

This can be very selective and reduces by-catch through the use of appropriate gear for the target species. Formal training in humane fish handling and fish welfare for recreational anglers, who may be participating in studies, is considered an ethical responsibility at Murdoch University. As Cooke & Suski (2004, 2005) and Rowland et al. (2008) demonstrate, the use of appropriate tackle greatly reduces trauma to the fish. Therefore most of the work at Murdoch University uses either jigs fitted with a large single barbless hook, or non-offset barbless circle hooks if using bait, and appropriate line strengths.

Non-extractive—such as sonar, video or visual observation

These techniques are considered to have little impact on the fish, although the potential for harm must always be considered. Accurate numbers involved may be very difficult to obtain.

Cadavers from other sources

Often researchers accompany commercial fishers, or they obtain samples from them. There may be limited opportunities for researchers to impact positively on the methods that are used by these industries, although it is encouraged wherever possible.

Numbers

One of the most confronting aspects of fish and fisheries research for ethics committee members is the large numbers of animals involved. Why are

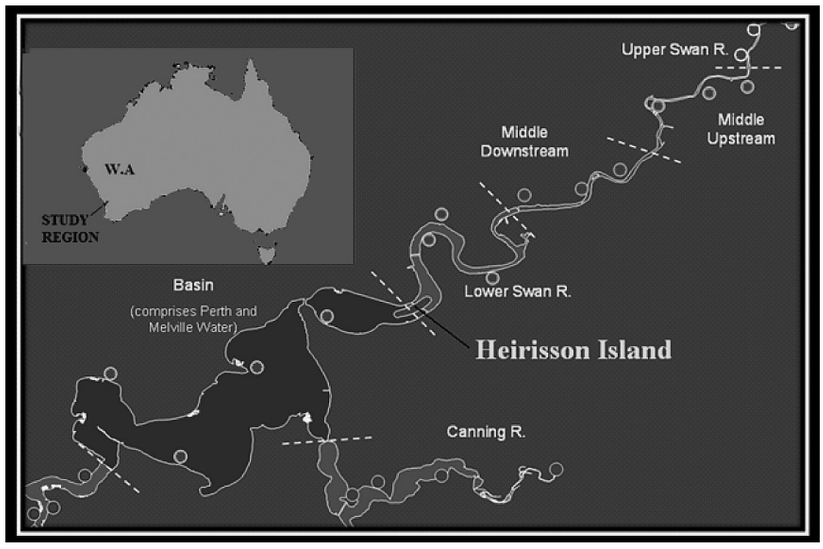

Fig. 1 Diagram of the Swan River Estuary. Dotted lines divide regions and circles represent replicate sites.
Courtesy Fiona Valesini

the numbers so high? It is often due to the clumped distribution of fish where schools can number in the thousands. For example, the senior author and colleagues have netted schools that, based on subsamples, contained over 30,000 Perth herring. Another contribution to high numbers is the nature of community studies. For example, a study currently being conducted on the Swan River aims to compare the community structure now with that based on historical data, and determine the effects of large-scale developments and other anthropomorphic effects over the last 25 years. To be scientifically rigorous, the current study must use a similar experimental design. Thus, 23 sites will be used; giving replicates in each of the regions of the estuary (Fig. 1). Each of these sites will be sampled using both gill nets and seine nets together, at day and night sessions, during each of the four seasons. Therefore, if on average 100 fish were caught at each sampling time by each method, it is possible that 23×2×2×4×100 or a total of 36,800 fish will be caught in a year's sampling.

Community studies include a high number of replicates as Figure 1 illustrates.

We have mentioned the technique of *subsampling*. This describes the situation when only representative samples of the animals that are netted or captured are actually retained. The use of this technique reduces the number of fish that are euthanased, and also reduces the stress on all fish as processing times are much faster. There are a number of methods that can be used for subsampling. In a population survey, 50 of each species may be more than adequate. However, if the particular fish being studied is very long-lived then it may well not be enough. To ensure that representations from enough stages of development are obtained it may be appropriate for the subsampling number to be much greater than 50. In this technique, fish are quickly identified and counted into a bucket of water and once the AEC-agreed subsampling number is reached, the rest are returned live to their normal habitat. In the hands of experienced investigators, this is likely to be the easiest and safest option. Where less experienced personnel or particularly vulnerable fish are involved, it may be worthwhile considering an anaesthetic bath (e.g., Aqui S) for fish being sorted, to reduce stress. Another subsampling technique is to utilise a visual estimate rather than conducting time-consuming counting, especially when community studies are being done and large numbers are captured. For example, investigators may return three-quarters of the catch to the water and keep the rest. The species density is fairly consistent throughout the net and hence it remains a representative sample.

Many fish numbers are actually estimates based on weights of known counts. Obviously all fish that are caught must be reported as they have been subjected to the stress of capture, even if many of them have been rapidly returned to the water, or sometimes not even removed from it. However, this leads to very high numbers of animals being reported and if the

numbers are released without supporting them with an understanding of what they actually represent, then it can create a false impression of the commitment to Reduction by an institution. While striving towards a decrease in the actual total numbers of animals used, the principles of Reduction and Refinement mean that research must be well tailored to answering the right questions and to minimising the number of animals actually retained or euthanased in each project.

At Murdoch University, the majority of fish that are caught and killed are stored, to enable future work by Honours and PhD students and research groups either at our institution or elsewhere. Another commitment to the principle of Reduction by Murdoch University is an attempt at modelling through the identification of major habitat drivers. One of our research groups is involved in an international search for an adequate model that can be used for surveying populations into the future. While we are using a number of animals today, once an adequate model is proven, the numbers of fish required in a "trawl and assess" community survey will potentially be drastically reduced. A model which could predict and identify habitat drivers may decrease the number of replicates and regions in an estuary, as well as the number, variety and size of nets required.

Solutions:

All experimental designs should be checked by statisticians, and researchers must fully explain to the AEC how they have reached the estimate of animals required.

- Researchers should endeavour to process as many fish on site as possible and return them safely to the water. Much of the work previously done back in the laboratory on dead fish can be safely and quickly done on live fish in the field. If this is not possible then use subsampling.
- Plan for likely by-catch and be ready to respond appropriately. Use suitable techniques to minimise by-catch.
- Keep the AEC informed about unexpectedly large captures that will affect total approved numbers.

Euthanasia

There are a number of published guides for euthanasia of fish e.g., American Veterinary Medical Association (AVMA), ANZCCART. There are also some studies that are being done in the commercial world of fish farming where emphasis may not be so much on animal welfare but on meat quality and flavour, and market value.

In fact, in the field it is almost impossible to have one approved method that can be used in all situations. The quickest is usually Ike-jimi (brain destruction or pithing), cervical dislocation or gill bleeding. The skill of the operator needs to be considered along with how many animals are involved and the subsequent use of the cadaver. For example, Ike Jimi often causes damage to the otoliths and is therefore less appropriate should these stones be required for aging the fish. It is also important to consider the OHS risks to the operator along with ease of disposal. The anaesthetic MS222 is therefore difficult to use in field situations.

One common technique is anaesthesia using sedation such as Aqui-S, followed by a physical method of euthanasia. While this is showing itself to be a reliable method in a number of different situations, it may not be entirely practical in all applications.

The use of an ice slurry remains a functional and effective method of sedation and euthanasia, provided that steps are taken to ensure it is only used with the right criteria. These include using crushed ice with enough water to ensure that the slurry consistency is maintained; ensuring it stays below 4°C; only using the technique for warm or tropical fish; and ensuring that any bagged samples are prefilled with the ice slurry.

Further research in pain perception in fish will help the search for appropriate euthanasia techniques.

Fish handling

The priorities for fish handling are similar whether the animals are to be euthanased or returned to the water. It is important for processing to be rapid and for handling to be minimal. For those fish that are to be retained, prompt and humane euthanasia is crucial.

When conducting research in the field, Murdoch University follows the following guidelines to enhance the survivability of fish:

- Handle fish only when necessary and keep handling times to a minimum.
- Be prepared, work quickly and have enough people for the task.

- Keep fish in water as much as possible. This includes, where possible, making measurements on fish while they are in the water. Do as much laboratory work as possible on site, rather than killing fish just to measure them at leisure later.
- Aid respiration. If fish are removed from the water, keep them in a bucket of water. If they are on deck, utilise a deck hose with highly oxygenated water to pass water over the gills. An alternative is to passively "swim" them at the side of the boat to aid water flow over the gills. Not only will this aid respiration but it prevents the gills collapsing and sticking together, and reduces injury.
- Provide UV protection, especially for the eyes, by a light cloth when feasible.
- Protect from predators. Avoid returning fish one by one to the water.
- Reduce the effects of barotraumas with weights (Rowland et al. 2008). [A Fisheries Research and Development Corporation (FRDC) funded DVD explaining this technique is available from the senior author.]

Pain in fishes

"..fish have the sense organs and the sensory processing systems required to perceive harmful stimuli and, probably, the central nervous systems necessary to experience at least some of the adverse states that we associate with pain in mammals. Hence our working position that fish have the capacity to perceive painful stimuli and that these are, at least, strongly aversive." (FSBI 2002). This view is currently shared by researchers and Animal Ethics Committee members at Murdoch University.

Other papers in these Proceedings explore the emerging understanding of pain and fish central nervous system in more detail.

Reporting

A problem faced by all institutions conducting fish research or teaching is reporting. In Western Australia and South Australia, fish are not considered "animals" under Animal Welfare legislation. This means that some institutions involved in using fish for scientific purposes are not required to utilise an AEC nor to report their use of fish. This does not necessarily mean that the use of fish by these institutions is unethical or inhumane, but it does make national and state figures unreliable and non-comparable across and within these States.

Quite often, researchers report over-runs of fish use in their annual reports. Should this be acceptable? In some cases the regions under investigation have never been sampled before so numbers for approval were always a guide and vulnerable to underestimation. In other cases there may be unexpectedly "good" conditions for a particular species—this can be seen with climatic changes such as too much rain, or not enough rain, which can result in breeding numbers varying dramatically both within and between years. For example, in an ongoing study involving the senior author, prolonged early winter rains allowed the colonisation of a reservoir by two new species. In addition, the ensuing late winter and early spring months were very mild resulting in particularly good recruitment. Sampling during the following summer resulted in over 100,000 fish being captured or observed (Howard Gill unpubl. data).

It is good practice for researchers to keep an AEC informed during the year regarding animal numbers and they are required to notify the AEC immediately if they may exceed approved figures. In practice, it may be the last session of the year that puts the project over its approved total. Researchers should, at minimum, provide the reason why this occurred and estimate a reasonable expectation of animal numbers for the future. AECs and researchers should be prepared that numbers may subsequently come in massively under the estimate—or even rise again. For example, in the case just discussed, the investigators informed the AEC and revised their estimates; however, the following year saw very heavy but late winter rains and an unseasonably cold spring, resulting in very poor recruitment with numbers caught being slightly under the original (unrevised) estimates. This example clearly demonstrates that even for groups with a wealth of experience, estimations are just that.

In general, when higher numbers than expected are encountered, the increase should be predominantly in the "fish caught" figure and not in the "fish retained" figure. Two possible exceptions would be when the abundant species is noxious, and therefore must by law be euthanased (e.g., gambusia), or if the abundant species is sensitive and susceptible to stress and early death (e.g., atherinids).

The Principle of Reduction

To summarise then, the principle of Reduction can be found in the following areas:

- reduction in the type and number of by-catch;
- reduction in the number of animals killed for research, by using appropriate techniques in the field;
- working towards Reduction in the future by developing modelling;
- use of stored killed fish from community samples for future species studies;
- requiring accountability from researchers for the number of animals used.

Priorities

We have identified the following areas we would like to see further dialogue or work undertaken in:

- Ongoing and targeted education—ANZCCART Fact Sheets, AEC members and researchers, commercial and recreational fishers.
- Separation of "Fish" into specific groups in the Code such as Teleost/Non bony fish; tropical/temperate; short/long lived. There is a need for discussion on what would be appropriate grouping, but the aim would be to provide the ability to talk about welfare recommendations for certain groups rather than a very large, diverse group.
- Further research into pain and welfare; the relationship between health, injury and welfare; and the role of behavioural expression. This would also provide guidance for further research into humane euthanasia techniques.
- Continued research into modelling and possible Reduction techniques.
- Shared Standard Operating Procedures—best practice for field research not just laboratory fish use or commercial fish use.
- Cohesive legislation with regards to fish and animal welfare. It is possible the Australian Animal Welfare Strategy (AAWS) will provide some solutions in Australia.
- Wider uptake of the use of retained specimens, especially between institutions.

Conclusion

As can be seen from the above discussion the very nature of research into wild fish brings with it dilemmas not normally associated with work on laboratory animals or even most other types of wildlife study, in particular the fact that fish are so diverse (and thus sampling methods and euthanasia techniques that may be suitable for some species, will be far from suitable for others), and the fact that the overall numbers of animals sampled can be extremely large. At Murdoch University, we have therefore attempted to look at ways of reducing our impact on both target species and by-catch and have developed a series of priorities we believe will not only improve the welfare of wild fishes used in research but also potentially aid other AECs and researchers in their work.

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